

Octet® System Data Acquisition User Guide

Release 11.0

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Table of Contents

Chapter 1:	
Welcome.....	11
<i>About Octet Systems</i>	<i>12</i>
<i>Conventions and Symbols Used in This Guide</i>	<i>12</i>
<i>Octet Systems Safety Information.....</i>	<i>13</i>
<i>Getting Started</i>	<i>13</i>
<i>Product Labeling Definitions</i>	<i>14</i>
<i>Consignes de securite des systemes Octet14</i>	
<i>Avant de commencer</i>	<i>14</i>
<i>Définitions de l'étiquetage des produits15</i>	
<i>Sicherheitshinweise für Octet-systeme..</i>	<i>15</i>
<i>Erste Schritte.....</i>	<i>15</i>
<i>Definitionen der</i>	
<i>Produktkennzeichnungen.....</i>	<i>16</i>
<i>Pall ForteBio Technical Support.....</i>	<i>17</i>
Chapter 2:	
Octet System Specifications and Site Requirements	19
<i>Octet RED96 System Specifications and Site Requirements</i>	<i>20</i>
<i>Instrument Identification and Safety Labeling</i>	<i>20</i>
<i>System Specifications</i>	<i>21</i>
<i>Octet RED384 System Specifications and Site Requirements</i>	<i>23</i>
<i>Instrument Identification and Safety Labeling</i>	<i>23</i>
<i>System Specifications</i>	<i>25</i>
<i>Octet QK^e System Specifications and Site Requirements</i>	<i>28</i>
<i>Instrument Identification and Safety Labeling.....</i>	<i>28</i>
<i>System Specifications.....</i>	<i>29</i>
<i>Octet QK System Specifications and Site Requirements</i>	<i>31</i>
<i>Octet QK384 System Specifications and Site Requirements</i>	<i>33</i>
<i>Instrument Identification and Safety Labeling.....</i>	<i>34</i>
<i>System Specifications.....</i>	<i>35</i>
<i>Octet HTX System Specifications and Site Requirements</i>	<i>38</i>
<i>Instrument Identification and Safety Labeling.....</i>	<i>39</i>
<i>System Specifications.....</i>	<i>40</i>
<i>Octet K2 System Specifications and Site Requirements</i>	<i>42</i>
<i>Instrument Identification and Safety Labeling.....</i>	<i>44</i>
<i>System Specifications.....</i>	<i>45</i>
<i>Octet RED96^e System Specifications and Site Requirements</i>	<i>46</i>
<i>Instrument Identification and Safety Labeling.....</i>	<i>48</i>
<i>System Specifications.....</i>	<i>49</i>
<i>Microplate Evaporation Cover.....</i>	<i>51</i>
<i>Intended Use</i>	<i>52</i>

Chapter 3:	Chapter 4:
Getting Started..... 55	21 CFR Part 11 Compliance81
<i>User Safety Guidelines and Warnings ... 56</i>	<i>FDA 21 CFR Part 11 Final Rule Compliance82</i>
<i>Directives et mises en garde relatives à la sécurité des utilisateurs..... 57</i>	<i>Overview of FDA 21 CFR Part 11 Compliance Features83</i>
<i>Sicherheitsrichtlinien und -hinweise für den Benutzer 58</i>	<i>Primary Data Integrity83</i>
<i>Installing Octet Data Acquisition Software59</i>	<i>Administratively Controlled Application Access83</i>
<i>Starting the Octet System and Data Acquisition Software 62</i>	<i>Audit Trail84</i>
<i>Software Overview 62</i>	<i>Administratively Controlled Electronic Signatures.....84</i>
<i>Main Menu and Toolbar 63</i>	<i>Automatic User Log Out (Idle Timeout)85</i>
<i>View Menu..... 65</i>	<i>Passwords.....85</i>
<i>Status Bar 69</i>	<i>Other Data Integrity Features86</i>
<i>Instrument Status Window..... 70</i>	<i>Octet CFR Software Overview.....86</i>
<i>Experiment Wizard 71</i>	<i>ForteBio FB Server Module.....86</i>
<i>Octet System Data Acquisition Options . 71</i>	<i>Selecting a Server Location87</i>
<i>Setting the Plate Temperature 74</i>	<i>Starting a User Session89</i>
<i>Changing the Plate Temperature for Individual Experiments..... 74</i>	<i>Accessing Compliance Features92</i>
<i>Defining a New Default Sample Plate Temperature..... 75</i>	<i>Experiment and Method File Compliance92</i>
<i>Monitoring Experiments Remotely..... 76</i>	<i>Verifying Digital Signatures.....92</i>
<i>Managing Biosensor Types 79</i>	<i>Viewing the Audit Trail.....94</i>
<i>Viewing Available Biosensor Types .. 79</i>	<i>Viewing Event Details.....95</i>
<i>Adding a Biosensor Type 80</i>	<i>Changing Projects During a User Session96</i>
<i>Removing a Biosensor Type 80</i>	<i>Changing Your Password96</i>
	<i>Locking/Unlocking the Application ..97</i>

<i>Logging Off the Application.....</i>	<i>97</i>	<i>Designating Standards</i>	<i>140</i>
<i>Installing the ForteBio FB Server</i>		<i>Designating Unknowns.....</i>	<i>145</i>
<i>Module</i>	<i>99</i>	<i>Designating Controls or Reference</i>	
<i>Database Backup and ForteBio FB Server</i>		<i>Wells</i>	<i>148</i>
<i>Module Upgrade.....</i>	<i>103</i>	<i>Annotating Samples.....</i>	<i>149</i>
<i>Administrator Account Setup</i>	<i>104</i>	<i>Replicate Groups</i>	<i>151</i>
<i>Starting an Administrator User</i>		<i>Managing Sample Plate Definitions ...</i>	<i>155</i>
<i>Session</i>	<i>107</i>	<i>Exporting a Plate Definition.....</i>	<i>155</i>
<i>Accessing Administrator Options... ..</i>	<i>110</i>	<i>Importing a Plate Definition</i>	<i>156</i>
<i>Group Administration.....</i>	<i>120</i>	<i>Printing a Sample Plate Definition .</i>	<i>157</i>
<i>Project Administration.....</i>	<i>123</i>	<i>Managing Assay Parameter Settings ..</i>	<i>158</i>
<i>Constants Tab</i>	<i>125</i>	<i>Modifying Assay Parameter Settings</i>	<i>158</i>
<i>Events Log.....</i>	<i>127</i>	<i>Viewing User-Modifiable Assay Parameter</i>	
<i>Accessing the ForteBio FB Server</i>		<i>Settings</i>	<i>158</i>
<i>Configuration Module</i>	<i>129</i>	<i>Assigning Biosensors to Samples</i>	<i>164</i>
<i>Server Testing.....</i>	<i>130</i>	<i>Biosensor Assignment in Single-Analyte</i>	
<i>Restarting the ForteBio FB Server</i>		<i>Experiments</i>	<i>164</i>
<i>Module</i>	<i>131</i>	<i>Biosensor Assignment in Multiple Analyte</i>	
Chapter 5:		<i>Experiments</i>	<i>167</i>
Quantitation Experiments:		<i>Biosensor Regeneration.....</i>	<i>179</i>
Octet K2 System.....	133	<i>Using Partial Biosensor Trays</i>	<i>180</i>
<i>Introduction.....</i>	<i>134</i>	<i>Reviewing Experiments.....</i>	<i>182</i>
<i>Starting a Quantitation Experiment ...</i>	<i>135</i>	<i>Saving Experiments</i>	<i>182</i>
<i>Starting an Experiment Using the</i>		<i>Running a Quantitation Experiment... ..</i>	<i>184</i>
<i>Experiment Wizard</i>	<i>135</i>	<i>Loading the Biosensor Tray and Sample</i>	
<i>Defining the Sample Plate</i>	<i>137</i>	<i>Plate</i>	<i>184</i>
<i>Designating Samples</i>	<i>137</i>	<i>Starting an Experiment</i>	<i>185</i>
<i>Selecting Wells in the Sample Plate</i>		<i>Run Experiment Window Settings.. ..</i>	<i>187</i>
<i>Map</i>	<i>139</i>		

Stopping an Experiment	191	Starting an Experiment Using the Experiment Wizard	211
Managing Runtime Binding Charts	191	Defining the Sample Plate	213
Opening a Runtime Binding Chart. .	192	Designating Samples	213
Viewing Reference-Subtracted Data	192	Selecting Wells in the Sample Plate Map	215
Viewing Inverted Data	194	Designating Standards	215
Magnifying the Runtime Binding Chart	194	Designating Unknowns	220
Scaling a Runtime Binding Chart ...	195	Designating Controls or Reference Wells	223
Adding a Runtime Binding Chart Title	195	Annotating Samples	224
Selecting a Runtime Binding Chart Legend	195	Replicate Groups	226
Viewing Multiple Runtime Binding Charts	196	Managing Sample Plate Definitions ...	230
Exporting or Printing the Runtime Binding Chart	196	Exporting a Plate Definition	230
Managing Experiment Method Files ...	197	Importing a Plate Definition	231
Custom Quantitation Assays	198	Printing a Sample Plate Definition .	232
Defining a Custom Assay	198	Managing Assay Parameter Settings ..	234
Editing Assay Parameters	200	Modifying Assay Parameter Settings	234
Selecting a Custom Assay	206	Viewing User-Modifiable Assay Parameter Settings	234
Multi-Step Advanced Quantitation Experiments	207	Assigning Biosensors to Samples	239
Chapter 6:		Biosensor Assignment in Single-Analyte Experiments	239
Quantitation Experiments:		Biosensor Assignment in Multiple Analyte Experiments	243
Octet RED96, RED96^e, QK^e and QK. .	209	Biosensor Regeneration	254
Introduction	210	Using Partial Biosensor Trays	255
Starting a Quantitation Experiment ...	211	Reviewing Experiments	257

<i>Saving Experiments</i>	257
<i>Running a Quantitation Experiment</i> ...	259
<i>Loading the Biosensor Tray and Sample Plate</i>	259
<i>Starting an Experiment</i>	261
<i>Run Experiment Window Settings</i> ..	263
<i>Stopping an Experiment</i>	267
<i>Managing Runtime Binding Charts</i>	267
<i>Opening a Runtime Binding Chart</i> ..	268
<i>Viewing Reference-Subtracted Data</i>	268
<i>Viewing Inverted Data</i>	270
<i>Magnifying the Runtime Binding Chart</i>	270
<i>Scaling a Runtime Binding Chart</i> ...	271
<i>Adding a Runtime Binding Chart Title</i>	271
<i>Selecting a Runtime Binding Chart Legend</i>	271
<i>Viewing Multiple Runtime Binding Charts</i>	272
<i>Exporting or Printing the Runtime Binding Chart</i>	272
<i>Managing Experiment Method Files</i> ...	273
<i>Custom Quantitation Assays</i>	274
<i>Defining a Custom Assay</i>	274
<i>Editing Assay Parameters</i>	276
<i>Selecting a Custom Assay</i>	282
<i>Multi-Step Advanced Quantitation Experiments</i>	283

Chapter 7:	
Quantitation Experiments:	
Octet RED384, QK384 and HTX	285
<i>Introduction</i>	286
<i>Starting a Quantitation Experiment</i> ...	287
<i>Starting an Experiment Using the Experiment Wizard</i>	287
<i>Defining the Sample Plate</i>	289
<i>Read Head Configuration and Plate Layout</i>	290
<i>Changing the Plate Format</i>	294
<i>Designating Samples</i>	295
<i>Selecting Wells in the Sample Plate Map</i>	298
<i>Designating Standards</i>	298
<i>Designating Unknowns</i>	303
<i>Designating Controls or Reference Wells</i>	306
<i>Annotating Samples</i>	307
<i>Replicate Groups</i>	309
<i>Managing Sample Plate Definitions</i> ...	313
<i>Exporting a Plate Definition</i>	313
<i>Importing a Plate Definition</i>	314
<i>Printing a Sample Plate Definition</i> .	315
<i>Working with a Reagent Plate</i>	316
<i>Saving a Reagent Plate Definition</i> ..	318
<i>Printing a Reagent Plate Definition</i> .	318
<i>Managing Assay Parameter Settings</i> ..	319

Modifying Assay Parameter Settings	319	Viewing Multiple Runtime Binding Charts	357
Viewing User-Modifiable Assay Parameter Settings	319	Exporting or Printing the Runtime Binding Chart	357
Assigning Biosensors to Samples	325	Managing Experiment Method Files	358
Biosensor Assignment in Single-Analyte Experiments	325	Custom Quantitation Assays	359
Biosensor Assignment in Multiple Analyte Experiments	329	Defining a Custom Assay	359
Biosensor Regeneration	340	Editing Assay Parameters	361
Using Partial Biosensor Trays	341	Selecting a Custom Assay	367
Reviewing Experiments	343	Multi-Step Advanced Quantitation Experiments	368
Saving Experiments	343	Octet RED384 and QK384	368
Running a Quantitation Experiment	345	Octet HTX	369
Loading the Biosensor Tray, Sample and Reagent Plates	345	Chapter 8:	
Starting an Experiment	346	Kinetics Experiments:	
Run Experiment Window Settings	348	Octet K2 System	375
Stopping an Experiment	352	Introduction	376
Managing Runtime Binding Charts	352	Starting a Basic Kinetics Experiment	377
Opening a Runtime Binding Chart	353	Starting an Experiment Using the Experiment Wizard	377
Viewing Reference-Subtracted Data	354	Defining the Sample Plate	378
Viewing Inverted Data	355	Designating Samples	379
Magnifying the Runtime Binding Chart	355	Selecting Wells in the Sample Plate Map	379
Scaling a Runtime Binding Chart	355	Designating Well Types	380
Adding a Runtime Binding Chart Title	356	Entering Sample Information	382
Selecting a Runtime Binding Chart Legend	356	Replicate Groups	387
		Editing the Sample Table	392

<i>Managing Sample Plate Definitions ...</i>	393	<i>Magnifying the Runtime Binding Chart.....</i>	432
<i>Exporting a Plate Definition.....</i>	394	<i>Scaling a Runtime Binding Chart... </i>	432
<i>Importing a Plate Definition.....</i>	395	<i>Adding a Runtime Binding Chart Title</i>	432
<i>Printing a Sample Plate Definition..</i>	396	<i>Selecting a Runtime Binding Chart Legend</i>	432
<i>Defining a Kinetic Assay</i>	397	<i>Viewing Multiple Runtime Binding Charts.....</i>	433
<i>Defining Step Types.....</i>	397	<i>Exporting or Printing the Runtime Binding Chart.....</i>	433
<i>Building an Assay.....</i>	401	<i>Managing Experiment Method Files ...</i>	434
<i>Assigning Biosensors to Samples.....</i>	411	<i>Epitope Binning</i>	435
<i>Reviewing Experiments.....</i>	418	<i>Starting an Experiment</i>	436
<i>Saving Experiments</i>	419	Chapter 9:	
<i>Saving an Experiment to the Template Folder</i>	419	Kinetics Experiments:	
<i>Running a Kinetics Experiment</i>	420	Octet RED96, RED96e, QK^e and QK .	445
<i>Loading the Biosensor Tray and Sample Plate.....</i>	420	<i>Introduction</i>	446
<i>Starting the Experiment.....</i>	420	<i>Starting a Basic Kinetics Experiment ...</i>	447
<i>Run Experiment Window Settings ..</i>	423	<i>Starting an Experiment Using the Experiment Wizard</i>	447
<i>Stopping an Experiment</i>	426	<i>Defining the Sample Plate.....</i>	448
<i>Managing the Runtime Binding Chart .</i>	427	<i>Designating Samples</i>	448
<i>Opening the Runtime Binding Chart</i>	428	<i>Selecting Wells in the Sample Plate Map.....</i>	449
<i>Viewing Reference-Subtracted Data</i>	428	<i>Designating Well Types</i>	450
<i>Viewing Inverted Data</i>	429	<i>Entering Sample Information</i>	451
<i>Aligning Data by a Selected Step ...</i>	429	<i>Replicate Groups</i>	457
<i>Aligning Data to a Specific Time....</i>	430	<i>Editing the Sample Table</i>	462
<i>Extending or Skipping an Assay Step</i>	431		
<i>Terminating a Step to Begin the Next Step</i>	431		

<i>Managing Sample Plate Definitions ...</i>	463	<i>Magnifying the Runtime Binding Chart.....</i>	505
<i>Exporting a Plate Definition.....</i>	464	<i>Scaling a Runtime Binding Chart... </i>	505
<i>Importing a Plate Definition.....</i>	465	<i>Adding a Runtime Binding Chart Title</i>	505
<i>Printing a Sample Plate Definition..</i>	466	<i>Selecting a Runtime Binding Chart Legend</i>	505
<i>Defining a Kinetic Assay</i>	467	<i>Viewing Multiple Runtime Binding Charts.....</i>	506
<i>Defining Step Types.....</i>	467	<i>Exporting or Printing the Runtime Binding Chart.....</i>	506
<i>Building an Assay.....</i>	471	<i>Managing Experiment Method Files ...</i>	507
<i>Assigning Biosensors to Samples.....</i>	481	<i>Epitope Binning</i>	508
<i>Reviewing Experiments.....</i>	490	<i>Starting an Experiment</i>	509
<i>Saving Experiments</i>	491	Chapter 10:	
<i>Saving an Experiment to the Template Folder</i>	492	Kinetics Experiments:	
<i>Running a Kinetics Experiment</i>	492	Octet RED384, QK384 and HTX	517
<i>Loading the Biosensor Tray and Sample Plate.....</i>	492	<i>Introduction</i>	518
<i>Starting the Experiment.....</i>	493	<i>Starting a Basic Kinetics Experiment: Octet RED384 and QK384</i>	519
<i>Run Experiment Window Settings ..</i>	497	<i>Starting an Experiment Using the Experiment Wizard</i>	519
<i>Stopping an Experiment</i>	499	<i>Defining the Sample Plate</i>	520
<i>Managing the Runtime Binding Chart .</i>	500	<i>Managing Sample Plate Definitions</i>	540
<i>Opening the Runtime Binding Chart</i>	501	<i>Working with a Reagent Plate.....</i>	543
<i>Viewing Reference-Subtracted Data</i>	501	<i>Defining a Kinetic Assay</i>	547
<i>Viewing Inverted Data</i>	502	<i>Assigning Biosensors to Samples...</i>	561
<i>Aligning Data by a Selected Step ...</i>	503	<i>Starting a Basic Kinetics Experiment: Octet HTX.....</i>	571
<i>Aligning Data to a Specific Time....</i>	503	<i>Starting an Experiment</i>	571
<i>Extending or Skipping an Assay Step</i>	504		
<i>Terminating a Step to Begin the Next Step</i>	504		

<i>Read Head Configuration and Plate Layout</i>	572	<i>Viewing Multiple Runtime Binding Charts</i>	605
<i>Tab 1 (Plate Definition)</i>	574	<i>Exporting or Printing the Runtime Binding Chart</i>	606
<i>Tab 2 (Assay Definition)</i>	581	<i>Managing Experiment Method Files</i> ...	607
<i>Tab 3 (Sensor Assignment)</i>	588	<i>Epitope Binning</i>	608
<i>Reviewing Experiments</i>	589	<i>Starting an Experiment: Octet RED384 or QK384</i>	608
<i>Saving Experiments</i>	590	<i>Starting an Experiment: Octet HTX</i> .	615
<i>Saving an Experiment to the Template Folder</i>	591	Chapter 11:	
<i>Running a Kinetics Experiment</i>	591	Maintenance	625
<i>Loading the Biosensor Tray, Sample, and Reagent Plates</i>	591	<i>Troubleshooting and Service</i>	626
<i>Starting the Experiment</i>	592	<i>Octet K2, RED96, RED96^e and QK^e Systems</i>	626
<i>Run Experiment Window Settings</i> ..	595	<i>Cleaning the Octet Instrument</i>	626
<i>Stopping an Experiment</i>	599	<i>Emptying the Waste Container</i>	628
<i>Managing the Runtime Binding Chart</i> .	599	<i>Replacing Fuses</i>	629
<i>Opening the Runtime Binding Chart</i>	600	<i>Octet RED384 and Octet QK384 Systems</i>	629
<i>Viewing Reference-Subtracted Data</i>	600	<i>Cleaning the Octet Instrument</i>	629
<i>Viewing Inverted Data</i>	602	<i>Cleaning the Biosensor Pickup Tips</i> .	631
<i>Aligning Data by a Selected Step</i> ...	602	<i>Replacing Fuses</i>	632
<i>Aligning Data to a Specific Time</i>	603	Appendix A:	
<i>Extending or Skipping an Assay Step</i>	603	Using Octet384 and HTX Systems with an Automation Interface	633
<i>Magnifying the Runtime Binding Chart</i>	604	<i>Overview</i>	634
<i>Scaling a Runtime Binding Chart</i> ...	604	<i>Design of the Automation Interface</i> ...	634
<i>Adding a Runtime Binding Chart Title</i>	605	<i>Automation Interface Control Setup</i>	634
<i>Selecting a Runtime Binding Chart Legend</i>	605		

<i>Automation Client Example</i>	
<i>Application</i>	<i>636</i>
<i>Automation Commands.....</i>	<i>637</i>
<i>Typical Automation Session.....</i>	<i>639</i>
<i>Advanced Automation Session.....</i>	<i>641</i>
<i>Automation API.H</i>	<i>644</i>
<i>Analysis Automation API.....</i>	<i>649</i>

CHAPTER 1:

Welcome



About Octet Systems	12
Conventions and Symbols Used in This Guide	12
Octet Systems Safety Information	13
Consignes de securite des systemes Octet	14
Sicherheitshinweise für Octet-systeme	15
Pall ForteBio Technical Support	17

ABOUT OCTET SYSTEMS

Octet systems enable real-time quantitation or kinetic characterization of biomolecular interactions. Each system includes:

- Octet instrument
- Computer
- Hardware accessories
- Octet Software Modules—Data Acquisition and Data Analysis For more details on the Data Analysis software, see the *Octet System Data Analysis User Guide*.

Table 1-1: Octet System Software Functions

Octet Software	Functions
Data Acquisition 	<ul style="list-style-type: none"> • Define quantitation or kinetic experiments and save them for future use. • Define custom assays. • Run experiments and acquire binding data. • View and save binding data.
Data Analysis 	<ul style="list-style-type: none"> • Analyze binding data and view analysis results. • Export or copy analysis results. • Generate reports of quantitation or kinetic results.

For information on preparing samples for quantitation or kinetics experiments, please see the appropriate Pall ForteBio biosensor product instructions.

CONVENTIONS AND SYMBOLS USED IN THIS GUIDE



NOTE: Presents pertinent details on a topic. For example, general information, tips or alternate options.






IMPORTANT: Indicates the assay or procedure will not work if the guidelines provided are not properly followed.



WARNING & CAUTION: *Inform the user that specific actions could cause irreversible consequences or damage. To prevent hazards, the manual should be read before operating the equipment.*

Table 1: Octet Instrument Labels

Symbol	Definition
	Electrical hazard Danger électrique Elektrische Gefahr
	Heat/hot Chaleur/Chaud Hitze/Heiß
	Fuse Fusible Sicherung

OCTET SYSTEMS SAFETY INFORMATION

Getting Started

All users must read the following safety information.



WARNING: *Do not operate the Octet system in any other way than described in the user manual. Failure to comply may expose you to hazards that can lead to personal injury and may cause damage to the equipment.*









WARNING: *Octet systems should only be installed, relocated, and/or moved by trained Pall ForteBio personnel. To obtain more information, please contact Pall ForteBio Technical Support. Failure to comply with these instructions voids any existing warranty or service contract agreements. Pall ForteBio is not responsible for personal injury or damages caused by unqualified personnel installing, relocating and/or moving an Octet system.*

For more information on and safety precautions for the supplied computer and computer equipment, please refer to the manufacturer's documentation supplied with the computer packaging.

Product Labeling Definitions

Table 2: Label Definitions

Symbol	Definition
	The system complies with applicable European directives.
	The system complies with the requirements for electromagnetic compliance (EMC) in Australia and New Zealand.
	The electromagnetic interference from this system is under limits approved by the Federal Communications Commission (United States).
	Electrical and electronic equipment must not be disposed of as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of equipment.
	High voltage; potential electrical shock hazard.
	Keep hands clear of moving parts.

CONSIGNES DE SECURITE DES SYSTEMES OCTET

Avant de commencer

Tous les utilisateurs sont tenus de lire impérativement les consignes de sécurité suivantes.



WARNING: N'utilisez pas le système Octet pour un usage autre que celui décrit dans le manuel utilisateur. Le non-respect de cette consigne peut vous exposer à des risques susceptibles d'occasionner des blessures et d'endommager votre équipement.









WARNING: Seul le personnel qualifié de Pall ForteBio est habilité à installer, déménager et/ou transférer les systèmes Octet. Pour plus d'informations, veuillez contacter l'assistance technique de Pall ForteBio. Le non-respect de ces consignes pourra conduire à l'annulation de votre contrat de garantie ou d'assistance. Pall ForteBio décline toute responsabilité en cas de blessures ou de dommages consécutifs à une installation, un déménagement et/ou transfert d'un système Octet effectués par du personnel non qualifié.

Pour plus d'informations sur les mesures de sécurité concernant l'ordinateur et l'équipement informatique fournis, veuillez consulter la documentation du fabricant jointe à l'emballage du produit.

Définitions de l'étiquetage des produits

Table 3: Label Definitions

Symbole	Définition
	Ce système est conforme aux directives européennes en vigueur.
	Ce système répond aux exigences relatives à la compatibilité électromagnétique (CEM) en vigueur en Australie et en Nouvelle-Zélande.
	Les interférences électromagnétiques émises par ce système se situent dans les limites approuvées par la Federal Communications Commission (Commission fédérale des communications) américaine.
	Les équipements électriques et électroniques ne doivent pas être jetés comme des déchets municipaux non triés ; ils doivent faire l'objet d'une collecte sélective. Pour toute information concernant le démantèlement de vos équipements, veuillez contacter un représentant agréé.
	Haute tension : risque potentiel de choc électrique.
	Ne touchez pas les pièces mobiles.

SICHERHEITSHINWEISE FÜR OCTET-SYSTEME

Erste Schritte

Die folgenden Sicherheitshinweise sind von jedem Benutzer zu lesen.



WARNING: Bedienen Sie das Octet-System nur wie im Benutzerhandbuch beschrieben. Eine Missachtung kann Sie Gefahren aussetzen, die zu Personen- und Sachschäden führen können.









WARNING: Octet-Systeme sollten nur durch geschultes Personal von Pall ForteBio installiert, umgelagert und/oder bewegt werden. Für weitere Informationen wenden Sie sich bitte an den technischen Support von Pall ForteBio. Durch Nichtbeachtung dieser Hinweise werden alle bestehenden Gewährleistungen oder Dienstleistungsvereinbarungen nichtig. Pall ForteBio übernimmt keine Verantwortung für Personen- oder Sachschäden, die infolge der Installation, Umlagerung und/oder Bewegung eines Octet-Systems durch ungeschultes Personal entstehen.

Weitere Informationen und Sicherheitsmaßnahmen für den im Lieferumfang enthaltenen Computer samt Computerzubehör finden Sie in der Herstellerdokumentation, die mit der Computerverpackung geliefert wurde.

Definitionen der Produktkennzeichnungen

Table 4: Label Definitions

Symbol	Definition
	Das System erfüllt die geltenden europäischen Richtlinien.
	Das System erfüllt die Anforderungen für elektromagnetische Verträglichkeit (EMV) in Australien und Neuseeland.
	Die elektromagnetische Störausstrahlung dieses Systems unterschreitet die von der Federal Communications Commission (Vereinigte Staaten) genehmigten Grenzwerte.
	Elektrische und elektronische Geräte dürfen nicht mit dem gewöhnlichen, unsortierten Hausmüll entsorgt werden, sondern sind getrennt zu entsorgen. Informationen zur Stilllegung der Geräte erhalten Sie von einem autorisierten Vertreter des Herstellers.
	Hochspannung; Stromschlaggefahr.
	Hände von beweglichen Teilen fernhalten.

PALL FORTEBIO TECHNICAL SUPPORT

You can contact Pall ForteBio technical support at:

Pall ForteBio, LLC
47661 Fremont Boulevard
Fremont, CA 94538
USA
Tel: +1-650-322-1360
Fax: +1-650-322-1370
E-mail: fortebio_support@pall.com

CHAPTER 2:

Octet System Specifications and Site Requirements

Octet RED96 System Specifications and Site Requirements	20
Octet RED384 System Specifications and Site Requirements	23
Octet QK ^e System Specifications and Site Requirements	28
Octet QK System Specifications and Site Requirements	31
Octet QK384 System Specifications and Site Requirements	33
Octet HTX System Specifications and Site Requirements	38
Octet K2 System Specifications and Site Requirements	42
Octet RED96 ^e System Specifications and Site Requirements	46
Microplate Evaporation Cover	51

OCTET RED96 SYSTEM SPECIFICATIONS AND SITE REQUIREMENTS

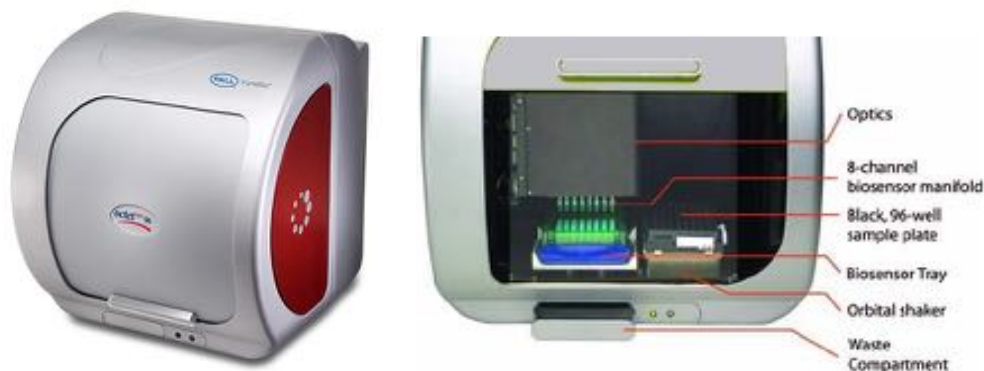


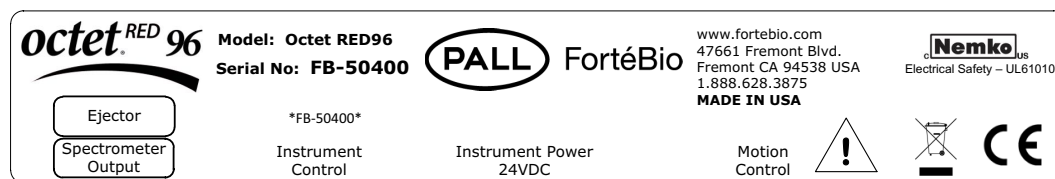
Figure 2-1: Octet RED96 Instrument—Door Closed (Left) or Open (Right)



IMPORTANT: Using 96-well half-area plates on the Octet RED96 system will result in non-optimal system performance. Pall ForteBio cannot guarantee results within the optimal performance specifications of the system when these plates are used.

Instrument Identification and Safety Labeling

Please see “Octet Systems Safety Information” on page 13 for definitions of symbols.



Octet RED96 System Rear Panel Label

System Specifications

Table 2-1: Octet RED96 System Specifications

Item	Description
Equipment Classifications	<ul style="list-style-type: none"> Product Classification: Class 1: Detachable power cord Installation/Overvoltage Category: Category II Pollution Degree: Degree 2 EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}
Environmental	<ul style="list-style-type: none"> Storage Temperature: -20 to 70 °C Optimum Operating Temperature: 22 ± 4 °C Safe Operating Temperature: 15 to 30 °C Humidity: Non-condensing, 10 to 80% Relative Humidity Indoor Use Only Operating Altitude: 0 to 2,000 meters Not for use in an environment with an explosive atmosphere
Compliance	<ul style="list-style-type: none"> Nemko NRTL/C CE compliance as indicated on the Instrument Identification and Safety Label.
Capabilities	<ul style="list-style-type: none"> Protein quantitation Kinetic and affinity analyses (k_{obs}, k_a, k_d, K_D) Binding specificity and cooperativity Kinetic screening of proteins, peptides, and other biomolecules Small molecule and fragments screening and kinetic analysis Recommended analyte molecular weight of 150 Da or higher
Sampling Format	<ul style="list-style-type: none"> Required plate: 96-well, black, flat bottom polypropylene microplate (Greiner Bio-One, #655209) or similar, SBS standard microplate Single sample plate capacity
Sampling Volume	180–220 µL/well (96-well plate)
Sample Types	Purified samples, common culture media, crude lysates

Table 2-1: Octet RED96 System Specifications (Continued)

Item	Description
Biosensor Type	Disposable, single-use fiber optic biosensors with optional reuse by regeneration and/or re-racking
Biosensor Tray Type	8 x 12 format 96-biosensor tray, green color
Optics and Mechanics	<ul style="list-style-type: none"> 8-channel biosensor manifold Optical interferometer Eight spectrometers (one dedicated spectrometer per biosensor)
Throughput	<ul style="list-style-type: none"> Up to 8 biosensors in parallel, maximum of 96 tests unattended One 96-well plate and one biosensor tray at once
Orbital Flow Capacity	Static or 100–1,500 rpm
Temperature Range	(Ambient + 4 °C)–40 °C, 1 °C increments
Dimensions	18.6" H x 17" W x 20.8" D (47 cm H x 43 cm W x 53 cm D)
Weight	63 lb (28.6 kg)
Electrical Requirements	<ul style="list-style-type: none"> Mains: 100-120/200-240 VAC, 50/60 Hz, 4 A max Power consumption: 120 W (240 W peak)



IMPORTANT: Use the power cords provided by Pall ForteBio or a suitable AC cord with ratings of 60 C, 300 V, 16 AWG or better.

OCTET RED384 SYSTEM SPECIFICATIONS AND SITE REQUIREMENTS

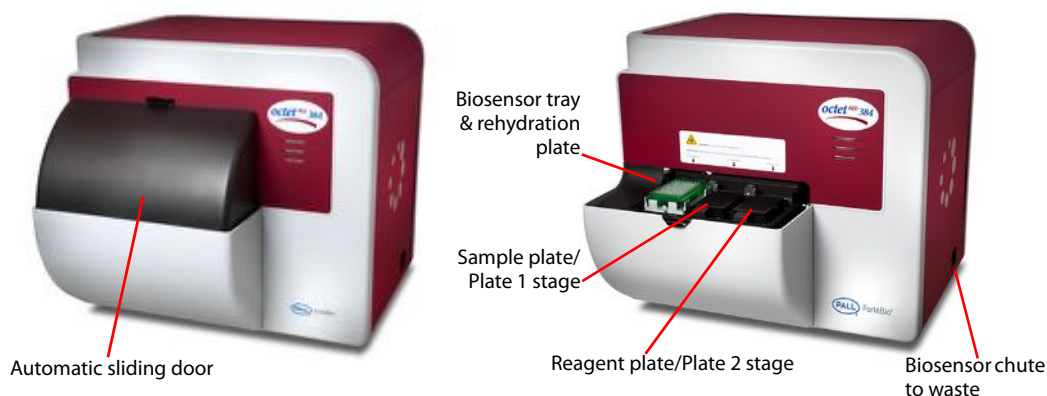


Figure 2-2: Octet RED384 Instrument—Door Closed (Left) or Open (Right).



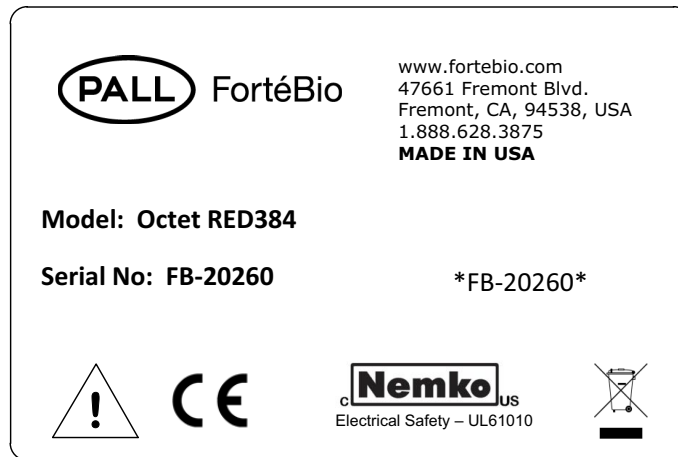
NOTE: In Octet Data Acquisition software Release 8.0 or later, the Sample plate and Reagent plate are referred to as Plate 1 and Plate 2.



WARNING: Movement of the instrument presents a high risk of system damage and risk of personal injury, and should only be performed by qualified Pall ForteBio service personnel. To obtain more information, please contact Pall ForteBio technical support. Failure to comply with these instructions voids any existing warranty or service contract agreements. Pall ForteBio is not responsible for personal injury or damages caused by unqualified personnel relocating and/or moving the system.

Instrument Identification and Safety Labeling

Please see “Octet Systems Safety Information” on page 13 for definitions of symbols.



Octet RED384 System Rear Panel Label

System Specifications

Table 2-2: Octet RED384 System Specifications

Item	Description
Equipment Classifications	<ul style="list-style-type: none"> • Product Classification: Class 1: Detachable power cord • Installation/Overvoltage Category: Category II • Pollution Degree: Degree 2 • EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}
Environmental	<ul style="list-style-type: none"> • Storage Temperature: -20 to 70 °C • Optimum Operating Temperature: 22 ± 4 °C • Safe Operating Temperature: 15 to 30 °C • Humidity: Non-condensing, 10 to 80% Relative Humidity • Indoor Use Only • Operating Altitude: 0 to 2,000 meters • Not for use in an environment with an explosive atmosphere
Compliance	<ul style="list-style-type: none"> • Nemko NRTL/C • CE compliance as indicated on the Instrument Identification and Safety Label.
Capabilities	<ul style="list-style-type: none"> • Protein quantitation • Kinetic and affinity analyses (k_{obs}, k_a, k_d, K_D) • Binding specificity and cooperativity • Kinetic screening • Small molecule kinetic analysis

Table 2-2: Octet RED384 System Specifications (Continued)

Item	Description
Sampling Format	<ul style="list-style-type: none"> Required plates: <ul style="list-style-type: none"> 96-well, black, flat bottom polypropylene microplate (Greiner Bio-One, #655209) or similar, SBS standard microplate 384-well black, flat-bottom polypropylene (Greiner Bio-One, #781209) 384-well black, tilted-bottom polypropylene (Pall ForteBio, #18-5076 or #18-5080), SBS standard microplate Two plate stations Test volume: <ul style="list-style-type: none"> 180–300 μL in a 96-well plate, non-destructive and recoverable 80–130 μL in a 384-well plate, non-destructive and recoverable 40–100 μL in a 384-well tilted bottom microplate (384TW), non-destructive and recoverable
Sample Types	Purified samples, common culture media, crude lysates
Biosensor Type	Disposable, single-use fiber optic biosensors with optional reuse by regeneration and/or re-racking
Biosensor Tray Type	8 x 12 format 96-biosensor tray, green color
Automation	<ul style="list-style-type: none"> Up to 16 biosensors in parallel Ability to integrate the Octet instrument with a laboratory-automated robotic system for automated plate and biosensor tray handling
Optics and Mechanics	<ul style="list-style-type: none"> 16-channel biosensor manifold Optical interferometer Sample plate platform temperature range: from 4 °C above ambient to 40 °C 16 spectrometers (one dedicated spectrometer per biosensor)
Throughput	<ul style="list-style-type: none"> Up to 16 biosensors in parallel, maximum of 384 tests unattended Two microplates, either 96- or 384-well at once. Only one plate can be used for samples. The second plate is used for reagents.

Table 2-2: Octet RED384 System Specifications (Continued)

Item	Description
Orbital Flow Capacity	Static or 100–1,500 rpm
Dimensions	30.1" H x 31.5" W x 31.4" D (76.5 cm H x 80 cm W x 79.8 cm D)
Weight	150 lb (68 kg)
Electrical Requirements	<ul style="list-style-type: none"> Mains: 100-120/200-240 VAC, 50/60 Hz, 4 A max Power consumption: 195 W (240 W peak)



IMPORTANT: Use the power cords provided by Pall ForteBio or a suitable AC cord with ratings of 60 C, 300 V, 16 AWG or better.

Table 2-3: Sensor Offset and Well Volumes for Octet RED384 and Octet QK384

Sensor Offset (mm)	Recommended Minimum Fill Volume (μL)		
	96-well plate (Greiner Bio-One)	384-well plate (Greiner Bio-One)	384-well tilted bottom plate (Pall ForteBio, 384TW)
3	200	80	40
4	200	80	60
5	225	100	80
6	250	120	100
7	300	130	100

OCTET QK^e SYSTEM SPECIFICATIONS AND SITE REQUIREMENTS

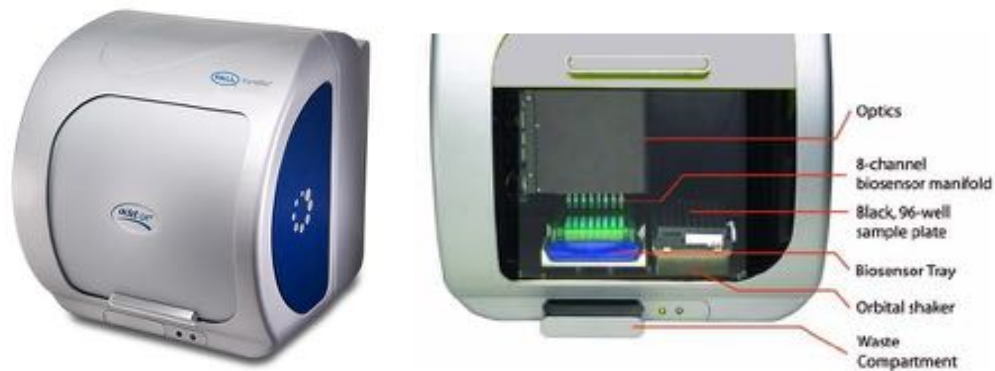
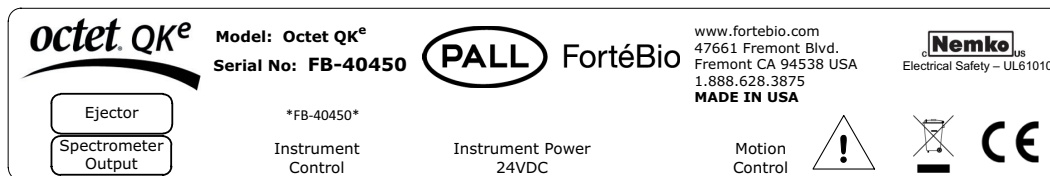


Figure 2-3: Octet QK^e Instrument—Door Closed (Left) or Open (Right)

Instrument Identification and Safety Labeling

Please see “Octet Systems Safety Information” on page 13 for definitions of symbols.



Octet QK^e System Rear Panel Label

System Specifications

Table 2-4: Octet QK^e System Specifications

Item	Description
Equipment Classifications	<ul style="list-style-type: none"> Product Classification: Class 1: Detachable power cord Installation/Overvoltage Category: Category II Pollution Degree: Degree 2 EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}
Environmental	<ul style="list-style-type: none"> Storage Temperature: -20 to 70 °C Optimum Operating Temperature: 22 ± 4 °C Safe Operating Temperature: 15 to 30 °C Humidity: Non-condensing, 10 to 80% Relative Humidity Indoor Use Only Operating Altitude: 0 to 2,000 meters Not for use in an environment with an explosive atmosphere
Compliance	<ul style="list-style-type: none"> Nemko NRTL/C CE compliance as indicated on the Instrument Identification and Safety Label.
Capabilities	<ul style="list-style-type: none"> Protein quantitation Kinetic and affinity analyses (k_{obs}, k_a, k_d, K_D) Binding specificity and cooperativity Kinetic screening of proteins, peptides and other biomolecules Biosensor re-racking Recommended analyte molecular weight of 5,000 Da or higher
Sampling Format	<ul style="list-style-type: none"> Required plate: 96-well, black, flat bottom polypropylene microplate (Greiner Bio-One, #655209), SBS standard microplate Single sample plate capacity
Sample Volume	180–220 µL/well (96-well plate)

Table 2-4: Octet QK[®] System Specifications (Continued)

Item	Description
Sample Types	Purified samples, common culture media, crude lysates
Biosensor Type	Disposable, single-use fiber optic biosensors with optional reuse by regeneration and/or re-racking
Biosensor Tray Type	8 x 12 format 96-biosensor tray, green color
Optics and Mechanics	<ul style="list-style-type: none"> 8-channel biosensor manifold Optical interferometer One spectrometer (shared by eight biosensors)
Throughput	<ul style="list-style-type: none"> Up to eight biosensors in parallel, maximum of 96 tests unattended One 96-well plate and one biosensor tray at once
Orbital Flow Capacity	Static or 100–1,500 rpm
Temperature Range	(Ambient + 4 °C)–40 °C, 1 °C increments
Dimensions	18.6" H x 17" W x 20.8" D (47 cm H x 43 cm W x 53 cm D)
Weight	54 lb (24.5 kg)
Electrical Requirements	<ul style="list-style-type: none"> Mains: 100-120/200-240 VAC, 50/60 Hz, 4 A max Power consumption: 120 W (240 W peak)



IMPORTANT: Use the power cords provided by Pall ForteBio or a suitable AC cord with ratings of 60 C, 300 V, 16 AWG or better.

OCTET QK SYSTEM SPECIFICATIONS AND SITE REQUIREMENTS



Figure 2-4: Octet QK Instrument—Door Closed (Left) or Open (Right)


Table 2-5: Octet QK System Specifications

Item	Description
Equipment Classifications	<ul style="list-style-type: none">• Product Classification: Class 1: Detachable power cord• Installation/Overvoltage Category: Category II• Pollution Degree: Degree 2• EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}
Environmental	<ul style="list-style-type: none">• Storage Temperature: -20 to 70 °C• Optimum Operating Temperature: 22 ± 4 °C• Safe Operating Temperature: 15 to 30 °C• Humidity: Non-condensing, 10 to 80% Relative Humidity• Indoor Use Only• Operating Altitude: 0 to 2,000 meters• Not for use in an environment with an explosive atmosphere
Compliance	<ul style="list-style-type: none">• CSA• CE compliance as indicated on the Instrument Identification and Safety Label.

Table 2-5: Octet QK System Specifications (Continued)

<i>Item</i>	<i>Description</i>
Capabilities	<ul style="list-style-type: none"> Protein quantitation Kinetic and affinity analyses (k_{obs}, k_a, k_d, K_D) Binding specificity and cooperativity Kinetic screening of proteins, peptides, and other biomolecules Recommended analyte molecular weight of 10,000 Da or higher
Sampling Format	<ul style="list-style-type: none"> Required plate: 96-well, black, flat bottom polypropylene microplate (Greiner Bio-One, #655209), SBS standard microplate Single sample plate capacity
Sample Volume	180–220 μ L/well (96-well plate)
Sample Types	Purified samples, common culture media, crude lysates
Biosensor Type	Disposable, single-use fiber optic biosensors with optional reuse by regeneration
Biosensor Tray Type	8 x 12 format 96-biosensor tray, green color
Optics and Mechanics	<ul style="list-style-type: none"> 8-channel biosensor manifold Optical interferometer One spectrometer (shared by eight biosensors)
Throughput	<ul style="list-style-type: none"> Up to 8 biosensors in parallel, maximum of 96 tests unattended One 96-well plate and one biosensor tray at once
Orbital Flow Capacity	Static or 100–1,500 rpm
Temperature Range	(Ambient + 4 °C)–40 °C, 1 °C increments
Dimensions	18.6" H x 17" W x 20.8" D (47 cm H x 43 cm W x 53 cm D)
Weight	50 lb (23 kg)
Electrical Requirements	<ul style="list-style-type: none"> Mains: 100-120/200-240 VAC, 50/60 Hz, 4 A max Power consumption: 120 W (240 W peak)

Table 2-5: Octet QK System Specifications (Continued)

Item	Description
	IMPORTANT: Use the power cords provided by Pall ForteBio or a suitable AC cord with ratings of 60 C, 300 V, 16 AWG or better.

OCTET QK384 SYSTEM SPECIFICATIONS AND SITE REQUIREMENTS

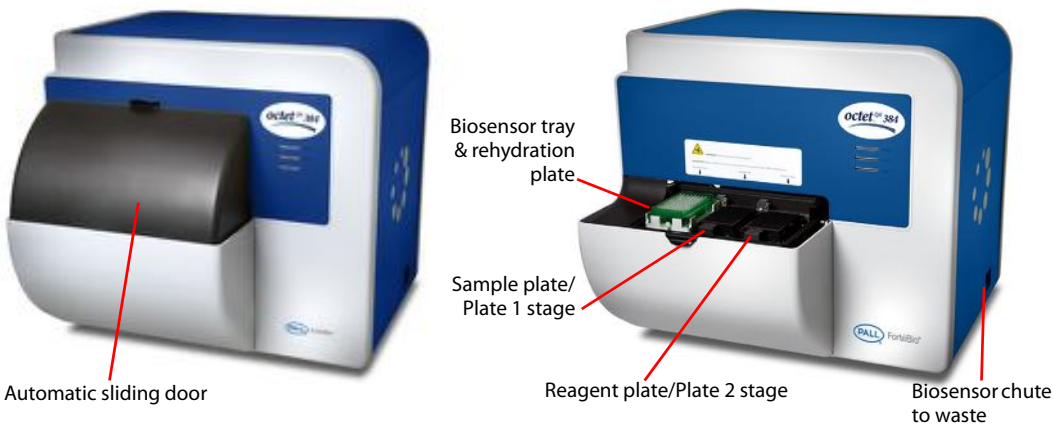



Figure 2-5: Octet QK384 Instrument—Door Closed (Left) or Open (Right)

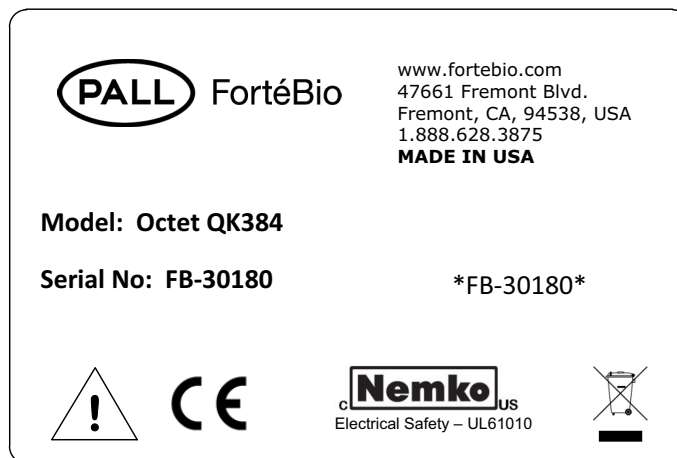
	NOTE: In Octet Data Acquisition software Release 8.0 or later, the Sample plate and Reagent plate are referred to as Plate 1 and Plate 2.
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WARNING: Movement of the instrument presents a high risk of system damage and risk of personal injury, and should only be performed by qualified Pall ForteBio service personnel. To obtain more information, please contact Pall ForteBio technical support. Failure to comply with these instructions voids any existing warranty or service contract agreements. Pall ForteBio is not responsible for personal injury or damages caused by unqualified personnel relocating and/or moving the system.

Instrument Identification and Safety Labeling

Please see “Octet Systems Safety Information” on page 13 for definitions of symbols.



Octet QK384 System Rear Panel Label

System Specifications

Table 2-6: Octet QK384 System Specifications

Item	Description
Equipment Classifications	<ul style="list-style-type: none"> • Product Classification: Class 1: Detachable power cord • Installation/Overvoltage Category: Category II • Pollution Degree: Degree 2 • EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}
Environmental	<ul style="list-style-type: none"> • Storage Temperature: -20 to 70 °C • Optimum Operating Temperature: 22 ± 4 °C • Safe Operating Temperature: 15 to 30 °C • Humidity: Non-condensing, 10 to 80% Relative Humidity • Indoor Use Only • Operating Altitude: 0 to 2,000 meters • Not for use in an environment with an explosive atmosphere
Compliance	<ul style="list-style-type: none"> • Nemko NRTL/C • CE compliance as indicated on the Instrument Identification and Safety Label.
Capabilities	<ul style="list-style-type: none"> • Protein quantitation • Kinetic and affinity analyses (k_{obs}, k_a, k_d, K_D) • Binding specificity and cooperativity • Kinetic screening

Table 2-6: Octet QK384 System Specifications (Continued)

Item	Description
Sampling Format	<ul style="list-style-type: none"> Required plates: <ul style="list-style-type: none"> 96-well, black, flat bottom polypropylene microplate (Greiner Bio-One, #655209) or similar, SBS standard microplate 384-well black, flat-bottom polypropylene (Greiner Bio-One, #781209) 384-well black, tilted-bottom polypropylene microplate (Pall ForteBio, #18-5076 or #18-5080), SBS standard microplate Two plate stations Test volume: <ul style="list-style-type: none"> 180–300 μL in a 96-well plate, non-destructive and recoverable 80–130 μL in a 384-well plate, non-destructive and recoverable 40–100 μL in a 384-well tilted bottom microplate (384TW), non-destructive and recoverable
Sample Types	Purified samples, common culture media, crude lysates
Biosensor Type	Disposable, single-use fiber optic biosensors with optional reuse by regeneration and/or re-racking
Biosensor Tray Type	8 x 12 format 96-biosensor tray, green color
Automation	<ul style="list-style-type: none"> Up to 16 biosensors in parallel Ability to integrate the Octet instrument with a laboratory-automated robotic system for automated plate and biosensor tray handling
Optics and Mechanics	<ul style="list-style-type: none"> 16-channel biosensor manifold Optical interferometer Sample plate platform temperature range: From 4 °C above ambient to 40 °C 2 spectrometers (one dedicated spectrometer per eight biosensors)
Throughput	<ul style="list-style-type: none"> Up to 16 biosensors in parallel, maximum of 384 tests unattended Two microplates, either 96- or 384-well at once. Only one plate can be used for samples. The second plate is used for reagents.

Table 2-6: Octet QK384 System Specifications (Continued)

Item	Description
Orbital Flow Capacity	Static or 100–1,500 rpm
Dimensions	30.1" H x 31.5" W x 31.4" D (76.5 cm H x 80 cm W x 79.8 cm D)
Weight	150 lb (68 kg)
Electrical Requirements	<ul style="list-style-type: none"> Mains: 100-120/200-240 VAC, 50/60 Hz, 4 A max Power consumption: 195 W (240 W peak)



IMPORTANT: Use the power cords provided by Pall ForteBio or a suitable AC cord with ratings of 60 C, 300 V, 16 AWG or better.

Table 2-7: Sensor Offset and Well Volumes for Octet RED384 and Octet QK384

Sensor Offset (mm)	Recommended Minimum Fill Volume (μL)		
	96-well plate (Greiner Bio-One)	384-well plate (Greiner Bio-One)	384-well tilted bottom plate (Pall ForteBio, 384TW)
3	200	80	40
4	200	80	60
5	225	100	80
6	250	120	100
7	300	130	100

OCTET HTX SYSTEM SPECIFICATIONS AND SITE REQUIREMENTS

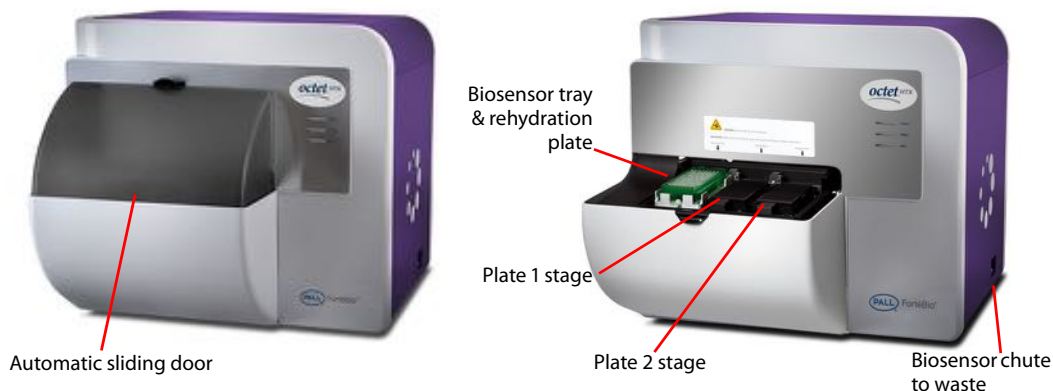


Figure 2-6: Octet HTX Instrument—Door Closed (Left) or Open (Right).



NOTE: In Octet Data Acquisition software Release 8.0 or later, the Sample plate and Reagent plate are referred to as Plate 1 and Plate 2.



WARNING: Movement of the instrument presents a high risk of system damage and risk of personal injury, and should only be performed by qualified Pall ForteBio service personnel. To obtain more information, please contact Pall ForteBio technical support. Failure to comply with these instructions voids any existing warranty or service contract agreements. Pall ForteBio is not responsible for personal injury or damages caused by unqualified personnel relocating and/or moving the system.

Instrument Identification and Safety Labeling

Please see “Octet Systems Safety Information” on page 13 for definitions of symbols.



Octet HTX System Rear Panel Label

System Specifications

Table 2-8: Octet HTX System Specifications

Item	Description
Equipment Classifications	<ul style="list-style-type: none"> Product Classification: Class 1: Detachable power cord Installation/Overvoltage Category: Category II Pollution Degree: Degree 2 EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}
Environmental	<ul style="list-style-type: none"> Storage Temperature: -20 to 70 °C Optimum Operating Temperature: 22 ± 4 °C Safe Operating Temperature: 15 to 30 °C Humidity: Non-condensing, 10 to 80% Relative Humidity Indoor Use Only Operating Altitude: 0 to 2,000 meters Not for use in an environment with an explosive atmosphere
Compliance	<ul style="list-style-type: none"> Nemko NRTL/C, CB Scheme CE compliance as indicated on the Instrument Identification and Safety Label.
Capabilities	<ul style="list-style-type: none"> Protein quantitation Kinetic and affinity analyses (k_{obs}, k_a, k_d, K_D) Binding specificity and cooperativity Kinetic screening Small molecule kinetic analysis

Table 2-8: Octet HTX System Specifications (Continued)

<i>Item</i>	<i>Description</i>
Sampling Format	<ul style="list-style-type: none"> Required plates: <ul style="list-style-type: none"> 96-well, black, flat bottom polypropylene microplate (Greiner Bio-One, #655209) or similar, SBS standard microplate 384-well black, flat-bottom polypropylene (Greiner Bio-One, #781209) 384-well black, tilted-bottom polypropylene (Pall ForteBio, #18-5076 or #18-5080), SBS standard microplate Two plate stations Test volume: <ul style="list-style-type: none"> 180–300 μL in a 96-well plate, non-destructive and recoverable 80–130 μL in a 384-well plate, non-destructive and recoverable 40–100 μL in a 384-well tilted bottom microplate (384TW), non-destructive and recoverable
Sample Types	Purified samples, common culture media, crude lysates
Biosensor Type	Disposable, single-use fiber optic biosensors with optional reuse by regeneration and/or re-racking
Biosensor Tray Type	8 x 12 format 96-biosensor tray, green color
Automation	<ul style="list-style-type: none"> Up to 96 biosensors in parallel Ability to integrate the Octet instrument with a laboratory-automated robotic system for automated plate and biosensor tray handling
Optics and Mechanics	<ul style="list-style-type: none"> 8, 16, 32, 48 and 96-channel biosensor manifold Optical interferometer Sample plate platform temperature range: from 4 °C above ambient to 40 °C 16 spectrometers (selectable: one dedicated spectrometer per biosensor, up to one dedicated spectrometer per six biosensors).
Throughput	<ul style="list-style-type: none"> Up to 96 biosensors in parallel, maximum of 384 tests unattended. Two microplates, either 96- or 384-well at once. Either or both plates may be used for samples or reagents.

Table 2-8: Octet HTX System Specifications (Continued)

Item	Description
Orbital Flow Capacity	Static or 100–1,500 rpm
Dimensions	30.1" H x 31.5" W x 31.4" D (76.5 cm H x 80 cm W x 79.8 cm D)
Weight	200 lb (90.7 kg)
Electrical Requirements	<ul style="list-style-type: none"> Mains: 100-120/200-240 VAC, 50/60 Hz, 4 A max Power consumption: 195 W (240 W peak)



IMPORTANT: Use the power cords provided by Pall ForteBio or a suitable AC cord with ratings of 60 C, 300 V, 16 AWG or better.

Table 2-9: Sensor Offset and Well Volumes for the Octet HTX System

Sensor Offset (mm)	Recommended Minimum Fill Volume (μL)		
	96-well plate (Greiner Bio-One)	384-well plate (Greiner Bio-One)	384-well tilted bottom plate (Pall ForteBio, 384TW)
3	200	80	40
4	200	80	60
5	225	100	80
6	250	120	100
7	300	130	100

OCTET K2 SYSTEM SPECIFICATIONS AND SITE REQUIREMENTS

The Octet K2 System is a benchtop instrument that should be installed on a standard, non-flammable laboratory bench with a sufficient weight capacity.

The shipping weight of the Octet K2 system (instrument, computer, and accessories ship together) is 180 lbs (81.6 kg), and measures 48" x 32" x 46" (121.9 cm x 81.3 cm x 116.8 cm).

Contents of the system as shipped include:

- The Octet K2 instrument
- Package of 10 disposable tray liners for spent biosensors
- Software Installation CD

- Instrument Settings Backup CD
- Octet mouse pad
- Pall ForteBio Software License agreement
- Communication cable set to connect instrument to computer workstation
- Dell computer with included Dell power cord, mouse, keyboard, and monitor connection adapters
- Dell monitor with monitor cables and power cord
- Instrument power cord or cords dependent on end user country

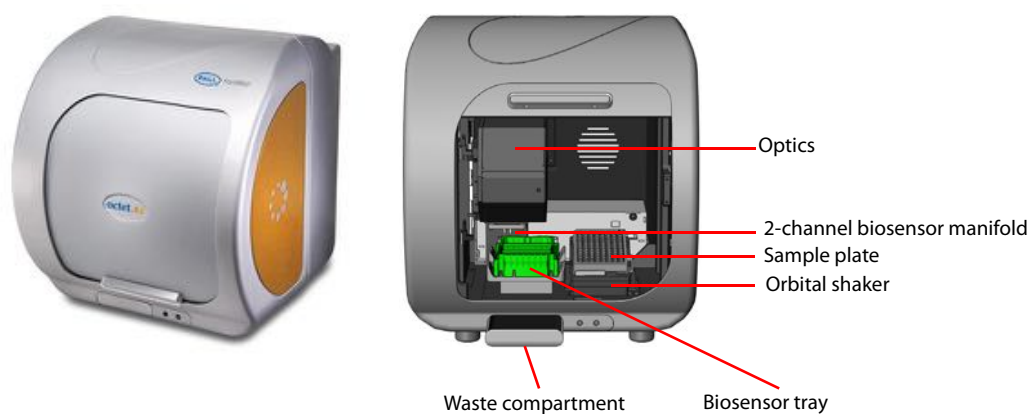


Figure 2-7: Octet K2 Instrument—Door Closed (Left) or Open (Right).

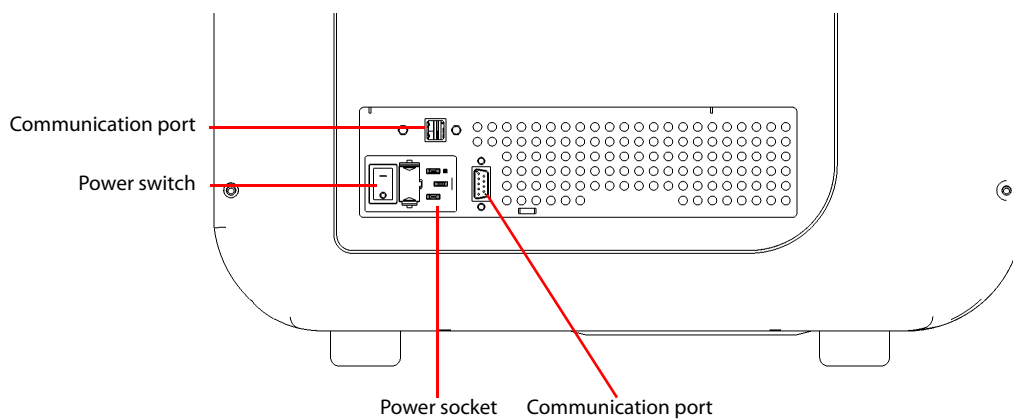


Figure 2-8: Octet K2 Instrument — Rear view



WARNING: Movement of the instrument presents a high risk of system damage and risk of personal injury, and should only be performed by qualified Pall ForteBio service personnel. To obtain more information, please contact Pall ForteBio technical support. Failure to comply with these instructions voids any existing warranty or service contract agreements. Pall ForteBio is not responsible for personal injury or damages caused by unqualified personnel relocating and/or moving the system.

Instrument Identification and Safety Labeling

Please see "Octet Systems Safety Information" on page 13 for definitions of symbols.




Figure 2-9: Octet K2 System Rear Panel Label

System Specifications

Table 2-10: Octet K2 System Specifications

Item	Description
Equipment Classifications	<ul style="list-style-type: none"> Product Classification: Class 1: Detachable power cord Installation/Overvoltage Category: Category II Pollution Degree: Degree 2 EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}
Environmental	<ul style="list-style-type: none"> Storage Temperature: -20 to 70 °C Optimum Operating Temperature: 22 ± 4 °C Safe Operating Temperature: 15 to 30 °C Humidity: Non-condensing, 10 to 80% Relative Humidity Indoor Use Only Operating Altitude: 0 to 2,000 meters Not for use in an environment with an explosive atmosphere
Compliance	<ul style="list-style-type: none"> Nemko NRTL/C, CB Scheme CE compliance as indicated on the Instrument Identification and Safety Label.
Capabilities	<ul style="list-style-type: none"> Protein quantitation Kinetic and affinity analyses (k_{obs}, k_a, k_d, K_D) Binding specificity and cooperativity Kinetic analysis of proteins, peptides, and other biomolecules Small molecule and fragment kinetic analysis Recommended analyte molecular weight of 150 Da or higher
Sampling Format	<ul style="list-style-type: none"> Required plate: 96-well, black, flat bottom polypropylene microplate (Greiner Bio-One, #655209) or similar, SBS standard microplate Single sample plate capacity
Sampling Volume	180–220 µL/well (96-well plate)
Sample Types	Purified samples, common culture media, crude lysates

Table 2-10: Octet K2 System Specifications (Continued)

Item	Description
Biosensor Type	Disposable, single-use fiber optic biosensors with optional reuse by regeneration and/or re-racking
Biosensor Tray Type	8 x 12 format 96-biosensor tray, green color
Optics and Mechanics	<ul style="list-style-type: none"> • 2-channel biosensor manifold • Optical interferometer • 2 spectrometers (one dedicated spectrometer per biosensor)
Throughput	<ul style="list-style-type: none"> • Up to 2 biosensors in parallel, maximum of 96 tests unattended, subject to total assay time • One 96-well plate and one biosensor tray at once
Orbital Flow Capacity	Static or 400–1,500 rpm
Temperature Range	(Ambient + 4 °C)–40 °C, 1 °C increments
Dimensions	18.6" H x 17" W x 20.8" D (47 cm H x 43 cm W x 53 cm D)
Weight	58 lb (26.3 kg)
Electrical Requirements	<ul style="list-style-type: none"> • Mains: 100-120/200-240 VAC, 50/60 Hz, 4 A max • Power consumption: 100 W (240 W peak)
 IMPORTANT: Use the power cords provided by Pall ForteBio or a suitable AC cord with ratings of 60 C, 300 V, 16 AWG or better.	

OCTET RED96e SYSTEM SPECIFICATIONS AND SITE REQUIREMENTS

The Octet RED96e system is a benchtop instrument that should be installed on a standard, non-flammable laboratory bench with a sufficient weight capacity.

The shipping weight of the Octet RED96e system (instrument, computer, and accessories ship together) is 180 lbs (81.6 kg), and measures 48" x 32" x 46" (121.9 cm x 81.3 cm x 116.8 cm).

Contents of the system as shipped include:

- The Octet RED96e instrument
- Package of 10 disposable tray liners for spent biosensors
- Package of 3 evaporation covers
- Software Installation CD

- Instrument Settings Backup CD
- Octet mouse pad
- Pall ForteBio Software License agreement
- Communication cable set to connect instrument to computer workstation
- Dell computer with included Dell power cord, mouse, keyboard, and monitor connection adapters
- Dell monitor with monitor cables and power cord
- Instrument power cord or cords dependent on end user country

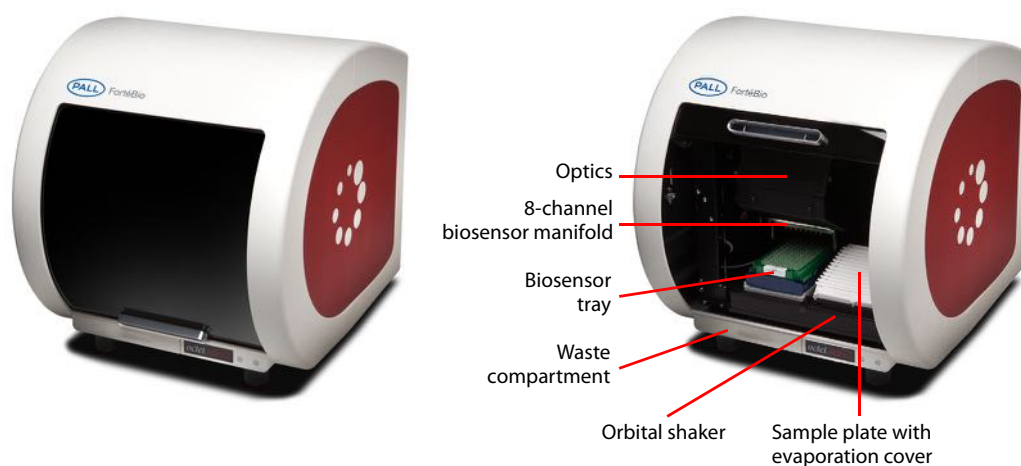


Figure 2-10: Octet RED96e Instrument—Door Closed (Left) or Open (Right)

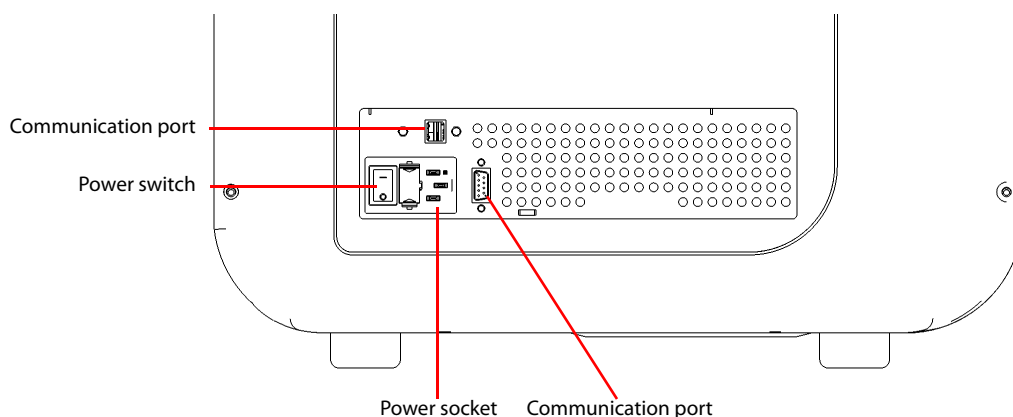


Figure 2-11: Octet RED96e Instrument — Rear view



WARNING: Movement of the instrument presents a high risk of system damage and risk of personal injury, and should only be performed by qualified Pall ForteBio service personnel. To obtain more information, please contact Pall ForteBio technical support. Failure to comply with these instructions voids any existing warranty or service contract agreements. Pall ForteBio is not responsible for personal injury or damages caused by unqualified personnel relocating and/or moving the system.



IMPORTANT:

Using 96-well half-area plates on the Octet RED96e system will result in non-optimal system performance. Pall ForteBio cannot guarantee results within the optimal performance specifications of the system when these plates are used.

Do not block the air inlet and outlet vents on the rear and bottom side of the instrument.

Instrument Identification and Safety Labeling

Please see “Octet Systems Safety Information” on page 13 for definitions of symbols.

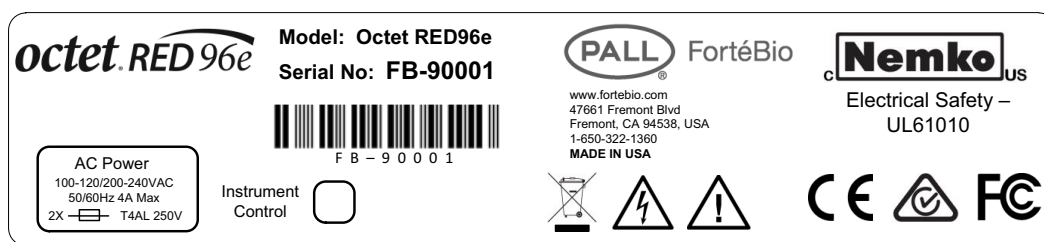


Figure 2-12: Octet RED96e System Rear Panel Label

System Specifications

Table 2-11: Octet RED96e System Specifications


Item	Description
Equipment Classifications	<ul style="list-style-type: none"> Product Classification: Class 1: Detachable power cord Installation/Overvoltage Category: Category II Pollution Degree: Degree 2 EMC Classification: Group I, Class A, ISM Equipment (EN55011, emissions), {EN61326, immunity}
Environmental	<ul style="list-style-type: none"> Storage Temperature: -20 to 70 °C Optimum Operating Temperature: 15 to 40 °C. <div>  <p>NOTE: For optimal performance, the environmental temperature change should be less than 2 °C per hour.</p> </div> <ul style="list-style-type: none"> Safe Operating Temperature: 15 to 30 °C Humidity: Non-condensing, 10 to 80% Relative Humidity Indoor Use Only Operating Altitude: 0 to 2,000 meters Not for use in an environment with an explosive atmosphere
Compliance	<ul style="list-style-type: none"> Nemko NRTL/C, CB Scheme CE compliance as indicated on the Instrument Identification and Safety Label.
Capabilities	<ul style="list-style-type: none"> Protein quantitation Kinetic and affinity analyses (k_{obs}, k_a, k_d, K_D) Binding specificity and cooperativity Kinetic screening of proteins, peptides, and other biomolecules Small molecule and fragments screening and kinetic analysis Recommended analyte molecular weight of 150 Da or higher

Table 2-11: Octet RED96e System Specifications (Continued)

Item	Description
Sampling Format	<ul style="list-style-type: none"> Required plate: 96-well, black, flat bottom polypropylene microplate (Greiner Bio-One, #655209) or similar, SBS standard microplate Single sample plate capacity
Sampling Volume	180–220 µL/well (96-well plate)
Sample Types	Purified samples, common culture media, crude lysates
Biosensor Type	Disposable, single-use fiber optic biosensors with optional reuse by regeneration and/or re-racking
Biosensor Tray Type	8 x 12 format 96-biosensor tray, green color
Optics and Mechanics	<ul style="list-style-type: none"> 8-channel biosensor manifold Optical interferometer Eight spectrometers (one dedicated spectrometer per biosensor)
Throughput	<ul style="list-style-type: none"> Up to 8 biosensors in parallel, maximum of 96 tests unattended One 96-well plate and one biosensor tray at once
Orbital Flow Capacity	Static or 100–1,500 rpm
Temperature Range	15–40 °C, 1 °C increments
Dimensions	19.5" H x 22" W x 18.2" D (49 cm H x 56 cm W x 46 cm D)
Weight	72 lb (32.7 kg)

Table 2-11: Octet RED96e System Specifications (Continued)

Item	Description
Electrical Requirements	<ul style="list-style-type: none">Mains: 100-120/200-240 VAC, 50/60 Hz, 4 A maxPower consumption: 200 W (300 W peak)

**IMPORTANT:**

Use the power cords provided by Pall ForteBio or a suitable AC cord with ratings of 60 C, 300 V, 16 AWG or better.

We recommend that the electrical circuit used for the system and computer not be connected to equipment with high intermittent power draws such as refrigerators, freezers, compressors, or vacuum pumps. If your site has a history of power outages, spikes, and/or drops, we highly encourage you to power the instrument and computer through an on-line UPS. Your Pall ForteBio service representative can provide specifications for the recommended UPS system.

MICROPLATE EVAPORATION COVER



NOTE: The microplate evaporation cover can only be used on the Octet RED96e system.

- The evaporation cover was designed specifically for use with Greiner 96-well regular microplates (Part No. 655209)
- Intended to extend the length of total experiment time up to 12 hours
- Ideal for precious samples that can be fully recovered to perform additional analyses
- Single-use only and should not be cleaned or re-used as any processing may alter its structural integrity
- The covers can withstand the standard operating temperature of the Octet RED96e systems of 15–40°C
- They are mostly solvent resistant but should not be subjected to 100% DMSO
- All covers are individually wrapped and sold in a pack of 3

Intended Use

Before using the evaporation cover, ensure that the push bar is installed near the sensor pickers, as shown in Figure 2-13, otherwise the biosensors will crash into the microplate evaporation cover.

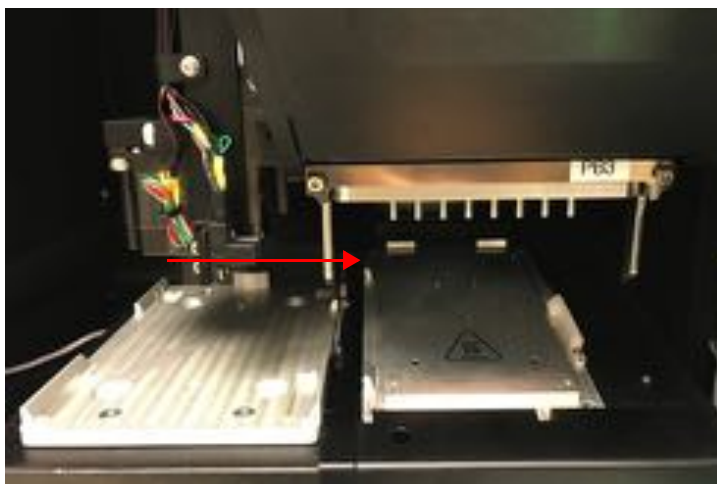


Figure 2-13: *Install Push Bar.*

For best results, place the 96-well microplate in the Octet RED96e instrument immediately after preparation and place the evaporation cover on it to prevent any evaporation and recover majority of the sample volume after the run.

After putting the cover evaporation cover on, make sure that all four corners are pressed down onto the plate. The LED light next to the plate will be solid blue if the evaporation cover is installed properly (Figure 2-14). If the cover is not installed properly, the LED light will blink and the experiment will not be able to start.

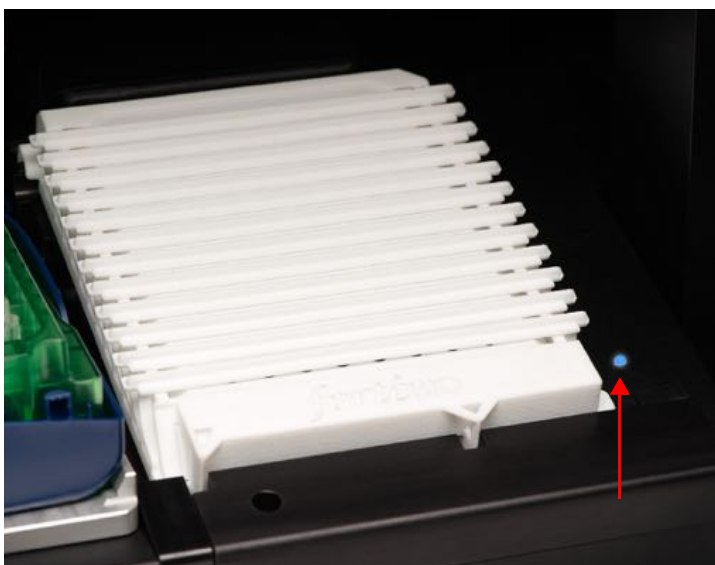


Figure 2-14: LED is Blue When Evaporation Cover is Installed Properly.

For your reference, we have printed the LED status information in the inside of the instrument below the home position of the sensor pickers (Figure 2-15).



Figure 2-15: LED Status Information.

Start the experiment after placing the biosensor tray on the tray holder and giving the samples enough time to equilibrate to the desired temperature.

During the experiment, the evaporation cover will open one column on the sample plate at a time, and enable eight biosensors to dip into the sample wells in that column (Figure 2-16). Following the column read, the panel in the evaporation cover will return to its original position. The microplate evaporation cover can extend the experiment run time to 12 hours with minimal sample evaporation so most of the samples can be recovered following the run.



Figure 2-16: Evaporation Cover Opened.

CHAPTER 3:

Getting Started

User Safety Guidelines and Warnings	56
Directives et mises en garde relatives à la sécurité des utilisateurs	57
Sicherheitsrichtlinien und -hinweise für den Benutzer	58
Installing Octet Data Acquisition Software	59
Software Overview	62
Octet System Data Acquisition Options	71
Setting the Plate Temperature	74
Monitoring Experiments Remotely	76
Managing Biosensor Types	79

USER SAFETY GUIDELINES AND WARNINGS



WARNING: Do not block, push objects into, or allow dust to accumulate in the air vents. Do not store an Octet system in a low airflow environment, such as a closed cabinet, while in operation. Restricting the airflow can damage the instrument or cause a fire.



WARNING: Connect the power cord between the product and a grounded AC outlet. Power connectors and power strips vary among countries. Using incompatible cables or improperly connecting cables to a power strip or electrical outlet may damage the equipment or cause a fire.



WARNING: Use only certified power cord sets having at least 16 AWG/3G (3 x 0.75mm²) cable with power plug and connector rated 250 V, 10 A.



WARNING: If the Octet system is not used as specified, injury to the user and/or damage to the instrument may result.



WARNING: Keep the area around the sample door clear and unobstructed.



NOTE: Do not position the Octet instrument in a way that makes it difficult to disconnect the power.



NOTE: Octet system and software installation should be performed by Pall ForteBio LLC personnel only.

DIRECTIVES ET MISES EN GARDE RELATIVES À LA SÉCURITÉ DES UTILISATEURS



WARNING: N'obtenez pas les ouïes d'aération, n'y insérez pas d'objets et ne laissez pas la poussière s'accumuler à l'intérieur. N'utilisez pas le système Octet dans un environnement mal ventilé (armoire fermée). Limiter la ventilation peut endommager l'instrument ou provoquer un incendie.



WARNING: À l'aide du cordon secteur, branchez le produit à une prise CC reliée à la terre. Les connecteurs d'alimentation et les blocs multiprises peuvent varier selon les pays. L'utilisation de câbles incompatibles ou le mauvais branchement des câbles à un bloc multiprise ou à une prise électrique peut endommager l'équipement ou provoquer un incendie.



WARNING: N'utilisez que des cordons secteur certifiés munis d'au moins un câble 16 AWG/3G (3 x 0,75 mm²) avec prise électrique et connecteur de 250 V, 10 A.



WARNING: Le non-respect des consignes d'utilisation du système Octet peut occasionner des blessures à l'utilisateur et/ou endommager l'instrument.



WARNING: Veillez à laisser la porte du compartiment échantillons accessible et dégagée.



NOTE: Do not position the Octet instrument in a way that makes it difficult to disconnect the power.



NOTE: Seul le personnel de Pall ForteBio LLC est habilité à procéder à l'installation du système et du logiciel Octet.

SICHERHEITSRICHTLINIEN UND -HINWEISE FÜR DEN BENUTZER



WARNING: Blockieren Sie niemals die Lüftungsöffnungen, stecken Sie keine Gegenstände in sie und lassen Sie keinen Staub in sie eintreten. Lagern Sie ein Octet-System während des Betriebs niemals in Umgebungen mit geringem Luftstrom, wie z. B. einem geschlossenen Schrank. Ein eingeschränkter Luftstrom kann zu Schäden am Gerät führen oder einen Brand verursachen.



WARNING: Schließen Sie das Netzkabel des Geräts an eine geerdete Wechselstrom-Steckdose an. Netzstecker und Steckerleisten unterscheiden sich von Land zu Land. Die Verwendung inkompatibler Kabel oder die unsachgemäße Verbindung von Kabeln mit einer Steckerleiste oder Steckdose kann zu Schäden am Gerät führen oder einen Brand verursachen.



WARNING: Verwenden Sie ausschließlich zugelassene Netzanschlusskabel mit mindestens 16 AWG/3G (3 x 0,75 mm²) und Netzstecker sowie einen Anschluss mit 250 V, 10 A.



WARNING: Ein nicht bestimmungsgemäßer Gebrauch des Octet-Systems kann zu Verletzungen des Benutzers und/oder Schäden am Gerät führen.



WARNING: Halten Sie den Bereich um die Probenklappe frei.



NOTE: Do not position the Octet instrument in a way that makes it difficult to disconnect the power.



NOTE: Die Installation des Octet-Systems und der dazugehörigen Software sollte ausschließlich durch Personal von Pall ForteBio LLC erfolgen.

INSTALLING OCTET DATA ACQUISITION SOFTWARE



NOTE: Version 8.2 or later of Octet Data Acquisition and Data Analysis CFR software require a new database schema and version 8.2 and 9.0 of the FB Server module. The new database schema is installed and configured during the version 8.2 software installation. Version 8.2 software will automatically check the version of the FB Server module in use and display a message if it is incompatible.

1. Insert the software CD into your CD drive.
 - If the Autoplay dialog box displays, choose to open the CD to view files.
 - If the Autoplay dialog box does not display, navigate to the CD using Windows Explorer.Optical drives are typically found under the **D:** or **E:** drive.
2. Double-click **DataAcquisition.exe** or **DataAcquisition-CFR.exe** depending on which version of software you need to install. This will launch the installation wizard (see Figure 3-1).



Figure 3-1: Octet Data Acquisition Software Setup Wizard

3. Click **Next** to display the Choose Install Location dialog box (Figure 3-2).

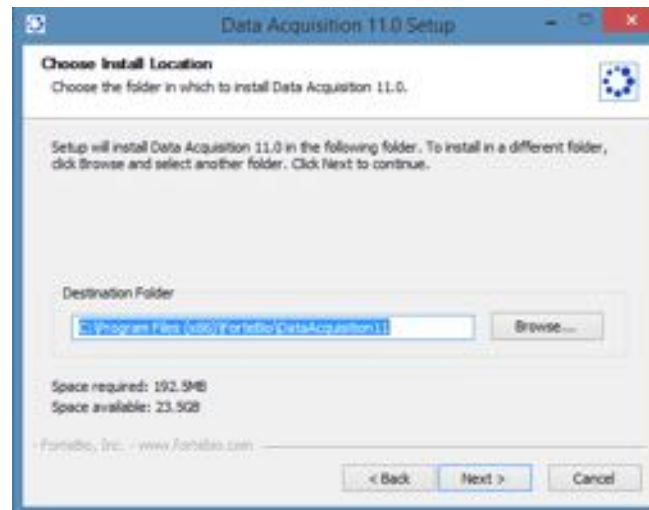


Figure 3-2: Choose Install Location Dialog Box

The default location for the software on the local machine is **C:\Program Files\ForteBio\DataAcquisition**.

4. Click **Next** to accept this path location.

The Choose Start Menu Folder dialog box displays (Figure 3-3).

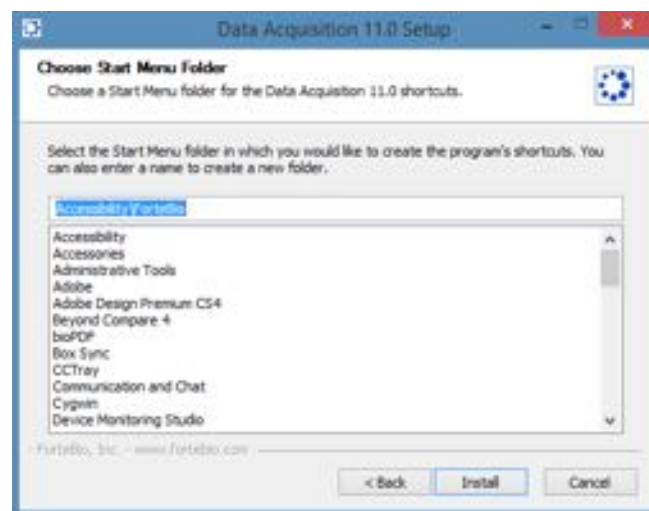


Figure 3-3: Choose Start Menu Folder Dialog Box

The default Start Menu folder is **ForteBio**.

5. Click **Install**.

The installation wizard takes a few seconds to install (Figure 3-4).

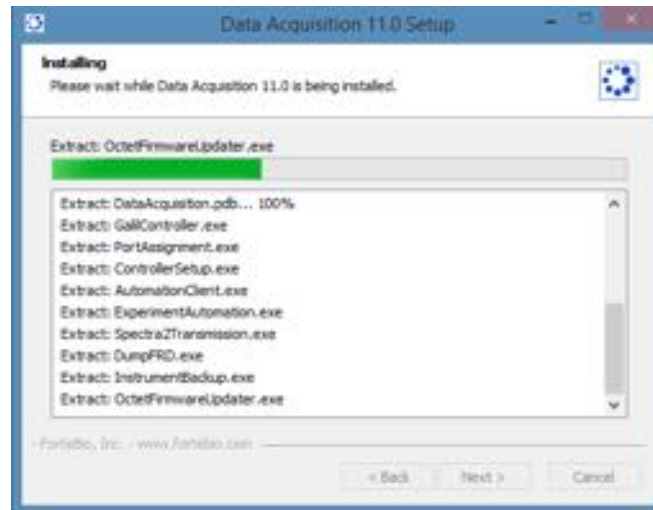


Figure 3-4: Installation Progress

The installation wizard displays the Completing the Data Acquisition Setup Wizard dialog box (Figure 3-5).



Figure 3-5: Completing the Data Analysis Setup

6. Click **Finish** to complete the installation.
7. If you are installing the CFR version of the software you will also need to install and setup the FB Server. Go to "Installing the ForteBio FB Server Module" on page 99 for those instructions.

STARTING THE OCTET SYSTEM AND DATA ACQUISITION SOFTWARE

To start the system and software:

1. Turn on the computer.
2. Turn the Octet instrument on using the power switch located on the external electrical box.



NOTE: The instrument requires a minimum one-hour warm-up time. Pall ForteBio also recommends leaving the instrument on for a minimum of eight hours prior to using it for the first time.

3. Launch the Octet System Data Acquisition software by double-clicking on the Data Acquisition desktop icon.

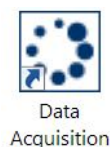


Figure 3-6: Desktop Icon



NOTE: When using the 21 CFR Part 11 version of the Octet System Data Acquisition software, users are required to log in and start a user session before the software will launch. Please refer to “Starting a User Session” on page 89 for more information.

SOFTWARE OVERVIEW

Launching the software displays the Octet System Data Acquisition software **Main Screen**. Screen components along with the default windows displayed are shown in Figure 3-7.

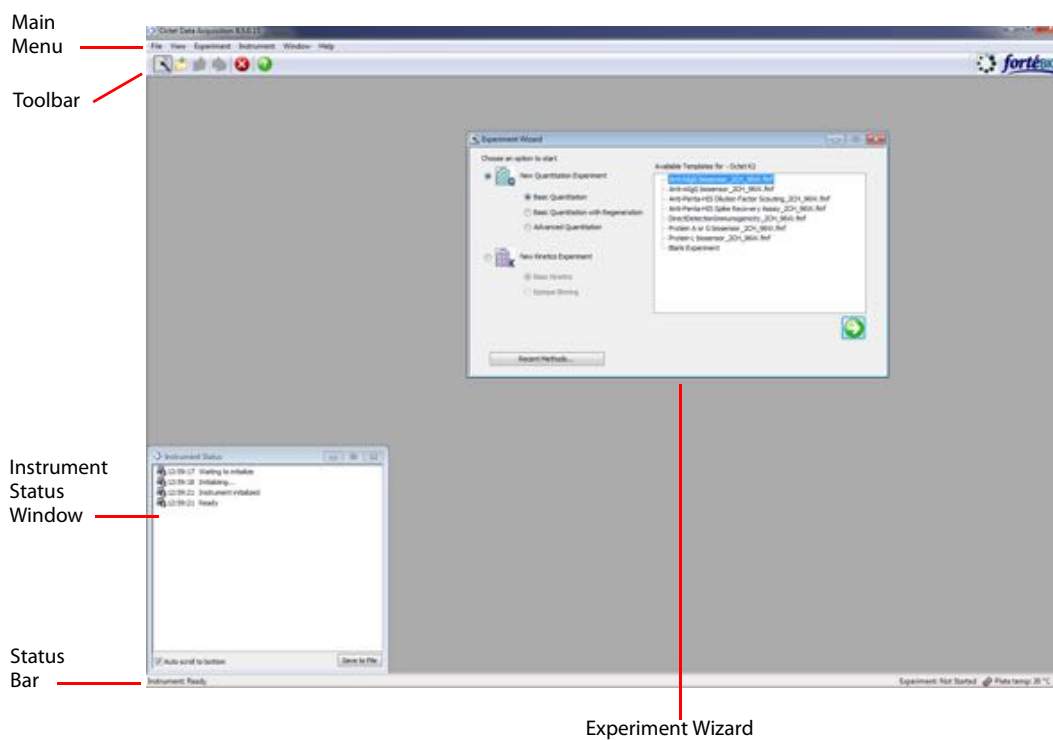


Figure 3-7: Main Screen

Main Menu and Toolbar

The Main Menu and Toolbar are located in the upper left of the **Main Screen** (Figure 3-8).

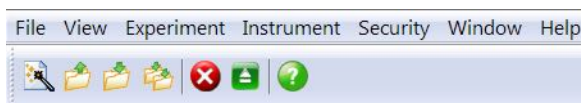


Figure 3-8: Main Menu and Toolbar



NOTE: The **Security** menu is only available in the 21 CFR Part 11 version of the Octet System Data Acquisition software.

File Menu

The **File** menu (Figure 3-9) allows users to open and save method files, view experiments, print files and set system and software options.

A method file (.fmf) contains sample plate configuration, sample plate table information,

sensor assignments and assay step information that allow the Octet instrument and software to run an experiment. A read-only copy of the method file will automatically be saved in the experiment folder. When the run is complete, the data in the experiment folder can then be reviewed.



NOTE: When using the 21 CFR Part 11 version of the Octet System Data Acquisition software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.

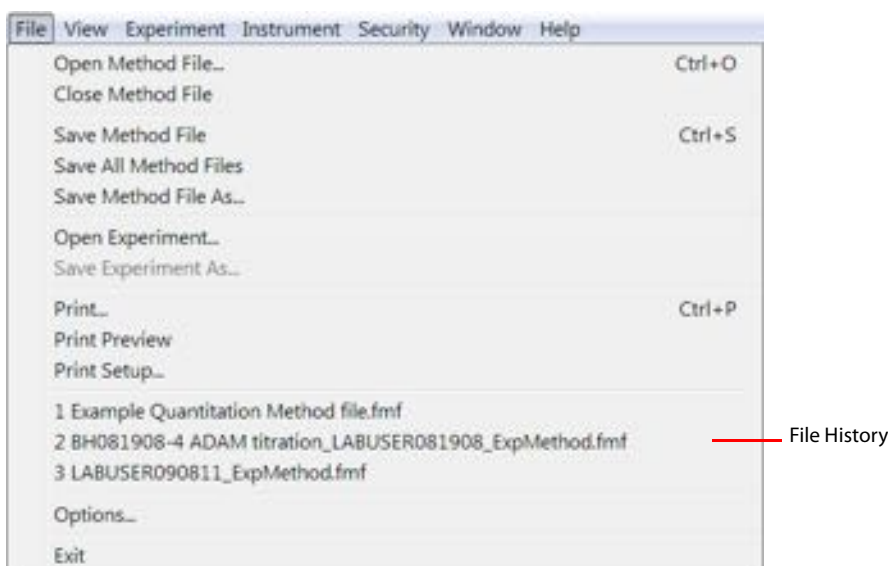


Figure 3-9: File Menu

Table 3-1: File Menu Commands

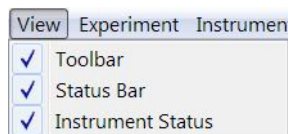
Menu Command	Toolbar Button	Function
Open Method File		Opens an experiment method file (.fmf).
Close Method File	N/A	Closes the active experiment method file but does not save changes.
Save Method File		Saves the active experiment method file (.fmf).
Save All Method Files		Saves all open method files (.fmf).

Table 3-1: File Menu Commands (Continued)

<i>Menu Command</i>	<i>Toolbar Button</i>	<i>Function</i>
Save Method File As	N/A	Allows the active experiment method file to be saved as a new file without overwriting the original method file.
Open Experiment	N/A	Opens an experiment folder.
Save Experiment	N/A	Saves the active experiment.
Print	N/A	Opens the Print dialog box to print a file.
Print Preview	N/A	Opens a print preview window of a method or assay definition file.
Print Setup	N/A	Opens the Print Setup dialog box to print a file.
File History	N/A	Displays a list of previously opened files.
Options	N/A	Opens the Options dialog box. Please refer to “Octet System Data Acquisition Options” on page 71 for more information on changing system and software options.
Exit	N/A	Closes the software.

View Menu

The **View** menu allows users to show or hide the **Toolbar** and status windows. A check mark next to the menu item indicates the option is currently shown.

**Figure 3-10:** View Menu**Table 3-2:** View Menu Commands

<i>Menu Command</i>	<i>Function</i>
Toolbar	Shows or hides the Toolbar .
Status Bar	Shows or hides the Status bar .
Instrument Status	Displays the Instrument Status window.

Experiment Menu

The **Experiment** menu provides access to the **Experiment Wizard**, assay and experiment options as well as experiment templates.

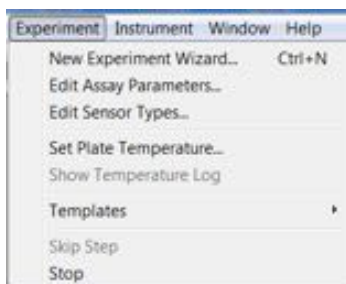


Figure 3-11: Experiment Menu

Table 3-3: Experiment Menu Commands



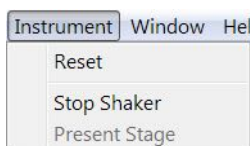
Menu Command	Toolbar Button	Function
New Experiment Wizard		Opens the Experiment Wizard .
Edit Assay Parameters	N/A	Opens the Edit Assay Parameters dialog box to define a new assay, edit an existing assay, or remove an assay from the quantitation application. See “Managing Assay Parameter Settings” on page 266 for more information.
Edit Sensor Types	N/A	Opens the Sensor Types dialog box to view current biosensor types, add new biosensor types and remove biosensor types. See “Managing Biosensor Types” on page 79 for more information.
Set Plate Temperature	N/A	Opens the Temperature Setting dialog box that displays the current sample plate temperature and allows users to change the current temperature setting of the instrument. See “Setting the Plate Temperature” on page 74 for more information. To set the default temperature, see “Defining a New Default Sample Plate Temperature” on page 75.


Table 3-3: Experiment Menu Commands (Continued)

<i>Menu Command</i>	<i>Toolbar Button</i>	<i>Function</i>
Templates	N/A	Allows users to select from a set of pre-defined quantitation or kinetics method templates.
Skip Step	N/A	Skips the step in the method that is currently executing (kinetics experiments only).
Stop		Stops the experiment. Data from the active biosensor is not saved, but all data prior to the active biosensor will be available.

Instrument Menu

The **Instrument** menu provides direct control of the Octet instrument.

**Figure 3-12: Instrument Menu****Table 3-4: Instrument Menu Commands**

<i>Menu Command</i>	<i>Toolbar Button</i>	<i>Function</i>
Reset	N/A	Resets the instrument and the log in the Instrument Status window .
Stop Shaker	N/A	Stops the sample plate shaker.
Present Stage		Presents the instrument stage that houses the biosensor tray, sample and reagent plates (Octet RED384 and Octet QK384 only).

Security Menu

The **Security** menu is only available in the 21 CFR Part 11 version of the Data Acquisition software. For complete details on menu options, please refer to “Accessing Compliance Features” on page 92.



Figure 3-13: Security Menu

Window Menu

The **Window** menu provides display options for the open windows in the **Main Screen**.

All open windows are listed at the bottom of the menu, and a check mark indicates the window that is currently active. To view another window, select it from the list.

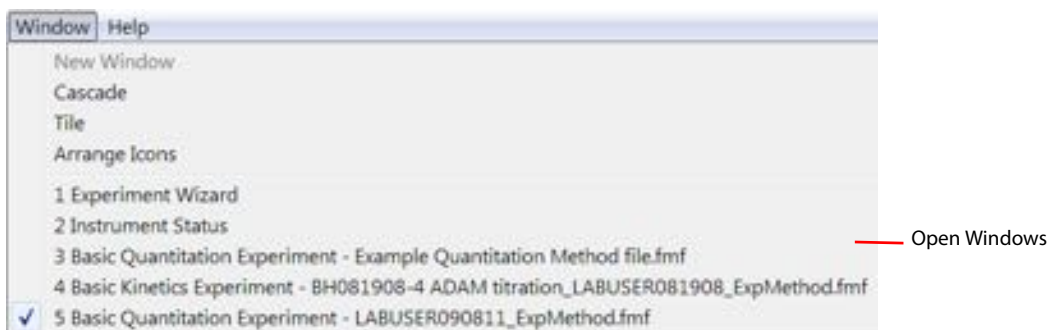


Figure 3-14: Window Menu

Table 3-5: Window Menu Commands

Menu Command	Function
New Window	Opens a new Runtime Binding Chart window.
Cascade	Organizes all windows in a cascade.
Tile	Tiles all windows vertically.
Arrange Icons	Arranges the minimized window icons in a row at the bottom of the screen.
Open Windows	Lists the windows currently open.

Help Menu

The **Help** menu provides access to software and instrument support information.

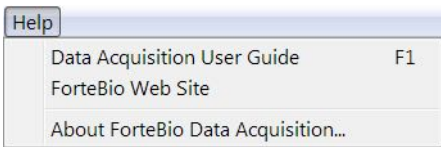



Figure 3-15: Help Menu

Table 3-6: Help Menu Commands

Menu Command	Toolbar Button	Function
Data Acquisition User Guide	N/A	Opens the online <i>Data Acquisition Software User Guide</i> .
ForteBio Web Site	N/A	Opens a web browser and displays the Pall ForteBio web page (www.fortebio.com).
About ForteBio Data Acquisition		Displays software, user and instrument information.



NOTE: Clicking on the Pall ForteBio logo in the upper right corner of the **Main Screen** also displays the **About ForteBio Data Acquisition** window.

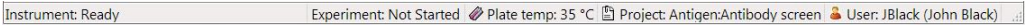
Status Bar

The **Status Bar** is located at the bottom of the **Main Screen** and displays current instrument and experiment status and plate temperature.



Figure 3-16: Status Bar

In the 21 CFR Part 11 version of the Data Acquisition software, the **Status Bar** will also display the User and Project name entered at login.



Instrument Status Window

The **Instrument Status** window displays a log of all instrument activity.

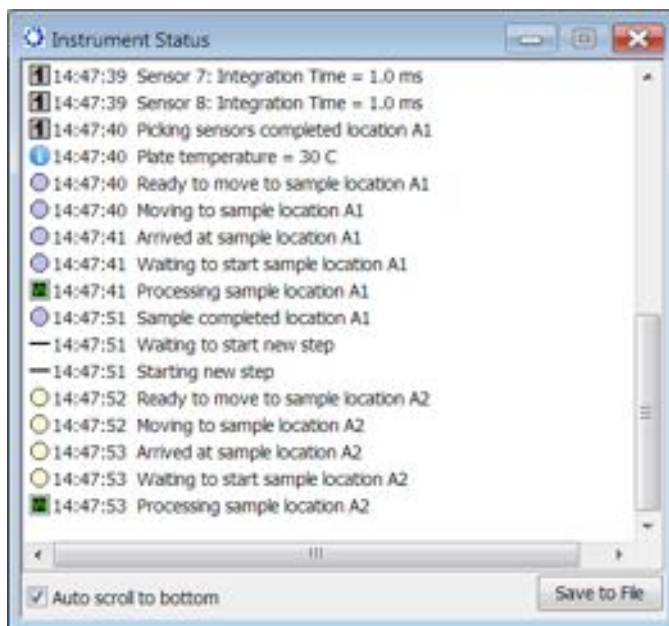


Figure 3-17: Instrument Status Window

Selecting the **Auto Scroll to bottom** check box will auto-scroll the log to display the most current events. Clicking **Save to File** will save a copy of the instrument log.



NOTES:

If a problem occurs during operation of the instrument, Pall ForteBio recommends saving a copy of the system log to better assist our technical support staff in diagnosing the issue.

The instrument log automatically resets when Octet System Data Acquisition software is closed.

Experiment Wizard

The **Experiment Wizard** guides users through the complete set up of an experiment. Using the wizard is described in detail in the Quantitation and Kinetics experiment chapters.

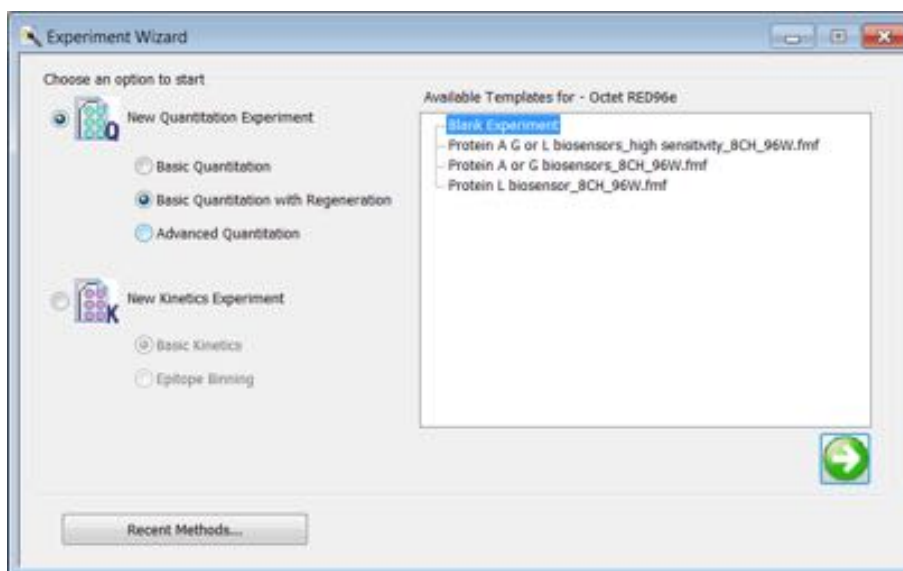


Figure 3-18: Experiment Wizard

OCTET SYSTEM DATA ACQUISITION OPTIONS

Acquisition options allow users to set system and data preferences for quantitation and kinetic data acquisition. To view these options (Figure 3-19), click **File > Options** from the **Main Menu**.

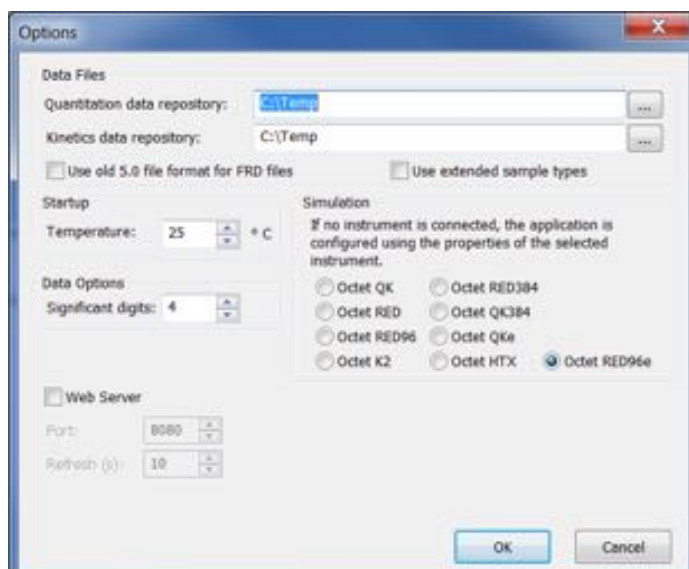


Figure 3-19: Options Dialog Box

Table 3-7: User Options



Item	Description
Data Files	
Quantitation data repository	<p>The default location where quantitation data files (.frd) are saved. Click ... (Browse) to select a different folder.</p> <hr/> <p> NOTE: Pall ForteBio recommends that the data be saved to the local machine first, then transferred to a network drive if needed.</p>
Kinetics data repository	<p>The default location where kinetics data files (.frd) are saved. Click ... (Browse) to select a different folder.</p> <hr/> <p> NOTE: Pall ForteBio recommends that the data be saved to the local machine first, then transferred to a network drive if needed.</p>

Table 3-7: User Options (Continued)




Item	Description
Use old 5.0 file format for FRD files	<p>Select this option to save data in the earlier Octet RED software 5.0 format.</p> <hr/> <p> NOTE: Saving data in the old file format produces larger files and may result in slower data analysis.</p> <hr/>
Use extended sample types	<p>Select this option to extend the sample types available in the right-click menu of the Sample Plate Map and Sample Plate Table to include negative and positive controls.</p> <hr/>
Startup	
Temperature	<p>User-defined default startup plate temperature. This temperature is used as the default setting for all experiments.</p> <hr/> <p> NOTE: This changes the startup plate temperature only, not the current plate temperature. The software must be restarted after entering the new value for the new setting to take affect.</p> <hr/>
Data Options	
Significant digits	<p>Specifies the number of significant digits the software uses for Molecular Weight, Concentration and Dilution values during data analysis.</p> <hr/> <p> NOTE: Six decimal places are recommended for the Protein A assay.</p> <hr/>

Table 3-7: User Options (Continued)

<i>Item</i>	<i>Description</i>
Simulation	If the workstation is not connected to an instrument, this option enables users to create and save an experiment to a method file (.fmf) using the properties of the selected instrument type.
Web Server	Selecting this option enables remote monitoring of the experiment using a web browser. See “Monitoring Experiments Remotely” on page 76 for more information.
Automation	Allows users to select the appropriate connection for automation interfaces used with OctetRED384 and OctetQK384 systems only. For more information, please refer to Appendix A, Using Octet384 and HTX Systems with an Automation Interface on page 633.

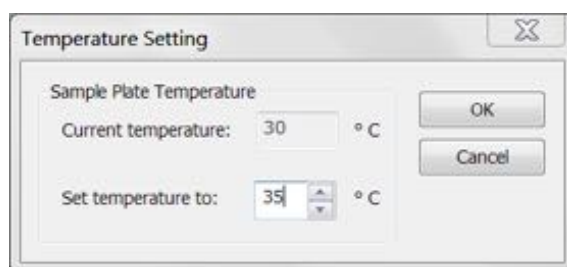
SETTING THE PLATE TEMPERATURE

The settable plate temperature can range from ambient plus 4 °C to a high of 40 °C. A factory-set default plate temperature of 25 °C is used as a system startup plate temperature and the experiment default temperature. This default value can be customized by the user. In addition, the plate temperature setting can be changed for individual experiments when needed. The current plate temperature displays in the **Status bar** at the bottom of the **Main Screen**.

Changing the Plate Temperature for Individual Experiments

To set the plate temperature to a value other than the default setting for a specific experiment:

1. From the **Main Menu**, click **Experiment > Set Plate Temperature**.
2. Click up or down in the **Set temperature to** field (Figure 3-20) to the desired value or enter a temperature and click **OK**.

**Figure 3-20: Temperature Setting**

3. Allow sufficient time for the sample plate to equilibrate to the new temperature before beginning an experiment. For experiments set to 25 or 30 °C from ambient, allow approximately 10 minutes for a plate at room temperature, or 15 minutes for a plate at ambient + 4 °C. For experiments set to 15 °C, allow approximately 20 minutes for plate at room temperature and for a plate at ambient + 4 °C. If the temperature is increased to 30 °C from a previous run at 15 °C, then 20 minutes should be sufficient time for the plate to equilibrate.



NOTE: If the Octet System Data Acquisition software is closed, the plate temperature will reset to the default startup value specified in the **Options** dialog box when the software is relaunched.

Defining a New Default Sample Plate Temperature

To define a new default temperature that will be used at startup and as the default plate temperature for all experiments:

1. From the **Main Menu**, click **File > Options**.
2. In the **Options** dialog box (Figure 3-21), select a new temperature in the **Startup** box and click **OK**. The plate temperature will then adjust to the new value, and this setting will be used as the new default startup temperature whenever the software is launched.

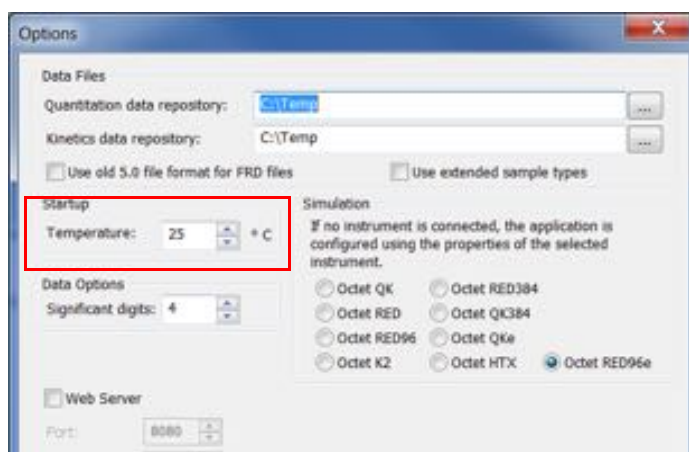


Figure 3-21: Setting the Default Startup Temperature in the Options Dialog Box

3. Allow sufficient time for the sample plate to equilibrate to the new temperature before beginning an experiment. For experiments set to 25 or 30 °C from ambient, allow approximately 10 minutes for a plate at room temperature, or 15 minutes for a plate at ambient + 4 °C. For experiments set to 15 °C, allow approximately 20 minutes for plate

at room temperature and for a plate at ambient + 4 °C. If the temperature is increased to 30 °C from a previous run at 15 °C, then 20 minutes should be sufficient time for the plate to equilibrate.



IMPORTANT: For the new default temperature value to take affect, you must restart the software.

MONITORING EXPERIMENTS REMOTELY

If the Octet system computer is connected to a local network, experiment progress can be monitored remotely from any networked computer, smartphone or mobile device using any web browser. In addition, instrument log files and previously run experiments can also be accessed remotely for review.

1. From the **Main Menu**, click **File > Options**.
2. In the **Options** dialog box (Figure 3-23), select the **Web Server** check box. Adjust the **Port** and **Refresh** settings and change the **Connect as** IP address if needed. The default **Refresh** rate of 10 will refresh the experiment view in the web browser every 10 seconds. Click **OK**.



NOTE: Pall ForteBio recommends using the **Port** and **Connect as** (IP address) settings shown as default in the **Web Server** box, as they are unique to your particular Octet system.

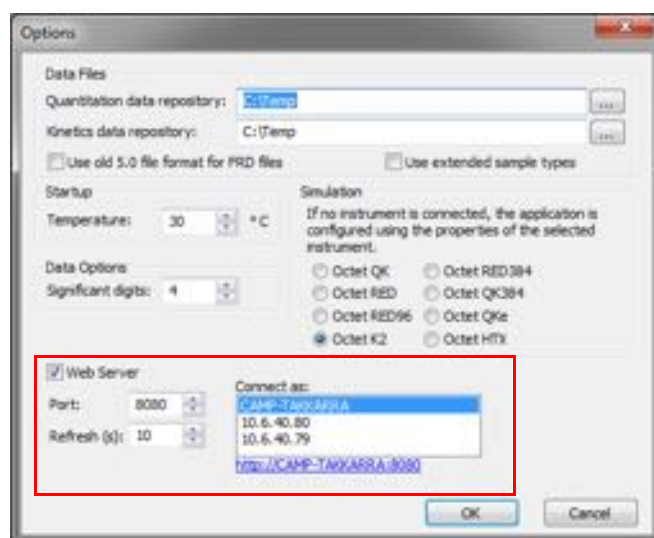


Figure 3-22: Selecting the Web Server in the Options Dialog Box

3. Click **File > Options** to access the **Options** dialog box again. A **Web Server URL** will now be listed under the **Connect as** box (Figure 3-23). Note this URL as it will be needed to access the experiment remotely.

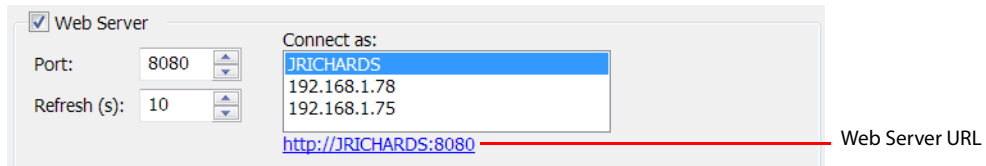


Figure 3-23: Web Server URL

4. Start the experiment in the Octet System Data Acquisition software as you normally would.
5. Open a web browser on a remote computer or device that is on the same network as the Octet system.



NOTE: The remote computer or device must be on the same network as the Octet system, or connected to the network the instrument is on via VPN.

6. Enter the **Web Server URL** in the browser window or click the **Web Server URL** link in the **Options** dialog box. The experiment in progress will display (Figure 3-24).

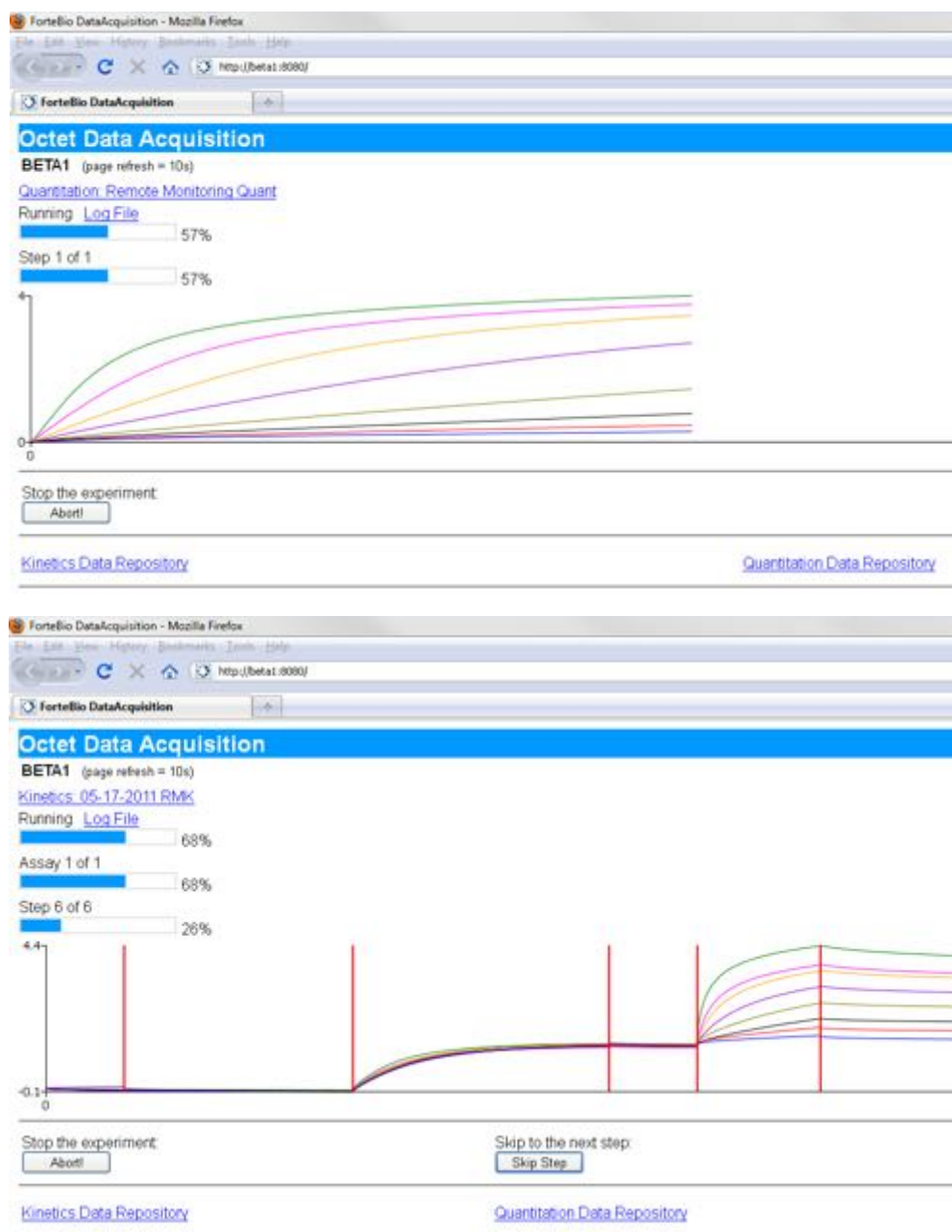


Figure 3-24: View of Quantitation Experiment (top) and Kinetics Experiment (bottom) via Web Browser

In the browser window, you can:

- Click the experiment name to view experiment details.
- Click **Log File** to display a log of current instrument activity.
- Click **Kinetics Data Repository** or **Quantitation Data Repository** to open and view previously run experiments.

MANAGING BIOSENSOR TYPES

The Octet System Data Acquisition software includes a default list of all the types of biosensors available for quantitation or kinetic analysis. The available biosensor types display in the **Sensor Assignment** tab. Users can add custom biosensors as needed.

Viewing Available Biosensor Types

To view the available types of biosensors, from the **Main Menu**, click **Experiment > Edit Sensor Types**.

The **Sensor Types** window will display (Figure 3-25).

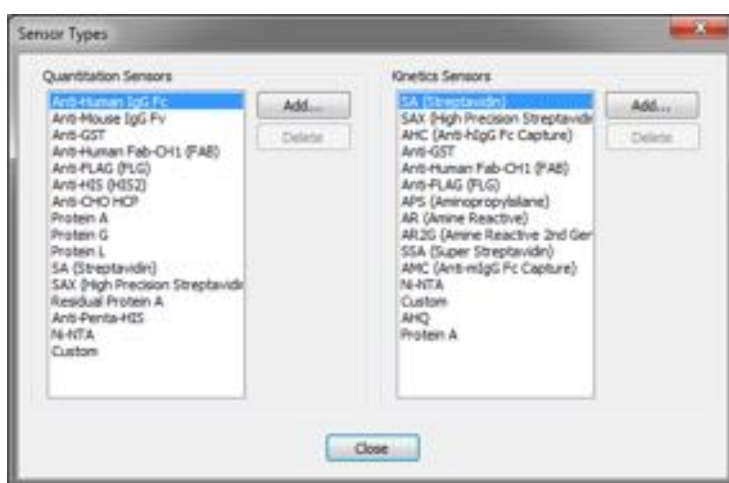


Figure 3-25: Sensor Types Dialog Box

Adding a Biosensor Type

To add a biosensor type:

1. From the **Main Menu**, click **Experiment > Edit Sensor Types**.
2. In the **Sensor Types** window (Figure 3-26), click **Add** next to the **Quantitation Sensors** or **Kinetic Sensors** box (depending on the type of biosensor that will be added).
3. In the **Add Sensor** dialog box, enter a name for the biosensor type and click **OK**.

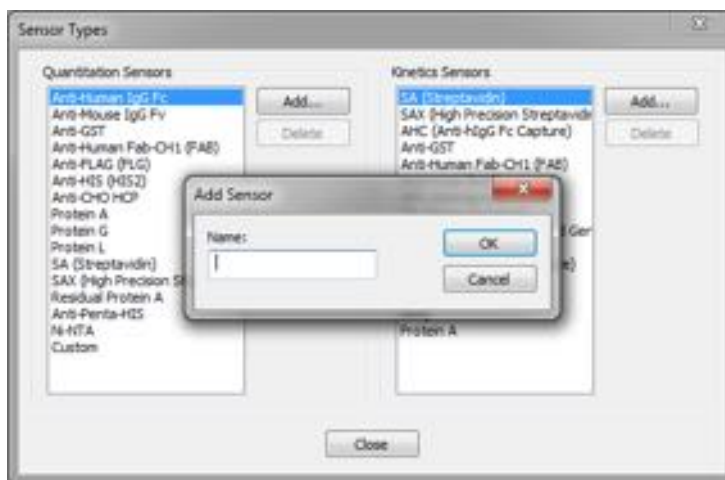


Figure 3-26: Adding a Biosensor Type

Removing a Biosensor Type

To remove a biosensor type, select the biosensor name in the **Quantitation Sensors** or **Kinetic Sensors** box and click **Delete**.



The default software biosensor types cannot be deleted. Only the biosensor types that users add to the system can be deleted.

CHAPTER 4:

21 CFR Part 11 Compliance

FDA 21 CFR Part 11 Final Rule Compliance 82

Overview of FDA 21 CFR Part 11 Compliance Features 83

Octet CFR Software Overview 86

ForteBio FB Server Module 86

Selecting a Server Location 87

Starting a User Session 89

Accessing Compliance Features 92

Administrator Options 99

FDA 21 CFR PART 11 FINAL RULE COMPLIANCE

Octet CFR software has features to allow users to produce electronic records that meet the requirements of the FDA 21 CFR Part 11 Final Rule. This chapter details how the features in Octet CFR software address the requirements for compliance with the FDA 21 CFR Part 11 Final Rule according to the following guidance provided by the FDA:

Subpart A: General Provisions

- Scope
- Implementation
- Definitions

Subpart B: Electronic Records

- Controls for closed systems
- Controls for open systems
- Signature manifestations
- Signature/record linking

Subpart C: Electronic Signatures

- General requirements
- Electronic signatures and controls
- Controls for identification codes/password



NOTE: The guidance provided represents the FDA stance on this topic that was current during the development and release of this version of Octet CFR software. For information, see <http://www.fda.gov/regulatoryinformation/guidances/ucm125067.htm>

Octet CFR software is comprised of three distinct software products: Octet Data Acquisition CFR software, Octet Data Analysis HT CFR Software and the ForteBio FB Server module. Octet Data Acquisition CFR software is used to define quantitation, kinetic or custom assays and to run and view experiments and binding data. Octet Data Analysis HT CFR software is used to analyze binding data and view analysis results. The ForteBio FB Server software manages the user database and stores Audit Trail data.

OVERVIEW OF FDA 21 CFR PART 11 COMPLIANCE FEATURES

Primary Data Integrity

The integrity of raw data is a primary design consideration of Octet CFR software. All data acquired using Octet Data Acquisition CFR software is time stamped and traceable to the user who initiated data acquisition. Acquired data is digitally signed and any modification will invalidate the data.

A single experimental data set is comprised of multiple files. Octet Data Acquisition CFR software creates a manifest file that keeps track of experiment files. Any modification or deletion of these files will invalidate the experiment.

Raw data are saved in binary format that cannot be easily edited. The method file (.fmf format) contains experiment settings and the experiment file (.efrd format) contains analysis settings. All files are digitally signed. Electronic statements and an Audit Trail are stored in the ForteBio FB Server database.

Data files created using Octet Data Acquisition CFR software are strictly bound to features that support FDA 21 CFR Part 11 regulations. As a result, these files cannot be opened or modified by the non-CFR version of Octet software to ensure the integrity of the acquired data is intact.

Administratively Controlled Application Access

Octet CFR software restricts the use of all features that can be used to acquire, modify, and analyze data, including exporting and saving the results as files. A user with no explicit privileges is considered as a Guest, and can only open and print data and method files created by the software.

Octet CFR software uses the ForteBio FB Server Monitor software to administer user settings. The software contains the following information for each user:

- User name
- User Identifier or ID (must be unique)
- Password
- One or more of the following permissions:
 - Manage users and user settings
 - Create and edit method template
 - Build multi-dataset
 - Edit preprocess settings
 - Edit analysis settings
 - Edit annotation or display properties
 - Convert Kinetic step or step type into Quantitation
 - Edit report pages
 - Sign document
 - Set commenting requirement

- Edit experiment info
- Edit sensor and sample info
- Include/exclude wells and sensors from analysis
- Run experiment
- Import analysis settings template to new dataset
- Export data and Excel report

Octet Acquisition and Octet Data Analysis HT CFR software must be linked to a ForteBio FB Server module to access and enforce the features under administrative control.

Audit Trail

Octet CFR software automatically generates time-stamped Audit Trails that record transactions that create, delete, or modify electronic records. In each instance, the Audit Trail records the date and time of the transaction, the computer and project name, the user ID of the person who was logged on, and information on the action performed. Additional information such as old and new values are also added for some Audit Trails that log changes in method file modifications and analysis settings.

Audit trails are recorded in the database managed by the ForteBio FB Server Monitor software. Each experiment has a unique identifier and all data-specific Audit Trails are logged with the experiment identifier. Audit trails can be filtered by experiment, user, machine, project or date for viewing and printing.

Octet Data Analysis HT CFR software also has an option to require users to enter comments or notes for each Audit Trail event. This option can be enabled and disabled using the Set Commenting Requirement permission.

Users can also add comments to an Audit Trail. Once logged, the Audit Trail cannot be deleted.

Administratively Controlled Electronic Signatures

Users who have been granted the Sign Document permission can access and electronically sign the experiment data. When experiment data is signed twice, it completely locks the data from further modification. Each electronic statement contains:

- User who signed the document
- Workstation or machine information
- ForteBio FB Server module information
- Project information
- Date and time
- Statement note

The author of the statement supplies the statement note. The electronic statement is produced when the signer agrees to the statement and Octet CFR software verifies the User ID and password combination of the signer.

Signed statements are listed sequentially in the Sign Document dialog. The Audit Trail is logged to the ForteBio FB Server Monitor software to record the action of signing a statement.

Automatic User Log Out (Idle Timeout)

The system administrator can set an option to have Octet CFR software automatically log out a user if the program is idle longer than a specified time. The user is automatically logged out after the specified time period even if Octet Data Acquisition CFR software is acquiring data from an Octet instrument.

After data acquisition has begun, Octet CFR software continues acquiring data until the experiment is finished and the data are saved, exported, and printed as set in Preferences, whether or not the user is logged on. If no users are logged on, data acquisition cannot be stopped manually.

Passwords

Expiration

The system administrator can set an option to have user passwords expire after a set period of time. If the system administrator activates the password expiration, then users are required to change their passwords at designated intervals. When expired, users are prompted to reset their passwords on the next login.

Requirements

The system administrator can set the minimum number of characters passwords must contain and the level of password complexity. At a higher level of complexity, passwords need to contain at least one alpha, one numeric, and one punctuation character. After logging on, users can change their password.

Security

The system administrator can set the maximum number of failed login attempts. If the user tries to log in with the wrong password and reaches the set number of tries, their account will be locked and this action will be logged into the Audit Trail.

The administrator can unlock the user and reset the user password.

If a user leaves the group or company, the system administrator can inactivate the user, thereby preventing any unauthorized use of the software.

Other Data Integrity Features

Overwriting Existing Files Prohibited

Existing method files cannot be overwritten using **File > Save As**. If the user attempts to save a record using the same name as a file that currently exists in the target directory, the user is notified that overwriting an existing file is prohibited, and that the file must be saved with a different name.

OCTET CFR SOFTWARE OVERVIEW

Octet Data Acquisition and Data Analysis software is available in an optional 21 CFR Part 11 version that enables users in GMP and GLP laboratories to comply with 21 CFR Part 11 regulations. This version of the software includes features such as user account management, audit trails and electronic signatures. In addition, the CFR version utilizes the ForteBio FB Server module to manage the information recorded during user sessions.

This chapter explains how to use the ForteBio FB Server module, compliance features and administrative functions specific to the CFR versions of the software.

FORTEBIO FB SERVER MODULE



NOTE: It is highly recommended that the ForteBio FB Server module not be installed on the computer connected to the Octet instrument. Instead, it should be configured on the administrator's computer that is connected to the server. This allows the administrator to control the users who have accounts and specific privileges in Octet Data Acquisition CFR software. Both the Octet system computer and the computer with the ForteBio FB Server module should be connected to the server.

When Octet Data Acquisition or Data Analysis CFR software is launched, users are prompted to log on to the ForteBio FB Server module. This initiates a user session where all system, software and user events are recorded. During user sessions, the ForteBio FB Server module manages and stores this recorded information. User sessions are closed when the user logs out or a set period of inactivity is reached. A new user session is initiated each time a user accesses the software.

**NOTE:**

Version 8.2 of Octet Data Acquisition and Data Analysis CFR software require version 8.2 of the ForteBio FB Server module. The software will automatically check the version of the ForteBio FB Server module in use and display a message if it is incompatible with version 8.2 software. Please contact your administrator to install version 8.2 of the ForteBio FB Server module if this happens.

SELECTING A SERVER LOCATION

**NOTES:**

Please contact your administrator to determine the ForteBio FB Server module host location that should be used.

Once the ForteBio FB Server module host location is selected, this location will be used as the default selection for the user account. It does not need to be reselected each time a new user session is initiated.

Users must select the host location of the ForteBio FB Server module during the login process. The ForteBio FB Server can be run on the local host computer where Octet Data Acquisition or Data Analysis CFR software is installed or from a network location.

1. Launch Octet Data Acquisition CFR software by double-clicking the desktop shortcut (Figure 4-1).



Figure 4-1: Octet Data Acquisition CFR Software Desktop Shortcut

The Login dialog box displays (Figure 4-2):

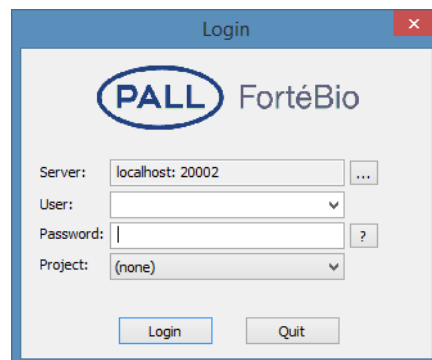


Figure 4-2: Login Dialog Box

2. Click on ... (**Browse**) to display the ForteBio FB Server dialog box (Figure 4-3).

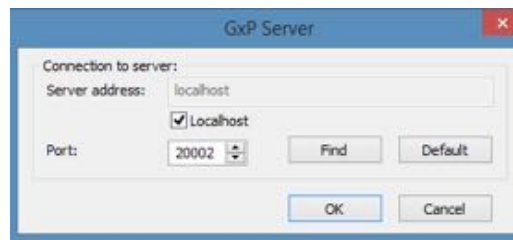


Figure 4-3: ForteBio FB Server Dialog Box

- **Choosing a remote host on same subnet**—If the ForteBio FB Server module is hosted on the same subnet, deselect the **Localhost** check box and click **Find**. A list of potential ForteBio FB Server module addresses will be listed (Figure 4-4). Choose the desired location from the list and click **OK**.

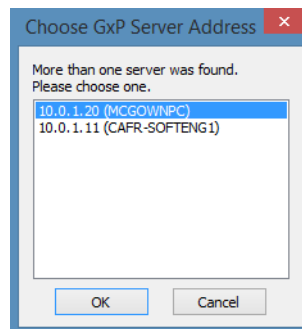


Figure 4-4: ForteBio FB Server Address Search Results

- **Choosing a remote host on another subnet**—If the ForteBio FB Server module is hosted on a different subnet, deselect the **Localhost** check box. Enter the IP address of the computer hosting the ForteBio FB Server module (Figure 4-5).

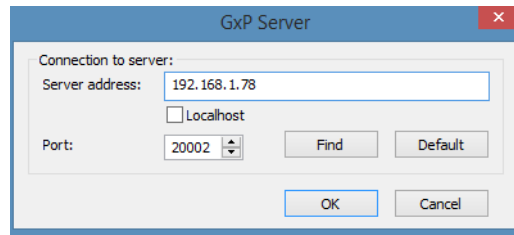


Figure 4-5: Manual Entry of Remote Host Address

- **Choosing the local host (not recommended)**—If an ForteBio FB Server has been installed on the local computer and is to be used as the ForteBio FB Server module host, select the **Localhost** check box. Change the **Port** number if needed.

When the ForteBio FB Server module host location has been selected or entered, click **OK** to save changes and exit the Authentication Server dialog box. The ForteBio FB Server module location will now be listed as the Server in the Login box (Figure 4-6).

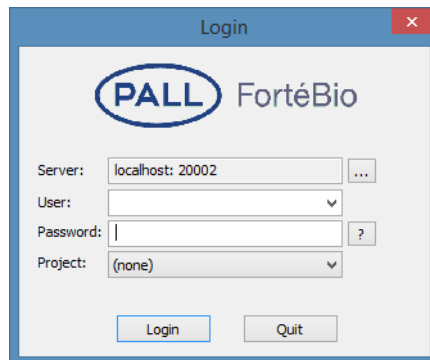


Figure 4-6: Login Dialog Box—Displaying ForteBio FB Server Location

You can now proceed to Step 3 in the next section or click **Quit**.

STARTING A USER SESSION



NOTE: Before starting your first user session, please contact your administrator to determine the ForteBio FB Server module host location that should be used.

1. Launch Octet Data Acquisition or Data Analysis CFR software by double-clicking the respective desktop shortcut.

The Login dialog box displays (Figure 4-7):

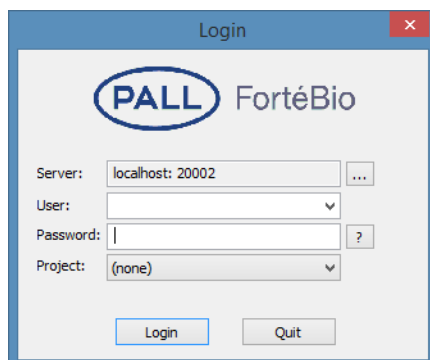


Figure 4-7: Login Dialog Box

2. Confirm that the **Server** location is correct. If not, please see “Selecting a Server Location” on page 87.
3. From the **User** drop down list, select your **user name** (Figure 4-8).



NOTE: To start an administrator session, select **Administrator** in the **User** drop down list.



Figure 4-8: User Name Selection

4. Enter your password in the **Password** text box. Click ? for a password reminder if needed (Figure 4-9).



Figure 4-9: Password Reminder

5. Select a project from the **Project** drop down list, if required (Figure 4-10).



Figure 4-10: Project Selection

6. Click **Login**.

Octet Data Acquisition or Data Analysis CFR software will now launch and start the user session. During the session, the user account and project selected at login display in the software status bar (Figure 4-11).

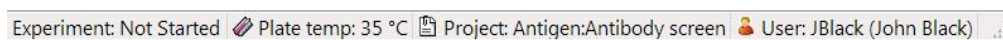


Figure 4-11: Status Bar

**NOTES:**

Software operation may be restricted based on your user privileges. For more information on user privileges, please contact your administrator.

User sessions are automatically locked after a period of inactivity which is set by the administrator. The Login dialog box will display and a message indicating the session has been locked will be shown. You can choose to log back into the session or log off at this time. User sessions will not be locked during experimental data acquisition.

ACCESSING COMPLIANCE FEATURES

The 21 CFR Part 11-compliant features provided in the CFR versions of Octet Data Acquisition and Data Analysis software can be accessed by clicking the **Security** menu (Figure 4-12) from the software's **Main Menu**:

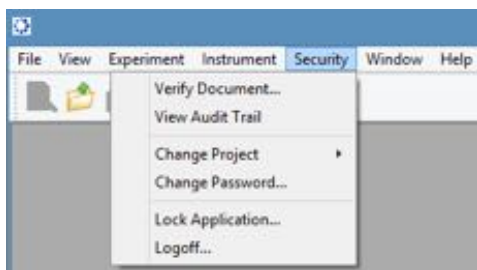


Figure 4-12: Security Menu



NOTE: Security menu options in Octet Data Acquisition and Data Analysis CFR software applications are identical.

Experiment and Method File Compliance

When using the CFR version of Octet Data Acquisition software, only 21 CFR Part 11-compliant experiments and method files generated using the CFR version of the software can be opened. Files generated using the non-compliant version of the software cannot be opened, and a message indicating this will be presented.

Verifying Digital Signatures

The electronic signature of method (.fmf) and data (.frd) files can be verified to ensure they were generated using 21 CFR Part 11-compliant software.



NOTE: When verifying digital signatures, both method (.fmf) and data (.frd) files can be selected in Octet Data Acquisition and Data Analysis CFR software.

1. Click **Security > Verify Document**.

The Verify Digital Signature dialog box (Figure 4-13) displays:

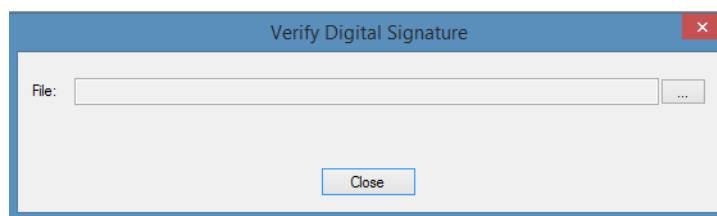


Figure 4-13: Verify Digital Signature

2. Click ... to display the Open dialog box (Figure 4-14), which allows you to browse for the desired .fmf or .frd file.

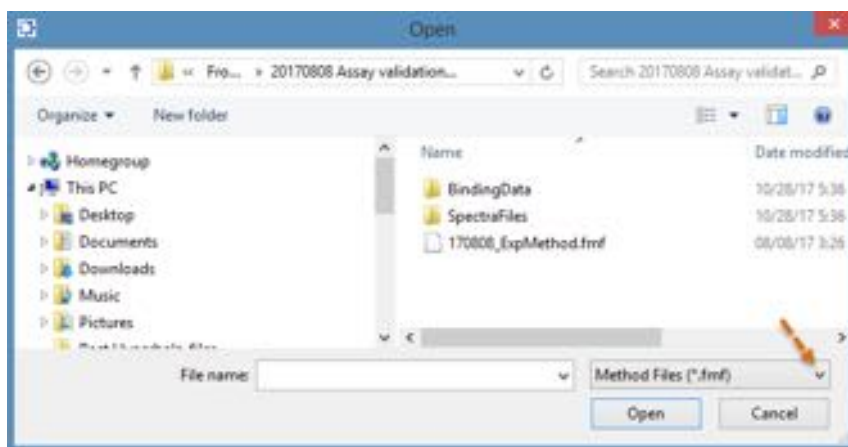


Figure 4-14: Open Dialog Box

To change the file type available for selection, click the down arrow in the file type box to display the menu (Figure 4-15), then select the desired format.

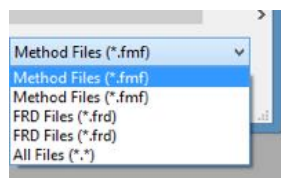


Figure 4-15: File Type Menu

3. Select a file type, then the desired file and click **Open**.

A message will display in the Verify Digital Signature dialog box indicating file compliance status: Compliant or Non-Compliant (Figure 4-16):

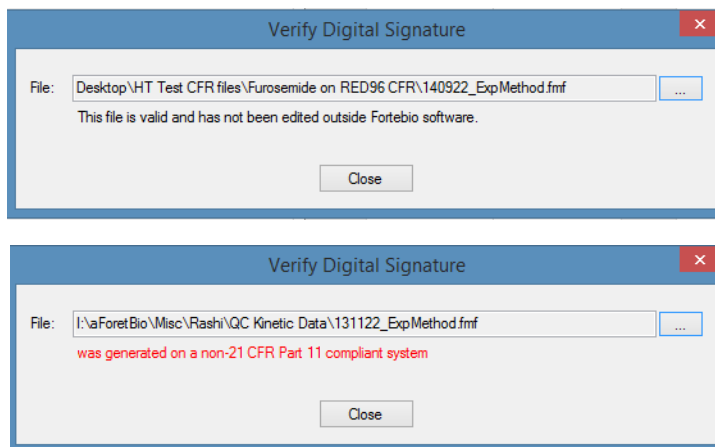


Figure 4-16: File Compliant (top), File Not a CFR Document (bottom)

Viewing the Audit Trail

The Audit Trail displays a historical log of user, system and software events recorded during user sessions. To view the Audit Trail, click **Security > View Audit Trail**. An example is shown in Figure 4-17.

Audit Trail					
From:	02/08/18	Project:	(any)	User:	DBean
To:	03/10/18	Experiment:	(any)	Machine:	MCGOWNPC
Date/Time	Project	Machine	Action	Description	
2018/03/10 10:30:23	Receptor Ligand Screen	MCGOWNPC	Method parameters changed	C:\Users\N	
2018/03/10 10:25:52	Cell Culture Screen	MCGOWNPC	Method parameters changed	C:\Users\N	
2018/03/10 10:25:52	Cell Culture Screen	MCGOWNPC	Method parameters changed	C:\Users\N	
2018/03/10 10:23:35	Antigen:Antibody Screen	MCGOWNPC	Method parameters changed	C:\Users\N	
2018/03/10 10:23:35	Antigen:Antibody Screen	MCGOWNPC	Method parameters changed	C:\Users\N	
2018/03/10 10:16:57	Antigen:Antibody Screen	MCGOWNPC	Save method file	C:\Users\N	
2018/03/10 10:15:05	Antigen:Antibody Screen	MCGOWNPC	Open method file	C:\Users\N	
2018/03/10 10:14:54	Antigen:Antibody Screen	MCGOWNPC	User login	DBean	
2018/03/10 10:06:35	Antigen:Antibody Screen	MCGOWNPC	User logout	DBean	

Figure 4-17: Audit Trail

For Users other than the Administrator, events shown in the Audit Trail are only those associated with the user account that is currently logged in. Administrators can view events associated with all Users. By default, the events initially displayed in the Audit Trail will be those associated with the project selected at login and the machine (computer) currently being used.

You can sort the events in the Audit Trail by clicking on any of the column headers.

You can also filter (limit) the events by selecting a particular project, Experiment, Machine and Users (Administrators only) from the corresponding drop down lists. For example, Figure 4-18 shows a drop down menu for selecting events associated with a Project.

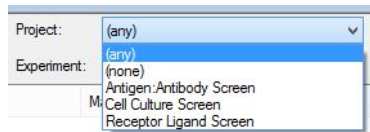


Figure 4-18: Selecting Events by Project

You can limit your search to a specific time period by choosing the start/stop day from the calendar drop down menus (Figure 4-19).

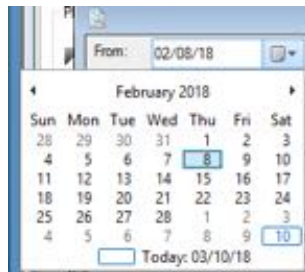


Figure 4-19: Selecting Events in a Time Period

The Audit Trail will then only display events for the entries and time period selected.

In addition to the specific project and machine selections, the following list options are also available:

- **(any)**—Displays events associated with all projects, experiments and/or machines for the user account. Administrators can view events associated with all Users.
- **(none)**—Displays all project and machine events not associated with a specific project (Project list only)

Viewing Event Details

If an action entailed a change in Method Parameters, you can view details of the change(s) by double-clicking on the individual action to display the Event Details box (Figure 4-20).

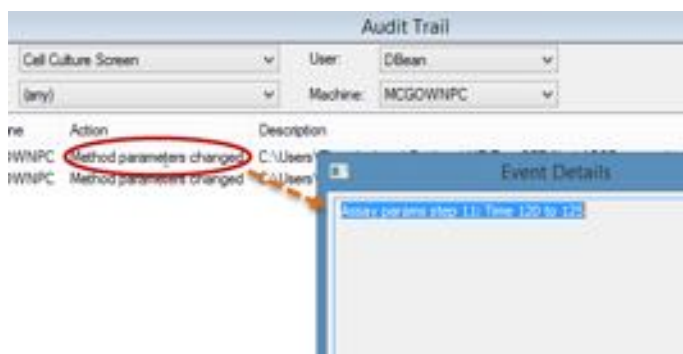


Figure 4-20: Viewing Event Details

Changing Projects During a User Session

During an active session, users can switch to another project in Octet Data Acquisition or Data Analysis CFR software without having to log out.

1. Click **Security > Change Projects**.

A list of projects assigned to your user account will be shown with the current active project highlighted (Figure 4-21):

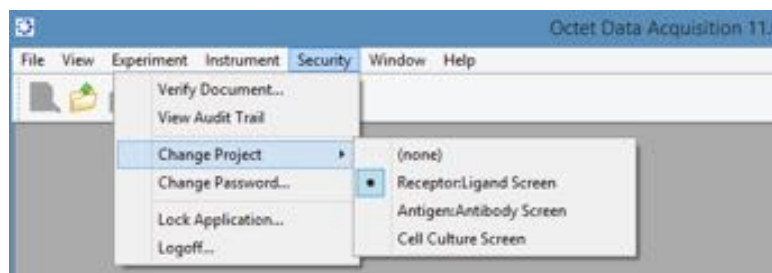


Figure 4-21: Changing Projects

2. Select the desired project from the list. The selected project will now become the active project for the user session.

Changing Your Password

1. Click **Security > Change Password** to display the Change Password dialog box (Figure 4-22).

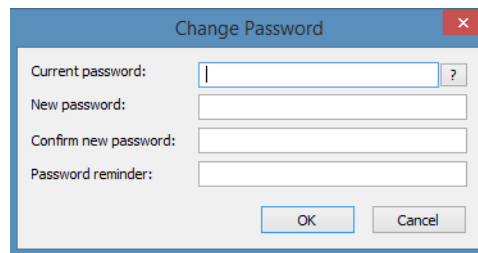


Figure 4-22: Change Password Dialog Box

2. Enter the **Current password** for your user account. Click ? for a password reminder.
3. Enter and re-enter your **new password**. If desired, enter a Password reminder.
4. Click **OK**.

Locking/Unlocking the Application

1. Click **Security > Lock Application**. The application Locked dialog box (Figure 4-23) appears and remains until you unlock it.

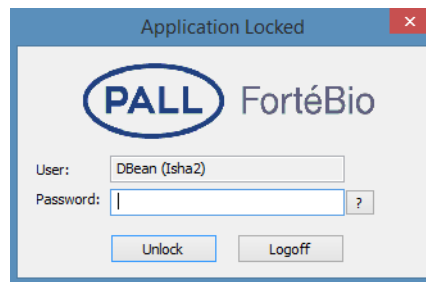


Figure 4-23: Application Locked Dialog Box

2. Enter your password and click **Unlock** or **Logoff**.

Logging Off the Application

1. Click **Security > Logoff**.
2. Click **OK** to *Are you sure you want to logoff?*.

The Login dialog box (Figure 4-24) appears and is available for other Users.

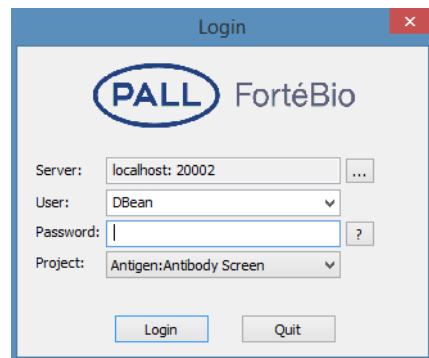


Figure 4-24: Login Dialog Box

3. **Quit** the application if desired.

ADMINISTRATOR OPTIONS

Installing the ForteBio FB Server Module

**NOTES:**

Recommended: *Install a single copy of the ForteBio FB Server module on the administrator's computer that is connected to the network.*

If needed: *Install additional copies of the ForteBio FB Server on other administrator computers connected to the network. Some companies may have multiple departments and choose to have multiple locations for the ForteBio FB Server. For added security, the ForteBio FB Server module should not be accessible to all users.*

Not recommended: *Install a copy on the local host computer where the Data Acquisition or Data Analysis HT 21 CFR Part 11 software is installed.*

Version 9.0, 10.0 and 11.0 of Octet Data Acquisition and Data Analysis CFR software require version 9.0, 10.0 and 11.0 of the ForteBio FB Server module, respectively. The software will automatically check the version of the ForteBio FB Server module in use and display a message if it is incompatible.

1. Navigate to the window listing the files located on the installation CD.
2. Double-click **ForteBio FB Server.exe** to launch the installer.
3. If prompted with the *Do you want the following program from an unknown publisher to make changes to this computer?* message, reply **Yes**.

The installation wizard should display (Figure 4-25).



Figure 4-25: ForteBio FB Server Software Setup Wizard

4. Click **Next** to display the Choose Components dialog box (Figure 4-26).

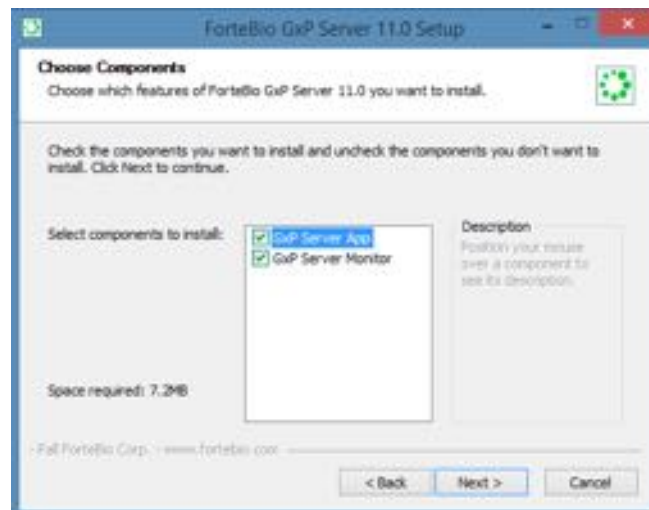


Figure 4-26: Choose Components

The default selection includes both the ForteBio FB Server and ForteBio FB Server Monitor. It is imperative to install both.

5. Click **Next** to display the Choose Install Location dialog box (Figure 4-27).

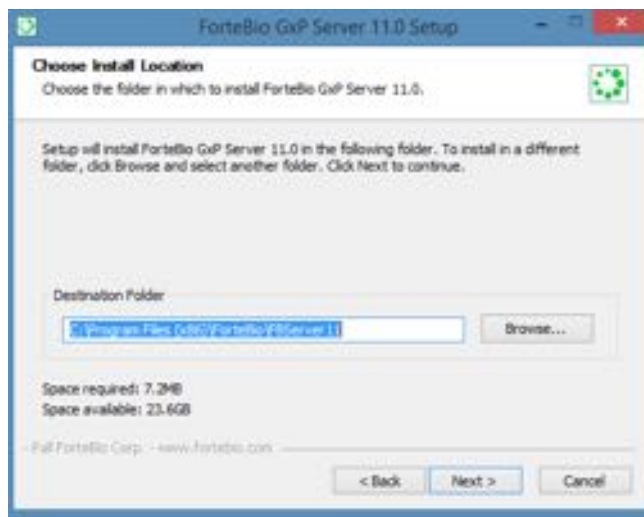


Figure 4-27: Choose Install Location

The default location for the software on the local machine is C:\Program Files\ForteBio\DataAnalysis.

6. Click **Next** to accept this path location.

The Choose Start Menu Folder dialog box displays (Figure 4-28).

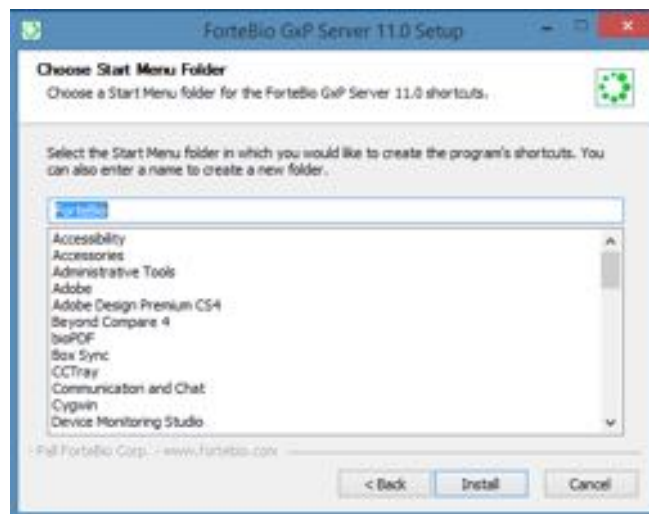


Figure 4-28: Choose Start Menu Folder Dialog Box

The default Start Menu folder is ForteBio.

7. Click **Install**.

The installation wizard takes a few seconds to install (Figure 4-29).

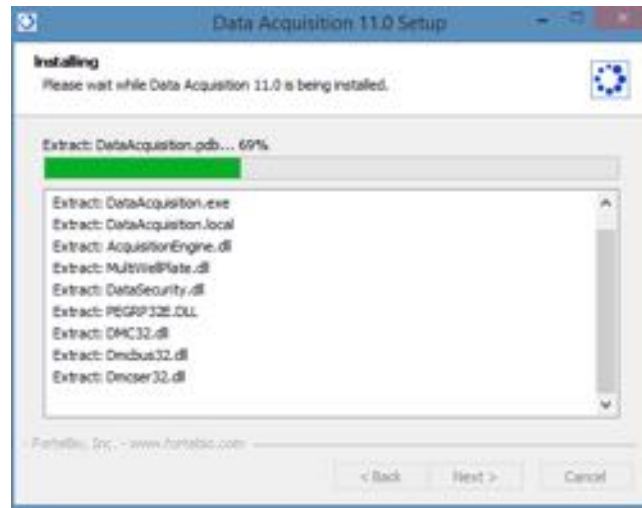


Figure 4-29: Installation Progress

The installation wizard displays the Completing the ForteBio FB Server Setup Wizard dialog box (Figure 4-30).



Figure 4-30: Completing the ForteBio FB Server Software Setup

8. Click **Finish** to complete the installation.

Database Backup and ForteBio FB Server Module Upgrade



IMPORTANT: It is strongly recommended that you make a backup copy of the existing database before installing and upgrading to ForteBio FB Server module version 11.x.

To backup your database and then upgrade your ForteBio FB Server module to version 11.0, follow the steps below. Once these steps are complete, the existing audit trail database that stores audit logs will be upgraded to the version 11.x schema.

Step 1: Backup the Version 8.0-10.0 Database

The database for the ForteBio FB Server module is a file-based database. So to make a backup of the database, all you need to do is make a copy of the database file and save it to an archival location. Since v11 software only works with ForteBio FB Server module v11.0, it is important to back-up the older database before installing the new ForteBio FB Server.

1. Go to the Windows® Task Manager and click the **Services** Tab.
2. Right-click **FBServer** and select **Stop** to stop the service.



NOTE: This will only be enabled if you have administrator privileges to your machine.

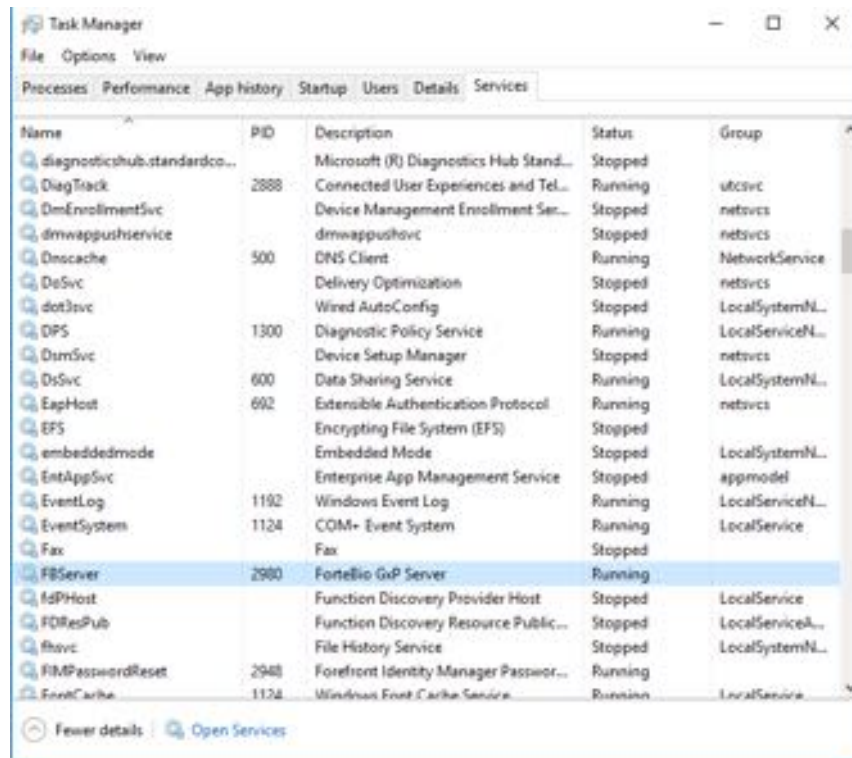


Figure 4-31: Windows Task Manager Services Tab

- Open Windows Explorer and browse to the hidden program data folder. Make a copy of the **FBEventLog.db** and **FBServer.db** files.
- Save the copies to another location.

Step 2: Upgrade the ForteBio FB Server Module v11.0

- From the Windows Start Menu, select **All Programs**. Scroll to the ForteBio FB Server folder and click on the folder to expand.
- Select **Uninstall ForteBio GxP Server**.
- Install ForteBio FB Server module v11.0 per the instructions in "Installing the ForteBio FB Server Module" on page 99.

When the ForteBio FB Server module software starts, it will automatically update the audit trail database.

Administrator Account Setup

- Launch the Octet Data Acquisition or Data Analysis CFR software by double-clicking on its desktop shortcut (Figure 4-32):

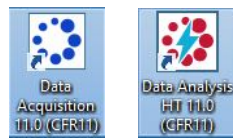


Figure 4-32: Octet Data Acquisition and Data Analysis HT CFR Software Desktop Shortcuts

The **Login** dialog box (Figure 4-33) will display:

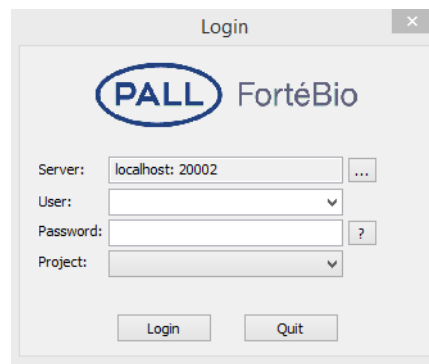


Figure 4-33: Login Dialog Box

2. Select a **Server** location by clicking ... (**Browse**).

The ForteBio FB Server dialog box (Figure 4-34) will display:

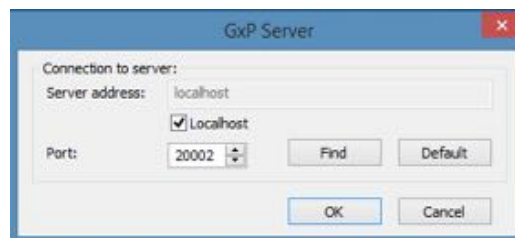


Figure 4-34: ForteBio FB Server Dialog Box

Click **Default** to recall the default server settings of localhost and Port 2002.

- **Local host**—If the local computer is to be used as the ForteBio FB Server module host, select the **Localhost** check box. Change the Port number if needed.
- **Remote host on same subnet**—If the ForteBio FB Server module is hosted on the same subnet, deselect the **Localhost** check box and click **Find**. A list of potential ForteBio FB Server module addresses will be listed (Figure 4-35). Choose the desired location from the list and click **OK**.

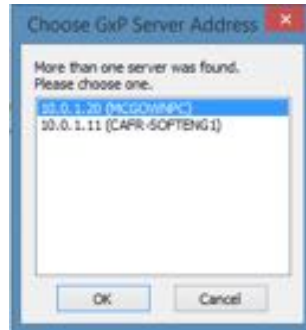


Figure 4-35: ForteBio FB Server Address Dialog Box

- **Remote host on another subnet**—If the ForteBio FB Server module is hosted on a different subnet, deselect the **Localhost** check box. Enter the IP address of the computer hosting the ForteBio FB Server module (Figure 4-36) and click **OK** to save changes and exit. The ForteBio FB Server module location will now be listed as the Server in the Login box.

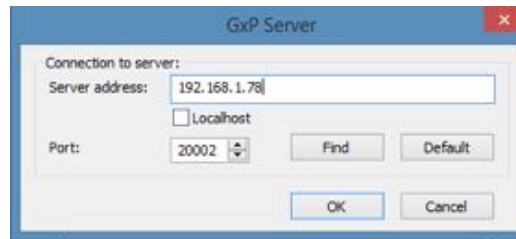


Figure 4-36: Manual Entry of Remote Host Address



NOTE: Once the ForteBio FB Server module host location is selected, this location will be used as the default selection for the administrator account. It does not need to be re-selected each time a new session is initiated.

3. From the **User** drop down list in the Login dialog box (Figure 4-37), select **Administrator**.



Figure 4-37: Administrator User Selection

4. Leave the Password blank and click **Login**. The Change Password dialog box will display (Figure 4-38).

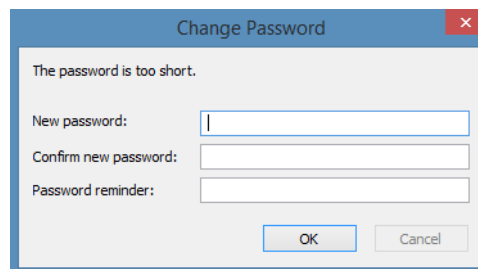


Figure 4-38: Change Password Dialog Box

5. Enter a **New password** and **Password reminder** (optional) and click **OK**.

The Octet Data Acquisition or Data Analysis software will now launch and initiate an administrator user session which will allow access to administration options.

Starting an Administrator User Session

Administrators initiate new user sessions the same way non-administrative users do.

1. Launch the Octet Data Acquisition or Data Analysis CFR software by double-clicking on the desktop shortcuts (Figure 4-39):

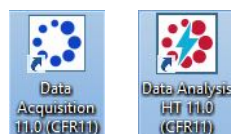


Figure 4-39: Octet Data Acquisition and Data Analysis HT CFR Software Desktop Shortcuts

The Login dialog box (Figure 4-40) will display:

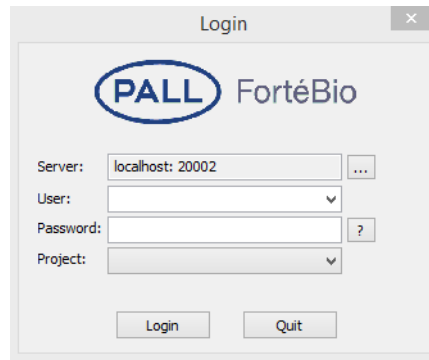


Figure 4-40: Login Dialog Box

2. Confirm that the Server location is correct. If not, please see “Administrator Account Setup” on page 104.
3. Select **Administrator** from the **User** drop down list (Figure 4-41).



Figure 4-41: Administrator User Name Selection

4. Enter your **Password**. Click ? for a password reminder if needed (Figure 4-42).



Figure 4-42: Password Reminder

5. Select a project from the **Project** drop down list (Figure 4-43), if required.



Figure 4-43: Project Selection

6. Click **OK**.

The Octet Data Acquisition or Data Analysis CFR software will now launch and start the administrator session. During the session, the administrator account and project selected at login display in the software status bar (Figure 4-44):

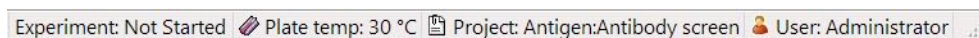


Figure 4-44: Status Bar



NOTE: Administrator and user sessions are automatically locked after a period of inactivity set using the `UserIdleMin` constant. Please see “Constants Tab” on page 125 for more information. The Login dialog box will display and a message indicating the session has been locked will be shown. You can choose to log back into the session or log off at this time. Administrator and user sessions will not be locked during experimental data acquisition.

Accessing Administrator Options

You can access administrator options in the ForteBio FB Server Monitor.

- **ForteBio FB Server module on network location**—Double-click on the **FBServerMonitor.exe** file (Figure 4-45) in the FBServer folder in the installed location:

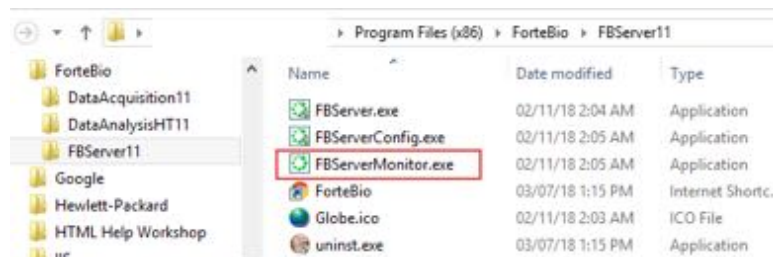


Figure 4-45: Accessing the ForteBio FB Server on the Network

- **ForteBio FB Server module on a local host computer** - Double-click the FB Server Monitor desktop shortcut (Figure 4-46):

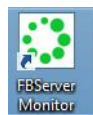


Figure 4-46: FB Server Monitor Desktop Shortcut



NOTE: Tools for testing server functionality are available in `FBServerConfig.exe`. Please see “Accessing the ForteBio FB Server Configuration Module” on page 129 for more information.

The ForteBio FB Server Monitor window will display (Figure 4-47):

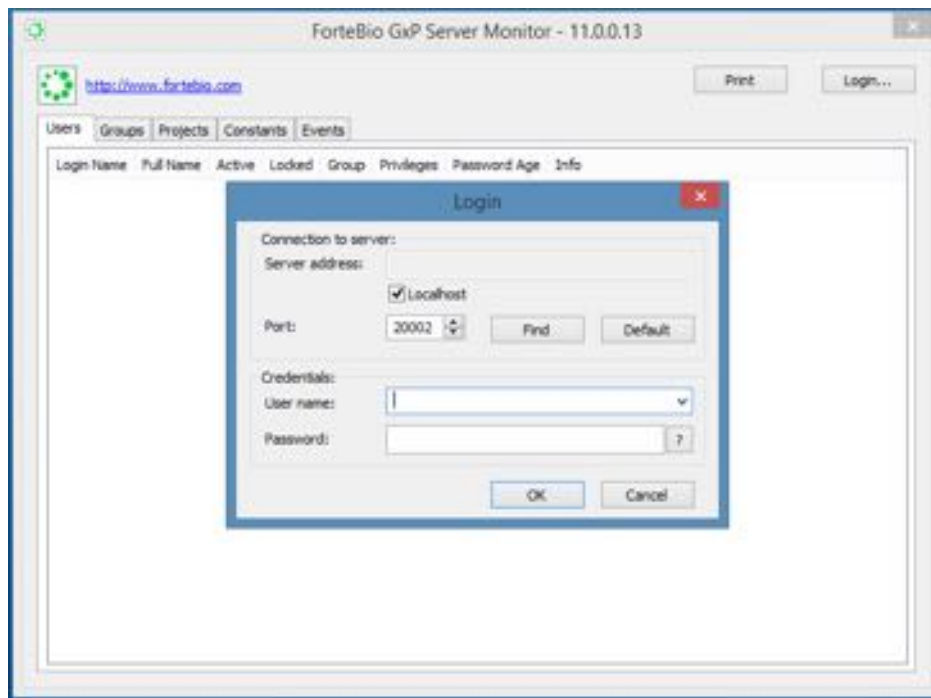


Figure 4-47: ForteBio FB Server Monitor with Login Dialog Box

From the **User name** drop down list, select **Administrator**. Enter your **Password**. Click '?' for a password reminder if needed. Click **OK** to dismiss the Login dialog which then displays the ForteBio FB Server Monitor Users Tab (Figure 4-48).

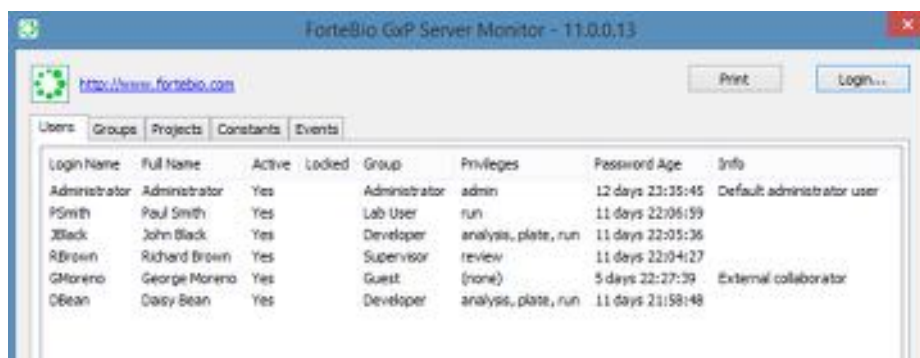


Figure 4-48: ForteBio FB Server Monitor Users Tab

Five tabs are available in the FB Server Monitor window:

- **Users Tab**—Allows user and password management and individual privileges selection
- **Groups Tab**—Allows user group management and group privileges selection

- **Projects Tab**—Allows project management and setup
- **Constants Tab**—Allows setup of password requirements, cached server credentials and screen lock due to inactivity.
- **Events Tab**—Displays event logs for individual user accounts, projects or machines

Click on a tab to view its information.

Each tab has a context-sensitive Tab menu that can be accessed by right-clicking in the tab window. The menu displayed depends on the tab currently selected and the position of the cursor when you right-click.

Contents of tabs can also be sorted. Clicking the header of a column sorts content alphabetically or chronologically, and data in other columns are also sorted to maintain data association.

Users Tab

The Users Tab (Figure 4-49) allows administrators to add and delete user accounts as well as set and change individual user account privileges and passwords. The columns contain information about each user. The table can be sorted by clicking on any column header. For example, the users in Figure 4-49 have been sorted alphabetically according to Group.



Login Name	Full Name	Active	Locked	Group	Privileges	Password Age	Info
Administrator	Administrator	Yes		Administrator	admin	12 days 23:35:45	Default administrator user
PSmith	Paul Smith	Yes		Lab User	run	11 days 22:06:59	
JBlack	John Black	Yes		Developer	analysis, plate, run	11 days 22:05:36	
RBrown	Richard Brown	Yes		Supervisor	review	11 days 22:04:27	
GMoreno	George Moreno	Yes		Guest	(none)	5 days 22:27:39	External collaborator
DBean	Daisy Bean	Yes		Developer	analysis, plate, run	11 days 21:58:48	

Figure 4-49: User Tab Information Sorted by Group

Creating a New User Account

1. Right-click in a blank area in the **Users Tab**. The Tab menu will appear (Figure 4-50).

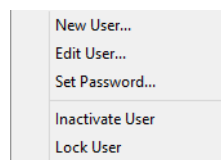


Figure 4-50: Users Tab Menu New User Option

2. Select **New User** to display the New User dialog box (Figure 4-51).

Figure 4-51: New User Dialog Box

3. **Assign Account Details.** Enter the user's **Login name**, **Full name**, **Information** (optional), **Password**, and **Password reminder** (optional).
4. **Assign to a User Group.** Select a user group from the **Group** drop down list. The following default group selections are available:
 - **Administrators**—can manage Users and Group settings including add, delete, edit and view all events
 - **Supervisors**—can review data and events
 - **Developers**—can create, run, save and export data
 - **Lab Users**—can only run experiments
 - **Guests**—have no explicit privileges, these must be assigned by the administrator

If other user groups have been created by an administrator, they will also be available for selection in the **Group** drop down box. For more information, please see "Creating a New User Group" on page 121.

5. **Modify Privileges.** The default privilege sets for each group type are shown in Table 4-1.

Table 4-1: Default User Group Privileges

Privilege Set	Administration	Analysis and Change	Review	Plate Settings	Run Experiment
Administrator	✓				
Supervisor			✓		
Developer		✓		✓	✓
Lab User			✓		
Guest					



NOTE: Analysis and Change, Review and Plate applies only Octet Data Analysis software, not Octet Data Analysis HT software.

Individual privilege sets for each user are shown Figure 4-52. To add/remove a specific privilege for a User, select/deselect the corresponding check box.

More details about each privilege are detailed in “Privilege Administration” on page 116 to aid in customizing privileges for each user, if needed.

6. **Options**—Select the **Password does not expire** check box if desired. This check box is deselected by default so that user account passwords expire according to the set PasswordTTL constant. For more information on setting constants please see “Constants Tab” on page 125.

Administrator	Supervisor
<p>Administrators can add/delete/edit users and groups</p> <p>Administration</p> <ul style="list-style-type: none"> <input checked="" type="checkbox"/> Manage users and user settings <p>Analysis and Change</p> <ul style="list-style-type: none"> <input type="checkbox"/> Create and edit method template <input type="checkbox"/> Build multi-dataset <input type="checkbox"/> Edit preprocess settings <input type="checkbox"/> Edit analysis settings <input type="checkbox"/> Edit annotation/display properties <input type="checkbox"/> Convert Kinetic step/step type into Quantitation <input type="checkbox"/> Edit report pages <input type="checkbox"/> (DATA ANALYSIS) Change <p>Review</p> <ul style="list-style-type: none"> <input type="checkbox"/> Sign document <input type="checkbox"/> Set commenting requirement <input type="checkbox"/> (DATA ANALYSIS) Review <p>Plate Settings</p> <ul style="list-style-type: none"> <input type="checkbox"/> Edit experiment info <input type="checkbox"/> Edit sensor and sample plate info <input type="checkbox"/> Include/exclude wells & sensors from analysis <input type="checkbox"/> (DATA ANALYSIS) Plate <p>Run Experiment</p> <ul style="list-style-type: none"> <input type="checkbox"/> Run experiment <input type="checkbox"/> Import analysis settings template to new dataset <input type="checkbox"/> Export data and Excel report 	<p>Supervisors can review data and events</p> <p>Administration</p> <ul style="list-style-type: none"> <input type="checkbox"/> Manage users and user settings <p>Analysis and Change</p> <ul style="list-style-type: none"> <input type="checkbox"/> Create and edit method template <input type="checkbox"/> Build multi-dataset <input type="checkbox"/> Edit preprocess settings <input type="checkbox"/> Edit analysis settings <input type="checkbox"/> Edit annotation/display properties <input type="checkbox"/> Convert Kinetic step/step type into Quantitation <input type="checkbox"/> Edit report pages <input type="checkbox"/> (DATA ANALYSIS) Change <p>Review</p> <ul style="list-style-type: none"> <input checked="" type="checkbox"/> Sign document <input checked="" type="checkbox"/> Set commenting requirement <input checked="" type="checkbox"/> (DATA ANALYSIS) Review <p>Plate Settings</p> <ul style="list-style-type: none"> <input type="checkbox"/> Edit experiment info <input type="checkbox"/> Edit sensor and sample plate info <input type="checkbox"/> Include/exclude wells & sensors from analysis <input type="checkbox"/> (DATA ANALYSIS) Plate <p>Run Experiment</p> <ul style="list-style-type: none"> <input type="checkbox"/> Run experiment <input type="checkbox"/> Import analysis settings template to new dataset <input type="checkbox"/> Export data and Excel report
<p>Developer</p> <p>Developers can create, run, save and export data</p> <p>Administration</p> <ul style="list-style-type: none"> <input type="checkbox"/> Manage users and user settings <p>Analysis and Change</p> <ul style="list-style-type: none"> <input checked="" type="checkbox"/> Create and edit method template <input checked="" type="checkbox"/> Build multi-dataset <input checked="" type="checkbox"/> Edit preprocess settings <input checked="" type="checkbox"/> Edit analysis settings <input checked="" type="checkbox"/> Edit annotation/display properties <input checked="" type="checkbox"/> Convert Kinetic step/step type into Quantitation <input checked="" type="checkbox"/> Edit report pages <input checked="" type="checkbox"/> (DATA ANALYSIS) Change <p>Review</p> <ul style="list-style-type: none"> <input type="checkbox"/> Sign document <input type="checkbox"/> Set commenting requirement <input type="checkbox"/> (DATA ANALYSIS) Review <p>Plate Settings</p> <ul style="list-style-type: none"> <input checked="" type="checkbox"/> Edit experiment info <input checked="" type="checkbox"/> Edit sensor and sample plate info <input checked="" type="checkbox"/> Include/exclude wells & sensors from analysis <input checked="" type="checkbox"/> (DATA ANALYSIS) Plate <p>Run Experiment</p> <ul style="list-style-type: none"> <input checked="" type="checkbox"/> Run experiment <input checked="" type="checkbox"/> Import analysis settings template to new dataset <input checked="" type="checkbox"/> Export data and Excel report 	<p>Lab User</p> <p>Lab Users can only run experiments</p> <p>Administration</p> <ul style="list-style-type: none"> <input type="checkbox"/> Manage users and user settings <p>Analysis and Change</p> <ul style="list-style-type: none"> <input type="checkbox"/> Create and edit method template <input type="checkbox"/> Build multi-dataset <input type="checkbox"/> Edit preprocess settings <input type="checkbox"/> Edit analysis settings <input type="checkbox"/> Edit annotation/display properties <input type="checkbox"/> Convert Kinetic step/step type into Quantitation <input type="checkbox"/> Edit report pages <input type="checkbox"/> (DATA ANALYSIS) Change <p>Review</p> <ul style="list-style-type: none"> <input type="checkbox"/> Sign document <input type="checkbox"/> Set commenting requirement <input type="checkbox"/> (DATA ANALYSIS) Review <p>Plate Settings</p> <ul style="list-style-type: none"> <input type="checkbox"/> Edit experiment info <input type="checkbox"/> Edit sensor and sample plate info <input type="checkbox"/> Include/exclude wells & sensors from analysis <input type="checkbox"/> (DATA ANALYSIS) Plate <p>Run Experiment</p> <ul style="list-style-type: none"> <input checked="" type="checkbox"/> Run experiment <input checked="" type="checkbox"/> Import analysis settings template to new dataset <input checked="" type="checkbox"/> Export data and Excel report
<p>Guest</p> <p>Guests have no explicit privileges</p> <p>Administration</p> <ul style="list-style-type: none"> <input type="checkbox"/> Manage users and user settings <p>Analysis and Change</p> <ul style="list-style-type: none"> <input type="checkbox"/> Create and edit method template <input type="checkbox"/> Build multi-dataset <input type="checkbox"/> Edit preprocess settings <input type="checkbox"/> Edit analysis settings <input type="checkbox"/> Edit annotation/display properties <input type="checkbox"/> Convert Kinetic step/step type into Quantitation <input type="checkbox"/> Edit report pages <input type="checkbox"/> (DATA ANALYSIS) Change <p>Review</p> <ul style="list-style-type: none"> <input type="checkbox"/> Sign document <input type="checkbox"/> Set commenting requirement <input type="checkbox"/> (DATA ANALYSIS) Review <p>Plate Settings</p> <ul style="list-style-type: none"> <input type="checkbox"/> Edit experiment info <input type="checkbox"/> Edit sensor and sample plate info <input type="checkbox"/> Include/exclude wells & sensors from analysis <input type="checkbox"/> (DATA ANALYSIS) Plate <p>Run Experiment</p> <ul style="list-style-type: none"> <input type="checkbox"/> Run experiment <input type="checkbox"/> Import analysis settings template to new dataset <input type="checkbox"/> Export data and Excel report 	

Figure 4-52: Default User Privileges

Privilege Administration



NOTE: All users will have the default privilege to print the audit trails.

Manage Users and User Settings

This privilege is mostly restricted to creating, modifying, inactivating users with specific privileges in the FB Monitor software.

Analysis and Change

Create and edit method templates: This privilege applies to modifying or creating new method files in Octet Data Acquisition software.

Build multi-dataset: This privilege applies to building mega-datasets by overlaying or appending multiple experiments for analysis in Octet Data Analysis HT software (Figure 4-53).

Linked Operations:

- Build dataset - append experiment
- Build dataset - overlay experiment
- Build dataset - move experiment
- Build dataset - delete experiment
- Print audit trail

Figure 4-53: Build Multi-Dataset

Edit preprocess settings: This privilege applies to pre-processing and correction settings during data analysis in Octet Data Analysis HT software (Figure 4-54).

Linked Operations:

- Modify reference sensor settings
- Modify reference sample settings
- Modify correction settings for kinetic analysis
- Modify correction settings for quantitative analysis
- Print audit trail

Figure 4-54: Edit Preprocess Settings

Edit annotation/display properties: This privilege applies to display settings, such as changing color of full traces or kinetic cycles, setting column order, etc. in Octet Data Analysis HT software (Figure 4-55).

Linked Operations:

- Modify color of kinetic cycles
- Set column orders in kinetic result table
- Modify color in quantitative analysis
- Modify sample alerts for quantitative settings
- Set column orders in quantitative table
- Modify EB color threshold settings
- Modify trace color
- Print audit trail

Figure 4-55: Edit Annotation/Display Properties

Convert kinetic step/step type into quantitation: This privilege applies to converting select step or step type in the Kinetics mode to Quantitation mode in Data Analysis HT software.

Edit report pages: This privilege only applies to the actions listed in Figure 4-56 in the Report module of Octet Data Analysis HT software.

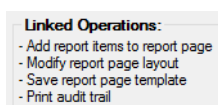


Figure 4-56: Edit Report Pages

Review

Sign document: This privilege applies to electronic signature that can be added to an analyzed dataset in the Data Analysis HT software to prevent any modification. More details about this feature can be found in the Data Analysis HT software manual on pg x.x

Set commenting requirement: This privilege applies to adding reasons/comments for making any changes to analysis settings that are added to the audit trail of the Data Analysis HT software. More details about this feature can be found in Chapter 9 of the Octet Data Analysis HT Software User Guide.

Plate Settings

Edit experiment info: This privilege applies to changing assay parameters in an existing method file in Octet Data Acquisition software. This privilege setting enables modifications in Assay Settings for Quantitation experiments that include assay type, step type, step time and shake speed. In Kinetics experiments, it enables modifications in step data and assay steps list tables under in the Assay Definition tab.

Edit sensor and sample plate info: This privilege applies to sample and biosensor information in an existing method file in Octet Data Acquisition software. This privilege setting enables modifications to the sample type, location, concentration, ID, replicate group, and dilution in the Plate Definition tab and sensor location and lot number in the Sensor Assignment tab.

Include/exclude wells and sensors from analysis: This privilege applies mainly to including and excluding data and/or biosensors from analysis in Octet Data Analysis HT software (Figure 4-57).

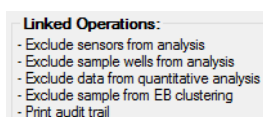


Figure 4-57: Include/Exclude Wells and Sensors from Analysis

Run Experiment

Run Experiment: This privilege enables the user to load a method file, change the experiment name, press GO and run experiments in Octet Data Acquisition software. It also allows the user to open a dataset and load the Report template in the software.

Import analysis settings template to new dataset: This privilege is adequate for a user who can only import or load existing analysis settings into a newly acquired dataset in Octet Data Analysis HT software. They are not able to make any modifications to the analysis settings.

Export data and Excel reports: This privilege enables the user to export all data (raw data, processed data) and Excel reports, including report points from Octet Data Acquisition and Data Analysis HT software.

Viewing and Changing User Account Settings

1. Position the cursor on the account and right-click to display the Tab menu (Figure 4-58).

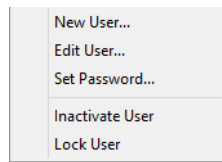


Figure 4-58: User Tab Menu

2. Select **Edit User**.
3. If needed, make changes to privileges by selecting/deselecting check boxes (Figure 4-59).

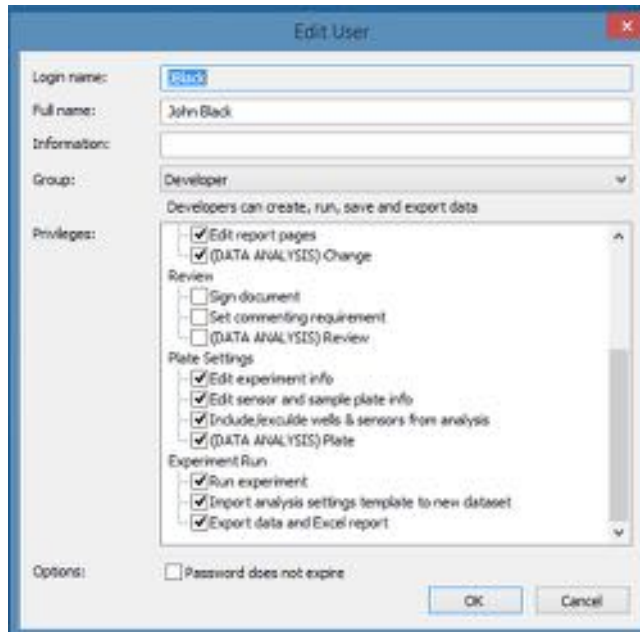


Figure 4-59: Edit User Dialog Box

4. Click **OK** to save changes and exit.

Inactivating a User Account

1. Position the cursor on the account and right-click to display the Tab menu (Figure 4-58).
2. Select **Inactivate User**.
3. Click **OK** to save changes and exit.

Changing User Account Passwords

1. Right-click on the user account and select **Set Password** from the Tab menu.
The Reset Password dialog box (Figure 4-60) will display:



Figure 4-60: Change Password Dialog Box

2. Enter the **New password** for the user account.
3. Re-enter the new password. Password reminder is optional.
4. Click **OK** to save changes and exit.



NOTE: Individual Users can also change their passwords by logging into the ForteBio FB Server Monitor with their Username and password. They can then change their password by right-clicking on their account, and following the same steps as described above.

Changing the Administrator Password

1. Right-click on the Administrator account and select **Set Password** from the Tab menu. The Change Password dialog box (Figure 4-61) will display:

Figure 4-61: Change Password Dialog Box

2. Enter the **Current password** then enter the **New Password**.
3. Re-enter the **New Password**. Password reminder is optional.
4. Click **OK** to save changes and exit.



NOTE: The Administrator password can also be changed within the Octet Data Acquisition application when logged in as administrator. Select **Change Password** from the Security menu then follow the prior steps.

Group Administration

The Groups Tab (Figure 4-62) allows administrators to add and delete user groups as well as set and change group privileges. The columns contain information about each user. The table can be sorted by clicking on any column header. For example, the Groups in Figure 4-62 have been sorted alphabetically according to Name.



Figure 4-62: Groups Tab

When a user account is assigned to a user group, the privileges defined in the group are also applied to the individual user account. The following default user groups are available and the detailed privileges are given above under User Account Administration.

- **Administrators** - Can manage Users and Group settings including add/delete/edit and view all events
- **Supervisors** - Can review data and events
- **Developers** - Can create, run, save and export data
- **Lab Users** - Can only run experiments
- **Guests** - Have no explicit privileges, these must be assigned by the administrator

Creating a New User Group

1. Right-click in a blank area in the **Groups** Tab to display the Tab menu (Figure 4-63).

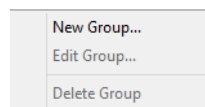


Figure 4-63: Group Tab Menu

2. Click **New Group** to display the New Group dialog box (Figure 4-64).

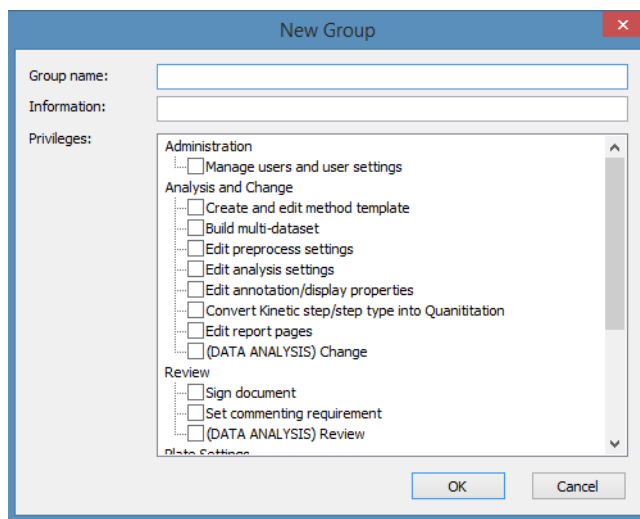


Figure 4-64: New Group Dialog Box

3. Enter the **Group name** and (if desired) **Information**.
4. **Assign Privileges** - Each group can be assigned specific privileges. Add group privileges by selecting or deselecting the check boxes next to each privilege. The categories include:
 - **Administration** - Can administer the user database
 - **Analysis and Change** - Can change methods and configuration values
 - **Review** - Can review changes and events
 - **Plate Settings** - Can change sample plate properties
 - **Run Experiment** - Can run experiments and analyses
5. Click **OK** to save changes and exit.

Viewing and Changing Group Settings

1. Right-click on a group to display the Tab menu (Figure B-45).

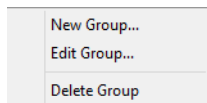


Figure 4-65: Group Tab Menu with All Options Active

2. Select **Edit Group** to display the Edit Group dialog box (Figure 4-66).

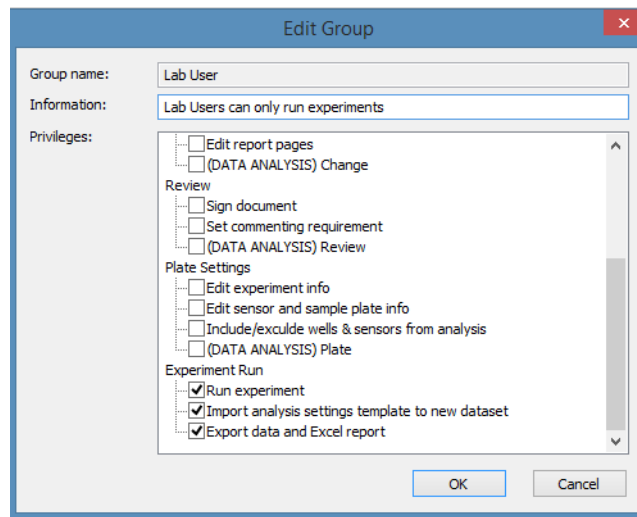


Figure 4-66: Edit Group Dialog Box

3. If needed, modify the group settings. For more details on individual settings, please refer to “Creating a New User Group” on page 121.
4. Click **OK** to save changes and exit.

Deleting a User Group

1. Right-click on the group to display the Tab menu and select **Delete Group**.
2. Click **OK** to save and exit.

Project Administration

The Projects Tab (Figure 4-67) allows administrators to add and delete user projects. Projects are selected when a new user session is initiated in Octet Data Acquisition or Data Analysis software, allowing all user, system and software events for a particular project to be monitored. The columns contain information about each user. The table can be sorted by clicking on any column header. For example, the Projects in Figure 4-67 have been sorted alphabetically according to Name.

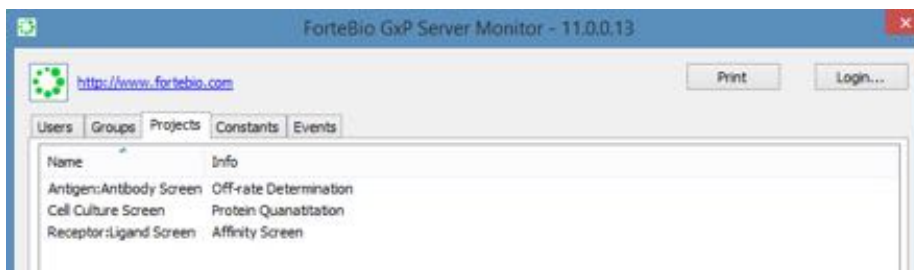


Figure 4-67: Projects Tab

Creating a New Project

1. Right-click in a blank area in the **Projects** Tab to display the Tab menu (Figure 4-68).

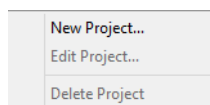


Figure 4-68: Projects Tab Menu.

2. Select **New Project** to display the New Project dialog box (Figure 4-69).

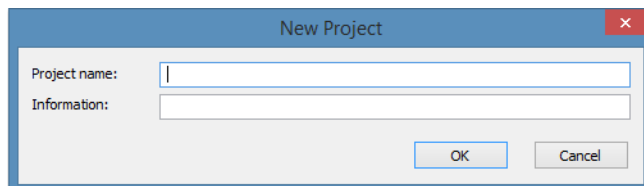


Figure 4-69: New Project Dialog Box

3. Enter the **Project name** and (if desired) **Information**.
4. Click **OK** to save and exit.

Viewing and Changing Project Settings

1. Right-click on a project to display the Tab menu (Figure 4-70).

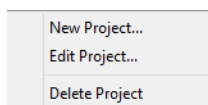


Figure 4-70: Projects Tab Menu with All Options Active

2. Select **Edit Project** to display the Edit Project dialog box (Figure 4-71).

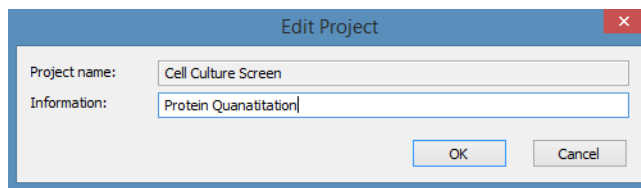


Figure 4-71: Edit Project Dialog Box

3. If needed, modify the **Project name** or **Information**.
4. Click **OK** to save changes and exit.

Deleting a Project

1. Right-click on the project to display the Tab menu and select **Delete Project**.
2. Click **OK** to save and exit.

Constants Tab

The Constants Tab (Figure 4-72) allows administrators to set ForteBio FB Server Monitor constant settings. Available administrator constants and their associated value ranges are shown in Table 4-2.



Figure 4-72: Constants Tab

Table 4-2: Administrator Constants

Constant	Description	Default Value	Value Range
CredentialsTTL	The number of days that the server settings are stored in the cache. This allows the software to operate in case the server is temporarily down. Data is saved on the local computer and, upon the next connection to the database, the cached events will automatically be uploaded to the database.	5	Minimum=0, no max value
PasswordMin-Length	Minimum number of characters that a password must contain.	0	Minimum=0, no max value
PasswordSecure	Level of password complexity. Setting the constant to 0 has no password restrictions. Setting the constant to 1 requires passwords to contain at least one alpha, one numeric, and one punctuation character.	0	0-1
PasswordTTL	Amount of time in days that a password is allowed to remain unchanged.	180	Minimum=0, no max value
UserIdleMin	Idle time in minutes allowed during a user session after which the session is automatically closed and requires the user to log back in.	15	Minimum=0, no max value
PasswordLock	Number of failed login attempts before the account is locked.	3	Minimum=3, no max value

Viewing and Changing Constants

1. Right-click on the constant to display the Tab menu which displays a single option: Edit Constant.
2. Click **Edit Constant** to display the Edit Constant dialog box (Figure 4-73).

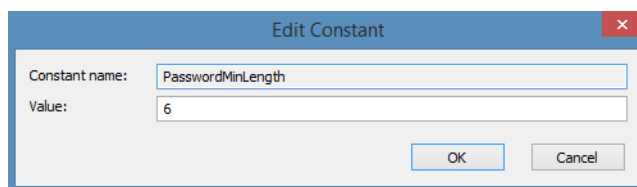


Figure 4-73: Edit Constant Dialog Box

3. If needed, modify the **Value**. For more information on value range, please see Table 4-2.
4. Click **OK** to save changes and exit.

Events Log

The Events Tab (Figure 4-74) allows administrators to view all the user, system and software event information recorded by the ForteBio FB Server module. Audit trails are stored on the ForteBio FB Server, not in individual files.

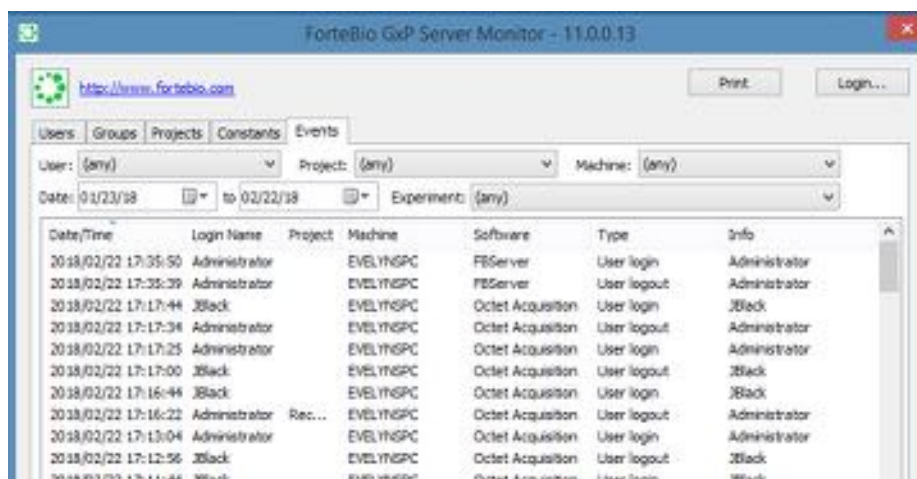


Figure 4-74: Events Tab



NOTE: ForteBio FB Server module version 8.2 and higher will also display event information recorded in BLItz Pro software for BLItz systems.

Events are tracked for individual user accounts, projects and machines. By default, a historical log of all events recorded on the active ForteBio FB Server module will display:

- **Date and Time** - When the event occurred
- **Login Name** - User name associated with the event
- **Project** - Name of project associated with the event

- **Machine** - Name of instrument used (includes both Octet and BLItz instruments for ForteBio FB Server module versions 8.2 and higher)
- **Software** - Which software the event was logged in (available in ForteBio FB Server module versions 8.2 and higher only, includes Octet Data Acquisition, Octet Data Analysis and BLItz Pro software events)
- **Type** - Event type
- **Info** - Any additional information recorded with the event

You can filter the Events Log according to User, Project, Machine and Experiment by selecting items in the corresponding drop down menus. For example, Figure 4-75 shows a drop down menu for selecting events by User Name.

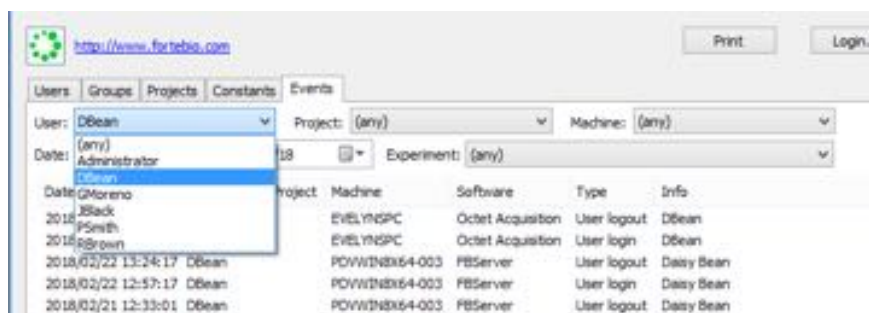


Figure 4-75: Selecting Events by User Name

You can also limit your search to a specific time period by choosing the start/stop day from the calendar drop down menus (Figure 4-76).

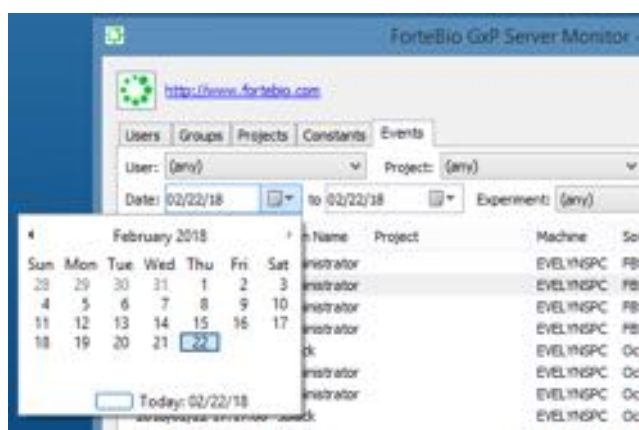


Figure 4-76: Events Displayed for User Name

If any action is a change in Method parameters, details about the changes can be viewed by double-clicking on the event which brings up the Event Details Box (Figure 4-77).

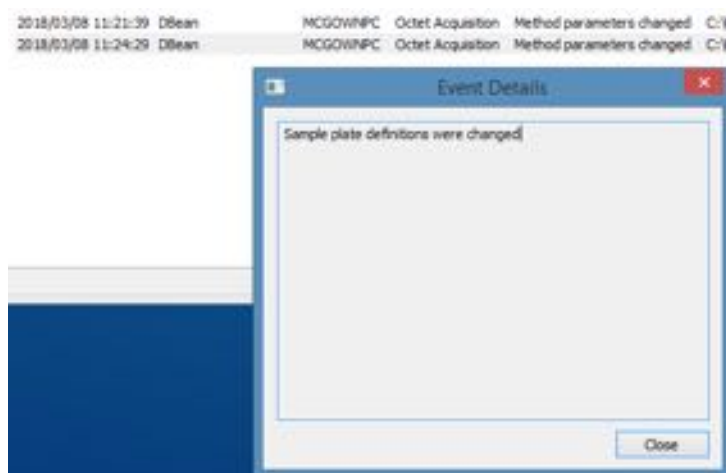


Figure 4-77: Event Details Describing Method Parameter Changes

Accessing the ForteBio FB Server Configuration Module

The ForteBio FB Server Configuration module allows an administrator to test and re-start the server.

- Double-click on the **FBServerConfig.exe** file in the FBServer folder in the installed location (Figure 4-78):

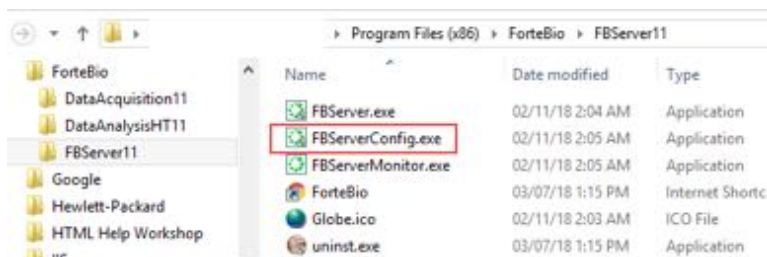


Figure 4-78: Accessing the ForteBio FB Server Configuration Module on the Network

The ForteBio FB Server Configuration window (Figure 4-79) will display:

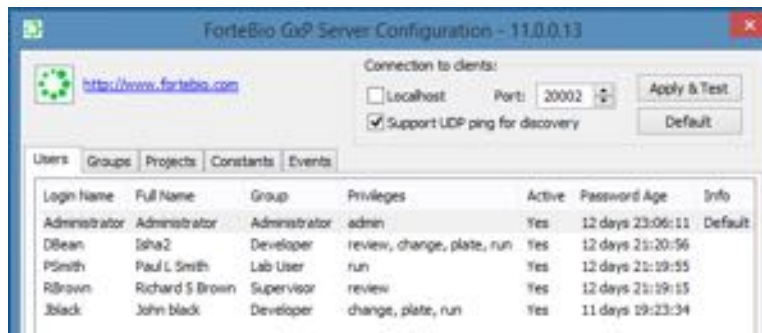


Figure 4-79: ForteBio FB Server Configuration Window



NOTE: Although the Configuration Window appears to be similar to that of the ForteBio FB Server Monitor, it does not offer the same functionality. Creation and use of the User, Groups, Projects, Constants and Events tabs should be done in the ForteBio FB Server Monitor and are described in “Accessing Administrator Options” on page 110.

Server Testing

The ForteBio FB Server module can be tested to ensure it is accessible and functioning properly.

1. In the **Connections to Clients** box (Figure 4-80), make changes to the server settings if needed.

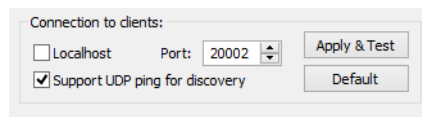


Figure 4-80: Connections to Clients Box

2. Click **Apply & Test**. If the ForteBio FB Server module is found and functioning properly, the message in Figure 4-81 will display:



Figure 4-81: Server Found

To return to the originally configured ForteBio FB Server module settings, click **Default** at any time.

Restarting the ForteBio FB Server Module

If the host location of the ForteBio FB Server module cannot be found during user login or if users are unable to login with valid credentials, the ForteBio FB Server module may be offline and need to be restarted.



NOTE: Pall ForteBio recommends contacting your IT department to confirm whether or not network or firewall settings may have been changed. This may also be preventing access to the ForteBio FB Server module.

- Double-click on the **FBServer.exe** file (Figure 4-82) in the FBServer folder from the installed location:

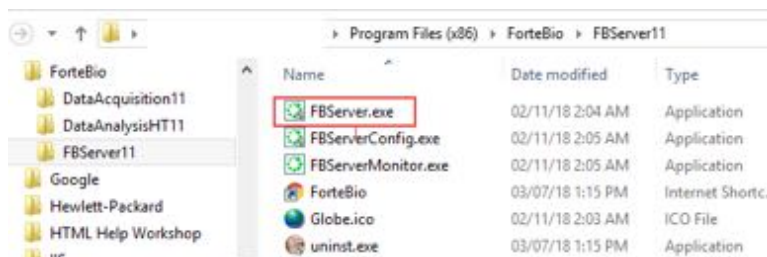


Figure 4-82: Restarting the ForteBio FBServer Module

CHAPTER 5:




Quantitation Experiments: Octet K2 System

Introduction	134
Starting a Quantitation Experiment	135
Defining the Sample Plate	137
Managing Sample Plate Definitions	155
Managing Assay Parameter Settings	158
Assigning Biosensors to Samples	164
Reviewing Experiments	182
Saving Experiments	182
Running a Quantitation Experiment	184
Managing Runtime Binding Charts	191
Managing Experiment Method Files	197
Custom Quantitation Assays	198
Multi-Step Advanced Quantitation Experiments	207

INTRODUCTION

A quantitation experiment enables you to determine analyte concentration within a sample using a reference set of standards. After starting the Octet system hardware and the Octet System Data Acquisition software, follow the steps (in Table 5-1) to set up and analyze a quantitation experiment.

Table 5-1: Setting Up and Analyzing a Quantitative Experiment

Software	Step	See
Data Acquisition 	1. Select a quantitation experiment in the Experiment wizard or open a method file (.fmf).	"Starting a Quantitation Experiment" on page 135
	2. Define a sample plate or import a sample plate definition.	"Defining the Sample Plate" on page 137
	3. Confirm or edit the assay settings.	"Managing Assay Parameter Settings" on page 158
	4. Assign biosensors to samples.	"Assigning Biosensors to Samples" on page 164
	5. Run the experiment.	"Running a Quantitation Experiment" on page 184
Data Analysis  or Data Analysis HT 	6. Analyze the binding data. 7. Generate a report.	<i>Octet System Data Analysis Software or Octet Data Analysis HT Software User Guide</i>

For more details on how to prepare the biosensors, see the appropriate biosensor product insert.

STARTING A QUANTITATION EXPERIMENT



IMPORTANT: Using 96-well half-area plates on the Octet K2 system will result in non-optimal system performance. Pall ForteBio cannot guarantee results within the optimal performance specifications of the system when these plates are used.



NOTE: Before starting an experiment, check the plate temperature displayed in the status bar. Confirm that the temperature is appropriate for your experiment and if not, set a new temperature. If the Octet System Data Acquisition software is closed, the plate temperature will reset to the default startup value specified in the **Options** dialog box when the software is relaunched.

You can start a quantitation experiment by one of the following methods:


- Launch the **Experiment Wizard**.
- Open a method file (.fmf) by clicking **File > Open Method File**. Method files may be saved and recalled using the **File** menu and are automatically saved when an experiment is run. For more details on method files see, “Managing Experiment Method Files” on page 197.
- On the menu bar, click **Experiment > Templates > Quantitation**.



NOTE: When using the 21 CFR Part 11 version of the Octet System Data Acquisition software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.

Starting an Experiment Using the Experiment Wizard

To start an experiment using the **Experiment Wizard**:

1. If the **Experiment Wizard** is not displayed when the software is launched, click the **Experiment Wizard** toolbar button  or click **Experiment > New Experiment Wizard (Ctrl+N)** from the **Main Menu**.
2. In the **Experiment Wizard**, select **New Quantitation Experiment** (see Figure 5-1 left).

3. Select a type of quantitation experiment (see Table 5-2 for options).

Table 5-2: Quantitation Experiment Selection

Quantitation Experiment	Description
Basic Quantitation	A standard quantitation assay.
Basic Quantitation with Regeneration	A standard quantitation assay that enables regeneration of biosensors.
Advanced Quantitation	A standard two- or three-step quantitation assay that enables signal amplification for higher detection sensitivity.

4. Optional: You can also click **Recent Methods** to display a list of recently used methods. You can open any method file from the list and use it with or without modifications to run a new experiment.

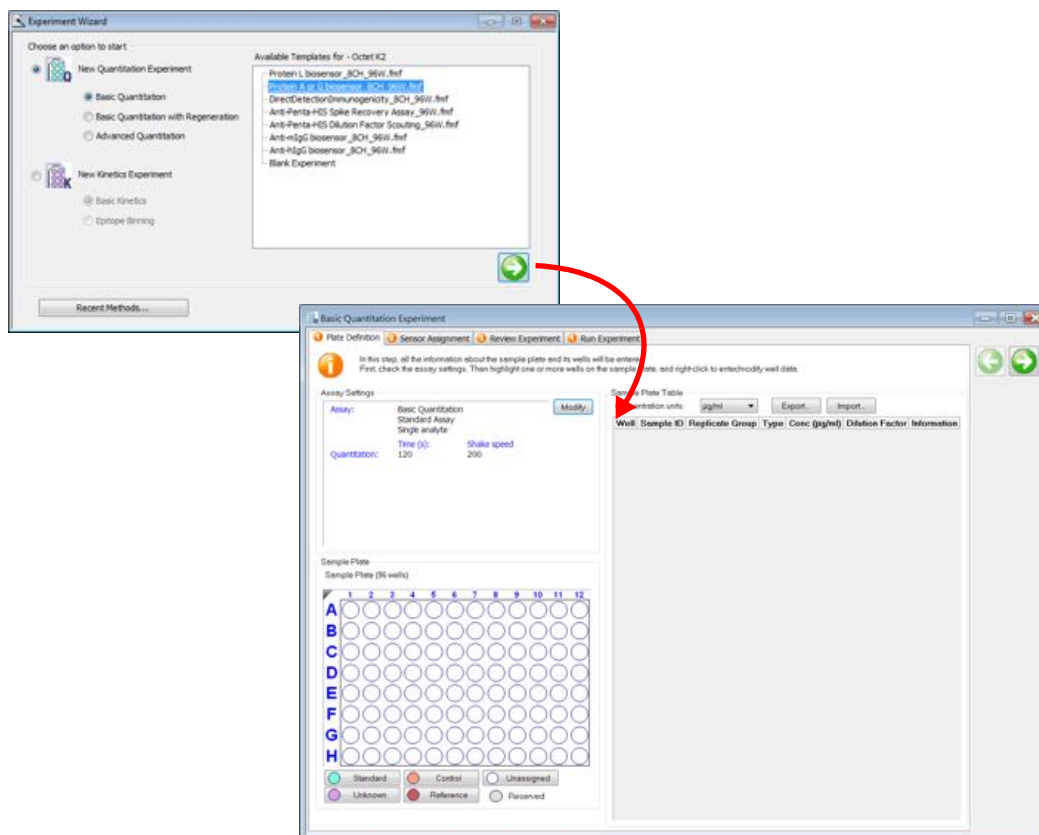


Figure 5-1: Selecting an Experiment Type in the Experiment Wizard

5. Click the  arrow.

The **Experiment** dialog box displays (Figure 5-1 right).

DEFINING THE SAMPLE PLATE

Table 5-3 lists the steps to define a sample plate.

Table 5-3: Defining a Sample Plate

Step	See Page
1. Designate the samples.	137
2. Annotate the samples (optional).	149
3. Save the sample plate definition (optional).	155

Designating Samples

Each well may be designated as a **Standard**, **Unknown**, **Control**, or **Reference**. A well may also remain **Unassigned** or be designated as **Reserved** by the system for Basic Quantitation with Regeneration and Advanced Quantitation experiments.



NOTE: It is important to define all of the wells that will be used in the assay. Only wells that are selected and defined using one of the sample types in Table 5-4 will be included in the assay.

Table 5-4: Types of Sample Wells







Icon	Description
 Standard	Contains an analyte of known concentration. Data from the well is used to generate a standard curve during analysis.
 Unknown	Contains an analyte of unknown concentration. The concentration of the analyte is calculated from the well data and the standard curve.
 Control	A control sample, either positive or negative, of known analyte composition. Data from the well is not used to generate a standard curve during analysis. <ul style="list-style-type: none"> Positive Control: A control sample that contains analyte of known concentration Negative Control: A control sample known not to contain analyte
 Reference	Provides a baseline signal which serves as a reference signal for Unknowns , Controls , and Standards . The reference signal can be subtracted during data acquisition in the Runtime Binding Chart and during data analysis.

Table 5-4: Types of Sample Wells

Icon	Description
 Unassigned	Not used during the experiment.
 Reserved	Used by the system during Basic Quantitation with Regeneration experiments and Advanced Quantitation multi-step experiments for Regeneration (R) , Neutralization (N) , Detection (D) , or Capture Antibody (C) . Reserved wells are not available for use as Standards , Unknowns , Controls , or References .

Reserved Wells

In a Basic Quantitation with Regeneration or an Advanced Quantitation experiment, the **Sample Plate Map** includes gray wells. These wells are reserved by the system and specify the location of particular sample types.









Reserved samples cannot be removed from the sample plate, but you can change their column or row location. To change the location of the two reserved wells (, , , or ) , right-click on the wells in the **Sample Plate Map** and select **Regeneration**, **Neutralization**, **Detection**, or **Capture Antibody**.

Table 5-5: Reserved Well Requirements

Reserved Well	Must Contain
 Regeneration	Regeneration buffer that is used to remove analyte from the biosensor (typically low pH, high pH, or high ionic strength).
 Neutralization	Neutralization buffer that is used to neutralize the biosensor after the regeneration step.
 Detection	Secondary antibody or precipitating substrate that is used with an enzyme-antibody conjugate to amplify the analyte signal. Sample concentrations are computed using the binding data from the detection wells.
 Capture Antibody	Capture antibody or molecule that is used to immobilize the specific molecule of interest onto the biosensor.

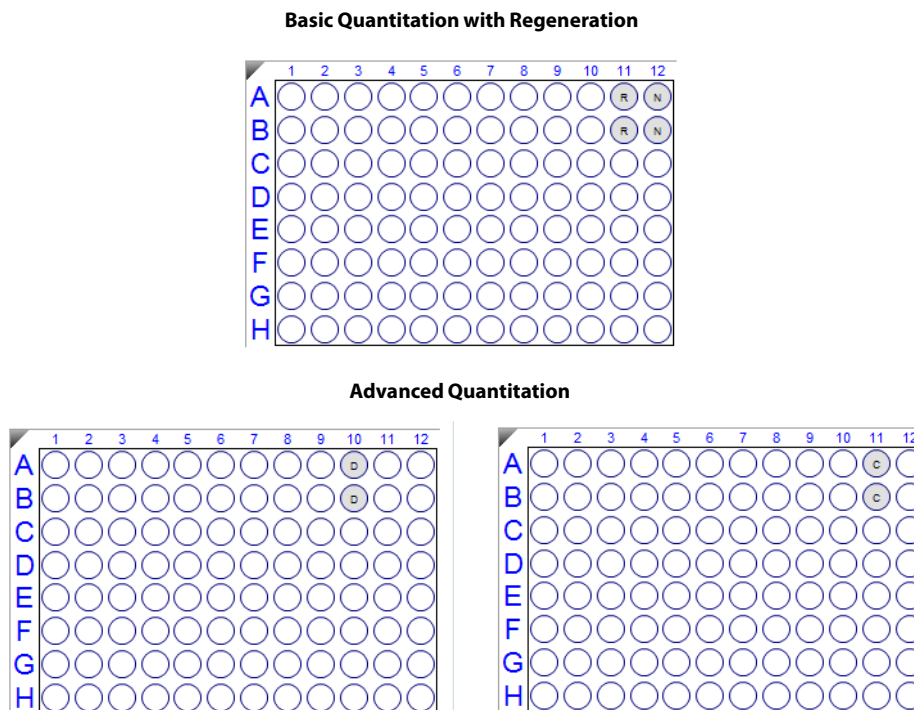


Figure 5-2: Default Locations for Reserved Wells in a 96-Well Sample Plate Map

Selecting Wells in the Sample Plate Map



NOTE: For the Octet K2 system, wells in sample plate are restricted to rows AB, CD, EF and GH. Sample wells cannot be designated in row pairs BC, DE and FG.

There are several ways to select wells in the **Sample Plate Map**:

- Click a column header or select adjacent column headers by click-hold-drag (Figure 5-3 left). To select non-adjacent columns, hold the **Ctrl** key and click the column header.
- Click a row header or select adjacent row headers by click-hold-drag (Figure 5-3, center).
- Click a well or draw a box around a group of wells (Figure 5-3, right).

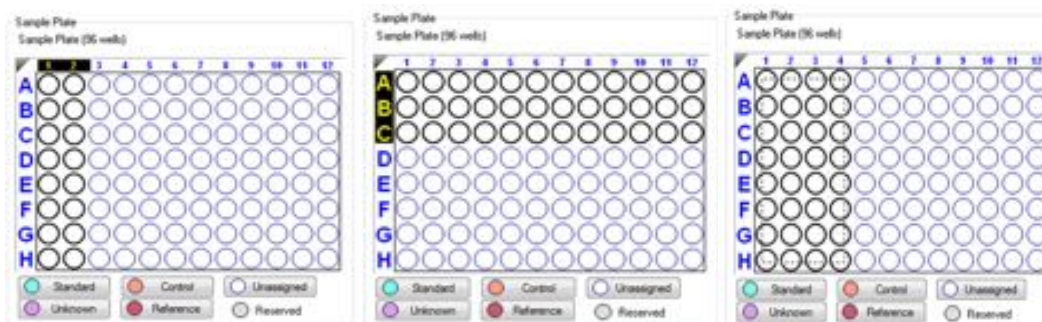


Figure 5-3: Selecting Wells in the Sample Plate Map



NOTE: Shift-clicking in the **Sample Plate Map** mimics the head of the instrument during the selection.

Designating Standards

To designate standards:

1. In the **Sample Plate Map**, select the wells to define as standards.
2. Click the **Standard** button below the **Sample Plate Map** (see Figure 5-3), or right-click and select **Standard**.

The standards are marked in the plate map and the **Sample Plate Table** is updated.

3. Select the concentration units for the standards using the **Concentration Units** drop-down list above the **Sample Plate Table**.

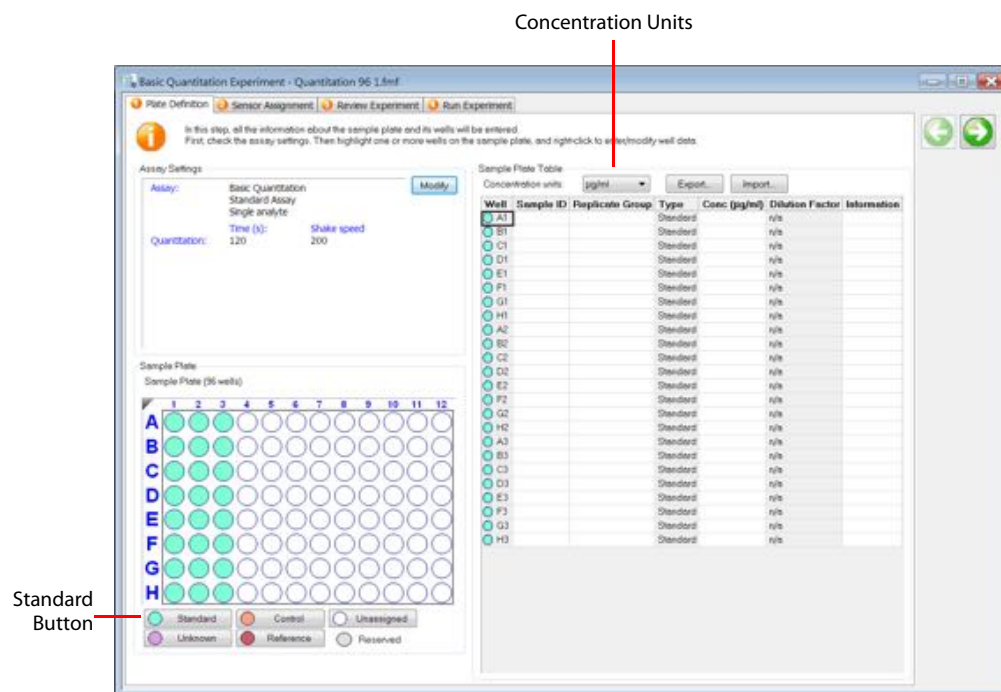


Figure 5-4: Plate Definition Window—Designating Standards

To remove a well designation, select the well(s) and click **Unassigned**. Or, right-click the well(s) and select **Clear Data**.

Assigning Standard Concentrations Using a Dilution Series

To assign standard concentrations using a dilution series:

1. In the **Sample Plate Map**, select the standard wells, right-click and select **Set Well Data**.

The **Set Well Data** dialog box displays (see Figure 5-5).

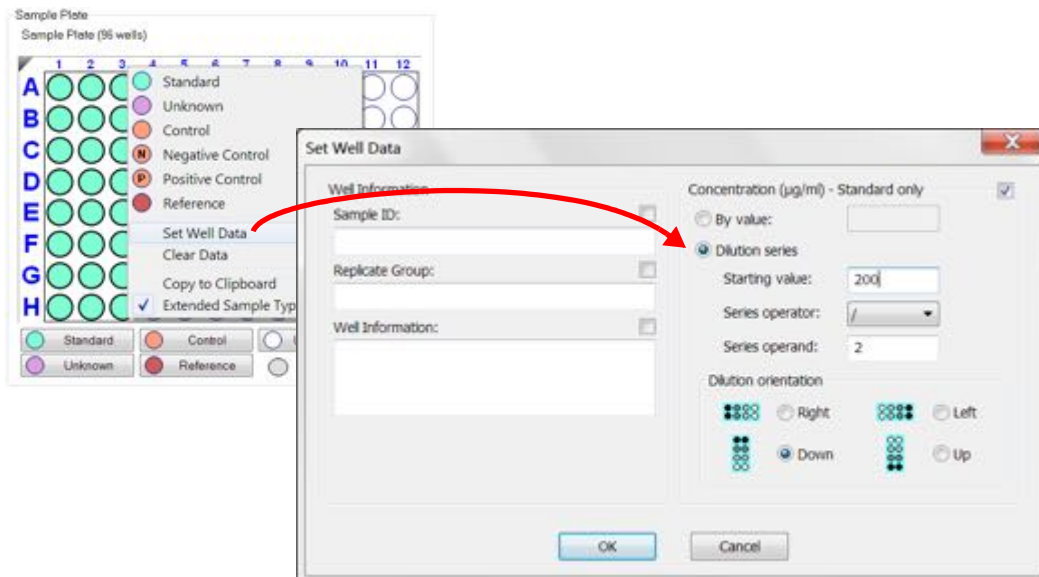


Figure 5-5: Sample Plate Map—Setting a Dilution Series

2. Select the **Dilution Series** option and enter the starting concentration value.
3. Select a series operator, enter an operand, and select the appropriate dilution orientation (see Figure 5-6).

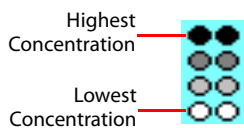


Figure 5-6: Concentration Representation in Dilution Series

4. Click **OK**.

The **Sample Plate Table** will display the standard concentrations entered.

Assigning a User-Specified Concentration to Standards

To assign a user-specified concentration to standards:

1. In the **Sample Plate Map**, select the standard wells, right-click and select **Set Well Data**.

The **Set Well Data** dialog box displays (see Figure 5-7).

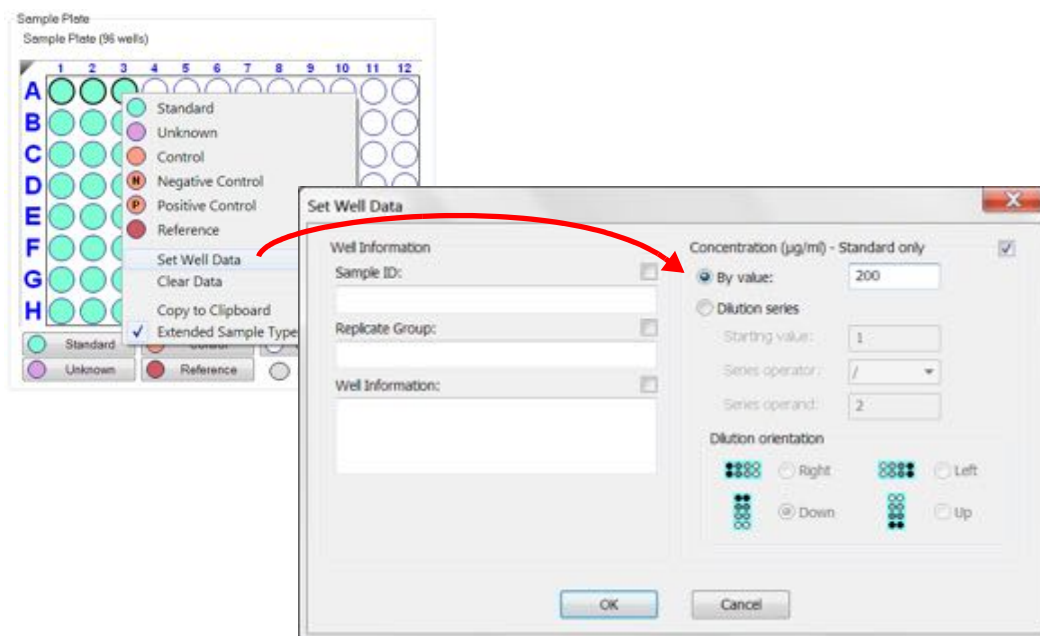


Figure 5-7: Sample Plate Map—Assigning a Standard Concentration

2. Select the **By value** option and enter the starting concentration value.
3. Click **OK**. The **Sample Plate Table** will display the standard concentrations entered.

Editing an Individual Standard Concentration

To enter or edit an individual standard concentration, in the **Conc** column of the **Sample Plate Table**, double-click the value and enter a new value (see Figure 5-8).

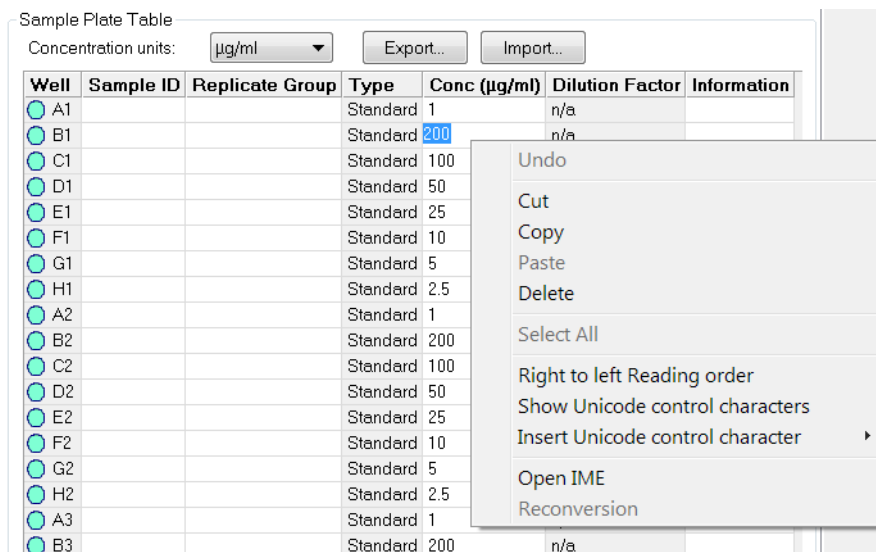


Figure 5-8: Sample Plate Table—Shortcut Menu of Edit Commands



NOTE: Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the **Sample Plate Table**. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the **Sample Plate Map** menu used to designate sample types.

Designating Unknowns

To designate unknowns in the **Sample Plate Map**, select the wells to define as unknown, right-click and select **Unknown**. The unknown wells are marked in the plate map and the sample plate table is updated (see Figure 5-9).

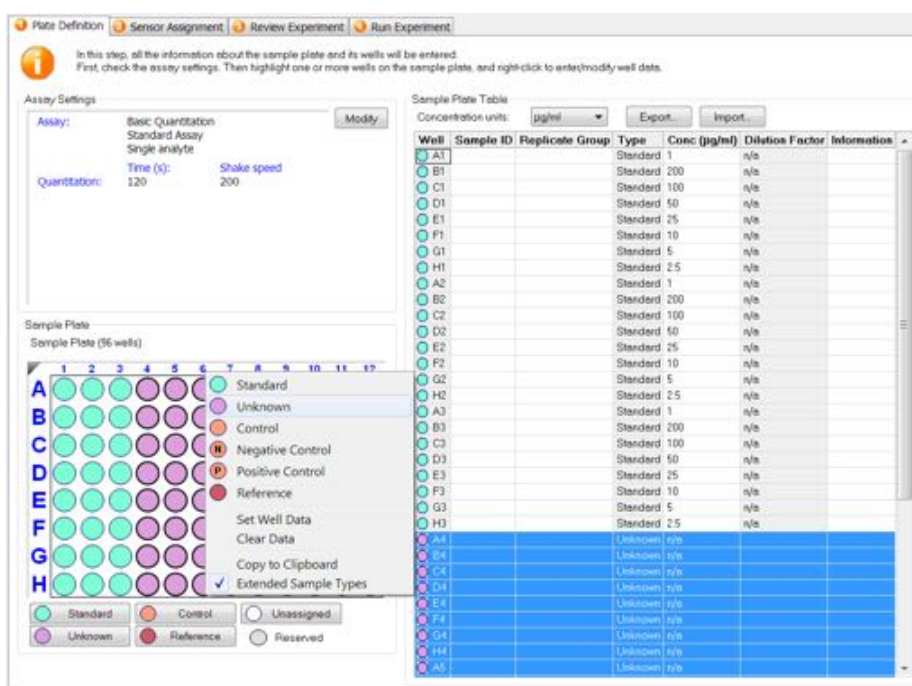


Figure 5-9: Plate Definition Window—Designate Unknown Wells

To remove a well designation, select the well(s) and click **Unassigned**. Or, right-click the well(s) and select **Clear Data**.

Assigning a Dilution Factor or Serial Dilution to Unknowns

To assign a dilution factor or serial dilution to unknowns:

1. In the **Sample Plate Map**, select the unknown wells (see Figure 5-9).
2. Right-click and select **Set Well Data**.

The **Set Well Data** dialog box displays (see Figure 5-10).

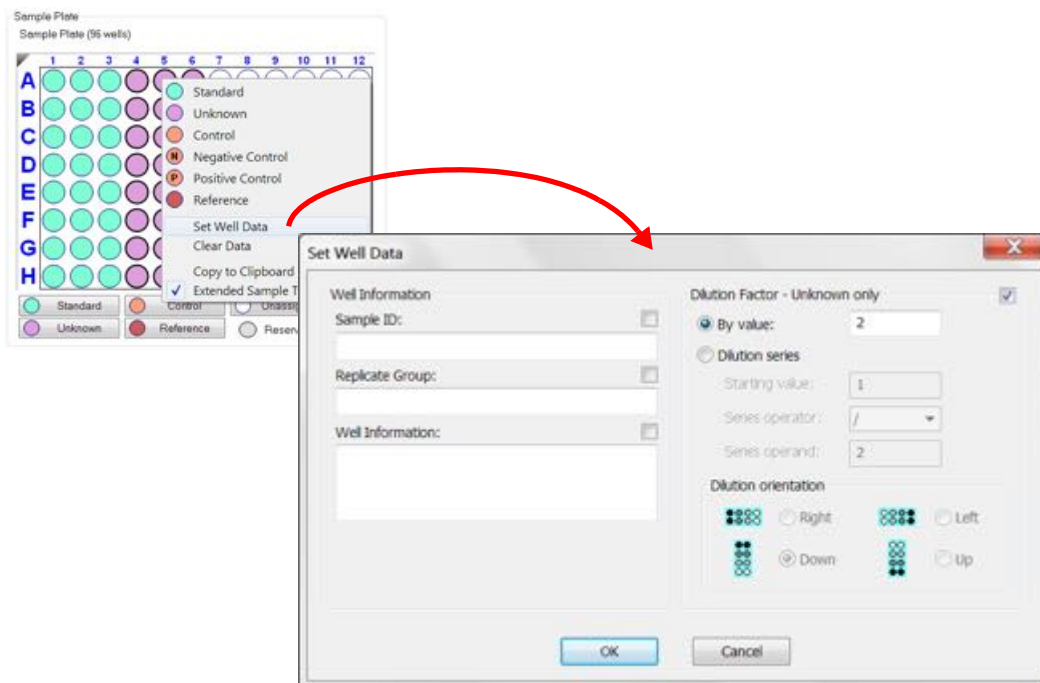


Figure 5-10: Sample Plate Map—Setting a Dilution Factor or a Serial Dilution

To assign a dilution factor to selected wells:

1. In the **Set Well Data** dialog box (see Figure 5-10), select the **By Value** option.
2. Enter the dilution factor value and click **OK**.

To assign a serial dilution to selected wells:

1. In the **Set Well Data** dialog box (see Figure 5-10), select the **Dilution series** option.
2. Enter the starting dilution, select a series operator, and enter a series operand.
3. Select the appropriate dilution orientation: (see Figure 5-11).

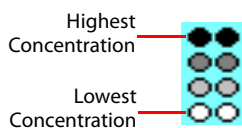


Figure 5-11: Concentration Representation in Dilution Series

4. Click **OK**.

The **Sample Plate Table** will display the dilution factors entered.

Editing a Dilution Factor in the Sample Plate Table

To edit a dilution factor in the **Sample Plate Table**:

1. In the **Set Well Data** dialog box (see Figure 5-10), double-click a cell in the **Dilution Factor** column for the desired unknown.
2. Enter the new value (the default dilution factor is 1)

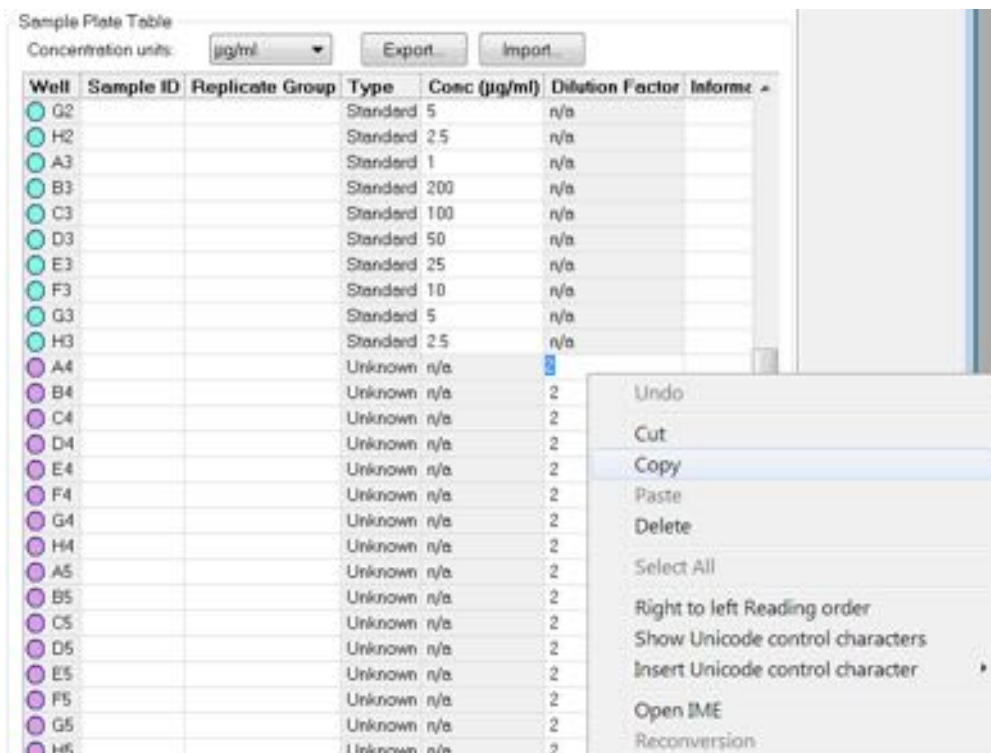


Figure 5-12: Sample Plate Table—Shortcut Menu of Edit Commands



NOTE: Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the **Sample Plate Table**. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the **Sample Plate Map** menu used to designate sample types.

Designating Controls or Reference Wells

Controls are samples of known concentration that are not used to generate a standard curve. A reference well contains sample matrix only, and is used to subtract non-specific binding of the sample matrix to the biosensor. During data analysis, data from reference wells can be subtracted from standards and unknowns to correct for background signal.

- To designate controls, select the control wells and click **Control** (below the **Sample Plate Map**), or right-click and select **Control**. Positive and Negative Control types can be assigned using right-click only if **extended sample types** is checked (Figure 5-13).
- To designate reference wells, select the reference wells and click the **Reference** button below the **Sample Plate Map**, or right-click the selection and choose **Reference**.

The wells are marked in the **Sample Plate Map** and the **Sample Plate Table** is updated (Figure 5-13).

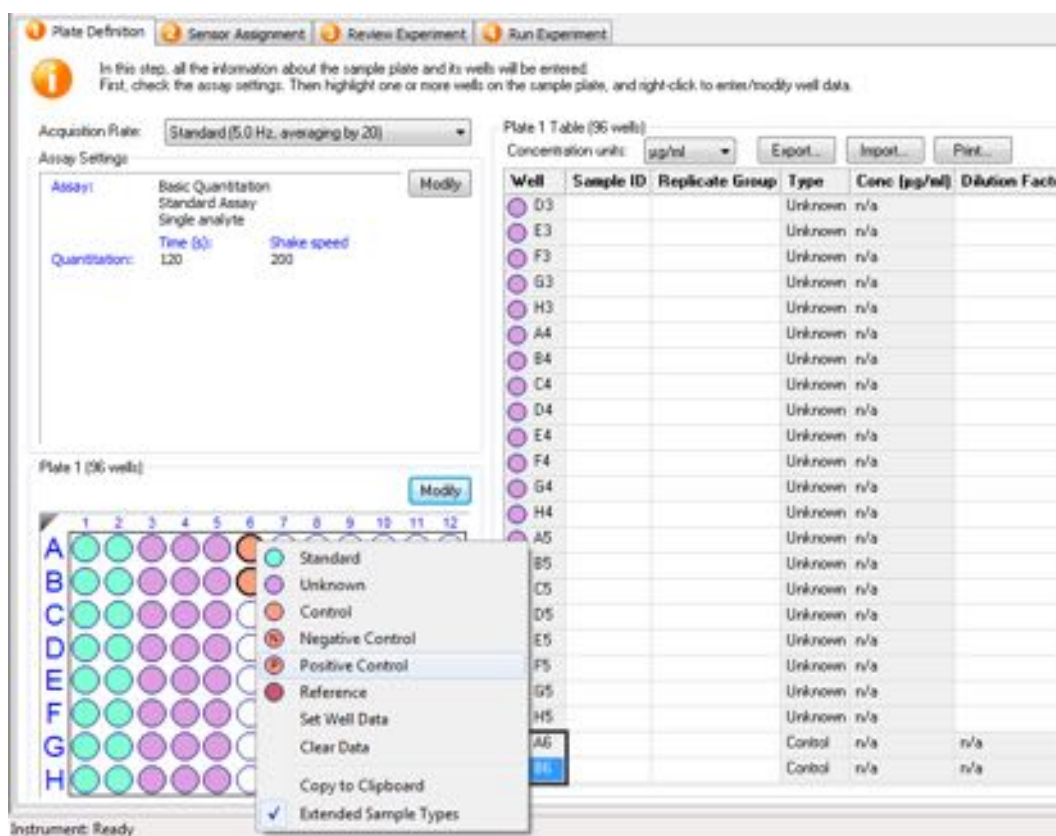


Figure 5-13: Designate Controls or Reference Wells



NOTE: Shift-clicking in the **Sample Plate Map** mimics the head of the instrument during the selection.

To remove a well designation, select the well(s) and click **Unassigned**. Or, right-click the well(s) and select **Clear Data**.

Annotating Samples

You can enter annotations (notes) for multiple samples in the **Sample Plate Map** or enter information for an individual sample in the **Sample Plate Table**. For greater clarity, annotation text may be displayed as the legend of the **Runtime Binding Chart** during data acquisition, but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it will not be available for display as a legend.

Annotating Wells in the Sample Plate Map

To annotate one or more wells:

1. In the **Sample Plate Map**, select the samples to annotate, right-click and select **Set Well Data**.
2. In the **Set Well Data** dialog box (see Figure 5-14), enter the **Sample ID** and/or **Well Information** and click **OK**.

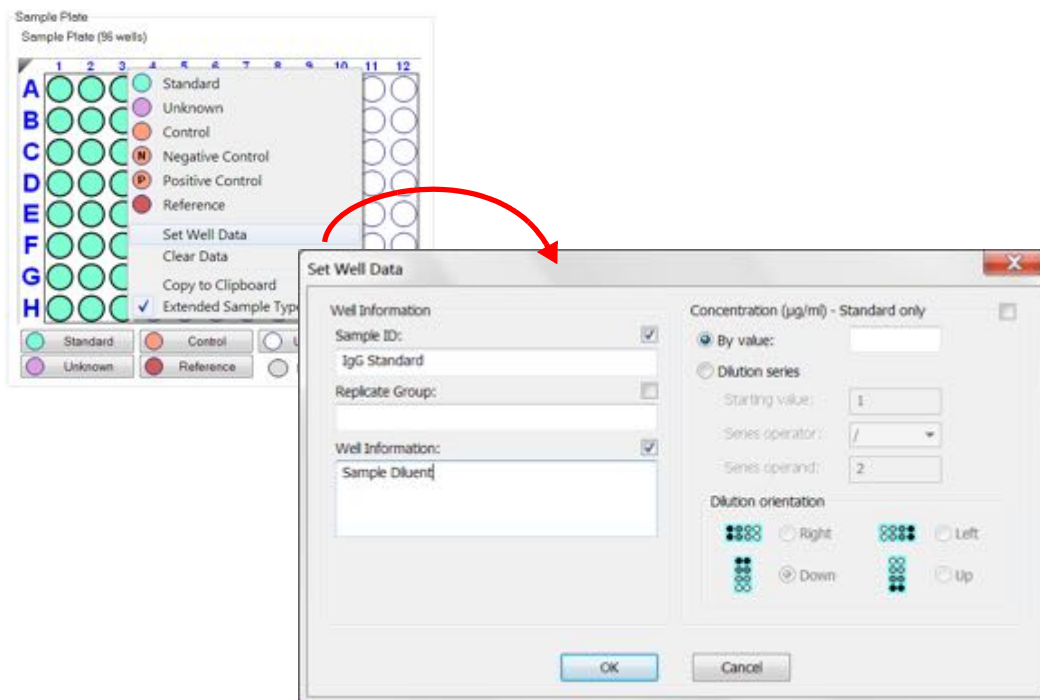


Figure 5-14: Adding Sample Annotations from the Sample Plate Map

Annotating Wells in the Sample Plate Table

To annotate an individual well in the **Sample Plate Table**:

1. Double-click the table cell for **Sample ID** or **Well Information**.

2. Enter the desired information in the respective field (see Figure 5-15).



NOTE: A series of Sample IDs may also be assembled in Excel and pasted into the **Sample Plate Table**.

Sample Plate Table

Concentration units: µg/ml Export Import

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	Dilution Factor	Information
G3	IgG Standard		Standard	5	n/a	Sample Diluent
H3	IgG Standard		Standard	2.5	n/a	Sample Diluent
A4	Ab1		Unknown	n/a	2	Sample Diluent
B4	Ab2		Unknown	n/a	2	Sample Diluent
C4	Ab3		Unknown	n/a	2	Sample Diluent
D4	Ab4		Unknown	n/a	2	Sample Diluent
E4	Ab5		Unknown	n/a	2	Sample Diluent
F4	Ab6		Unknown	n/a	2	Sample Diluent
G4	Ab7		Unknown	n/a	2	Sample Diluent
H4	Ab8		Unknown	n/a	2	Sample Diluent
A5	Ab1		Unknown	n/a	2	Sample Diluent
B5	Ab2		Unknown	n/a	2	Sample Diluent
C5	Ab3		Unknown	n/a	2	Sample Diluent
D5	Ab4		Unknown	n/a	2	Sample Diluent
E5	Ab5		Unknown	n/a	2	Sample Diluent
F5	Ab6		Unknown	n/a	2	Sample Diluent
G5	Ab7		Unknown	n/a	2	Sample Diluent
H5	Ab8		Unknown	n/a	2	Sample Diluent
A6	Ab1		Unknown	n/a	2	Sample Diluent
B6	Ab2		Unknown	n/a	2	Sample Diluent
C6	Ab3		Unknown	n/a	2	Sample Diluent
D6	Ab4		Unknown	n/a	2	Sample Diluent
E6	Ab5		Unknown	n/a	2	Sample Diluent
F6	Ab6		Unknown	n/a	2	Sample Diluent
G6	Ab7		Unknown	n/a	2	Sample Diluent
H6	Ab8		Unknown	n/a	2	Sample Diluent
A7	hlgG		Positive Control	n/a	n/a	10 µg/mL in
B7	hlgG		Positive Control	n/a	n/a	10 µg/mL
C7	hlgG		Positive Control	n/a	n/a	10 µg/mL
D7	hlgG		Positive Control	n/a	n/a	10 µg/mL
E7	hlgG		Positive Control	n/a	n/a	10 µg/mL

Figure 5-15: Adding Sample Annotations in the Sample Plate Table



NOTE: Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the **Sample Plate Table**. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the **Sample Plate Map** menu used to designate sample types.

Replicate Groups

When samples are assigned to a **Replicate Group**, the Octet System Data Analysis software will automatically calculate statistics for all samples in that group. The average binding rate, average concentration and corresponding standard deviation as well CV% are presented in the **Results** table for each group (see Figure 5-16).

Sensor...	Replicat...	BR Avg	BR SD	BR CV	Conc. Avg	Conc. SD	Conc. CV
Protein A	Group 1	0.66	0.01	1.5	604.5	17.8	2.9
Protein A	Group 1	0.66	0.01	1.5	604.5	17.8	2.9
Protein A	Group 1	0.66	0.01	1.5	604.5	17.8	2.9
Protein A	Group 1	0.66	0.01	1.5	604.5	17.8	2.9
Anti-Hu...	Group 2	0.6589	0.0052	0.8	602.5	9.15	1.5
Anti-Hu...	Group 2	0.6589	0.0052	0.8	602.5	9.15	1.5
Anti-Hu...	Group 2	0.6589	0.0052	0.8	602.5	9.15	1.5
Anti-Hu...	Group 2	0.6589	0.0052	0.8	602.5	9.15	1.5
Anti-Mo...	Group 3	0.6773	0.0087	1.3	635.3	15.4	2.4
Anti-Mo...	Group 3	0.6773	0.0087	1.3	635.3	15.4	2.4
Anti-Mo...	Group 3	0.6773	0.0087	1.3	635.3	15.4	2.4
Anti-Mo...	Group 3	0.6773	0.0087	1.3	635.3	15.4	2.4
Protein A	Group 4	0.6544	0.0073	1.1	594.6	12.9	2.2
Protein A	Group 4	0.6544	0.0073	1.1	594.6	12.9	2.2
Protein A	Group 4	0.6544	0.0073	1.1	594.6	12.9	2.2
Protein A	Group 4	0.6544	0.0073	1.1	594.6	12.9	2.2

Figure 5-16: Replicate Group Result Table Statistics



NOTE: Replicate Group information can also be entered in the Results table in the Octet System Data Analysis software.

Assigning Replicate Groups in the Sample Plate Map

To assign **Replicate Groups** in the **Sample Plate Map**:

1. Select the samples to group, right-click and select **Set Well Data**.
2. In the **Set Well Data** dialog box (see Figure 5-17), enter a name in the **Replicate Group** box and click **OK**.

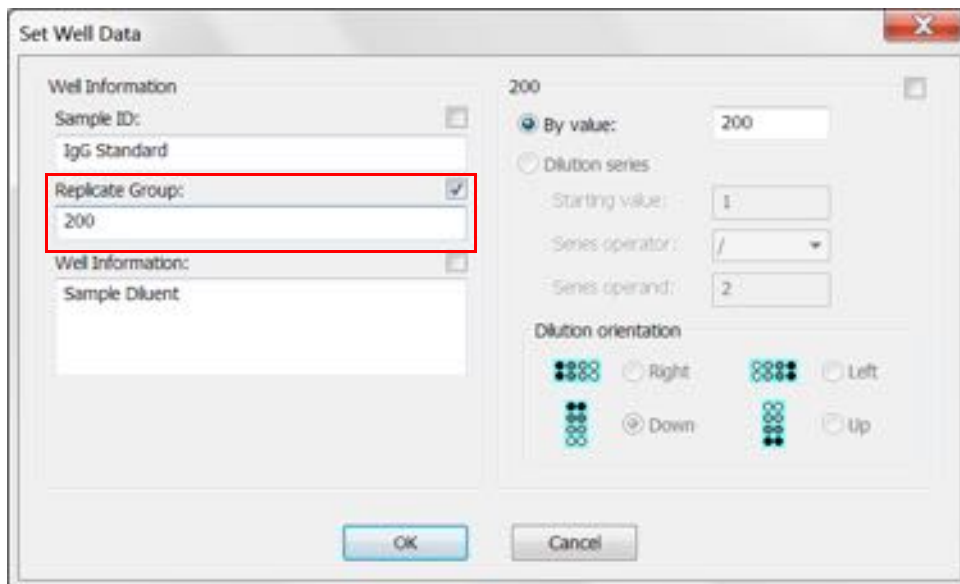


Figure 5-17: Add Replicate Group from the Sample Plate Map

3. Repeat the previous steps to assign new samples to the existing **Replicate Group**, or to designate another set of samples to a new **Replicate Group**. Multiple groups can be used in an experiment.



IMPORTANT: The Octet System Data Analysis software will only recognize and calculate statistics for samples that use the same **Replicate Group** names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.



NOTE: When performing a Multiple Analyte experiment, if the same **Replicate Group** name is used with different biosensor types, they will be treated as separate groups. Statistics for these groups will be calculated separately for each biosensor type.

Wells in the **Sample Plate Map** will show color-coded outlines as a visual indication of which wells are in the same group (see Figure 5-18).

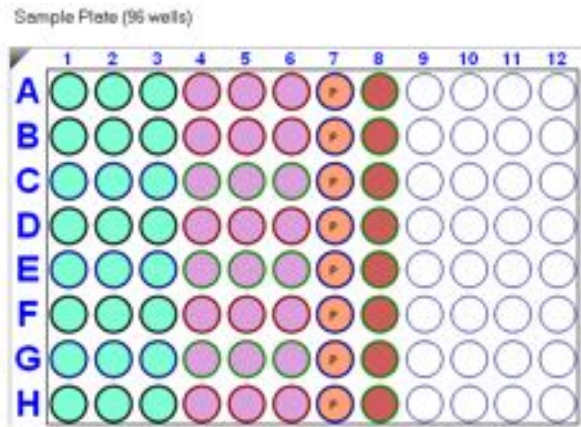


Figure 5-18: Replicate Groups Displayed in Sample Plate Map

The **Sample Plate Table** will update with the **Replicate Group** names entered (see Figure 5-19).

Sample Plate Table

Concentration units:

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	Dilution Factor
A1	IgG Standard	200	Standard	200	n/a
B1	IgG Standard	100	Standard	100	n/a
C1	IgG Standard	50	Standard	50	n/a
D1	IgG Standard	25	Standard	25	n/a
E1	IgG Standard	10	Standard	10	n/a
F1	IgG Standard	5	Standard	5	n/a
G1	IgG Standard	2.5	Standard	2.5	n/a
H1	IgG Standard	1	Standard	1	n/a
A2	IgG Standard	200	Standard	200	n/a
B2	IgG Standard	100	Standard	100	n/a
C2	IgG Standard	50	Standard	50	n/a
D2	IgG Standard	25	Standard	25	n/a
E2	IgG Standard	10	Standard	10	n/a
F2	IgG Standard	5	Standard	5	n/a
G2	IgG Standard	2.5	Standard	2.5	n/a
H2	IgG Standard	1	Standard	1	n/a
A3	IgG Standard	200	Standard	200	n/a
B3	IgG Standard	100	Standard	100	n/a
C3	IgG Standard	50	Standard	50	n/a
D3	IgG Standard	25	Standard	25	n/a
E3	IgG Standard	10	Standard	10	n/a
F3	IgG Standard	5	Standard	5	n/a
G3	IgG Standard	2.5	Standard	2.5	n/a
H3	IgG Standard	1	Standard	1	n/a
A4	Ab1	Ab1	Unknown	n/a	2
B4	Ab2	Ab2	Unknown	n/a	2
C4	Ab3	Ab3	Unknown	n/a	2
D4	Ab4	Ab4	Unknown	n/a	2
E4	Ab5	Ab5	Unknown	n/a	2
F4	Ab6	Ab6	Unknown	n/a	2
G4	Ab7	Ab7	Unknown	n/a	2

Figure 5-19: Replicate Groups in Sample Plate Table

Assigning Replicate Groups in the Sample Plate Table

To assign **Replicate Groups** in the **Sample Plate Table**:

1. Double-click the desired cell in the **Replicate Group** table column.
2. Enter a group name (see Figure 5-20).

Sample Plate Table

Concentration units:

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	Dilution Factor
A1	IgG Standard	200	Standard	200	n/a
B1	IgG Standard	100	Standard	100	n/a
C1	IgG Standard	50	Standard	50	n/a
D1	IgG Standard	25	Standard	25	n/a
E1	IgG Standard	10	Standard	10	n/a
F1	IgG Standard	5	Standard	5	n/a
G1	IgG Standard	2.5	Standard	2.5	n/a

Figure 5-20: Add Replicate Group from the Sample Plate Table



NOTE: Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the **Sample Plate Table**. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the **Sample Plate Map** menu used to designate sample types.

3. Repeat the previous steps to assign new samples to the existing **Replicate Group**, or to designate another set of samples to a new **Replicate Group**. Multiple groups can be used in an experiment.



IMPORTANT: The Octet System Data Analysis software will only recognize and calculate statistics for samples that use the same **Replicate Group** names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.



NOTE: When performing a Multiple Analyte experiment, if the same Replicate Group name is used with different biosensor types, they will be treated as separate groups. Statistics for these groups will be calculated separately for each biosensor type.

MANAGING SAMPLE PLATE DEFINITIONS



NOTE: After you define a sample plate, you can export and save the plate definition for future use.

Exporting a Plate Definition

To export a plate definition:

1. In the **Sample Plate Table** (see Figure 5-21), click **Export**.

Sample Plate Table

Concentration units: µg/ml Export... Import...

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	Dilution Factor
A1	IgG Standard	200	Standard	200	n/a
B1	IgG Standard	100	Standard	100	n/a
C1	IgG Standard	50	Standard	50	n/a
D1	IgG Standard	25	Standard	25	n/a
E1	IgG Standard	10	Standard	10	n/a
F1	IgG Standard	5	Standard	5	n/a

Figure 5-21: Export Button in Sample Plate Table

2. In the **Export Plate Definition** window (see Figure 5-22), select a folder, enter a name for the plate (.csv), and click **Save**.

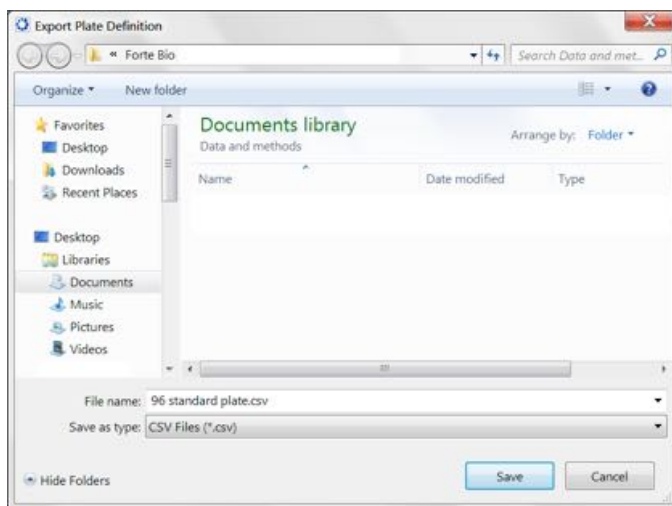


Figure 5-22: Export Plate Definition Window

Importing a Plate Definition

To import a plate definition:

1. In the **Sample Plate Table** (see Figure 5-23), click **Import**.

Sample Plate Table					
Concentration units:		$\mu\text{g/ml}$	Export...	Import...	
Well	Sample ID	Replicate Group	Type	Conc ($\mu\text{g/ml}$)	Dilution Factor
A1	IgG Standard	200	Standard	200	n/a
B1	IgG Standard	100	Standard	100	n/a
C1	IgG Standard	50	Standard	50	n/a
D1	IgG Standard	25	Standard	25	n/a
E1	IgG Standard	10	Standard	10	n/a
F1	IgG Standard	5	Standard	5	n/a

Figure 5-23: Import Button in Sample Plate Table

2. In the **Import Plate Definition** window (see Figure 5-24), select the plate definition (.csv), and click **Open**.

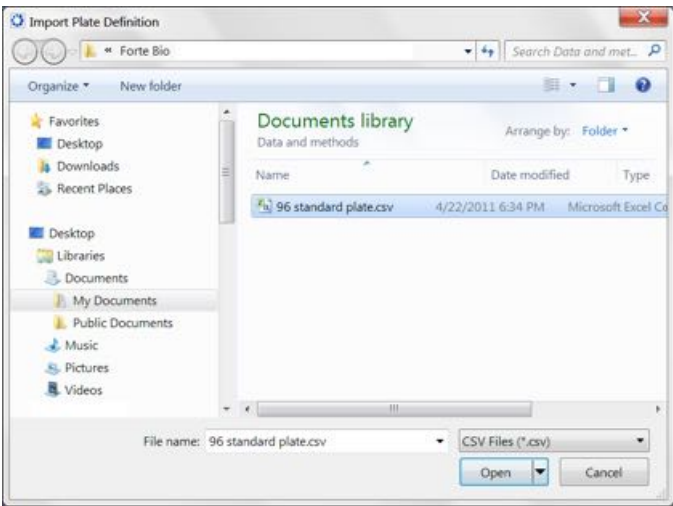


Figure 5-24: Import Plate Definition Window



NOTE: You can also create a .csv file for import. Figure 5-25 shows the appropriate column information layout.

	A	B	C	D	E	F	G
1	PlateWells	96					
2	Well	ID	Replicate Group	Group	Concentration (µg/ml)	Dilution	Information
3	A1	IgG Standard	200	Standard	200		Sample Diluent
4	B1	IgG Standard	100	Standard	100		Sample Diluent
5	C1	IgG Standard	50	Standard	50		Sample Diluent
6	D1	IgG Standard	25	Standard	25		Sample Diluent
7	E1	IgG Standard	10	Standard	10		Sample Diluent
8	F1	IgG Standard	5	Standard	5		Sample Diluent
9	G1	IgG Standard	2.5	Standard	2.5		Sample Diluent
10	H1	IgG Standard	1	Standard	1		Sample Diluent
11	A2	IgG Standard	200	Standard	200		Sample Diluent

Figure 5-25: Example Sample Plate File (.csv)

Printing a Sample Plate Definition

To print a plate definition:

1. In the **Sample Plate Map** (see Figure 5-26), click **Print**.

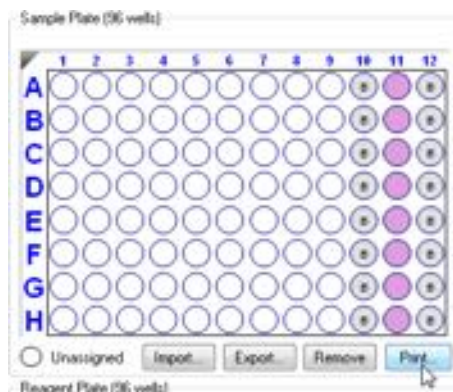


Figure 5-26: Sample Plate Print Button

The associated **Sample Plate Table** information will print.

MANAGING ASSAY PARAMETER SETTINGS

Modifying Assay Parameter Settings

You can modify the assay parameter settings during sample plate definition. However, the changes are only applied to the current experiment. To save modified parameter settings, you must define a new assay. For details on creating a new assay, see "Custom Quantitation Assays" on page 198.

Viewing User-Modifiable Assay Parameter Settings

To view the user-modifiable settings for an assay, click **Modify** in the **Assay Settings** box. The **Assay Parameters** box will display (Figure 5-27). The settings available are experiment-dependent.

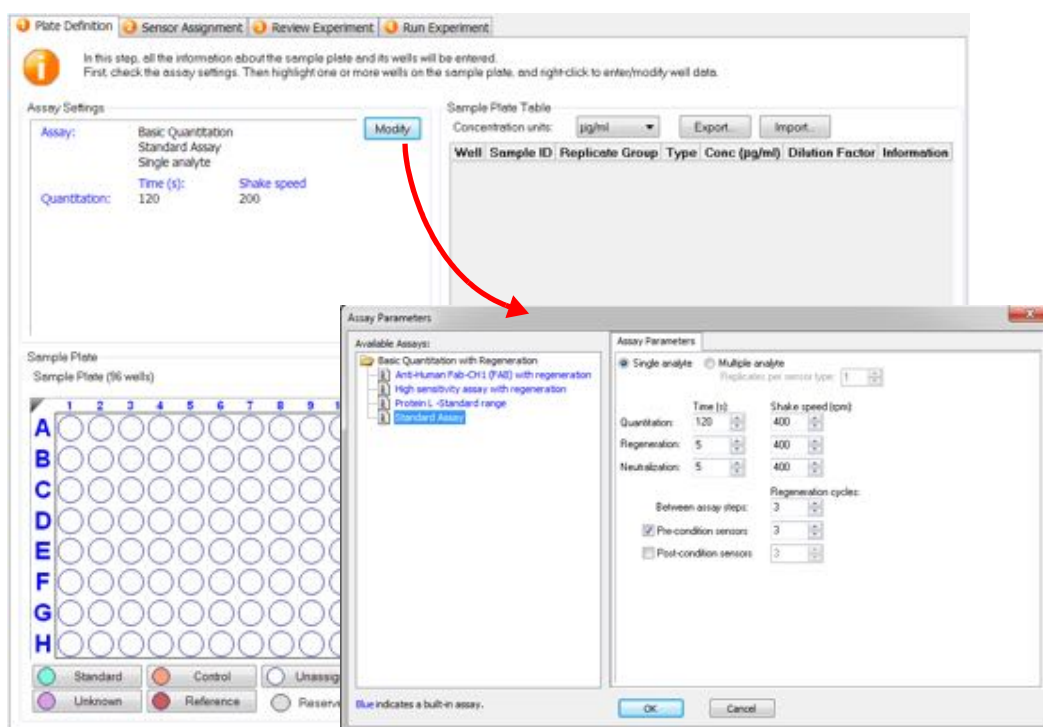


Figure 5-27: Modifying Assay Parameters

Basic Quantitation Assay Parameters

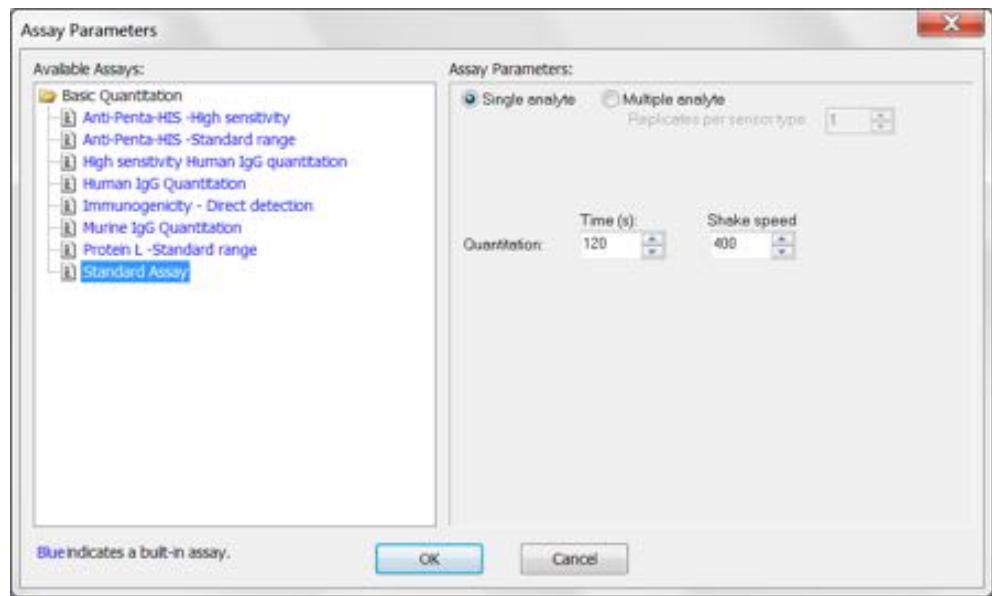



Figure 5-28: Assay Parameters—Basic Quantitation Assay

Table 5-6: Basic Quantitation Assay Parameters

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time (s)	The duration of data acquisition seconds while the biosensor is incubated in sample.
<div> NOTE: A subset of data points may be selected for processing during data analysis.</div>	
Quantitation Shake speed (rpm)	The sample shaking speed (rotations per minute).

Basic Quantitation with Regeneration Assay Parameters

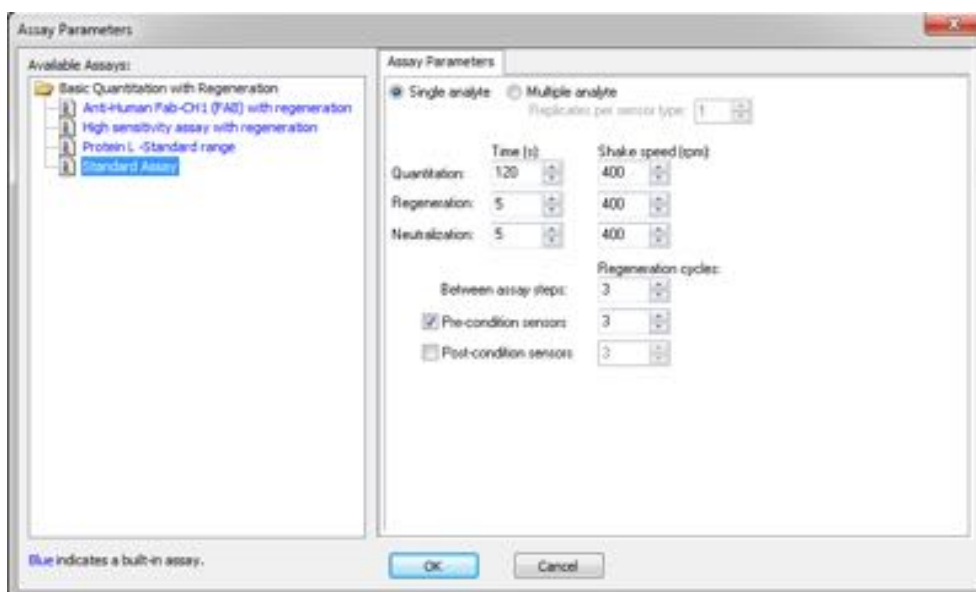


Figure 5-29: Assay Parameters—Basic Quantitation with Regeneration

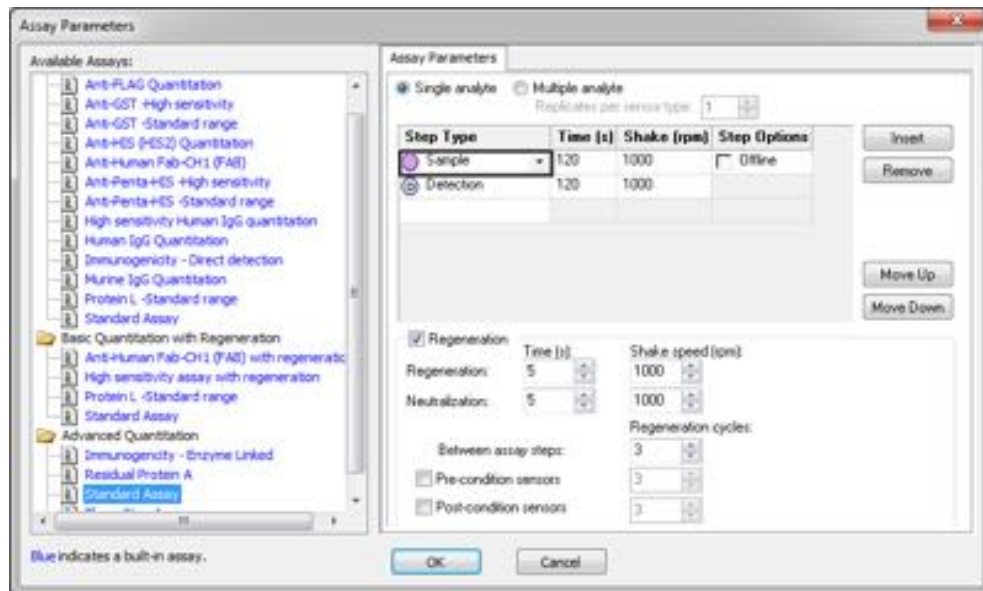
Table 5-7: Assay Parameters—Basic Quantitation with Regeneration

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time(s) and Shake speed (rpm)	The duration of data acquisition in seconds while the biosensor is incubated in sample and the sample shaking speed (rotations per minute).
<div>➔</div> NOTE: A subset of data points may be selected for processing during data analysis.	
Regeneration Time(s) and Shake speed (rpm)	The duration time and shaking speed of the regeneration step where the biosensor is incubated in regeneration buffer to remove bound analyte.
Neutralization Time(s) and Shake speed (rpm)	The duration time and shaking speed of the neutralization step where the biosensor is incubated in neutralization buffer after the regeneration step.

Table 5-7: Assay Parameters—Basic Quantitation with Regeneration

Parameter	Description
Pre-condition sensors	Performs a set of regeneration/neutralization steps prior to the start of the experiment. The pre-conditioning settings are equivalent to the time and rpm settings for the regeneration in the assay. For example, an acidic pre-conditioning buffer maximizes the binding competence of Pro-A biosensors.
Post-condition sensors	Post-conditions biosensors, allowing re-racked biosensors to be stored in a regenerated state.
Regeneration cycles	The number of regeneration-neutralization cycles that a biosensor undergoes before reuse.

Advanced Quantitation Assay Parameters

**Figure 5-30: Assay Parameters—Advanced Quantitation****Table 5-8: Advanced Quantitation Assay Parameters**

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.

Table 5-8: Advanced Quantitation Assay Parameters (Continued)



Parameter	Description
Sample Time(s) and Shake speed (rpm)	The duration of data acquisition in seconds while the biosensor is incubated in sample and the sample shaking speed (rotations per minute).
 NOTE: A subset of data points may be selected for processing during data analysis.	
Buffer Time(s) and Shake speed (rpm)	The duration of biosensor incubation in the first buffer in seconds and the sample shaking speed (rotations per minute).
Enzyme Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the enzyme solution and the sample shaking speed (rotations per minute).
2nd Buffer Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the second buffer solution and the sample shaking speed (rotations per minute).
Capture Antibody Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the first capture antibody solution and the shaking speed (rotations per minute).
2nd Antibody Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the secondary antibody solution and the shaking speed (rotations per minute).
Detection Time(s) & Shake speed (rpm)	The duration of data acquisition during the detection step in seconds in an advanced quantitation assay.
 NOTE: A subset of data points may be selected for processing during data analysis.	
Offline	Choose this option to incubate sample with biosensors outside the Octet system. Offline incubation is best performed on the Pall ForteBio Sidekick biosensor immobilization station.
Reuse Buffer	Allows buffer wells to be reused. If unselected, the number of buffer columns must equal the number of sample columns. If selected, the number of buffer columns may be less than the number of sample columns as the buffer columns are reused.
Regeneration Time(s) and Shake speed (rpm)	The duration time and shaking speed of the regeneration step where the biosensor is incubated in regeneration buffer to remove bound analyte.

Table 5-8: Advanced Quantitation Assay Parameters (Continued)

Parameter	Description
Neutralization Time(s) and Shake speed (rpm)	The duration time and shaking speed of the neutralization step where the biosensor is incubated in neutralization buffer after the regeneration step.
Pre-condition sensors	Performs a set of regeneration/neutralization steps prior to the start of the experiment. The pre-conditioning settings are equivalent to the time and rpm settings for the regeneration in the assay. For example, an acidic pre-conditioning buffer maximizes the binding competence of Protein A biosensors.
Post-condition sensors	Post-conditions biosensors, allowing re-racked biosensors to be stored in a regenerated state.
Regeneration cycles	The number of regeneration-neutralization cycles that a biosensor undergoes before reuse.



NOTE: In an Advanced Quantitation experiment, this option is only available if the first step (biosensor incubation in sample) is performed online.

ASSIGNING BIOSENSORS TO SAMPLES


After the sample plate is defined, biosensors must be assigned to the samples.

Biosensor Assignment in Single-Analyte Experiments

In a single analyte experiment, only one biosensor type is assigned to each sample and only one analyte is analyzed per experiment.



NOTE: For single analyte experiments, the **Single Analyte** option must be selected in the **Assay Parameters** dialog box. For more information, please see “Managing Assay Parameter Settings” on page 158.

Click the **Sensor Assignment** tab, or click the  arrow to access the Sensor Assignment window (see Figure 5-31).

The software generates a color-coded **Sensor Tray Map** and **Sample Plate Map** that shows how the biosensors are assigned to the samples by default.

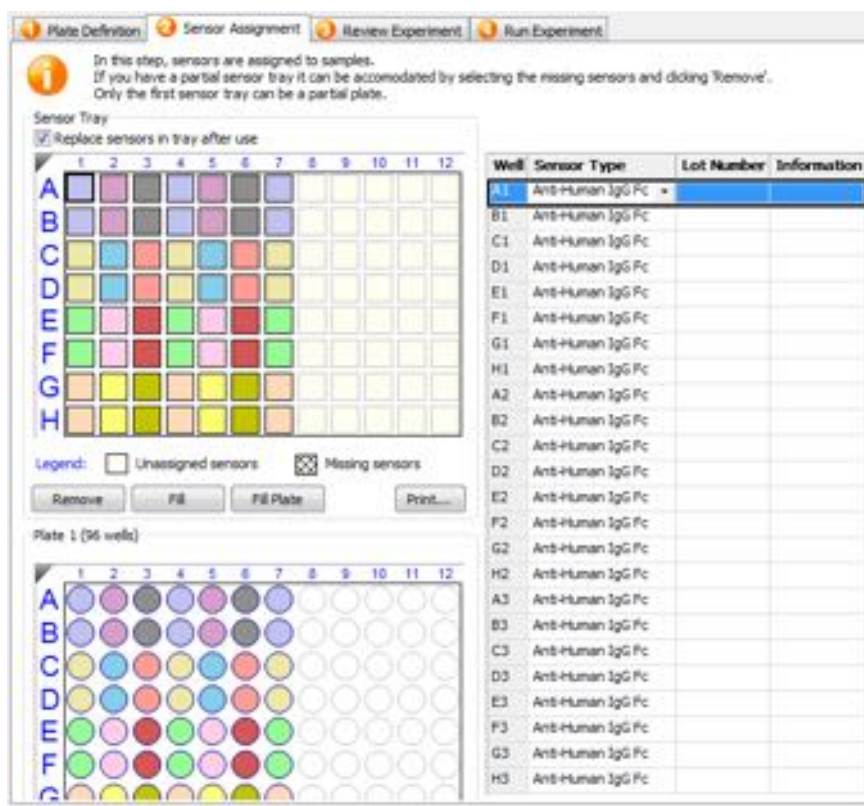


Figure 5-31: Sensor Assignment Window for Basic Quantitation without Regeneration

- Assign biosensors in one of two ways:
 - Select a column(s) in the **Sensor Tray Map**, right-click and select a biosensor type from the drop-down list).
 - Select a cell in the **Sensor Type** table column, click the down arrow and select a biosensor type from the drop-down list (see Figure 5-32).

All wells in the **Sensor Type** column will automatically populate with the biosensor type selected.

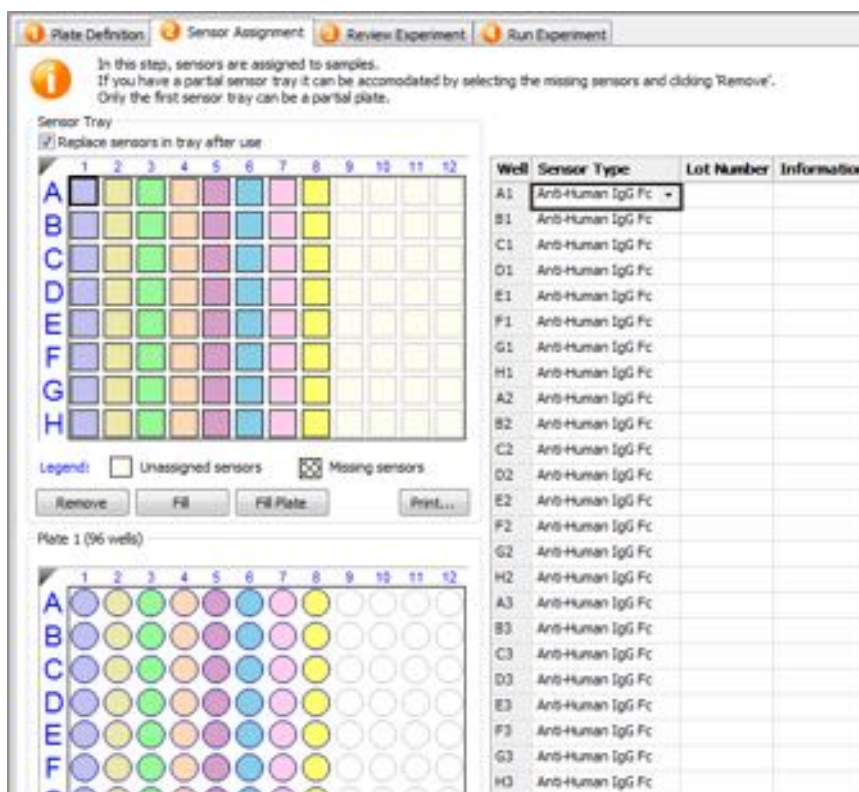


Figure 5-32: Changing Biosensor Types

- To designate reference biosensors, select the desired biosensors in the **Sensor Tray Map**, right-click and select **Reference**. The reference biosensors are marked with an **R**.



NOTE: Reference biosensors may also be designated in the **Runtime Binding Chart** during acquisition.

- Optional: Double-click in any cell in the **Lot Number** column to enter the biosensor lot number. All wells in the **Lot Number** column will automatically populate with the lot number entered.
- Optional: Double-click in a cell in the **Information** column to enter biosensor information for a particular cell.



NOTE: Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: For greater clarity, annotation text may be displayed as the legend of the **Runtime Binding Chart** during data acquisition but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it will not be available for display as a legend.

- Optional for the Octet K2 instrument only: After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace sensors in tray after use** check box (see Figure 5-33).

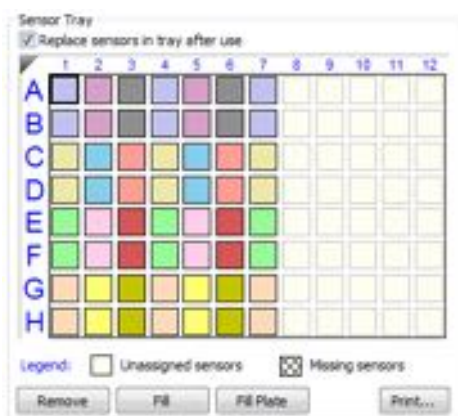


Figure 5-33: Replace Sensors in Tray After Use Check Box




NOTE: Biosensors can be regenerated up to a max of 11 times per experiment.

Biosensor Assignment in Multiple Analyte Experiments

In a multiple analyte experiment, more than one biosensor type is assigned to the same sample, allowing multiple analytes to be analyzed in a single experiment.



NOTE: For multiple analyte experiments, the **Multiple Analyte** option must be selected in the **Assay Parameters** dialog box. For more information, please see “Managing Assay Parameter Settings” on page 158.

Click the **Sensor Assignment** tab, or click the  arrow to access the Sensor Assignment window (see Figure 5-31).

The software generates a color-coded **Sensor Tray Map** and **Sample Plate Map** that shows how the biosensors are assigned to the samples by default. In the example shown in Figure 5-31, **one** replicate had been previously selected with the **Multiple Analyte** assay parameter option.

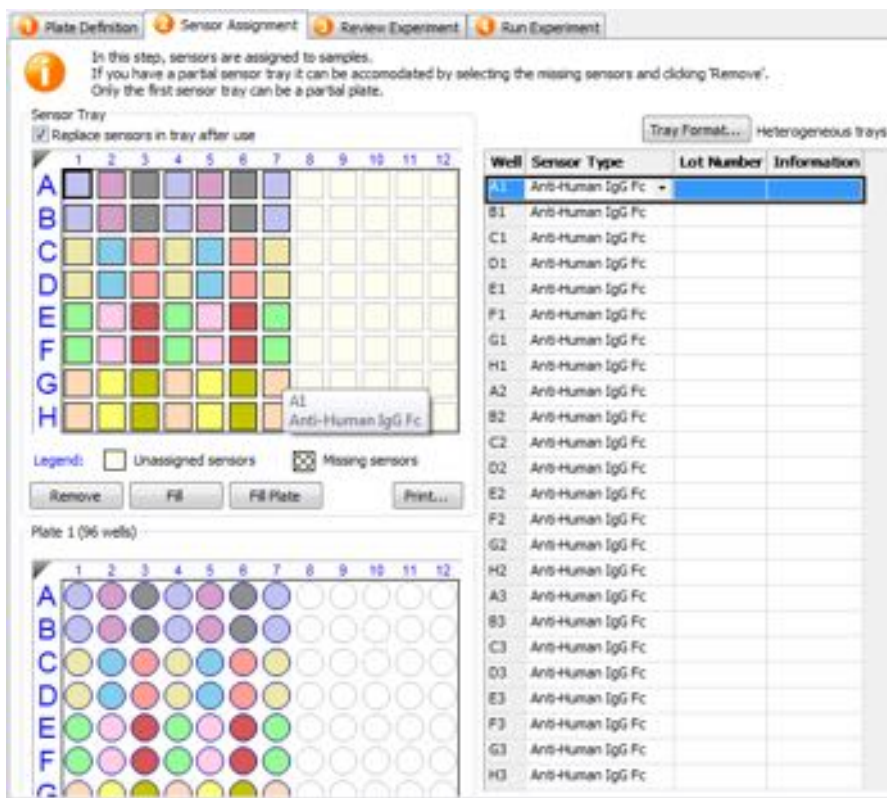


Figure 5-34: Sensor Assignment Window for Basic Quantitation Using the Multiple Analyte Option

There are two ways to assign biosensors:

- Select a set of wells in the **Sensor Tray Map**, right-click and select a biosensor type from the drop-down list.
- Select a cell in the **Sensor Type** table column, click the down arrow and select a biosensor type from the drop-down list (see Figure 5-35).

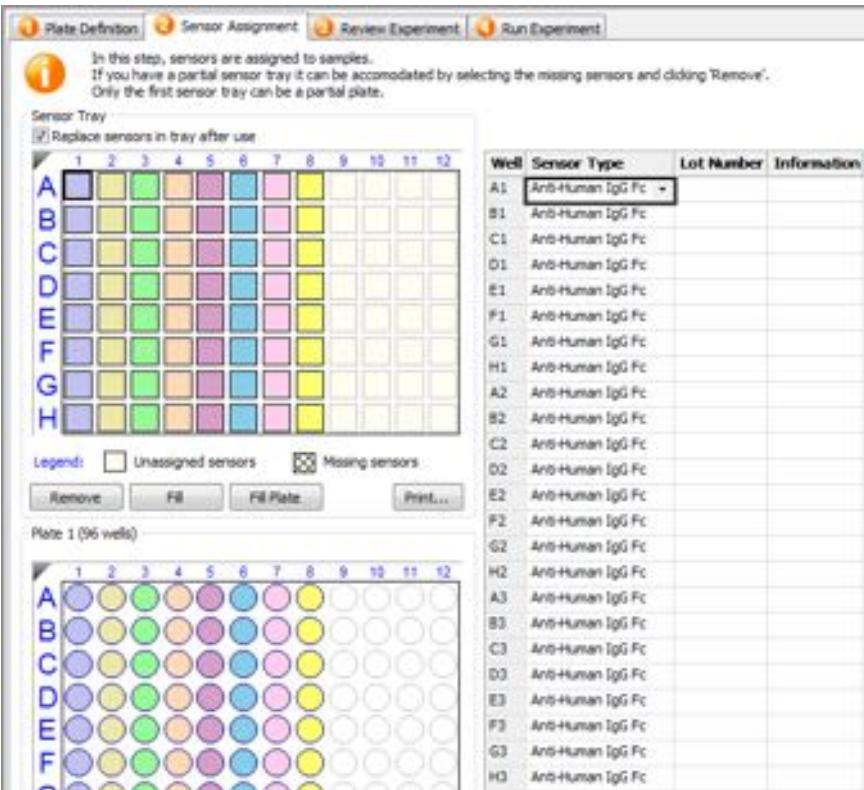


Figure 5-35: Changing Biosensor Types

Biosensor Assignment Using Heterogeneous Biosensor Trays

The default **Tray Format** is **Heterogeneous**. Heterogeneous biosensor trays contain a mixture of biosensor types.



NOTE: When using this **Heterogeneous** option, the order of biosensor types in each tray must be identical.

1. If Heterogeneous Trays is not displayed next to the **Tray Format** button, click the button.

The **Tray Format** dialog box displays (see Figure 5-36).

2. Select **Heterogeneous** and click **OK**.

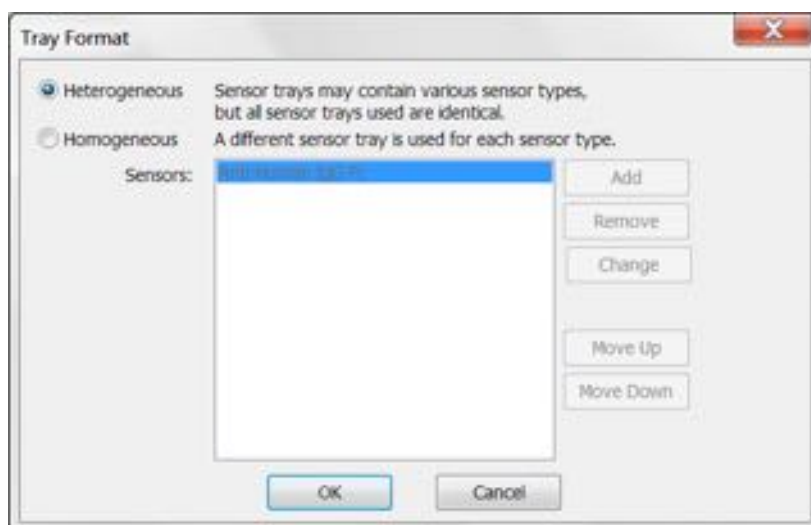


Figure 5-36: Tray Format Dialog Box

The Tray 1 **Sensor Tray Map** will be displayed by default.

3. Select **all** columns with default biosensor assignments in the **Sensor Tray Map**, right-click and select the first biosensor type to be used (see Figure 5-37).

The **Sensor Type** column will update accordingly.



Figure 5-37: Populating the Sensor Tray Map with First Biosensor Type

4. Select the sensors in the **Sensor Tray Map** that should contain the second biosensor type, right-click and select the second biosensor type (see Figure 5-39).

The **Sensor Type** column will update accordingly.

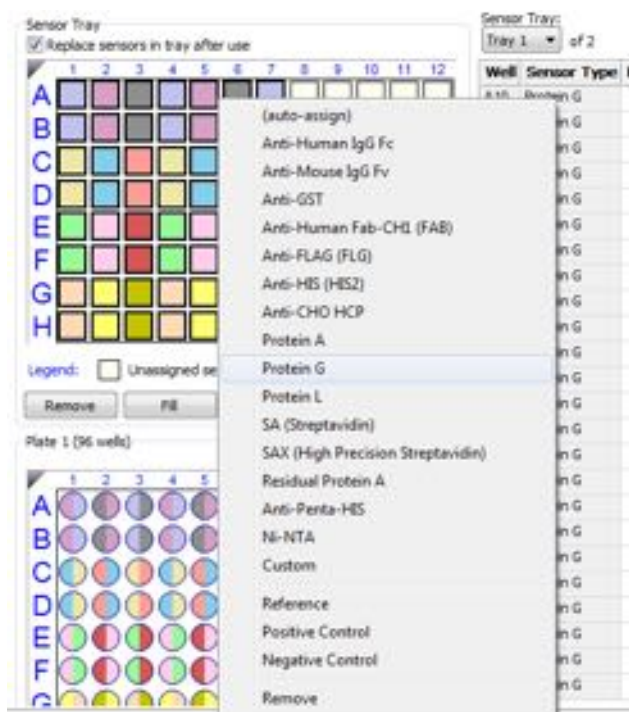


Figure 5-38: Populating the Sensor Tray Map with Second Biosensor Type

5. Repeat this sensor selection and assignment process for all other biosensor types to be used in the experiment. The software will automatically update the number of biosensor trays needed and biosensor assignments in all trays according to the column assignments made in Tray 1.

In the example shown in Figure 5-39, Protein A and Protein G biosensor types are used for a multiple analyte experiment using two replicates. Three heterogeneous biosensor trays will be needed for the experiment.



Figure 5-39: Biosensor Assignment using Heterogeneous Trays and Two Biosensor Types

- To view or change the biosensor assignments in another tray, click the **Sensor Tray** button and select a tray number from the drop down list.

The **Sensor Tray Map** and table for the tray selected will be shown and biosensor assignments can be changed as needed (see Figure 5-40).

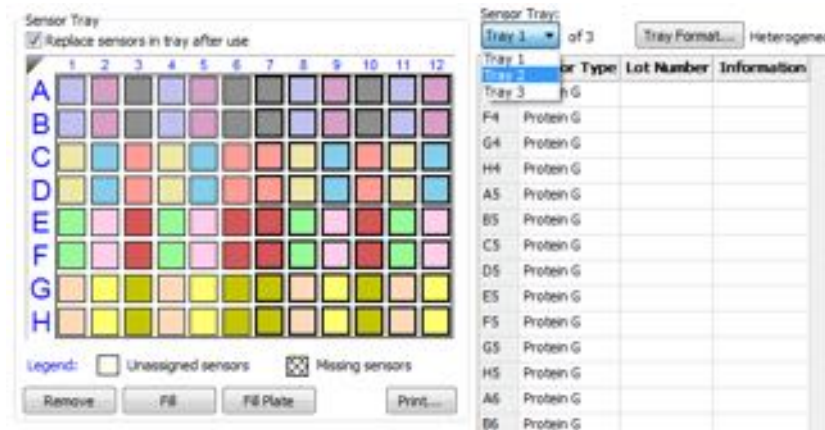


Figure 5-40: Tray Selection

7. To designate reference biosensors, select the desired biosensors in the **Sensor Tray Map**, right-click and select **Reference**.

The reference biosensors are marked with an **R**.



NOTE: Reference biosensors may also be designated in the **Runtime Binding Chart** during acquisition.

8. Optional: Double-click in any cell in the **Lot Number** column to enter a biosensor lot number. All wells in the **Lot Number** column for that biosensor type will automatically populate with the lot number entered.
9. Optional: Double-click in a cell in the **Information** column to enter biosensor information for a particular cell.



NOTE: Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: For greater clarity, annotation text may be displayed as the legend of the **Runtime Binding Chart** during data acquisition but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it will not be available for display as a legend.

10. Optional: After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace sensors in tray after use** check box (see Figure 5-41).



Figure 5-41: Replace Sensors in Tray After Use Check Box



NOTE: Biosensors can be regenerated up to a max of 11 times per experiment.

Biosensor Assignment Using Homogeneous Trays

Homogeneous biosensor trays contain only one biosensor type.



NOTE: Using the **Homogeneous** option will necessitate switching trays during the experiment.

1. Click **Tray Format**.

The **Tray Format** dialog box displays (see Figure 5-42) and the **Sensors** box will be populated with the default biosensor type.

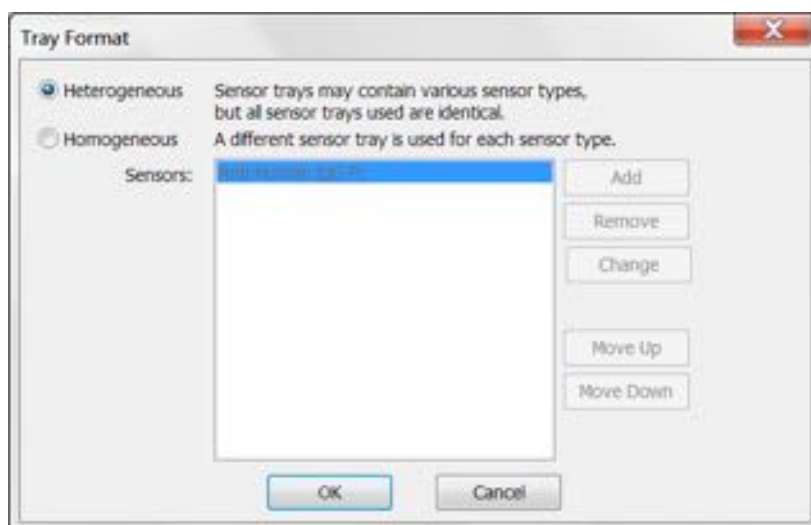


Figure 5-42: Tray Format Dialog Box

2. Select **Homogeneous**. Click **Add** to select the first biosensor type (see Figure 5-43).

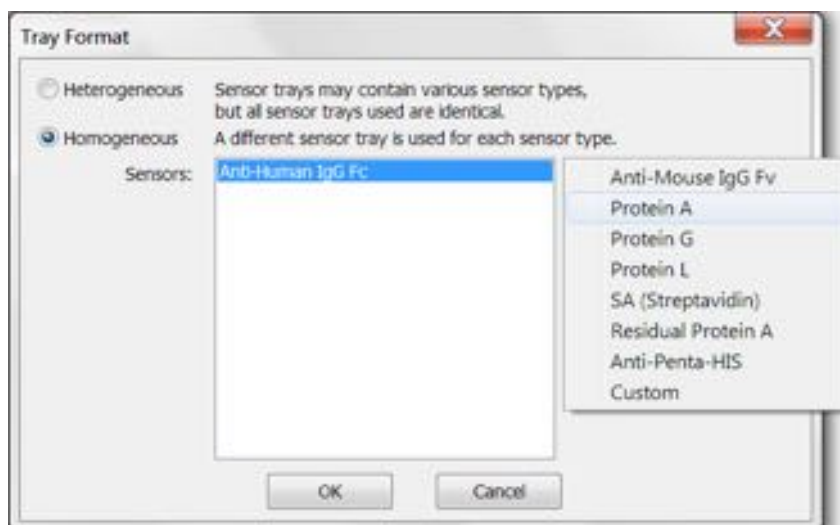


Figure 5-43: Selecting a Biosensor Type in the Tray Format Dialog Box

3. Repeat this step to add any additional biosensor types that will be used in the experiment. To remove a biosensor type, select a biosensor type in the **Sensor** box and click **Remove**.
4. Adjust the order of biosensor types as needed by selecting the biosensor type in the **Sensor** box and clicking **Move Up** or **Move Down**.

The order of biosensor types listed in the **Sensor** box will be used as the default tray assignment (see Figure 5-44).

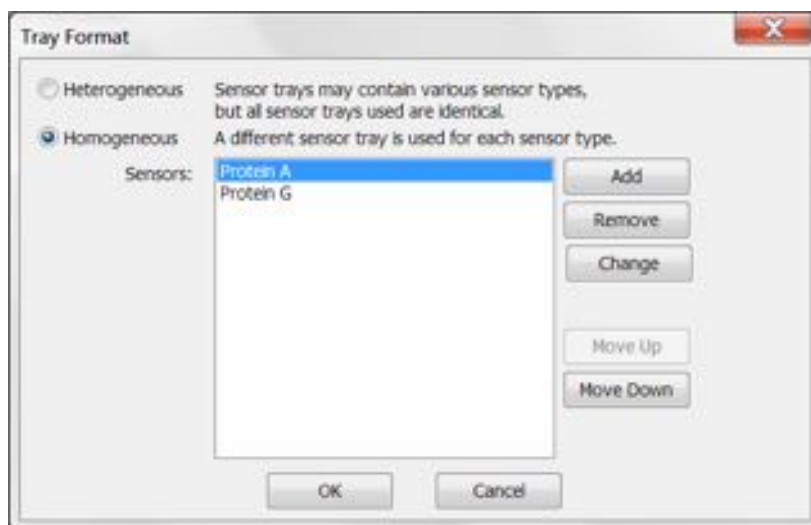


Figure 5-44: Biosensor Types List Order in Sensor Box

5. Click **OK**.

The software will automatically calculate the number of biosensor trays needed and assign biosensors types to each tray.

In the example shown in Figure 5-45, Protein A and Protein G biosensor types will be used for the multiple analyte experiment using two replicates. Four homogeneous biosensor trays (two for each biosensor type) will be needed for the experiment. The Tray 1 **Sensor Tray Map** will be displayed by default.



Figure 5-45: Biosensor Assignment using Homogeneous Trays and Two Biosensor Types

6. To view the biosensor assignments in another tray, click the **Sensor Tray** button and select a tray number from the drop down list.

The **Sensor Tray Map** and table for the tray selected will be shown (see Figure 5-40).

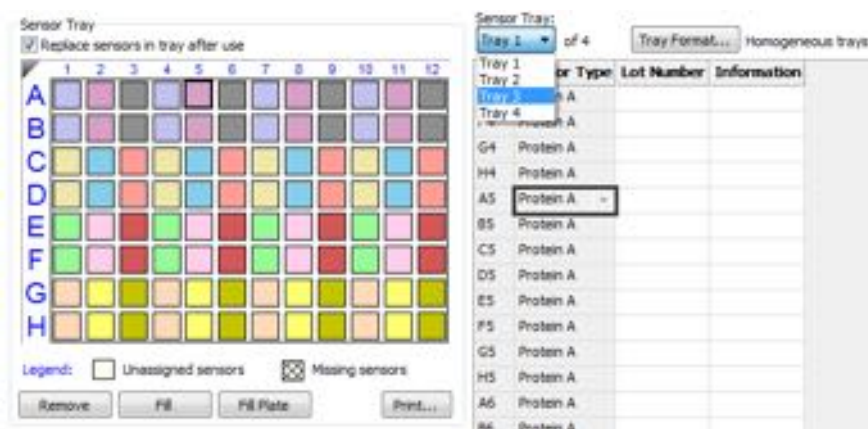


Figure 5-46: Tray Selection

7. To designate reference biosensors, select the desired biosensors in the **Sensor Tray Map**, right-click and select **Reference**.

The reference biosensors are marked with an **R**.



NOTE: Reference biosensors may also be designated in the **Runtime Binding Chart** during acquisition.

8. Optional: Double-click in any cell in the **Lot Number** column to enter a biosensor lot number.

All wells in the **Lot Number** column for the biosensor type selected will automatically populate with the lot number entered.

9. Optional: Double-click in a cell in the **Information** column to enter biosensor information for a particular cell.



NOTE: Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: For greater clarity, annotation text may be displayed as the legend of the **Runtime Binding Chart** during data acquisition but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it will not be available for display as a legend.

10. Optional: After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace sensors in tray after use** check box (see Figure 5-47).



Figure 5-47: Replace Sensors in Tray After Use Check Box



NOTE: Biosensors can be regenerated up to a max of 11 times per experiment.

Biosensor Regeneration

For Basic Quantitation with Regeneration experiments only, the **Sensor Assignment** tab includes the **Regenerations** parameter, which specifies the maximum number of regeneration cycles for each column of biosensors. The specified number of regeneration cycles determines the minimum number of cycles required for two sensors. This calculation may result in non-equal regeneration cycles for columns of biosensors. The fractional use of the regeneration and neutralization wells by two sensors is represented by a pie chart (Figure 5-48).

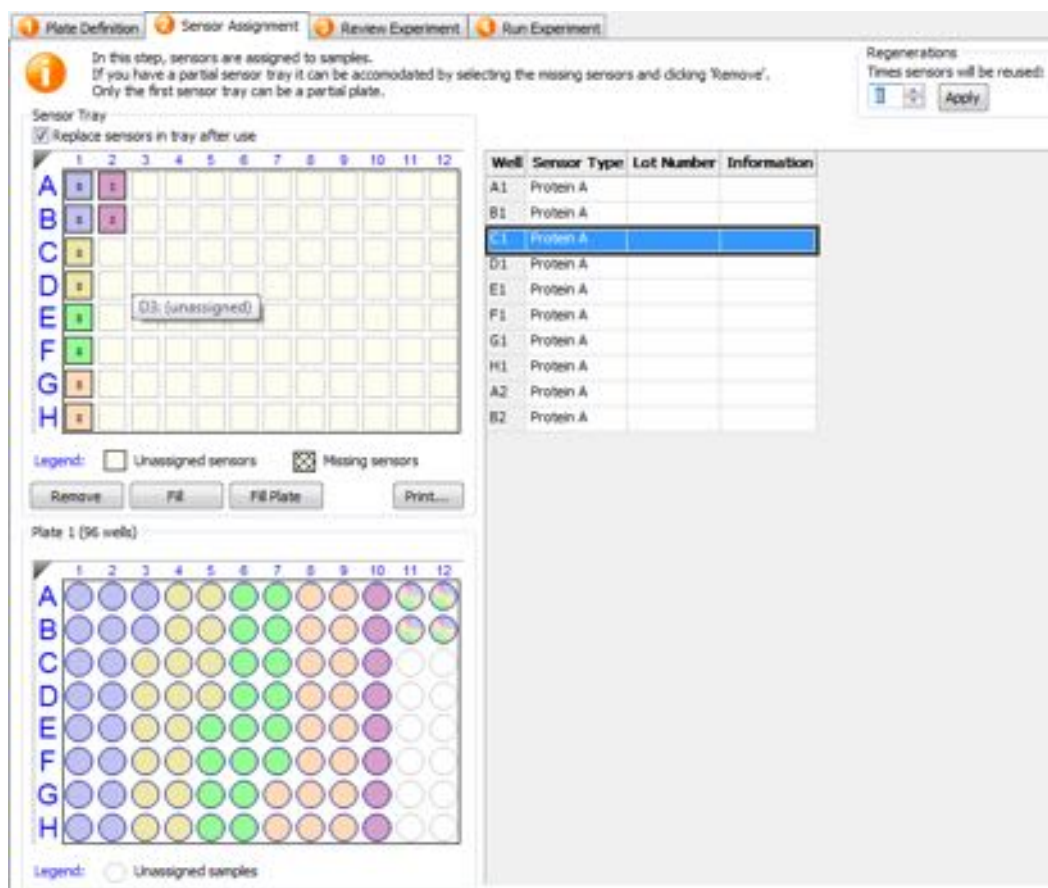


Figure 5-48: Fractional Use of Regeneration and Neutralization Wells

Using Partial Biosensor Trays

If you are using a partial tray of biosensors (some biosensors are missing), specify the missing columns in the **Sensor Tray Map**:

1. Select the column(s) without biosensors and click **Remove**, or right-click the selection and select **Remove**.

If the number of specified biosensors in the **Sensor Assignment** tab is less than the number required to perform the assay, the software automatically adds a second tray of biosensors and assigns the biosensors that are required for the assay.

2. To view the additional biosensor tray that is required for the assay, select Tray 2 from the **Sensor Tray** drop-down list (Figure 5-49). In the example shown, Tray 1 is a partial tray that does not contain enough biosensors for the assay. To designate a second tray, select Tray 2 from the **Sensor Tray** drop-down list (Figure 5-49 top). The **Sensor Tray Map** will then display the additional biosensors required for the assay (Figure 5-49 bottom).

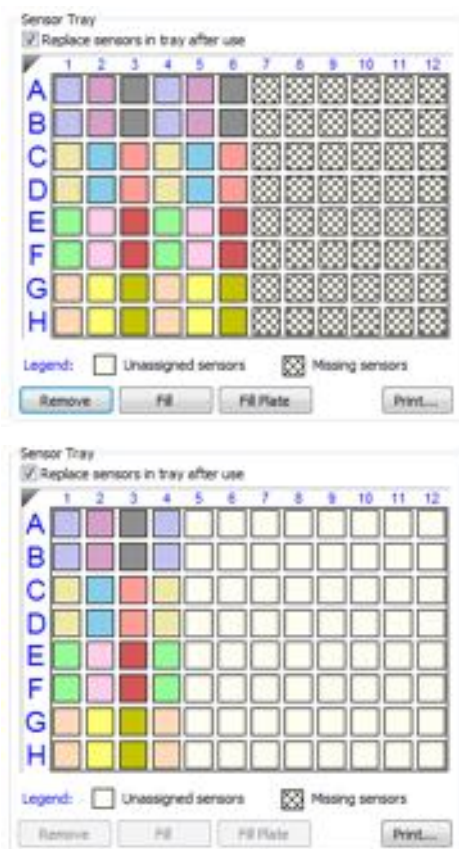


Figure 5-49: Example Assay Using One Partial Biosensor Tray and Biosensors from a Second Tray

To restore biosensors that have been removed, select the columns to restore and click **Fill**. To restore all sensors on the plate, click **Fill Plate**.





NOTE: If multiple biosensor trays are used, only the first biosensor tray can be a partial tray. During the experiment, the software prompts you to insert the appropriate tray in the Octet instrument.

REVIEWING EXPERIMENTS



NOTE: For optimal results, ensure total assay time is less than 3 hours.

Before running an experiment, you can review the sample plate layout and the biosensors assigned to each assay in the experiment.

In the **Review Experiment** window, move the slider left or right to highlight the biosensors and samples in an assay, or click the   arrows to select an assay.

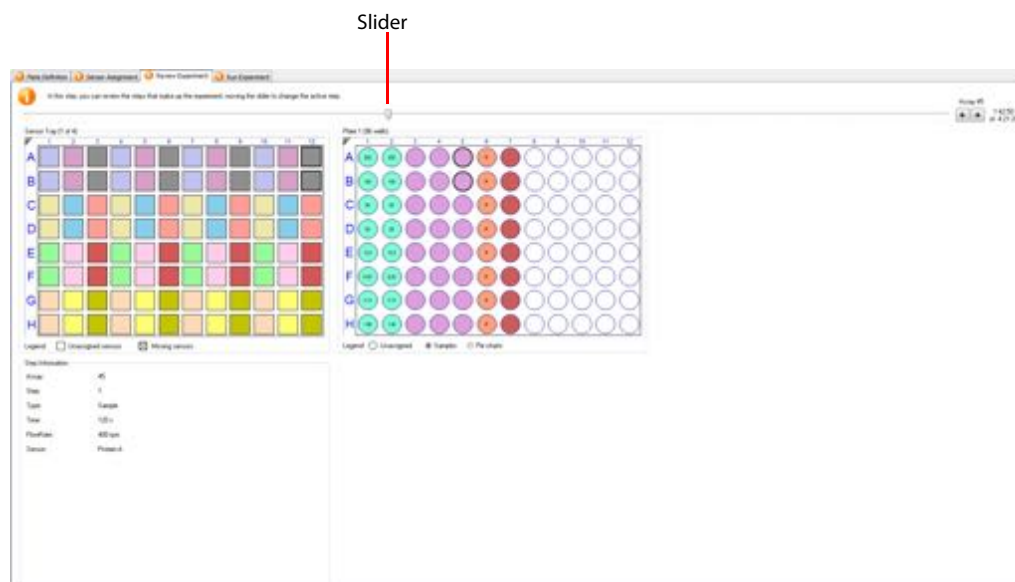




Figure 5-50: Review Experiment Window

SAVING EXPERIMENTS

After a run, the software automatically saves the experiment information that you specified (sample plate definition, biosensor assignment, assay settings) to an experiment method file (.fmf). If you set up an experiment, but do not start the run, you can manually save the experiment method.

To manually save an experiment method:

1. Click the **Save Method File** button , or on the main menu, click **File > Save Method File**. To save more than one open experiment, click the **Save All Methods Files** button .
2. In the **Save** dialog box, enter a name and location for the file, and click **Save**.



NOTE: If you edit a saved experiment and want to save it without overwriting the original file, select **File > Save Method File As** and enter a new name for the experiment.

Saving an Experiment to the Template Folder

If you save an experiment to the factory-installed Template folder, the experiment will be available on the menu bar. To view templates click **Experiment > Templates > Quantitation > Experiment Name** (see Figure 5-51).

Follow the steps above to save an experiment to the Template folder located at C:\Program Files\ForteBio\DataAcquisition\TemplateFiles.



IMPORTANT: Do not change the location of the Template folder. If the Template folder is not at the factory-set location, the software may not function properly.

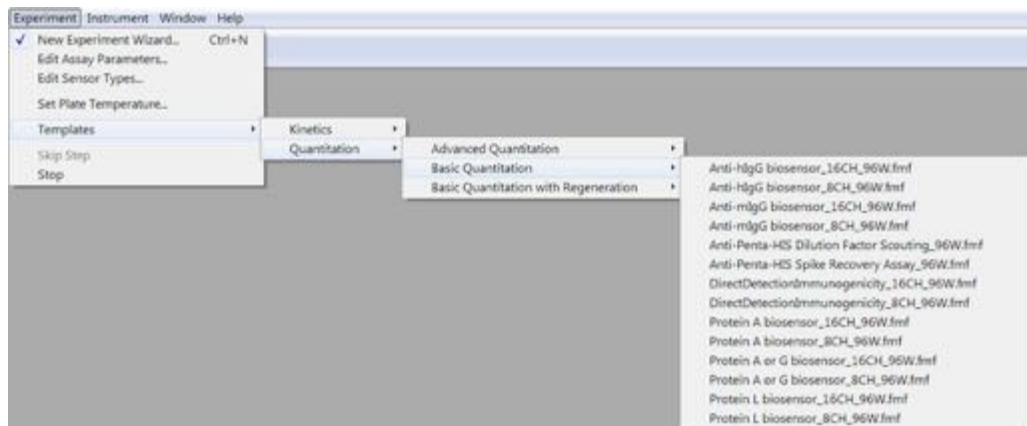


Figure 5-51: Experiments in the Template Folder

RUNNING A QUANTITATION EXPERIMENT



IMPORTANT: Before starting an experiment, ensure that the biosensors are properly rehydrated. For details on how to prepare the biosensors, see the appropriate biosensor product insert.

Loading the Biosensor Tray and Sample Plate

To load the biosensor tray and sample plate:

1. Open the Octet instrument door (lift the handle up).
2. Place the biosensor tray on the biosensor stage (left side) so that well A1 is located at the upper right corner (see Figure 5-52).
3. Place the sample plate on the sample stage (right side) so that well A1 is located at the upper right corner (see Figure 5-52).

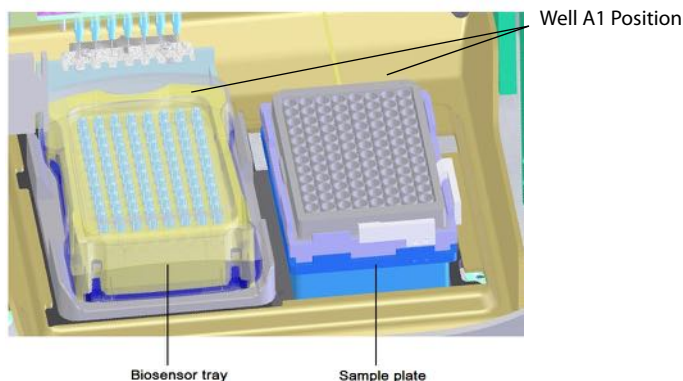


Figure 5-52: Biosensor Stage (left) and Sample Stage (right)




IMPORTANT: Ensure that the bottom of the sample plate and biosensor tray are flat on each stage.

4. Close the Octet instrument door.
5. Allow the plate to equilibrate.

The time required for temperature equilibration depends on the temperature that your application requires and the initial temperature of the sample plate. For specific biosensor rehydration times, see the appropriate biosensor product insert.

Starting an Experiment

To start the experiment:

1. Click the **Run Experiment** tab, or click the arrow  to access the Run Experiment window (see Figure 5-53).

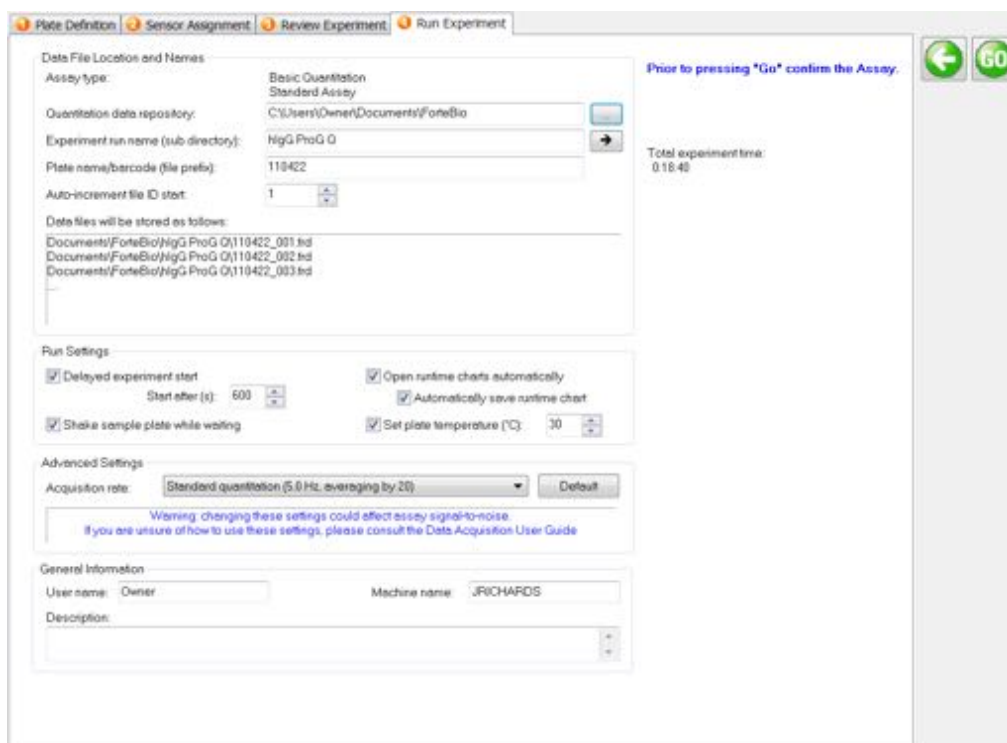


Figure 5-53: Run Experiment Window

2. Confirm the defaults or enter new settings. See “Run Experiment Window Settings” on page 187 for more information on experimental settings.



NOTE: If you delay the experiment start, you have the option to shake the plate until the experiment starts.

3. To start the experiment, click .

If you specified a delayed experiment start, a message box displays the remaining time until the experiment starts.

If you selected the **Open runtime charts automatically** option, the **Runtime Binding Chart** window displays the binding data in real-time and the experiment progress (see Figure 5-54).



NOTE: For more details about the **Runtime Binding Chart**, see “Managing Runtime Binding Charts” on page 191.

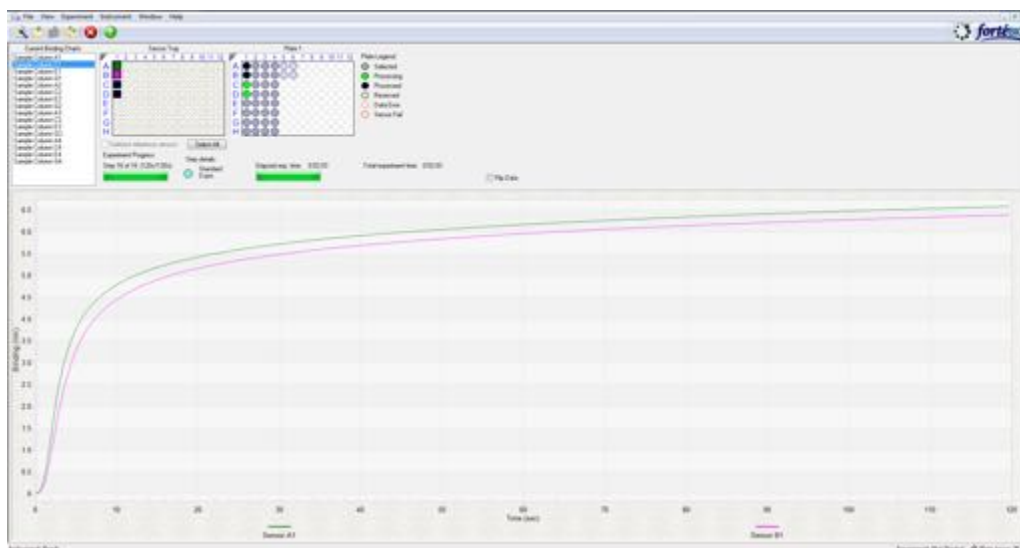


Figure 5-54: Runtime Binding Chart

4. Optional: Click **View > Instrument Status** to view the log file (see Figure 5-55).

The experiment temperature is recorded at the beginning of every experiment as well as each time the manifold picks up a new set of biosensors. Instrument events such as biosensor pick up, manifold movement, integration time, biosensor ejection and sample plate temperature are recorded in the log file.



WARNING: Do not open the Octet instrument door when an experiment is in progress. If the door is opened the data from the active acquisition step is lost. The data acquired in previous steps is saved, however the assay is aborted and cannot be restarted without ejecting the biosensors and starting from the beginning.



WARNING: N'ouvrez pas la porte de l'instrument Octet lorsqu'une analyse est en cours. En cas d'ouverture de la porte, les données issues de l'étape d'acquisition active seront perdues et cela entraînera l'échec de la procédure.



WARNING: Öffnen Sie die Instrumentenklappe des Octet-Systems nicht während eines laufenden Experiments. Wird die Klappe geöffnet, gehen die Daten des aktiven Erfassungsschritts verloren und das Experiment wird abgebrochen.

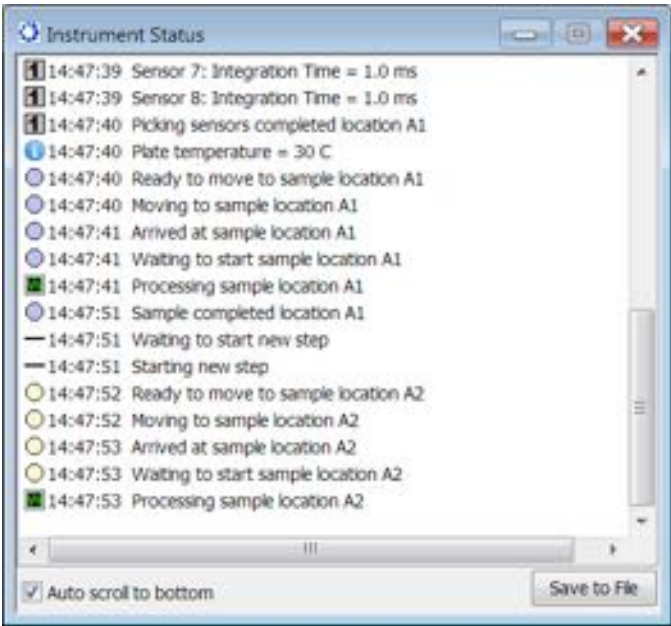


Figure 5-55: Instrument Status Log

Run Experiment Window Settings

The following **Data File Location and Name** settings are available on the **Run Experiment** Tab:

Table 5-9: Data File Location and Name

Item	Description
Assay type	The name of the selected assay.
Quantitation data repository	The location where quantitation data files (.frd) are saved. Click Browse to select another data location.



NOTE: It is recommended that you save the data to the local machine first, then transfer to a network drive.

Table 5-9: Data File Location and Name (Continued)

<i>Item</i>	<i>Description</i>
Experiment Run name (sub-directory)	Specifies a subdirectory name for the data files (.frd) that are created. The software generates one data file for each biosensor.
Plate name/barcode (file prefix)	A user-defined field where you can enter text or a barcode (barcode reader required).
2nd Plate name/barcode	A user-defined field where you can enter text or a barcode (barcode reader required) for a second plate.
Auto Increment File ID Start	Each file is saved with a number after the plate name. For example, if the Auto Increment File ID Start number is 1, the first file name is xxx_001.frd.

The following **Run Settings** are available on the **Run Experiment** Tab:

Table 5-10: Run Settings



<i>Item</i>	<i>Description</i>
Delayed experiment start	Specifies a time delay for the start of the experiment. Enter the number of seconds to wait before the experiment starts after you click  .
Start after	Enter the number of seconds to delay the start of the experiment.
Shake sample plate while waiting	If the experiment has a delayed start time, this setting shakes the plate until the experiment starts.
Open runtime charts automatically	Displays the Runtime Binding Chart for the current biosensor during data acquisition.
Automatically save runtime chart	Saves an image (.jpg) of the Runtime Binding Chart . The binding data (.frd) is saved as a text file, regardless of whether a chart image is created.



Table 5-10: Run Settings (Continued)

Item	Description
Set plate temperature (°C)	Specifies a plate temperature and enters the temperature in the dialog box. If not selected, the plate temperature is set to the default temperature specified in File > Options . The factory set default temperature is 30 °C.
<div> NOTE: If the actual plate temperature is not equal to the set plate temperature, a warning displays and the Octet System Data Acquisition software provides the option to wait until the set temperature is reached before proceeding with the run, continue without waiting until the set temperature is reached, or cancel the run.</div>	

Advanced settings are available for the Octet K2 system. The signal to noise ratio of the assay can be optimized by selecting different acquisition rates. The acquisition rate refers to the number of binding signal data points reported by the Octet system per second and is reported in Hertz (per second). A higher acquisition rate generates more data points per second and monitors faster binding events better than a slower acquisition rate. A lower acquisition rate allows the software enough time to perform more averages of the collected data. Typically, more averaging leads to reduced noise and thus, better signal-to-noise

ratios. Therefore, the frequency setting should be determined based on consideration of the binding rate, the amount of signal generated in your assay and some experimentation with the settings.

Table 5-11: Advanced Settings for Octet K2 System


Item	Description
Acquisition rate	<div>  NOTE: For the Octet K2 system, acquisition rate settings are available on the Plate Definition Tab. </div> <ul style="list-style-type: none"> High concentration quantitation (10 Hz, averaging by 5) — The average of 5 data frames is reported as one data point. 10 data points are reported per second. High sensitivity quantitation (2 Hz, averaging by 50)—The average of 50 data frames is reported as one data point. Two data points are reported per second. Standard quantitation (5 Hz, averaging by 20)—The average of 20 data frames is reported as one data point. Five data points are reported per second.
Sensor offset (mm)	Recommended sensor offset for quantitation—3 mm. <div>  NOTE: For more details on optimizing the sensor offset and acquisition rate please contact your local Pall ForteBio representative. </div>
Default	Sets acquisition rate and sensor offset to the defaults.

The following **General Settings** are available on the **Run Experiment** Tab:

Table 5-12: General Settings

Item	Description
Machine name	The computer name that controls the Octet instrument and acquires the data.
User name	The user logon name.
Description	A user-specified description of the assay or assay purpose. The description is saved with the method file (.fmf).

Stopping an Experiment

To stop an experiment in progress, click  or click **Experiment > Stop**.

The experiment is aborted. The data for the active biosensor is lost, the biosensor is ejected into the waste tray, and the event is recorded in the experimental log.



NOTE: After the experiment is run, the software automatically saves the experiment method (.fmf).

MANAGING RUNTIME BINDING CHARTS

If the **Open runtime charts automatically** check box is selected in the Run Experiment window, the Runtime Binding Charts are automatically displayed when data acquisition starts (see Figure 5-56). The **Runtime Binding Chart** window displays the current step number, time remaining for the current step, (total) elapsed experimental time, and total experiment time.

The **Runtime Binding Chart** is updated at the start of each experimental step. The active two sensors are color-coded (A=green, B=magenta, C=orange, D=purple, E=olive, F= black, G=red, H=blue) within the **Sensor Tray Map**. Used sensors that are inactive are colored black. Active sample columns are colored green. Each data acquisition step is represented by **Sample Column X** in the **Current Binding Charts** box.

To selectively display acquisition data for a particular acquisition step:

1. Click the corresponding **Sample Column** number.
2. Select a sub-set of sensors for a displayed column under **Sensors to Chart** box (see Figure 5-56).



IMPORTANT: Do not close the **Runtime Binding Chart** window until the experiment is complete and all data is acquired. If the window is closed, the charts are not saved. To remove the chart from view, minimize the window. The Octet System Data Acquisition software saves the **Runtime Binding Chart** as displayed at the end of the experiment. For example, modifying a chart by hiding the data for a particular biosensor will cause this data not to be included in the bitmap image generated at the end of the run.

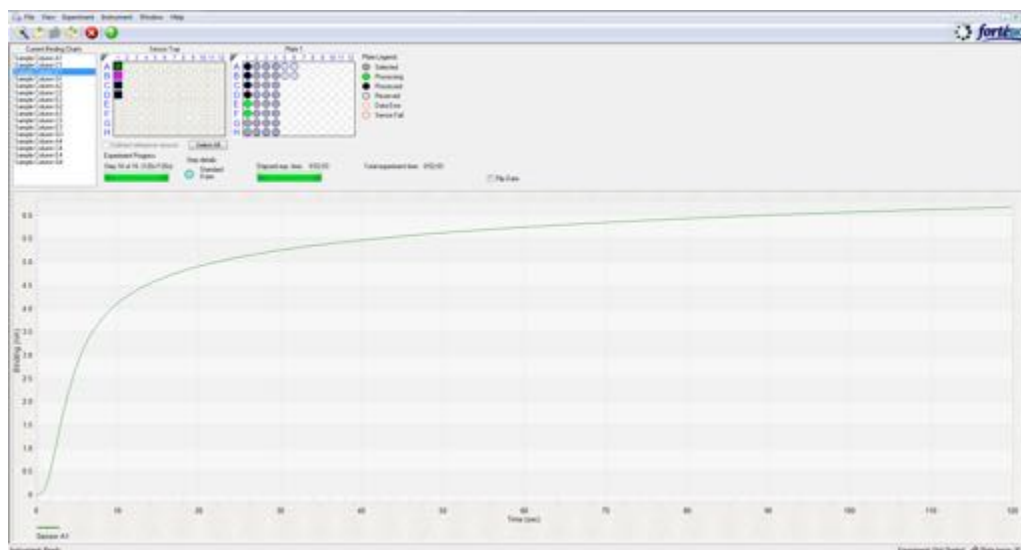


Figure 5-56: Runtime Binding Chart Window

Opening a Runtime Binding Chart

After an experiment is run, you can open and review the **Runtime Binding Chart** at any time:

1. Click **File > Open Experiment**.
2. In the dialog box that appears, select an experiment folder and click **Select**.

Viewing Reference-Subtracted Data

If the experiment includes reference biosensors, you can display reference-subtracted data during acquisition in the chart by clicking the **Subtract reference sensors** check box in the chart window. To view raw data, remove the check mark next to this option.

Reference biosensors can be designated:

- During experiment setup in the **Sensor Assignment** tab
- During acquisition in the Runtime Binding Chart **Sensors to Chart** box
- During analysis in the **Data Selection** tab

Designating a Reference Biosensor During Acquisition

To designate a reference biosensor during acquisition:

1. In the **Sensors to Chart** list or the **Sensor Tray**, right-click a biosensor and select **Reference** (see Figure 5-58).

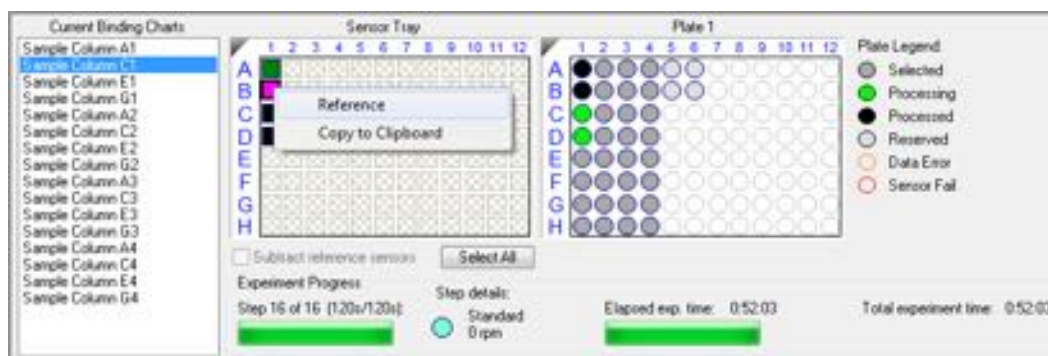


Figure 5-57: Designating a Reference Biosensor in the Runtime Binding Chart

The selected biosensor will be shown with an **R** in the **Sensors to Chart** list and **Sensor Tray** (see Figure 5-58).

2. Click the **Subtract reference sensors** check box (see Figure 5-58).

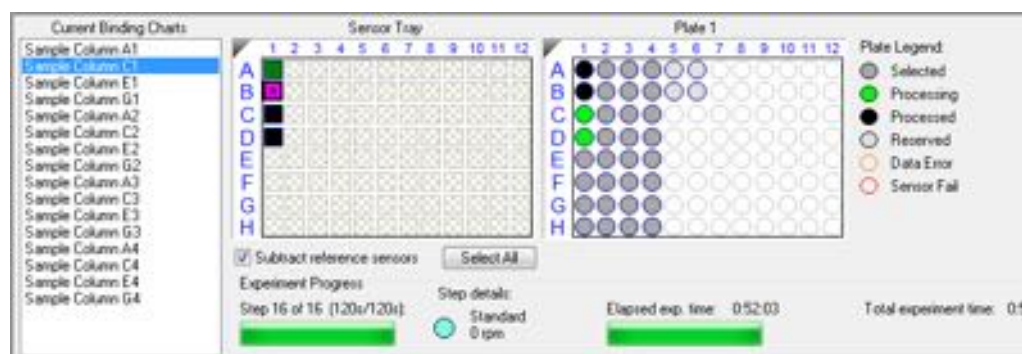


Figure 5-58: Subtract Reference Sensors check box in the Runtime Binding Chart



NOTE: Subtracting reference data in the **Runtime Binding Chart** only makes a visual change to the data on the screen. The actual raw data is unaffected and the reference subtraction must be re-done in data analysis if needed.

Viewing Inverted Data

The data displayed in the **Runtime Binding Chart** can be inverted during real-time data acquisition or data analysis after the experiment has completed. To invert data, select the **Flip Data** check box (see Figure 5-59). Uncheck the box to return to the default data display.

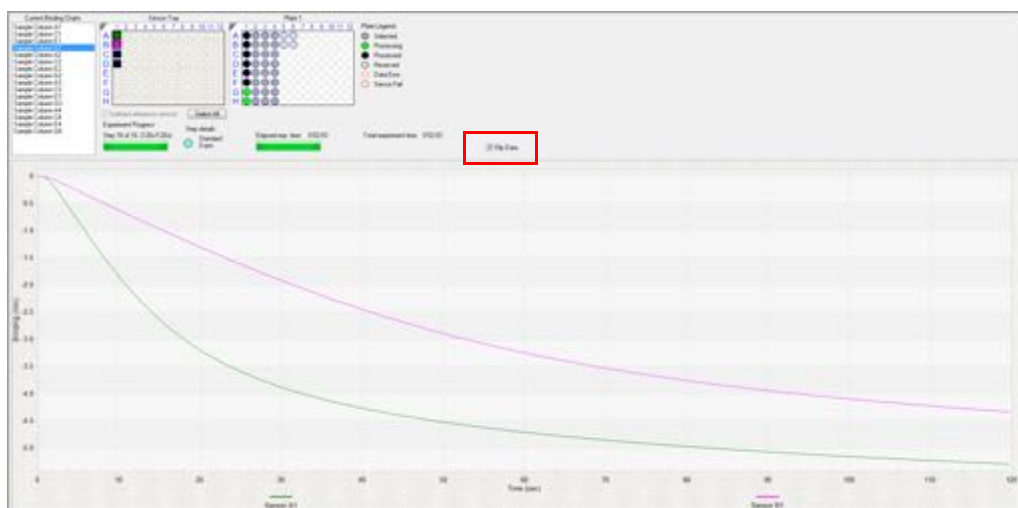


Figure 5-59: Data Inverted Using Flip Data Function

Magnifying the Runtime Binding Chart

To magnify the chart, press and hold the mouse button while you draw a box around the chart area to magnify.

To undo the magnification, right-click the chart and select **Undo Zoom**.

Scaling a Runtime Binding Chart

To scale the **Runtime Binding Chart**:

1. Right-click the chart and select **Properties**.
2. In the **Runtime Graph Properties** dialog box, select **Fullscale** or **Autoscale**.

Adding a Runtime Binding Chart Title

To add a **Runtime Binding Chart** title:

1. Right-click the chart and select **Properties**.
2. In the **Runtime Graph Properties** dialog box, enter a graph title or subtitle.

Selecting a Runtime Binding Chart Legend

To select a **Runtime Binding Chart** legend:

1. Right-click the chart and select **Properties**.
2. In the **Runtime Graph Properties** dialog box (see Figure 5-60), select one of the following legends:
 - Sensor Location
 - Sample ID
 - Sensor Information
 - Concentration/Dilution

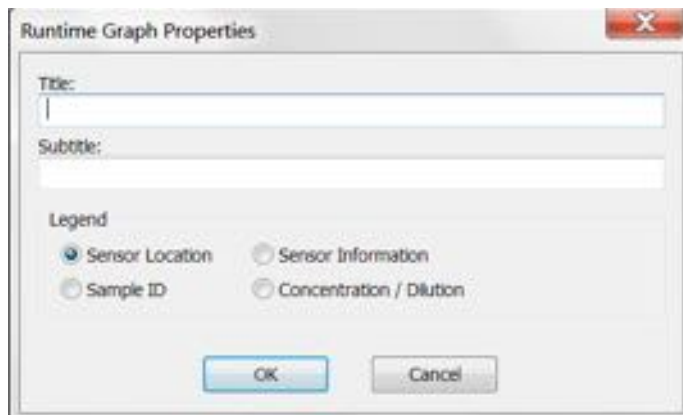


Figure 5-60: Selecting a Runtime Binding Chart Legend



NOTE: Text for **Sample ID**, **Sensor Information**, or **Concentration/Dilution** is taken from the **Plate Definition** and **Sensor Assignment** tabs, and must be entered before the experiment is started.

3. Click **OK**.

Viewing Multiple Runtime Binding Charts

To view multiple Runtime Binding Charts, click **Window > New Window**.

Exporting or Printing the Runtime Binding Chart

To export the **Runtime Binding Chart** as a graphic or data file:

- 1. Right-click the chart and select **Export Data**.
- 2. In the **Exporting** dialog box (see Figure 5-61), select the export options and click **Export**.

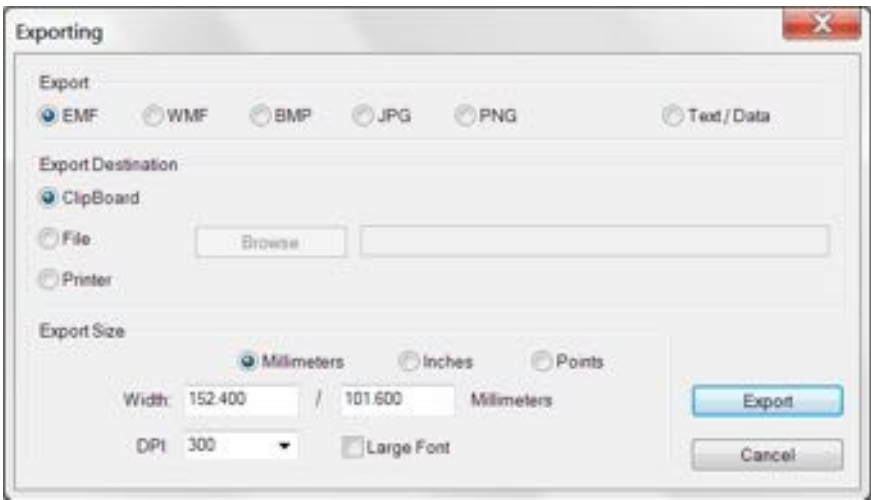


Figure 5-61: Exporting Dialog Box

Table 5-13: Runtime Binding Chart Export Options

Task	Export	Option	Export Destination	Result
	Text/ Data	EMF, WMF, BMP, JPG, or PNG		
Save the binding data	✓		Click File > Browse to select a folder and enter a file name.	Creates a tab-delimited text file of the numerical raw data from each biosensor. Open the file with a text editor such as Notepad.
Export the Runtime Binding Chart to a graphic file		✓	Click File > Browse to select a folder and enter a file name.	Creates a graphic image.

Table 5-13: Runtime Binding Chart Export Options (Continued)

Task	Export	Option	Export Destination	Result
Copy the Runtime Binding Chart		✓	Clipboard	Copies the chart to the system clipboard
Print the Runtime Binding Chart		✓	Printer	Opens the Print dialog box.

MANAGING EXPERIMENT METHOD FILES

After you run an experiment, the Octet System Data Acquisition software automatically saves the method file (.fmf), which includes the sample plate definition, biosensor assignment, and the run parameters. An experiment method file provides a convenient initial template for subsequent experiments. Open a method (.fmf) and edit it if necessary.



NOTE: When using the 21 CFR Part 11 version of the Octet System Data Acquisition software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.

Table 5-14: Managing Experiment Method Files

Menu Bar Command/ Toolbar Button	Description
File > Open Method File	Enables you to select and open a method file (.fmf)
File > Save Method File or	Saves one method file or all method files. Saves a method file before the experiment is run.
File > Save Method File As	Saves a method file to a new name so that the original file is not overwritten.

CUSTOM QUANTITATION ASSAYS

Defining a Custom Assay

To define a custom assay:

1. Click **Experiment > Edit Assay Parameters**.

The **Edit Assay Parameters** dialog box appears; see Figure 5-62.

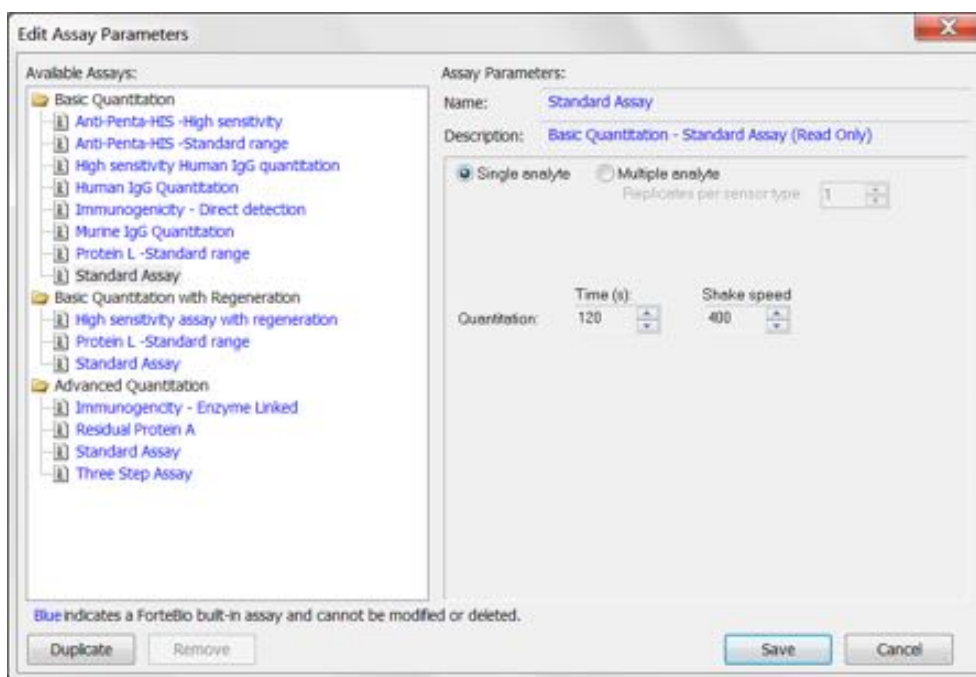


Figure 5-62: Edit Assay Parameters Dialog Box

2. In the directory tree of assays, select the type of standard assay to modify. For example, to define a new basic quantitation assay, in the Basic Quantitation folder, select **Standard Assay**.
3. Click **Duplicate**.
4. In the **New Assay** dialog box (see Figure 5-63 top), enter an **Assay name**.
5. Optional: In the **Assay Description**, enter information about the assay.
6. Click **Save**.

The new assay appears in the directory tree of available assays (see Figure 5-63 bottom).

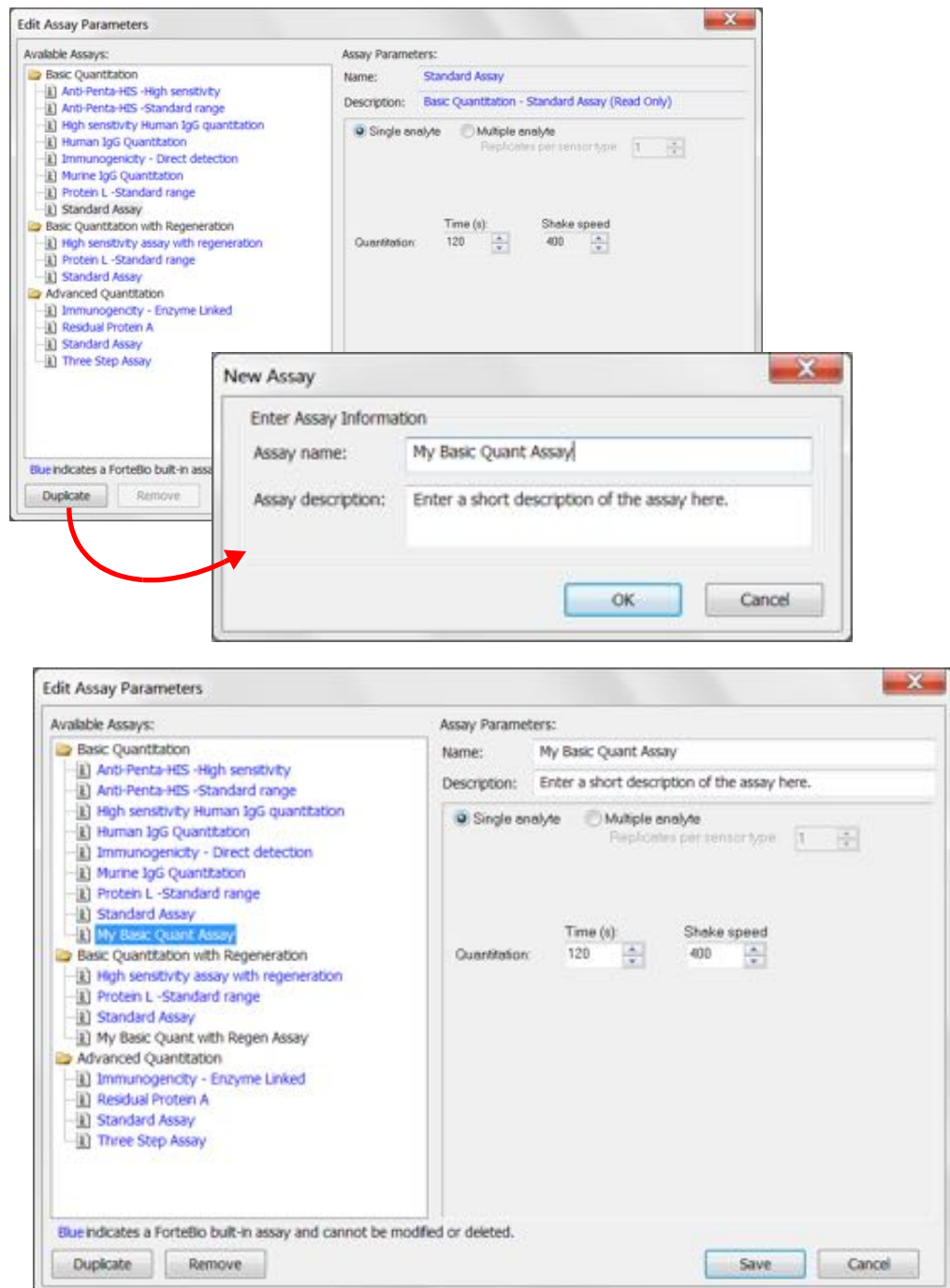


Figure 5-63: Defining a New Assay

Editing Assay Parameters

To edit assay parameters:

1. In the **Edit Assay Parameters** dialog box, confirm that the new assay is selected in **Available Assays** (see Figure 5-63 bottom).
2. Modify the assay parameters as needed. A complete list of parameters for each type of quantitation experiment follows this procedure.
3. Click **Save** to accept the new parameter values. The new assay is added to the system.



NOTE: Not all parameters are available for all of the assays.

Basic Quantitation Assay Parameters

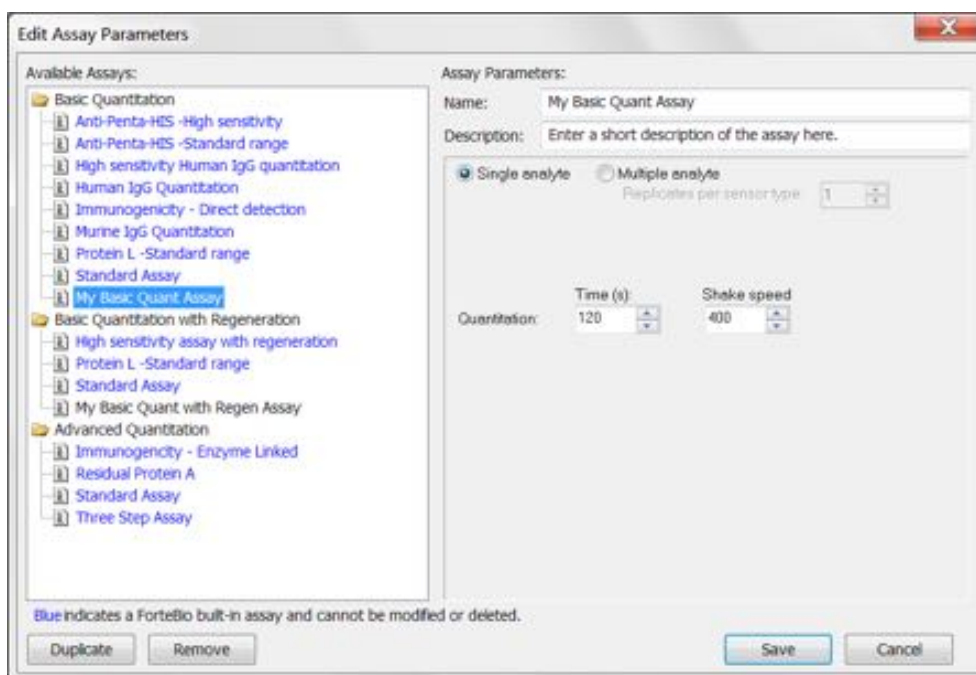



Figure 5-64: Assay Parameters—Basic Quantitation Assay

Table 5-15: Basic Quantitation Assay Parameters

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.

Table 5-15: Basic Quantitation Assay Parameters (Continued)

Parameter	Description
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time (s)	The duration of data acquisition seconds while the biosensor is incubated in sample.
<div> NOTE: A subset of data points may be selected for processing during data analysis.</div>	
Quantitation Shake speed (rpm)	The sample shaking speed (rotations per minute).

Basic Quantitation with Regeneration Assay Parameters

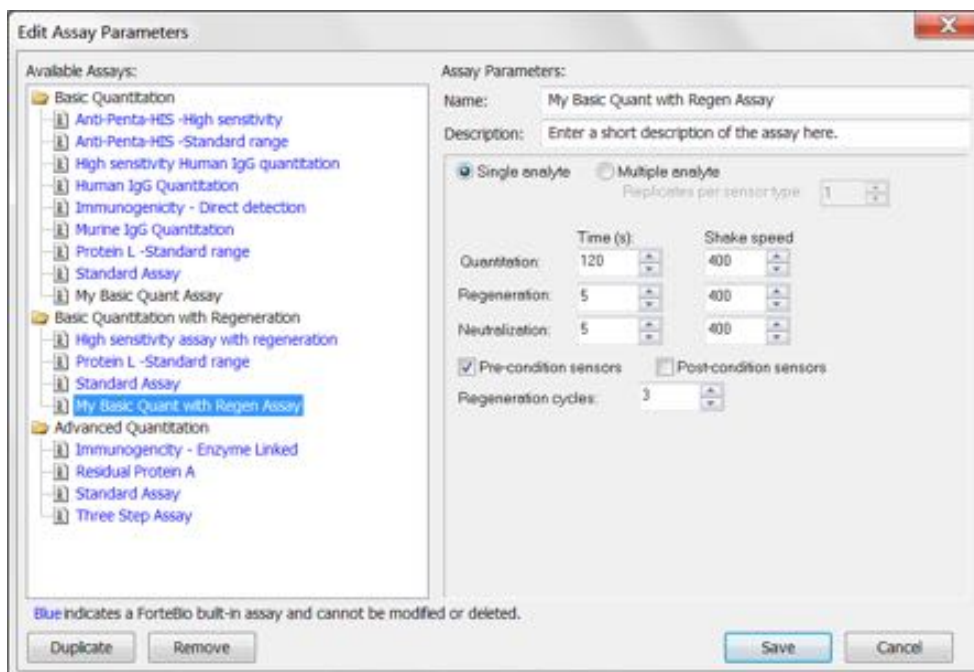


Figure 5-65: Assay Parameters—Basic Quantitation with Regeneration

Table 5-16: Assay Parameters—Basic Quantitation with Regeneration

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time(s) and Shake speed (rpm)	The duration of data acquisition in seconds while the biosensor is incubated in sample and the sample shaking speed (rotations per minute).
<div>➔</div> NOTE: A subset of data points may be selected for processing during data analysis.	
Regeneration Time(s) and Shake speed (rpm)	The duration time and shaking speed of the regeneration step where the biosensor is incubated in regeneration buffer to remove bound analyte.

Table 5-16: Assay Parameters—Basic Quantitation with Regeneration

Parameter	Description
Neutralization Time(s) and Shake speed (rpm)	The duration time and shaking speed of the neutralization step where the biosensor is incubated in neutralization buffer after the regeneration step.
Pre-condition sensors	Performs a set of regeneration/neutralization steps prior to the start of the experiment. The pre-conditioning settings are equivalent to the time and rpm settings for the regeneration in the assay. For example, an acidic pre-conditioning buffer maximizes the binding competence of Pro-A biosensors.
Post-condition sensors	Post-conditions biosensors, allowing re-racked biosensors to be stored in a regenerated state.
Regeneration cycles	The number of regeneration-neutralization cycles that a biosensor undergoes before reuse.

Advanced Quantitation Assay Parameters

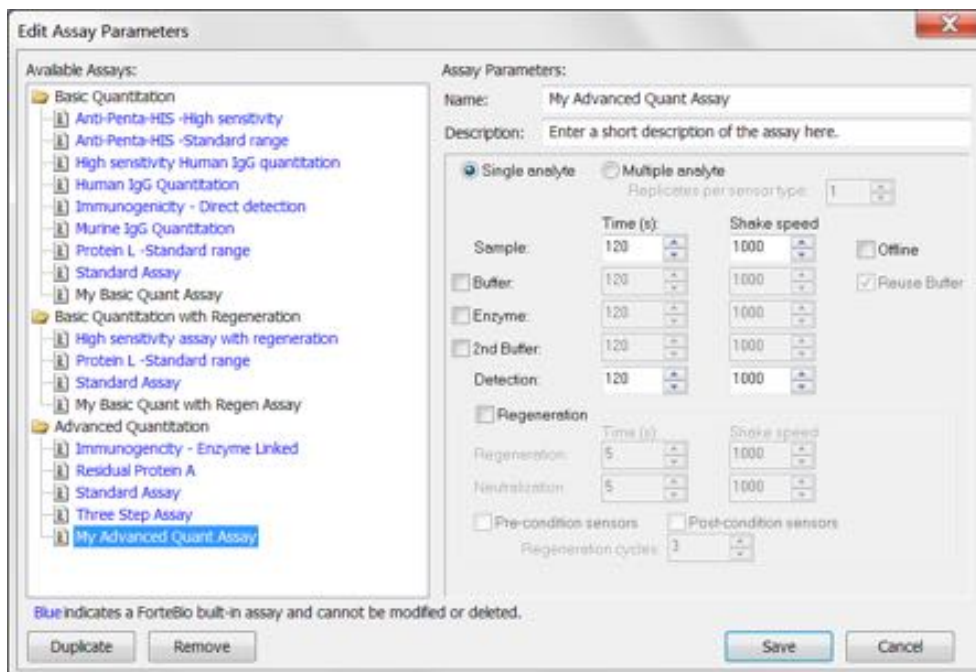


Figure 5-66: Assay Parameters—Advanced Quantitation

Table 5-17: Advanced Quantitation Assay Parameters

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Sample Time(s) and Shake speed (rpm)	The duration of data acquisition in seconds while the biosensor is incubated in sample and the sample shaking speed (rotations per minute).
<div>➔</div> NOTE: A subset of data points may be selected for processing during data analysis.	
Buffer Time(s) and Shake speed (rpm)	The duration of biosensor incubation in the first buffer in seconds and the sample shaking speed (rotations per minute).

Table 5-17: Advanced Quantitation Assay Parameters


Parameter	Description
Enzyme Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the enzyme solution and the sample shaking speed (rotations per minute).
2nd Buffer Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the second buffer solution and the sample shaking speed (rotations per minute).
Capture Antibody Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the first capture antibody solution and the shaking speed (rotations per minute).
2nd Antibody Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the secondary antibody solution and the shaking speed (rotations per minute).
Detection Time(s) & Shake speed (rpm)	The duration of data acquisition during the detection step in seconds in an advanced quantitation assay.
<div>  NOTE: A subset of data points may be selected for processing during data analysis. </div>	
Offline	Choose this option to incubate sample with biosensors outside the Octet system. Offline incubation is best performed on the Pall ForteBio Sidekick biosensor immobilization station.
Reuse Buffer	Allows buffer wells to be reused. If unselected, the number of buffer columns must equal the number of sample columns. If selected, the number of buffer columns may be less than the number of sample columns as the buffer columns are reused.
Regeneration Time(s) and Shake speed (rpm)	The duration time and shaking speed of the regeneration step where the biosensor is incubated in regeneration buffer to remove bound analyte.
Neutralization Time(s) and Shake speed (rpm)	The duration time and shaking speed of the neutralization step where the biosensor is incubated in neutralization buffer after the regeneration step.
Pre-condition sensors	Performs a set of regeneration/neutralization steps prior to the start of the experiment. The pre-conditioning settings are equivalent to the time and rpm settings for the regeneration in the assay. For example, an acidic pre-conditioning buffer maximizes the binding competence of Protein A biosensors.

Table 5-17: Advanced Quantitation Assay Parameters

Parameter	Description
Post-condition sensors	Post-conditions biosensors, allowing re-racked biosensors to be stored in a regenerated state.
Regeneration cycles	The number of regeneration-neutralization cycles that a biosensor undergoes before reuse.



NOTE: In an Advanced Quantitation experiment, this option is only available if the first step (biosensor incubation in sample) is performed online.

Selecting a Custom Assay

You can select a custom assay when you define a sample plate.

To select a custom assay:

1. In the **Plate Definition** tab, click **Modify** in the **Assay Settings** box.

The **Edit Assay Parameters** dialog box displays (see Figure 5-67).

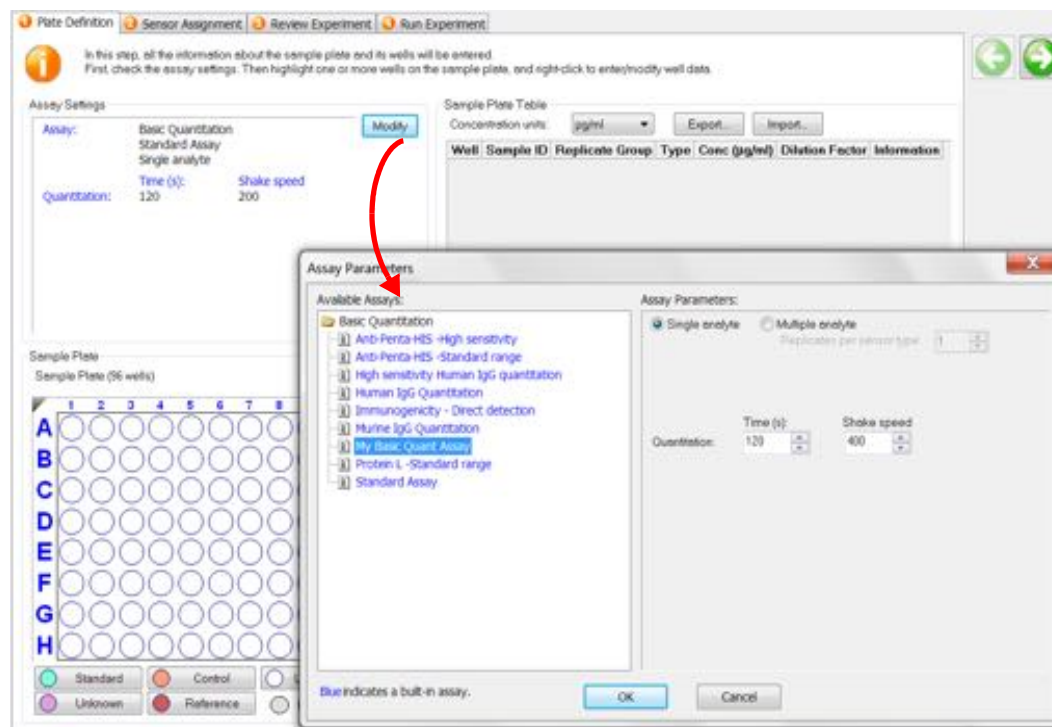


Figure 5-67: Selecting a Custom Assay

2. Select the custom assay from the directory tree and click **OK**.

MULTI-STEP ADVANCED QUANTITATION EXPERIMENTS

The multi-step selection interface for Advanced Quantitation methods increases the flexibility to add more assay steps prior to the Sample or Detection steps. In addition, all steps in an Advanced Quantitation assay may be viewed and analyzed in the Octet Data Analysis software.

After starting the Octet system and the Octet Data Acquisition software, follow the steps below to set up and run an Advanced Quantitation experiment. You can start an Advanced Quantitation experiment using one of the following options:

- Launch the **Experiment Wizard**.
- Open a method file (.fmf) by clicking **File > Open Method File**. Method files may be saved and recalled using the **File** menu and are automatically saved when an experiment is run.
- On the menu bar, click **Experiment > Templates > Quantitation > Advanced Quantitation**.

These options are explained further in “Starting an Experiment Using the Experiment Wizard” on page 135.



NOTE: The Sample plate and the Reagent plate are now referred to as “Plate 1” and “Plate 2” in the software.

1. To add or edit assay steps in Tab 1 (Plate Definition), click **Modify** in Assay Settings to display the Assay Parameters window. Click on the **Step Type** drop-down list or highlight the parameter you want to change:

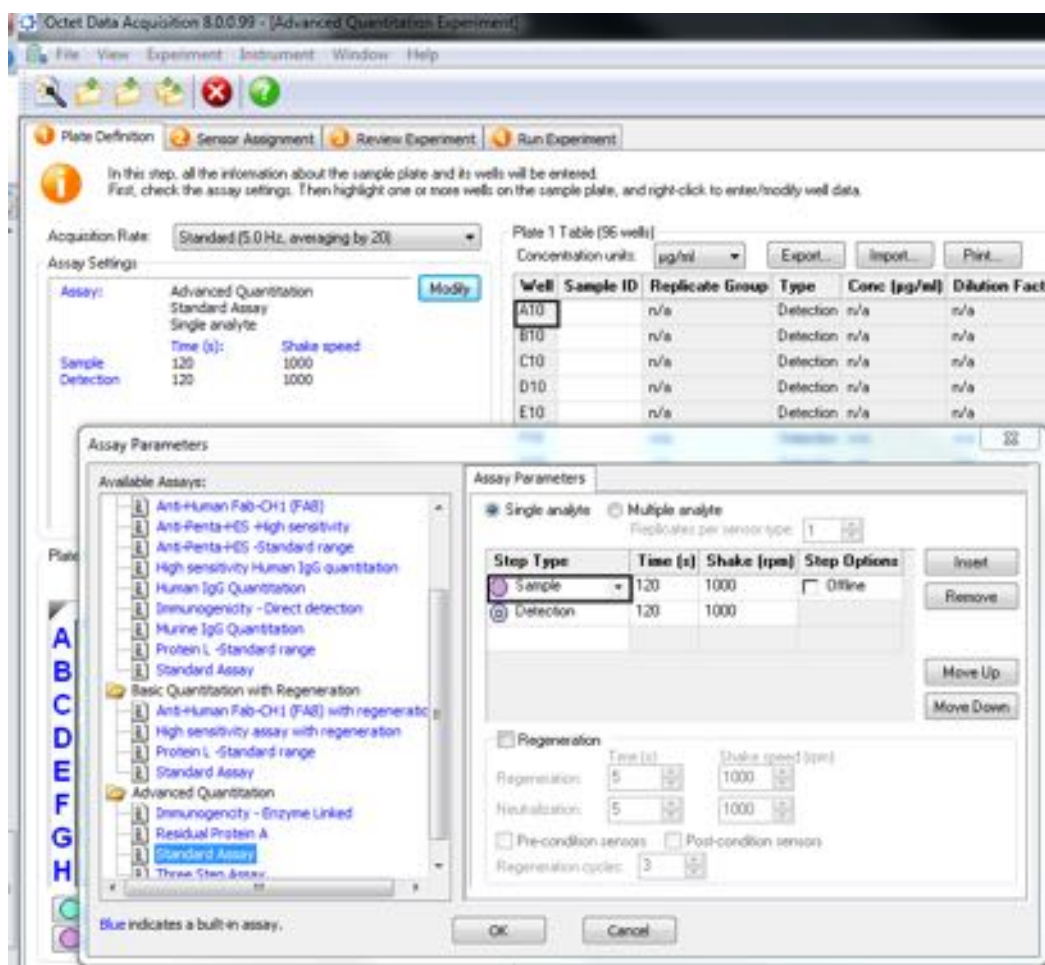


Figure 5-68: Assay Parameters Window.

To add or remove steps, click the **Insert** or **Remove** buttons. Individual steps may be re-organized using the **Move Up** or **Move Down** buttons. Click **OK** to save any changes.

2. Continue with the plate layout and sample well designation in Tab 1. For more details see “Defining the Sample Plate” on page 137, “Managing Sample Plate Definitions” on page 155 and “Managing Assay Parameter Settings” on page 158.
3. Proceed to Tab 2 (Sensor Assignment) and the remaining tabs as described starting with “Assigning Biosensors to Samples” on page 164 before running the Advanced Quantitation method.

CHAPTER 6:




Quantitation Experiments: Octet RED96, RED96e, QK^e and QK

Introduction	210
Starting a Quantitation Experiment	211
Defining the Sample Plate	213
Managing Sample Plate Definitions	230
Managing Assay Parameter Settings	234
Assigning Biosensors to Samples	239
Reviewing Experiments	257
Saving Experiments	257
Running a Quantitation Experiment	259
Managing Runtime Binding Charts	267
Managing Experiment Method Files	273
Custom Quantitation Assays	274
Multi-Step Advanced Quantitation Experiments	283

INTRODUCTION

A quantitation experiment enables you to determine analyte concentration within a sample using a reference set of standards. After starting the Octet system hardware and the Octet System Data Acquisition software, follow the steps (in Table 6-1) to set up and analyze a quantitation experiment.

Table 6-1: Setting Up and Analyzing a Quantitative Experiment

Software	Step	See
Data Acquisition 	1. Select a quantitation experiment in the Experiment wizard or open a method file (.fmf).	"Starting a Quantitation Experiment" on page 211
	2. Define a sample plate or import a sample plate definition.	"Defining the Sample Plate" on page 213
	3. Confirm or edit the assay settings.	"Managing Assay Parameter Settings" on page 234
	4. Assign biosensors to samples.	"Assigning Biosensors to Samples" on page 239
	5. Run the experiment.	"Running a Quantitation Experiment" on page 259
Data Analysis  or Data Analysis HT 	6. Analyze the binding data.	<i>Octet System Data Analysis Software or Octet Data Analysis HT Software User Guide</i>
	7. Generate a report.	

For more details on how to prepare the biosensors, see the appropriate biosensor product insert.

STARTING A QUANTITATION EXPERIMENT



IMPORTANT: Using 96-well half-area plates on the Octet RED96 or RED96e system will result in non-optimal system performance. Pall ForteBio cannot guarantee results within the optimal performance specifications of the system when these plates are used.



NOTE: Before starting an experiment, check the plate temperature displayed in the status bar. Confirm that the temperature is appropriate for your experiment and if not, set a new temperature. If the Octet System Data Acquisition software is closed, the plate temperature will reset to the default startup value specified in the **Options** dialog box when the software is relaunched.

You can start a quantitation experiment by one of the following methods:


- Launch the **Experiment Wizard**.
- Open a method file (.fmf) by clicking **File > Open Method File**. Method files may be saved and recalled using the **File** menu and are automatically saved when an experiment is run. For more details on method files see, “Managing Experiment Method Files” on page 273.
- On the menu bar, click **Experiment > Templates > Quantitation**.



NOTE: When using the 21 CFR Part 11 version of the Octet System Data Acquisition software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.

Starting an Experiment Using the Experiment Wizard

To start an experiment using the **Experiment Wizard**:

1. If the **Experiment Wizard** is not displayed when the software is launched, click the **Experiment Wizard** toolbar button  or click **Experiment > New Experiment Wizard (Ctrl+N)** from the **Main Menu**.
2. In the **Experiment Wizard**, select **New Quantitation Experiment** (see Figure 6-1 left).

3. Select a type of quantitation experiment (see Table 6-2 for options).

Table 6-2: Quantitation Experiment Selection

Quantitation Experiment	Description
Basic Quantitation	A standard quantitation assay.
Basic Quantitation with Regeneration	A standard quantitation assay that enables regeneration of biosensors.
Advanced Quantitation	A standard two- or three-step quantitation assay that enables signal amplification for higher detection sensitivity.

4. Optional: You can also click **Recent Methods** to display a list of recently used methods. You can open any method file from the list and use it with or without modifications to run a new experiment.

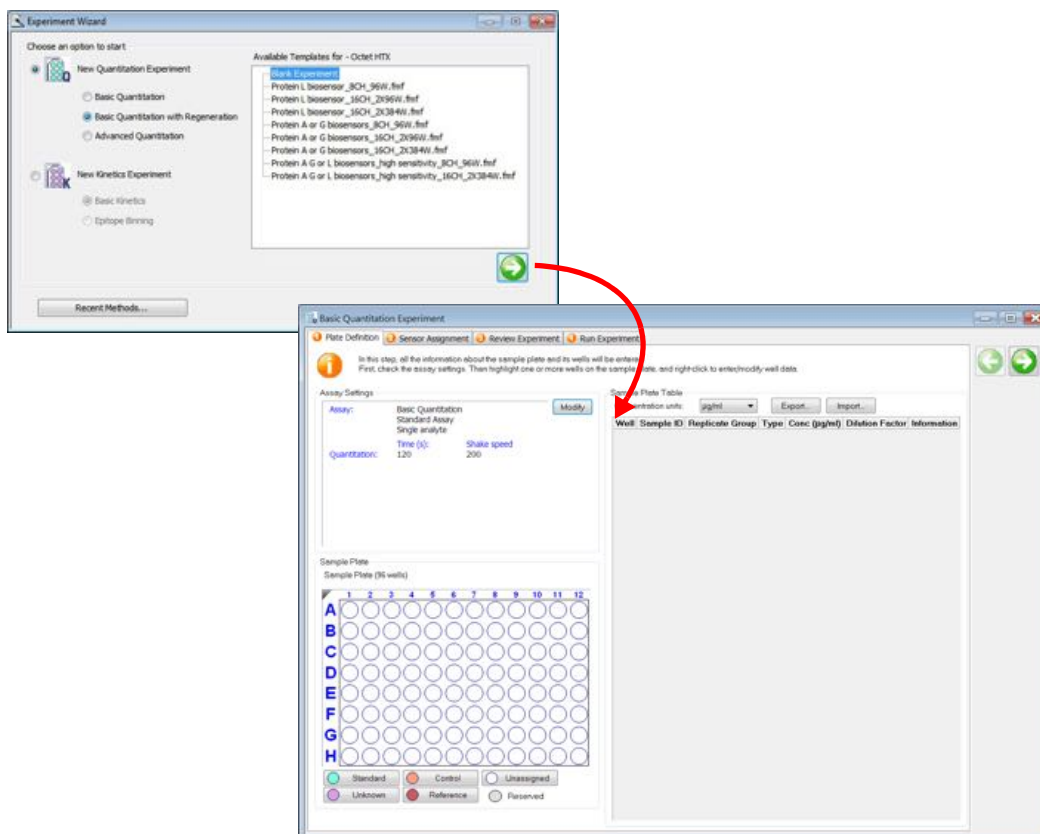


Figure 6-1: Selecting an Experiment Type in the Experiment Wizard (for Octet RED96)

5. Click the  arrow.

The **Experiment** dialog box displays (Figure 6-1 right).

DEFINING THE SAMPLE PLATE

Table 6-3 lists the steps to define a sample plate.

Table 6-3: Defining a Sample Plate

Step	See Page
1. Designate the samples.	213
2. Annotate the samples (optional).	224
3. Save the sample plate definition (optional).	230

Designating Samples

Each well may be designated as a **Standard**, **Unknown**, **Control**, or **Reference**. A well may also remain **Unassigned** or be designated as **Reserved** by the system for Basic Quantitation with Regeneration and Advanced Quantitation experiments.



NOTE: It is important to define all of the wells that will be used in the assay. Only wells that are selected and defined using one of the sample types in Table 6-4 will be included in the assay.

Table 6-4: Types of Sample Wells







Icon	Description
 Standard	Contains an analyte of known concentration. Data from the well is used to generate a standard curve during analysis.
 Unknown	Contains an analyte of unknown concentration. The concentration of the analyte is calculated from the well data and the standard curve.
 Control	A control sample, either positive or negative, of known analyte composition. Data from the well is not used to generate a standard curve during analysis. <ul style="list-style-type: none"> Positive Control: A control sample that contains analyte of known concentration Negative Control: A control sample known not to contain analyte
 Reference	Provides a baseline signal which serves as a reference signal for Unknowns , Controls , and Standards . The reference signal can be subtracted during data acquisition in the Runtime Binding Chart and during data analysis.

Table 6-4: Types of Sample Wells

Icon	Description
 Unassigned	Not used during the experiment.
 Reserved	Used by the system during Basic Quantitation with Regeneration experiments and Advanced Quantitation multi-step experiments for Regeneration (R) , Neutralization (N) , Detection (D) , or Capture Antibody (C) . Reserved wells are not available for use as Standards , Unknowns , Controls , or References .

Reserved Wells

In a Basic Quantitation with Regeneration or an Advanced Quantitation experiment, the **Sample Plate Map** includes gray wells. These wells are reserved by the system and specify the location of particular sample types.









Reserved samples cannot be removed from the sample plate, but you can change their column location. To change the location of a reserved column (, , , or ) right-click a column header in the **Sample Plate Map** and select **Regeneration**, **Neutralization**, **Detection**, or **Capture Antibody**.

Table 6-5: Reserved Well Requirements

Reserved Well	Must Contain
 Regeneration	Regeneration buffer that is used to remove analyte from the biosensor (typically low pH, high pH, or high ionic strength).
 Neutralization	Neutralization buffer that is used to neutralize the biosensor after the regeneration step.
 Detection	Secondary antibody or precipitating substrate that is used with an enzyme-antibody conjugate to amplify the analyte signal. Sample concentrations are computed using the binding data from the detection wells.
 Capture Antibody	Capture antibody or molecule that is used to immobilize the specific molecule of interest onto the biosensor.

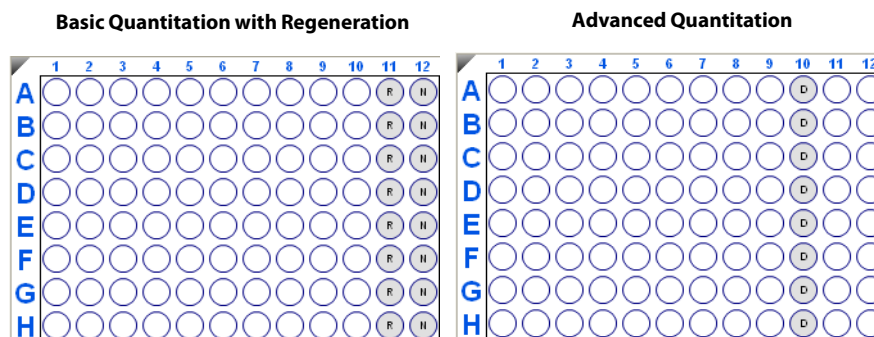


Figure 6-2: Default Locations for Reserved Wells in a 96-Well Sample Plate Map

Selecting Wells in the Sample Plate Map

There are several ways to select wells in the **Sample Plate Map**:

- Click a column header or select adjacent column headers by click-hold-drag (Figure 6-3 left). To select non-adjacent columns, hold the **Ctrl** key and click the column header.
- Click a row header or select adjacent row headers by click-hold-drag (Figure 6-3, center).
- Click a well or draw a box around a group of wells (Figure 6-3, right).

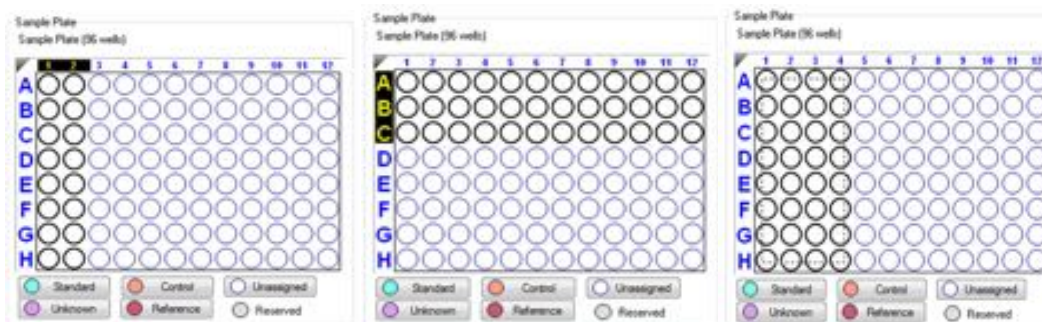


Figure 6-3: Selecting Wells in the Sample Plate Map



NOTE: Shift-clicking in the **Sample Plate Map** mimics the head of the instrument during the selection.

Designating Standards

To designate standards:

1. In the **Sample Plate Map**, select the wells to define as standards.
2. Click the **Standard** button below the **Sample Plate Map** (see Figure 6-3), or right-click and select **Standard**.

The standards are marked in the plate map and the **Sample Plate Table** is updated.

3. Select the concentration units for the standards using the **Concentration Units** drop-down list above the **Sample Plate Table**.

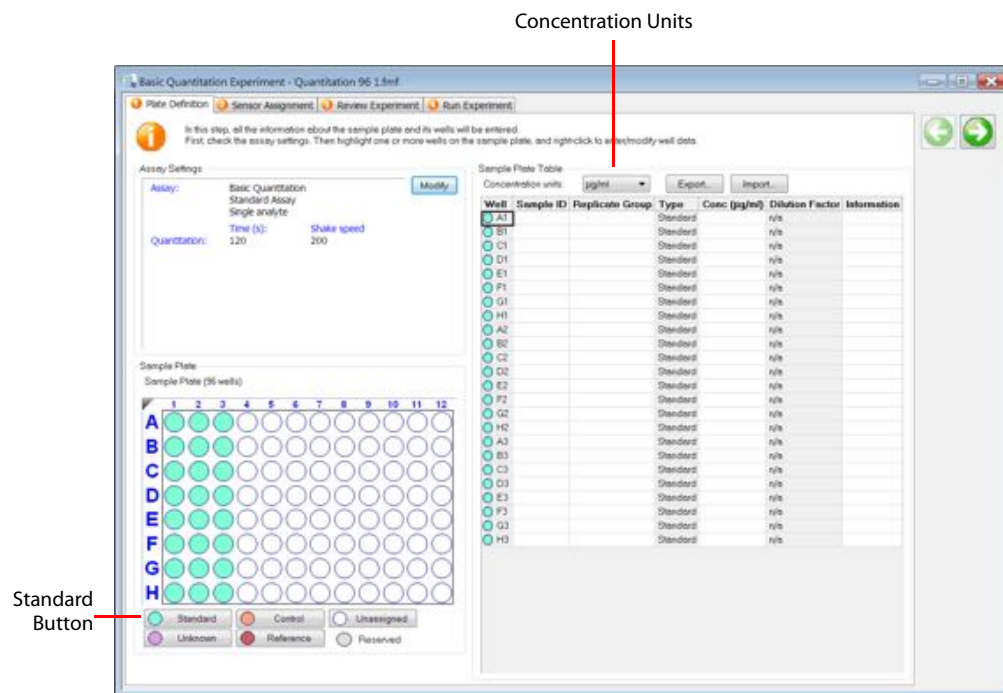


Figure 6-4: Plate Definition Window—Designating Standards

To remove a well designation, select the well(s) and click **Unassigned**. Or, right-click the well(s) and select **Clear Data**.

Assigning Standard Concentrations Using a Dilution Series

To assign standard concentrations using a dilution series:

1. In the **Sample Plate Map**, select the standard wells, right-click and select **Set Well Data**.

The **Set Well Data** dialog box displays (see Figure 6-5).

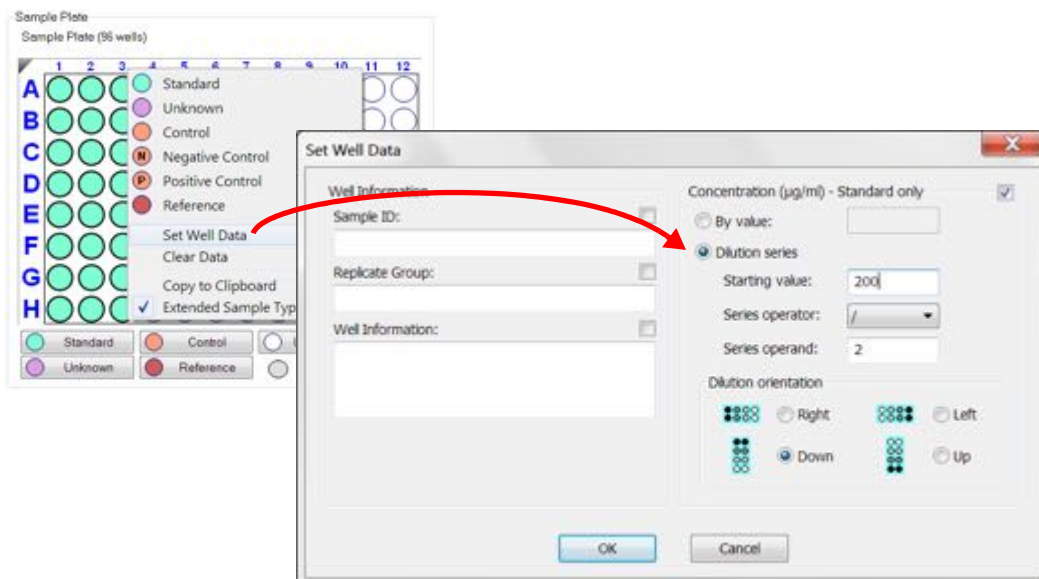


Figure 6-5: Sample Plate Map—Setting a Dilution Series

2. Select the **Dilution Series** option and enter the starting concentration value.
3. Select a series operator, enter an operand, and select the appropriate dilution orientation (see Figure 6-6).

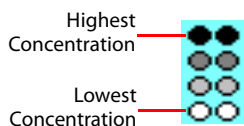


Figure 6-6: Concentration Representation in Dilution Series

4. Click **OK**.

The **Sample Plate Table** will display the standard concentrations entered.

Assigning a User-Specified Concentration to Standards

To assign a user-specified concentration to standards:

1. In the **Sample Plate Map**, select the standard wells, right-click and select **Set Well Data**.

The **Set Well Data** dialog box displays (see Figure 6-7).

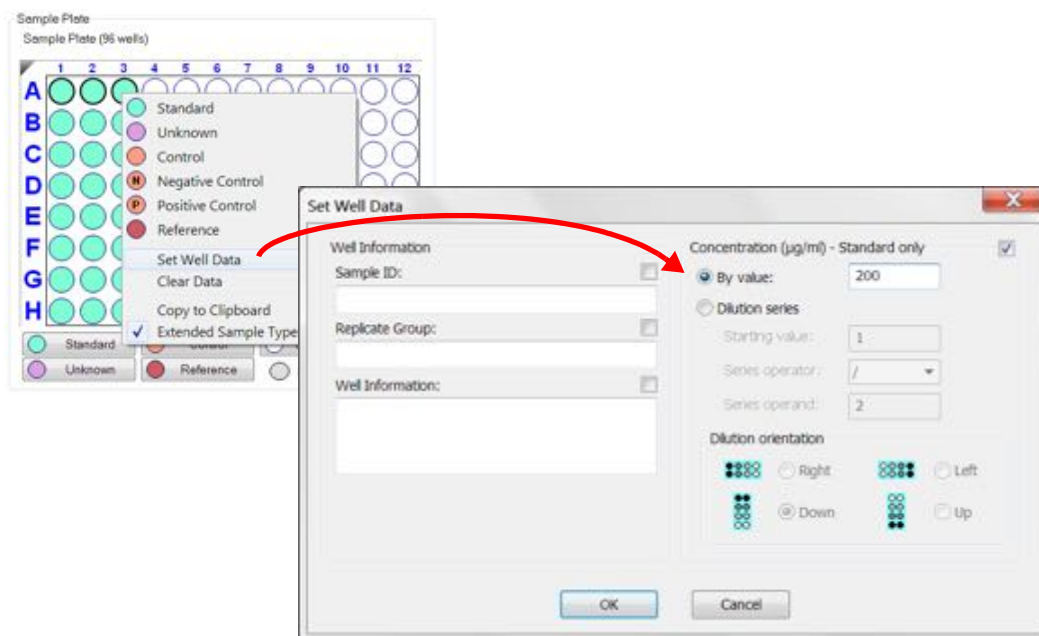


Figure 6-7: Sample Plate Map—Assigning a Standard Concentration

2. Select the **By value** option and enter the starting concentration value.
3. Click **OK**. The **Sample Plate Table** will display the standard concentrations entered.

Editing an Individual Standard Concentration

To enter or edit an individual standard concentration, in the **Conc** column of the **Sample Plate Table**, double-click the value and enter a new value (see Figure 6-8).

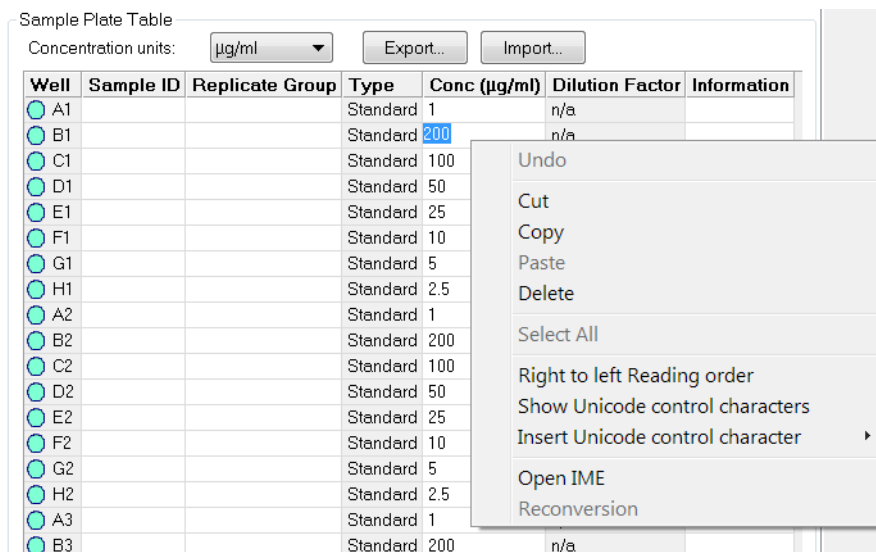


Figure 6-8: Sample Plate Table—Shortcut Menu of Edit Commands



NOTE: Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the **Sample Plate Table**. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the **Sample Plate Map** menu used to designate sample types.

Designating Unknowns

To designate unknowns in the **Sample Plate Map**, select the wells to define as unknown, right-click and select **Unknown**. The unknown wells are marked in the plate map and the sample plate table is updated (see Figure 6-9).

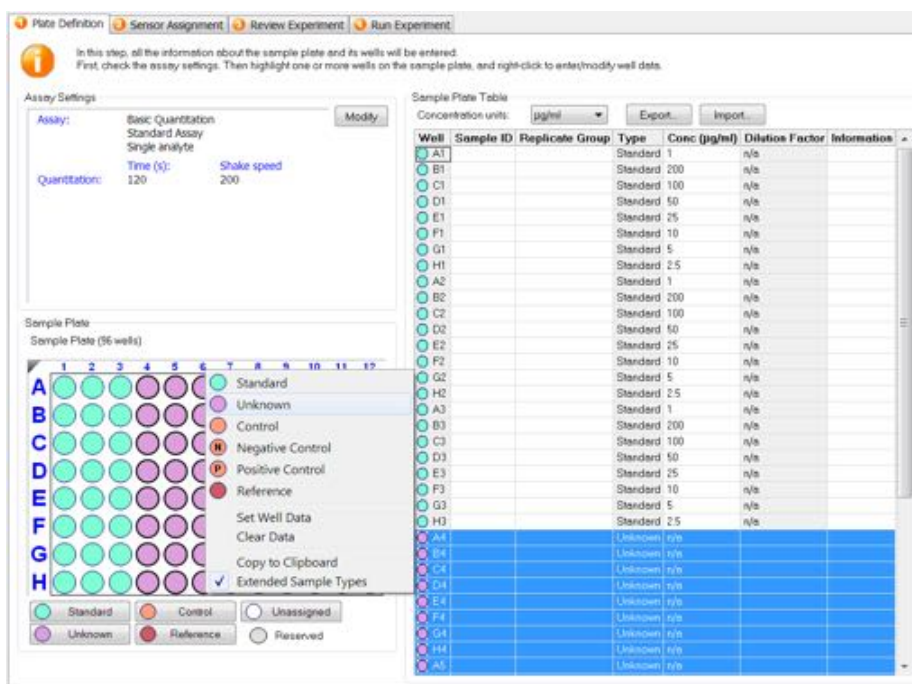


Figure 6-9: Plate Definition Window—Designate Unknown Wells

To remove a well designation, select the well(s) and click **Unassigned**. Or, right-click the well(s) and select **Clear Data**.

Assigning a Dilution Factor or Serial Dilution to Unknowns

To assign a dilution factor or serial dilution to unknowns:

1. In the **Sample Plate Map**, select the unknown wells (see Figure 6-9).
2. Right-click and select **Set Well Data**.

The **Set Well Data** dialog box displays (see Figure 6-10).

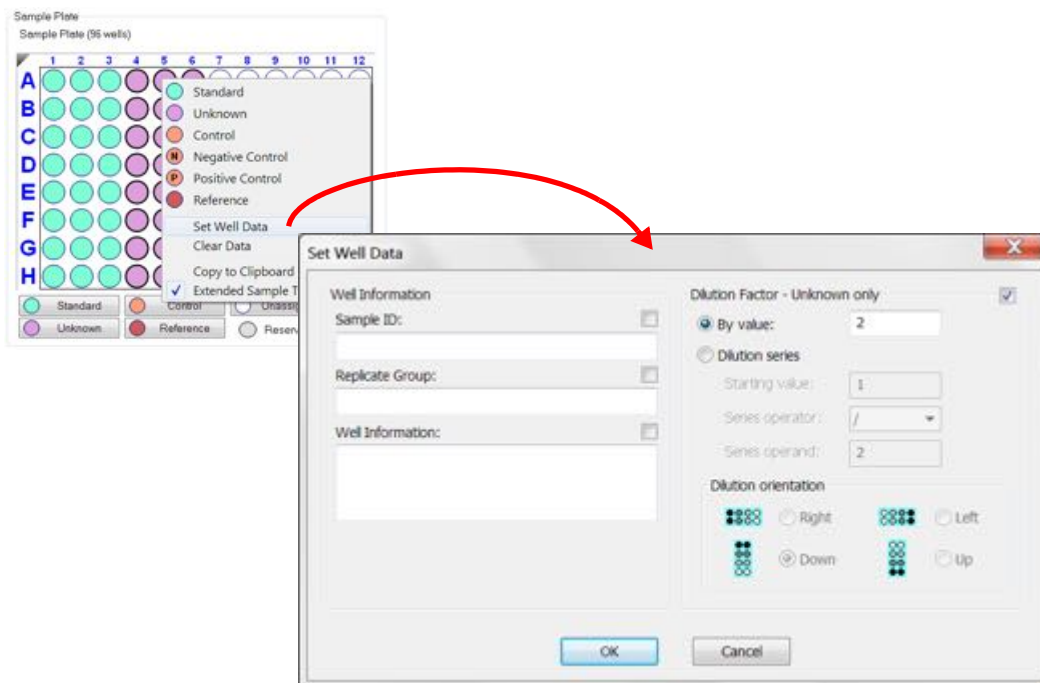


Figure 6-10: Sample Plate Map—Setting a Dilution Factor or a Serial Dilution

To assign a dilution factor to selected wells:

1. In the **Set Well Data** dialog box (see Figure 6-10), select the **By Value** option.
2. Enter the dilution factor value and click **OK**.

To assign a serial dilution to selected wells:

1. In the **Set Well Data** dialog box (see Figure 6-10), select the **Dilution series** option.
2. Enter the starting dilution, select a series operator, and enter a series operand.
3. Select the appropriate dilution orientation: (see Figure 6-11).

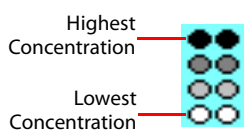


Figure 6-11: Concentration Representation in Dilution Series

4. Click **OK**.

The **Sample Plate Table** will display the dilution factors entered.

Editing a Dilution Factor in the Sample Plate Table

To edit a dilution factor in the **Sample Plate Table**:

1. In the **Set Well Data** dialog box (see Figure 6-10), double-click a cell in the **Dilution Factor** column for the desired unknown.
2. Enter the new value (the default dilution factor is 1)

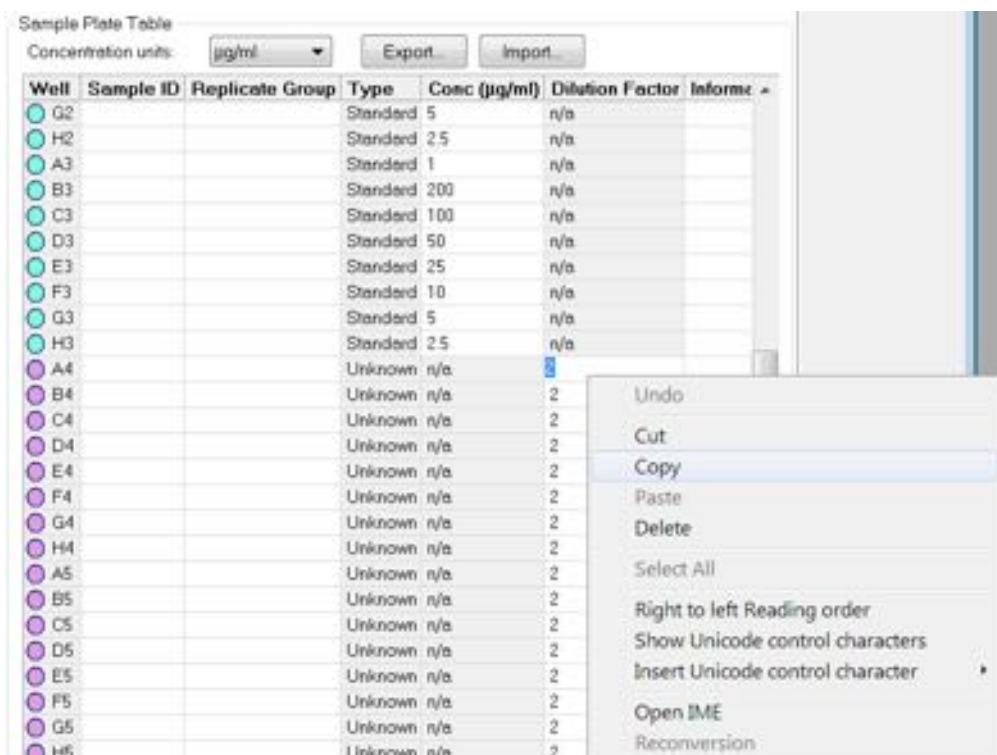


Figure 6-12: Sample Plate Table—Shortcut Menu of Edit Commands



NOTE: Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the **Sample Plate Table**. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the **Sample Plate Map** menu used to designate sample types.

Designating Controls or Reference Wells

Controls are samples of known concentration that are not used to generate a standard curve. A reference well contains sample matrix only, and is used to subtract non-specific binding of the sample matrix to the biosensor. During data analysis, data from reference wells can be subtracted from standards and unknowns to correct for background signal.

- To designate controls, select the control wells and click **Control** (below the **Sample Plate Map**), or right-click and select **Control**. Positive and Negative Control types can also be assigned using this menu.
- To designate reference wells, select the reference wells and click the **Reference** button below the **Sample Plate Map**, or right-click the selection and choose **Reference**.

The wells are marked in the **Sample Plate Map** and the **Sample Plate Table** is updated (see Figure 6-12).

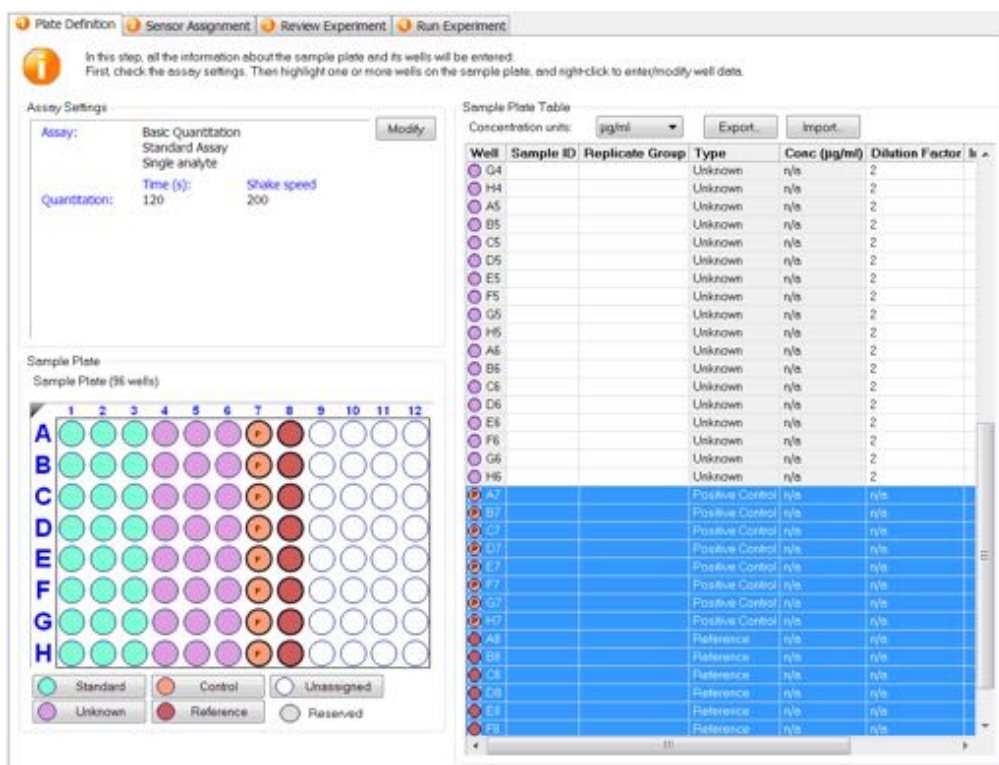


Figure 6-13: Designate Controls or Reference Wells



NOTE: Shift-clicking in the **Sample Plate Map** mimics the head of the instrument during the selection.

To remove a well designation, select the well(s) and click **Unassigned**. Or, right-click the well(s) and select **Clear Data**.

Annotating Samples

You can enter annotations (notes) for multiple samples in the **Sample Plate Map** or enter information for an individual sample in the **Sample Plate Table**. For greater clarity, annotation text may be displayed as the legend of the **Runtime Binding Chart** during data acquisition, but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it will not be available for display as a legend.

Annotating Wells in the Sample Plate Map

To annotate one or more wells:

1. In the **Sample Plate Map**, select the samples to annotate, right-click and select **Set Well Data**.
2. In the **Set Well Data** dialog box (see Figure 6-14), enter the **Sample ID** and/or **Well Information** and click **OK**.

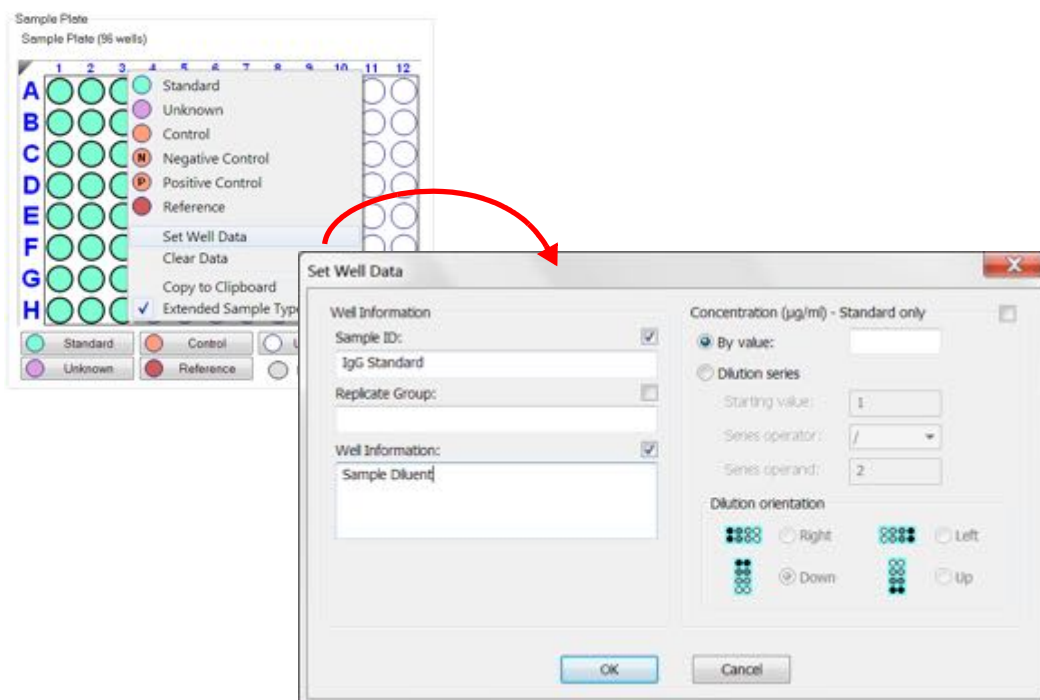


Figure 6-14: Adding Sample Annotations from the Sample Plate Map

Annotating Wells in the Sample Plate Table

To annotate an individual well in the **Sample Plate Table**:

1. Double-click the table cell for **Sample ID** or **Well Information**.
2. Enter the desired information in the respective field (see Figure 6-15).



NOTE: A series of Sample IDs may also be assembled in Excel and pasted into the **Sample Plate Table**.

Sample Plate Table

Concentration units: µg/ml Export... Import...

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	Dilution Factor	Information
G3	IgG Standard		Standard	5	n/a	Sample Diluent
H3	IgG Standard		Standard	2.5	n/a	Sample Diluent
A4	Ab1		Unknown	n/a	2	Sample Diluent
B4	Ab2		Unknown	n/a	2	Sample Diluent
C4	Ab3		Unknown	n/a	2	Sample Diluent
D4	Ab4		Unknown	n/a	2	Sample Diluent
E4	Ab5		Unknown	n/a	2	Sample Diluent
F4	Ab6		Unknown	n/a	2	Sample Diluent
G4	Ab7		Unknown	n/a	2	Sample Diluent
H4	Ab8		Unknown	n/a	2	Sample Diluent
A5	Ab1		Unknown	n/a	2	Sample Diluent
B5	Ab2		Unknown	n/a	2	Sample Diluent
C5	Ab3		Unknown	n/a	2	Sample Diluent
D5	Ab4		Unknown	n/a	2	Sample Diluent
E5	Ab5		Unknown	n/a	2	Sample Diluent
F5	Ab6		Unknown	n/a	2	Sample Diluent
G5	Ab7		Unknown	n/a	2	Sample Diluent
H5	Ab8		Unknown	n/a	2	Sample Diluent
A6	Ab1		Unknown	n/a	2	Sample Diluent
B6	Ab2		Unknown	n/a	2	Sample Diluent
C6	Ab3		Unknown	n/a	2	Sample Diluent
D6	Ab4		Unknown	n/a	2	Sample Diluent
E6	Ab5		Unknown	n/a	2	Sample Diluent
F6	Ab6		Unknown	n/a	2	Sample Diluent
G6	Ab7		Unknown	n/a	2	Sample Diluent
H6	Ab8		Unknown	n/a	2	Sample Diluent
A7	hlgG		Positive Control	n/a	n/a	10 µg/mL in
B7	hlgG		Positive Control	n/a	n/a	10 µg/mL
C7	hlgG		Positive Control	n/a	n/a	10 µg/mL
D7	hlgG		Positive Control	n/a	n/a	10 µg/mL
E7	hlgG		Positive Control	n/a	n/a	10 µg/mL

Figure 6-15: Adding Sample Annotations in the Sample Plate Table



NOTE: Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the **Sample Plate Table**. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the **Sample Plate Map** menu used to designate sample types.

Replicate Groups

When samples are assigned to a **Replicate Group**, the Octet System Data Analysis software will automatically calculate statistics for all samples in that group. The average binding rate, average concentration and corresponding standard deviation as well CV% are presented in the **Results** table for each group (see Figure 6-16).

Sensor...	Replicat...	BR Avg	BR SD	BR CV	Conc. Avg	Conc. SD	Conc. CV
Protein A	Group 1	0.66	0.01	1.5	604.5	17.8	2.9
Protein A	Group 1	0.66	0.01	1.5	604.5	17.8	2.9
Protein A	Group 1	0.66	0.01	1.5	604.5	17.8	2.9
Protein A	Group 1	0.66	0.01	1.5	604.5	17.8	2.9
Anti-Hu...	Group 2	0.6589	0.0052	0.8	602.5	9.15	1.5
Anti-Hu...	Group 2	0.6589	0.0052	0.8	602.5	9.15	1.5
Anti-Hu...	Group 2	0.6589	0.0052	0.8	602.5	9.15	1.5
Anti-Hu...	Group 2	0.6589	0.0052	0.8	602.5	9.15	1.5
Anti-Mo...	Group 3	0.6773	0.0087	1.3	635.3	15.4	2.4
Anti-Mo...	Group 3	0.6773	0.0087	1.3	635.3	15.4	2.4
Anti-Mo...	Group 3	0.6773	0.0087	1.3	635.3	15.4	2.4
Anti-Mo...	Group 3	0.6773	0.0087	1.3	635.3	15.4	2.4
Protein A	Group 4	0.6544	0.0073	1.1	594.6	12.9	2.2
Protein A	Group 4	0.6544	0.0073	1.1	594.6	12.9	2.2
Protein A	Group 4	0.6544	0.0073	1.1	594.6	12.9	2.2
Protein A	Group 4	0.6544	0.0073	1.1	594.6	12.9	2.2

Figure 6-16: Replicate Group Result Table Statistics



NOTE: Replicate Group information can also be entered in the Results table in the Octet System Data Analysis software.

Assigning Replicate Groups in the Sample Plate Map

To assign **Replicate Groups** in the **Sample Plate Map**:

1. Select the samples to group, right-click and select **Set Well Data**.
2. In the **Set Well Data** dialog box (see Figure 6-17), enter a name in the **Replicate Group** box and click **OK**.

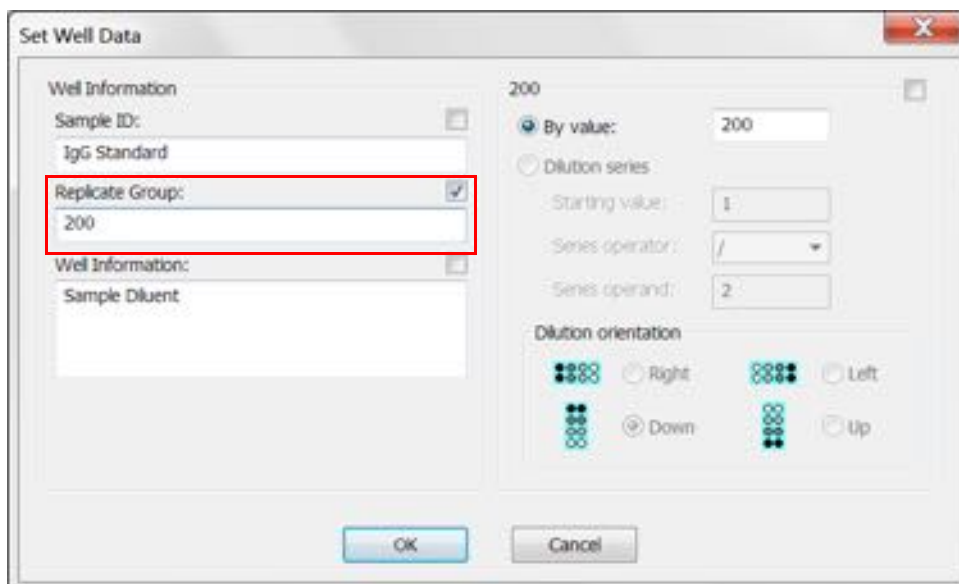


Figure 6-17: Add Replicate Group from the Sample Plate Map

3. Repeat the previous steps to assign new samples to the existing **Replicate Group**, or to designate another set of samples to a new **Replicate Group**. Multiple groups can be used in an experiment.



IMPORTANT: The Octet System Data Analysis software will only recognize and calculate statistics for samples that use the same **Replicate Group** names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.



NOTE: When performing a Multiple Analyte experiment, if the same **Replicate Group** name is used with different biosensor types, they will be treated as separate groups. Statistics for these groups will be calculated separately for each biosensor type.

Wells in the **Sample Plate Map** will show color-coded outlines as a visual indication of which wells are in the same group (see Figure 6-18).

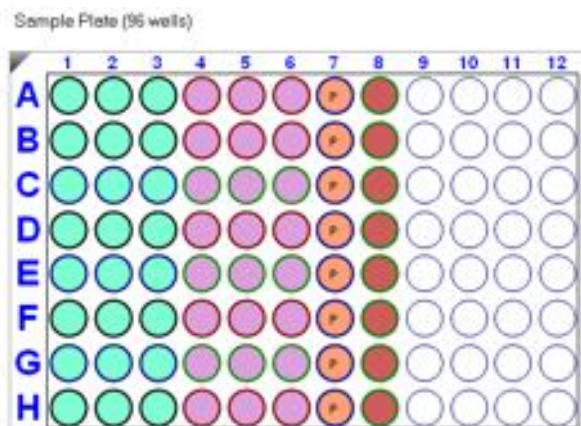


Figure 6-18: Replicate Groups Displayed in Sample Plate Map

The **Sample Plate Table** will update with the **Replicate Group** names entered (see Figure 6-19).

Sample Plate Table

Concentration units:

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	Dilution Factor
A1	IgG Standard	200	Standard	200	n/a
B1	IgG Standard	100	Standard	100	n/a
C1	IgG Standard	50	Standard	50	n/a
D1	IgG Standard	25	Standard	25	n/a
E1	IgG Standard	10	Standard	10	n/a
F1	IgG Standard	5	Standard	5	n/a
G1	IgG Standard	2.5	Standard	2.5	n/a
H1	IgG Standard	1	Standard	1	n/a
A2	IgG Standard	200	Standard	200	n/a
B2	IgG Standard	100	Standard	100	n/a
C2	IgG Standard	50	Standard	50	n/a
D2	IgG Standard	25	Standard	25	n/a
E2	IgG Standard	10	Standard	10	n/a
F2	IgG Standard	5	Standard	5	n/a
G2	IgG Standard	2.5	Standard	2.5	n/a
H2	IgG Standard	1	Standard	1	n/a
A3	IgG Standard	200	Standard	200	n/a
B3	IgG Standard	100	Standard	100	n/a
C3	IgG Standard	50	Standard	50	n/a
D3	IgG Standard	25	Standard	25	n/a
E3	IgG Standard	10	Standard	10	n/a
F3	IgG Standard	5	Standard	5	n/a
G3	IgG Standard	2.5	Standard	2.5	n/a
H3	IgG Standard	1	Standard	1	n/a
A4	Ab1	Ab1	Unknown	n/a	2
B4	Ab2	Ab2	Unknown	n/a	2
C4	Ab3	Ab3	Unknown	n/a	2
D4	Ab4	Ab4	Unknown	n/a	2
E4	Ab5	Ab5	Unknown	n/a	2
F4	Ab6	Ab6	Unknown	n/a	2
G4	Ab7	Ab7	Unknown	n/a	2

Figure 6-19: Replicate Groups in Sample Plate Table

Assigning Replicate Groups in the Sample Plate Table

To assign **Replicate Groups** in the **Sample Plate Table**:

1. Double-click the desired cell in the **Replicate Group** table column.
2. Enter a group name (see Figure 6-20).

Sample Plate Table

Concentration units:

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	Dilution Factor
A1	IgG Standard	200	Standard	200	n/a
B1	IgG Standard	100	Standard	100	n/a
C1	IgG Standard	50	Standard	50	n/a
D1	IgG Standard	25	Standard	25	n/a
E1	IgG Standard	10	Standard	10	n/a
F1	IgG Standard	5	Standard	5	n/a
G1	IgG Standard	2.5	Standard	2.5	n/a

Figure 6-20: Add Replicate Group from the Sample Plate Table



NOTE: Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the **Sample Plate Table**. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the **Sample Plate Map** menu used to designate sample types.

3. Repeat the previous steps to assign new samples to the existing **Replicate Group**, or to designate another set of samples to a new **Replicate Group**. Multiple groups can be used in an experiment.



IMPORTANT: The Octet System Data Analysis software will only recognize and calculate statistics for samples that use the same **Replicate Group** names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.



NOTE: When performing a Multiple Analyte experiment, if the same Replicate Group name is used with different biosensor types, they will be treated as separate groups. Statistics for these groups will be calculated separately for each biosensor type.

MANAGING SAMPLE PLATE DEFINITIONS



NOTE: After you define a sample plate, you can export and save the plate definition for future use.

Exporting a Plate Definition

To export a plate definition:

1. In the **Sample Plate Table** (see Figure 6-21), click **Export**.

Sample Plate Table

Concentration units: µg/ml Export... Import...

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	Dilution Factor ▲
A1	IgG Standard	200	Standard	200	n/a
B1	IgG Standard	100	Standard	100	n/a
C1	IgG Standard	50	Standard	50	n/a
D1	IgG Standard	25	Standard	25	n/a
E1	IgG Standard	10	Standard	10	n/a
F1	IgG Standard	5	Standard	5	n/a

Figure 6-21: Export Button in Sample Plate Table

2. In the **Export Plate Definition** window (see Figure 6-22), select a folder, enter a name for the plate (.csv), and click **Save**.

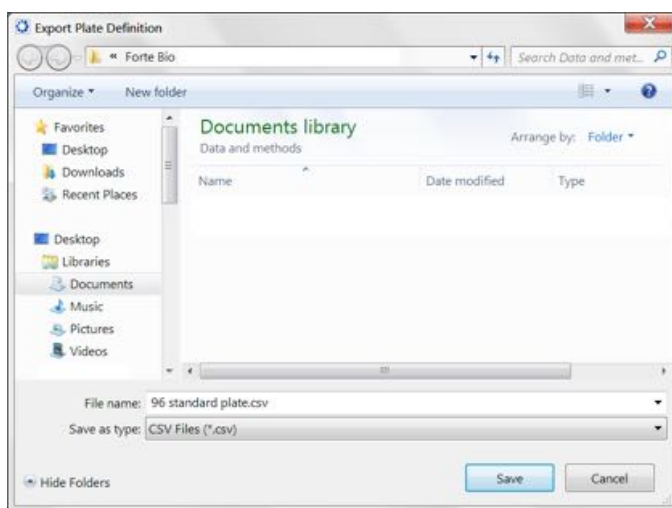


Figure 6-22: Export Plate Definition Window

Importing a Plate Definition

To import a plate definition:

1. In the **Sample Plate Table** (see Figure 6-23), click **Import**.

Sample Plate Table					
Concentration units:		$\mu\text{g/ml}$	Export...	Import...	
Well	Sample ID	Replicate Group	Type	Conc ($\mu\text{g/ml}$)	Dilution Factor
A1	IgG Standard	200	Standard	200	n/a
B1	IgG Standard	100	Standard	100	n/a
C1	IgG Standard	50	Standard	50	n/a
D1	IgG Standard	25	Standard	25	n/a
E1	IgG Standard	10	Standard	10	n/a
F1	IgG Standard	5	Standard	5	n/a

Figure 6-23: Import Button in Sample Plate Table

2. In the **Import Plate Definition** window (see Figure 6-24), select the plate definition (.csv), and click **Open**.

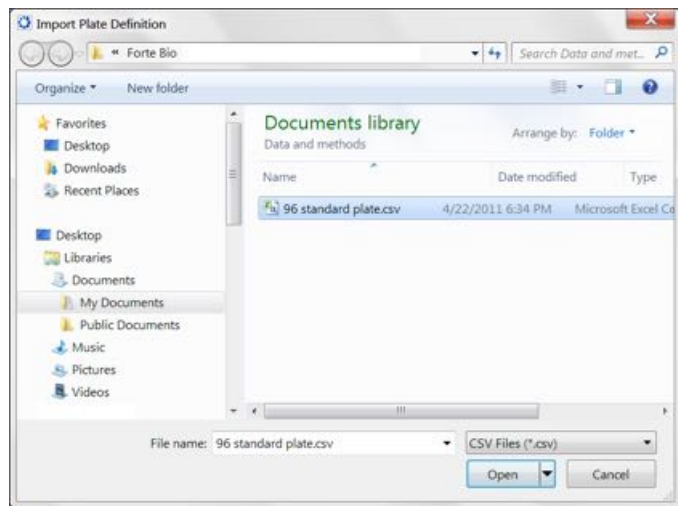


Figure 6-24: Import Plate Definition Window



NOTE: You can also create a .csv file for import. Figure 6-25 shows the appropriate column information layout.

	A	B	C	D	E	F	G
1	PlateWells	96					
2	Well	ID	Replicate Group	Group	Concentration (µg/ml)	Dilution	Information
3	A1	IgG Standard	200	Standard	200		Sample Diluent
4	B1	IgG Standard	100	Standard	100		Sample Diluent
5	C1	IgG Standard	50	Standard	50		Sample Diluent
6	D1	IgG Standard	25	Standard	25		Sample Diluent
7	E1	IgG Standard	10	Standard	10		Sample Diluent
8	F1	IgG Standard	5	Standard	5		Sample Diluent
9	G1	IgG Standard	2.5	Standard	2.5		Sample Diluent
10	H1	IgG Standard	1	Standard	1		Sample Diluent
11	A2	IgG Standard	200	Standard	200		Sample Diluent

Figure 6-25: Example Sample Plate File (.csv)

Printing a Sample Plate Definition

To print a plate definition:

- 1. In the **Sample Plate Map** (see Figure 6-26), click **Print**.

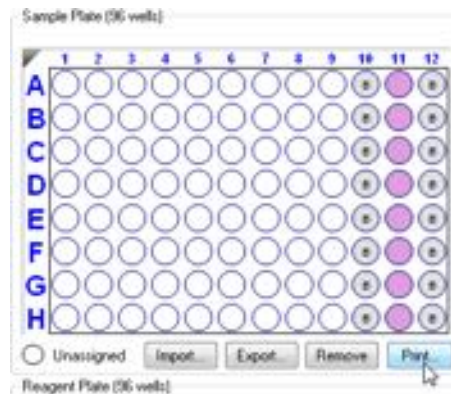


Figure 6-26: Sample Plate Print Button

The associated **Sample Plate Table** information will print.

MANAGING ASSAY PARAMETER SETTINGS

Modifying Assay Parameter Settings

You can modify the assay parameter settings during sample plate definition. However, the changes are only applied to the current experiment. To save modified parameter settings, you must define a new assay. For details on creating a new assay, see “Custom Quantitation Assays” on page 274.

Viewing User-Modifiable Assay Parameter Settings

To view the user-modifiable settings for an assay, click **Modify** in the **Assay Settings** box. The **Assay Parameters** box will display (Figure 6-27). The settings available are experiment-dependent.

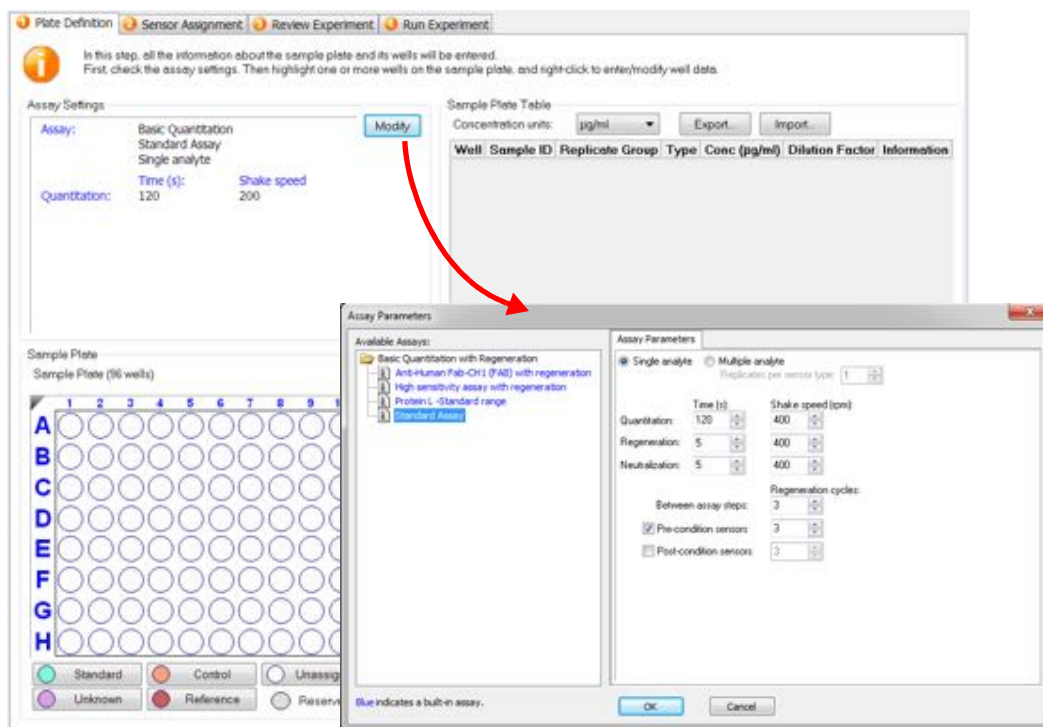


Figure 6-27: Modifying Assay Parameters

Basic Quantitation Assay Parameters

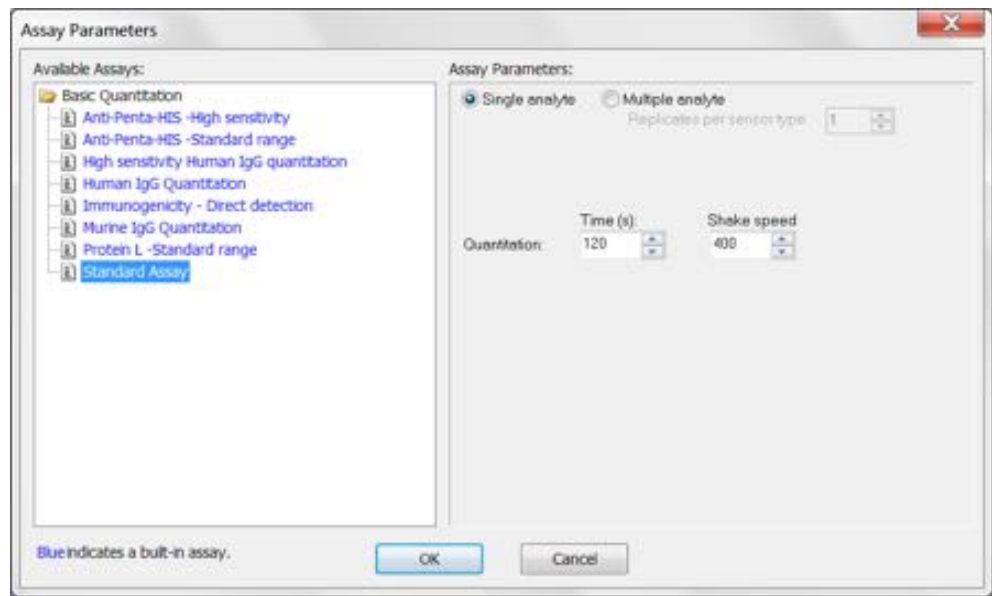



Figure 6-28: Assay Parameters—Basic Quantitation Assay

Table 6-6: Basic Quantitation Assay Parameters

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time (s)	The duration of data acquisition seconds while the biosensor is incubated in sample.
<div> NOTE: A subset of data points may be selected for processing during data analysis.</div>	
Quantitation Shake speed (rpm)	The sample shaking speed (rotations per minute).

Basic Quantitation with Regeneration Assay Parameters

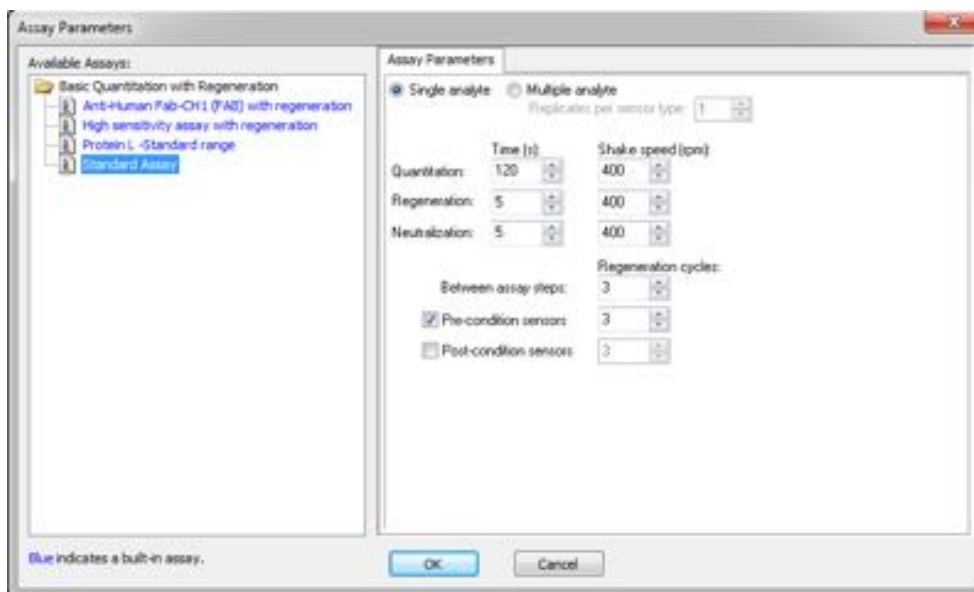


Figure 6-29: Assay Parameters—Basic Quantitation with Regeneration

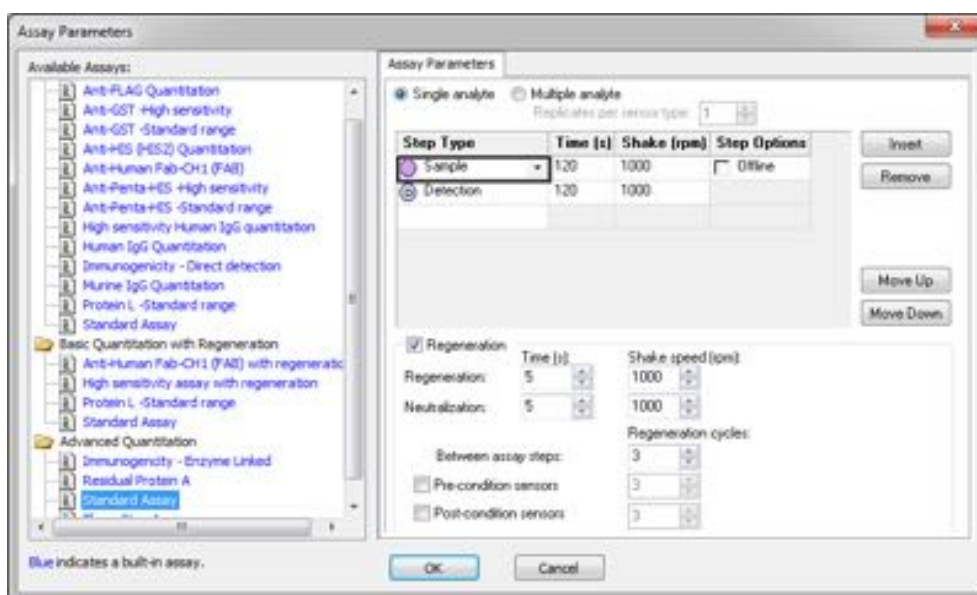
Table 6-7: Assay Parameters—Basic Quantitation with Regeneration

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time(s) and Shake speed (rpm)	The duration of data acquisition in seconds while the biosensor is incubated in sample and the sample shaking speed (rotations per minute).
<div>➔</div> NOTE: A subset of data points may be selected for processing during data analysis.	
Regeneration Time(s) and Shake speed (rpm)	The duration time and shaking speed of the regeneration step where the biosensor is incubated in regeneration buffer to remove bound analyte.
Neutralization Time(s) and Shake speed (rpm)	The duration time and shaking speed of the neutralization step where the biosensor is incubated in neutralization buffer after the regeneration step.

Table 6-7: Assay Parameters—Basic Quantitation with Regeneration

Parameter	Description
Pre-condition sensors	Performs a set of regeneration/neutralization steps prior to the start of the experiment. The pre-conditioning settings are equivalent to the time and rpm settings for the regeneration in the assay. For example, an acidic pre-conditioning buffer maximizes the binding competence of Pro-A biosensors.
Post-condition sensors	Post-conditions biosensors, allowing re-racked biosensors to be stored in a regenerated state.
Regeneration cycles	The number of regeneration-neutralization cycles that a biosensor undergoes before reuse.

Advanced Quantitation Assay Parameters

**Figure 6-30:** Assay Parameters—Advanced Quantitation**Table 6-8:** Advanced Quantitation Assay Parameters

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.

Table 6-8: Advanced Quantitation Assay Parameters (Continued)



Parameter	Description
Sample Time(s) and Shake speed (rpm)	The duration of data acquisition in seconds while the biosensor is incubated in sample and the sample shaking speed (rotations per minute).
 NOTE: A subset of data points may be selected for processing during data analysis.	
Buffer Time(s) and Shake speed (rpm)	The duration of biosensor incubation in the first buffer in seconds and the sample shaking speed (rotations per minute).
Enzyme Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the enzyme solution and the sample shaking speed (rotations per minute).
2nd Buffer Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the second buffer solution and the sample shaking speed (rotations per minute).
Capture Antibody Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the first capture antibody solution and the shaking speed (rotations per minute).
2nd Antibody Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the secondary antibody solution and the shaking speed (rotations per minute).
Detection Time(s) & Shake speed (rpm)	The duration of data acquisition during the detection step in seconds in an advanced quantitation assay.
 NOTE: A subset of data points may be selected for processing during data analysis.	
Offline	Choose this option to incubate sample with biosensors outside the Octet system. Offline incubation is best performed on the Pall ForteBio Sidekick biosensor immobilization station.
Reuse Buffer	Allows buffer wells to be reused. If unselected, the number of buffer columns must equal the number of sample columns. If selected, the number of buffer columns may be less than the number of sample columns as the buffer columns are reused.
Regeneration Time(s) and Shake speed (rpm)	The duration time and shaking speed of the regeneration step where the biosensor is incubated in regeneration buffer to remove bound analyte.

Table 6-8: Advanced Quantitation Assay Parameters (Continued)

Parameter	Description
Neutralization Time(s) and Shake speed (rpm)	The duration time and shaking speed of the neutralization step where the biosensor is incubated in neutralization buffer after the regeneration step.
Pre-condition sensors	Performs a set of regeneration/neutralization steps prior to the start of the experiment. The pre-conditioning settings are equivalent to the time and rpm settings for the regeneration in the assay. For example, an acidic pre-conditioning buffer maximizes the binding competence of Protein A biosensors.
Post-condition sensors	Post-conditions biosensors, allowing re-racked biosensors to be stored in a regenerated state.
Regeneration cycles	The number of regeneration-neutralization cycles that a biosensor undergoes before reuse.



NOTE: In an Advanced Quantitation experiment, this option is only available if the first step (biosensor incubation in sample) is performed online.

ASSIGNING BIOSENSORS TO SAMPLES

After the sample plate is defined, biosensors must be assigned to the samples.

Biosensor Assignment in Single-Analyte Experiments

In a single analyte experiment, only one biosensor type is assigned to each sample and only one analyte is analyzed per experiment.



NOTE: For single analyte experiments, the **Single Analyte** option must be selected in the **Assay Parameters** dialog box. For more information, please see “Managing Assay Parameter Settings” on page 234.

Click the **Sensor Assignment** tab, or click the  arrow to access the Sensor Assignment window (see Figure 6-31).

The software generates a color-coded **Sensor Tray Map** and **Sample Plate Map** that shows how the biosensors are assigned to the samples by default.

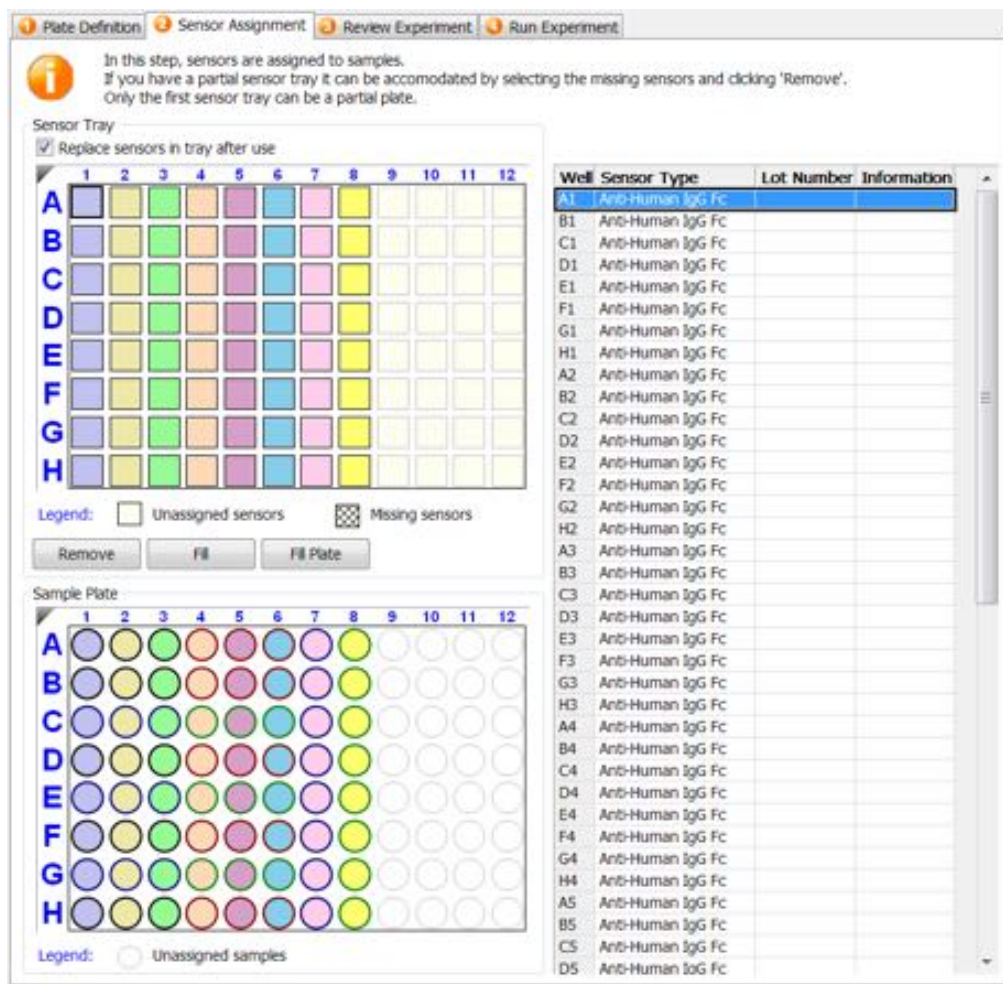


Figure 6-31: Sensor Assignment Window for Basic Quantitation without Regeneration

- Assign biosensors in one of two ways:
 - Select a column(s) in the **Sensor Tray Map**, right-click and select a biosensor type from the drop-down list.
 - Select a cell in the **Sensor Type** table column, click the down arrow and select a biosensor type from the drop-down list (see Figure 6-31).

All wells in the **Sensor Type** column will automatically populate with the biosensor type selected.

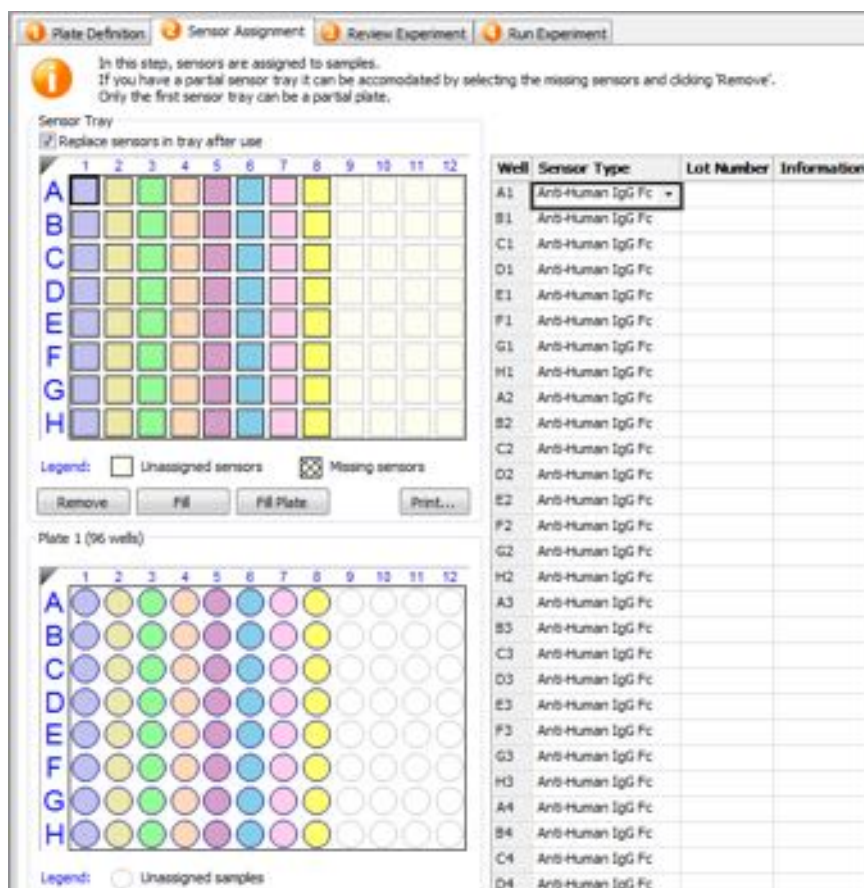


Figure 6-32: Changing Biosensor Types

- To designate reference biosensors, select the desired biosensors in the **Sensor Tray Map**, right-click and select **Reference**. The reference biosensors are marked with an **R**.



NOTE: Reference biosensors may also be designated in the **Runtime Binding Chart** during acquisition.

- Optional: Double-click in any cell in the **Lot Number** column to enter the biosensor lot number. All wells in the **Lot Number** column will automatically populate with the lot number entered.
- Optional: Double-click in a cell in the **Information** column to enter biosensor information for a particular cell.



NOTE: Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: For greater clarity, annotation text may be displayed as the legend of the **Runtime Binding Chart** during data acquisition but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it will not be available for display as a legend.

- Optional for the Octet RED96 and RED96e instrument only: After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace sensors in tray after use** check box (see Figure 6-33).

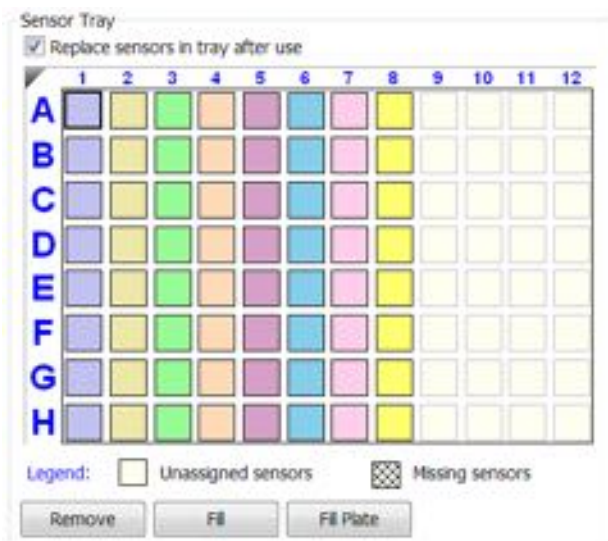


Figure 6-33: Replace Sensors in Tray After Use Check Box




NOTE: Biosensors can be regenerated up to a max of 11 times per experiment.

Biosensor Assignment in Multiple Analyte Experiments

In a multiple analyte experiment, more than one biosensor type is assigned to the same sample, allowing multiple analytes to be analyzed in a single experiment.



NOTE: For multiple analyte experiments, the **Multiple Analyte** option must be selected in the **Assay Parameters** dialog box. For more information, please see “Managing Assay Parameter Settings” on page 234.

Click the **Sensor Assignment** tab, or click the  arrow to access the Sensor Assignment window (see Figure 6-31).

The software generates a color-coded **Sensor Tray Map** and **Sample Plate Map** that shows how the biosensors are assigned to the samples by default. In the example shown in Figure 6-31, **one** replicate had been previously selected with the **Multiple Analyte** assay parameter option.

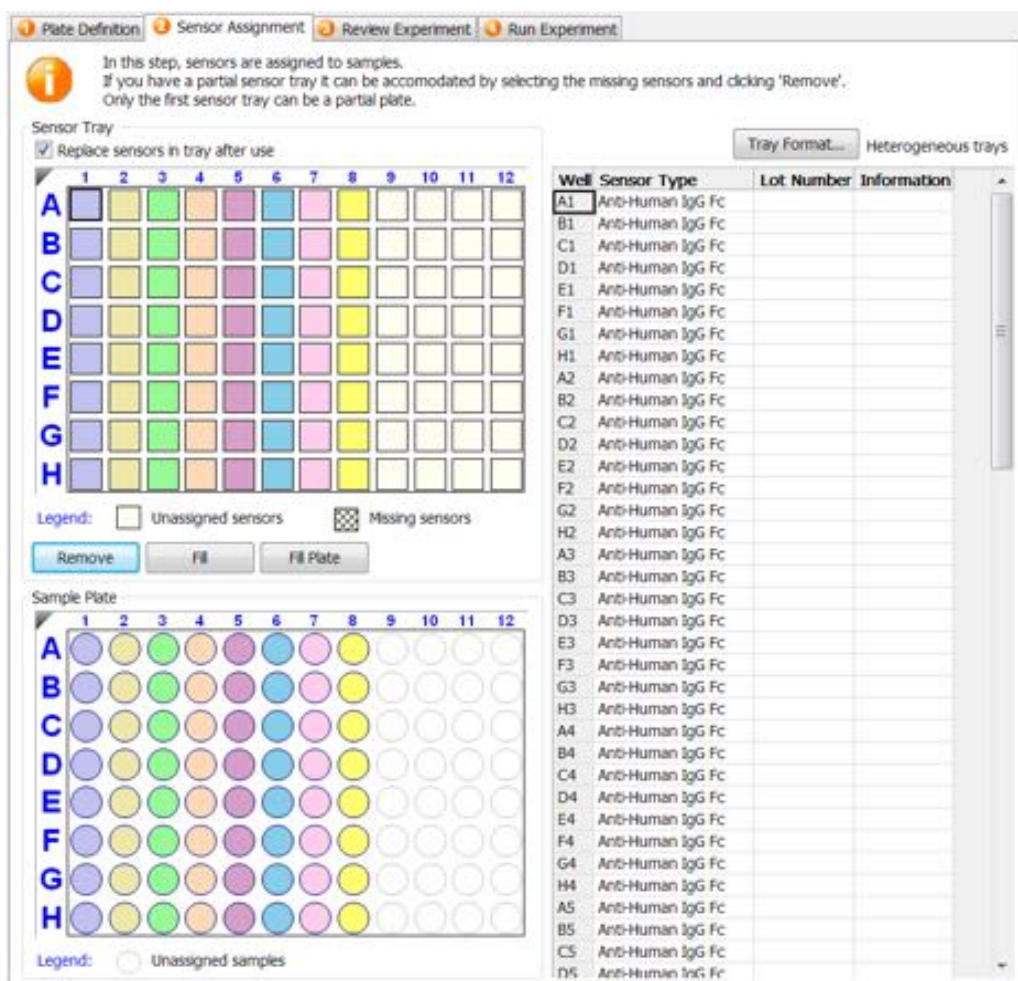


Figure 6-34: Sensor Assignment Window for Basic Quantitation Using the Multiple Analyte Option

There are two ways to assign biosensors:

- Select a column in the **Sensor Tray Map**, right-click and select a biosensor type from the drop-down list.
- Select a cell in the **Sensor Type** table column, click the down arrow and select a biosensor type from the drop-down list (see Figure 6-35).

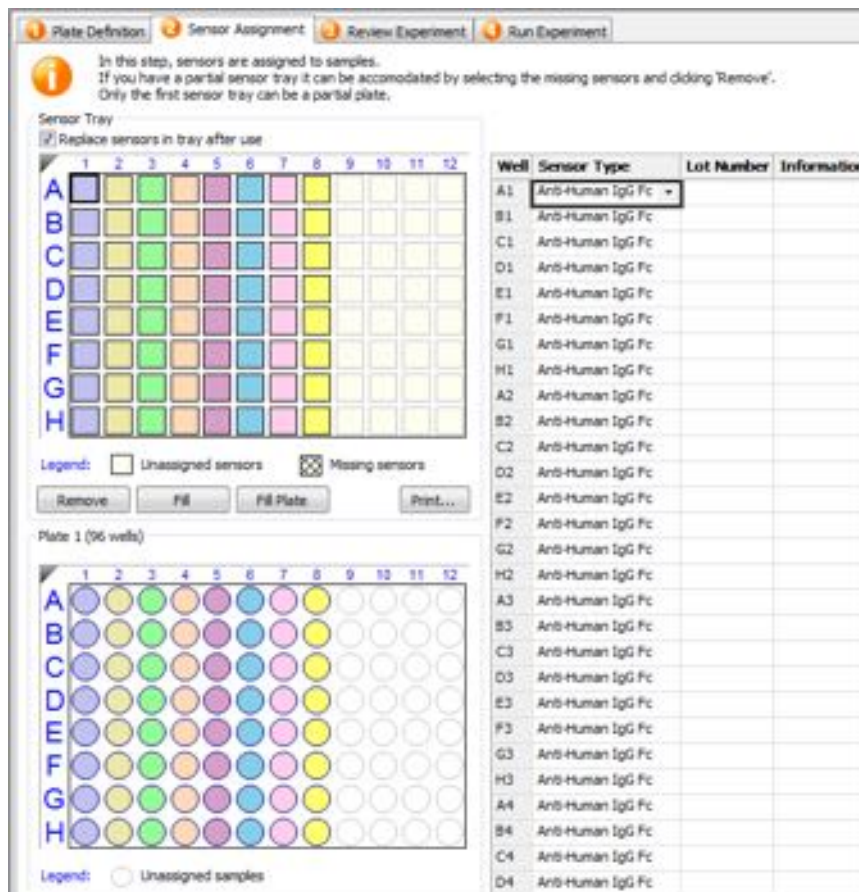


Figure 6-35: Changing Biosensor Types

Biosensor Assignment Using Heterogeneous Biosensor Trays

The default **Tray Format** is **Heterogeneous**. Heterogeneous biosensor trays contain a mixture of biosensor types.



NOTE: When using this **Heterogeneous** option, the order of biosensor types in each tray must be identical.

1. If Heterogeneous Trays is not displayed next to the **Tray Format** button, click the button.

The **Tray Format** dialog box displays (see Figure 6-36).

2. Select **Heterogeneous** and click **OK**.

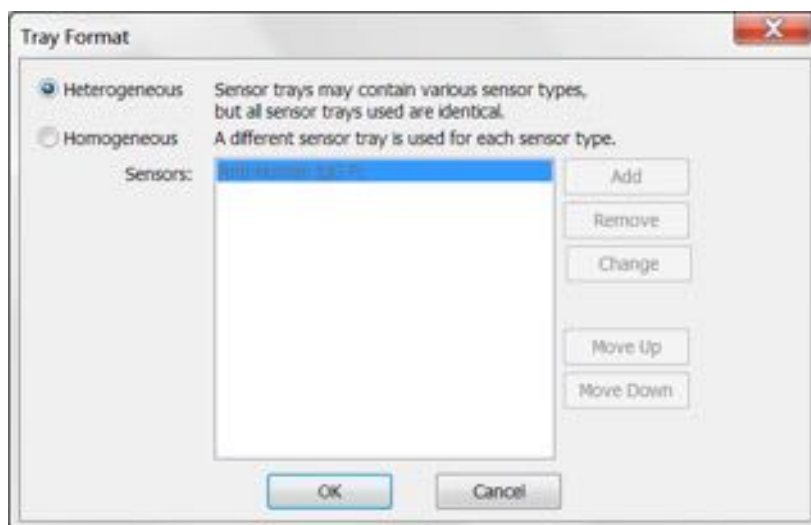


Figure 6-36: Tray Format Dialog Box

The Tray 1 **Sensor Tray Map** will be displayed by default.

3. Select **all** columns with default biosensor assignments in the **Sensor Tray Map**, right-click and select the first biosensor type to be used (see Figure 6-37).

The **Sensor Type** column will update accordingly.



Figure 6-37: Populating the Sensor Tray Map with First Biosensor Type

4. Select the columns in the **Sensor Tray Map** that should contain the second biosensor type, right-click and select the second biosensor type (see Figure 6-39).

The **Sensor Type** column will update accordingly.

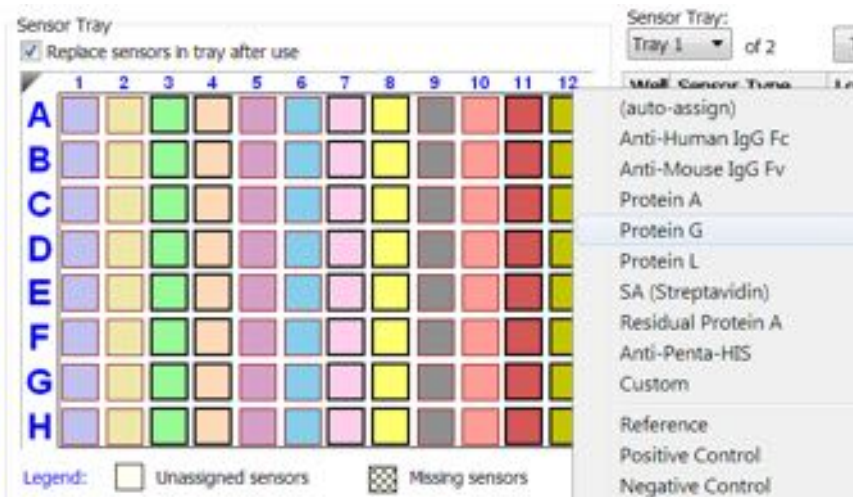


Figure 6-38: Populating the Sensor Tray Map with Second Biosensor Type

5. Repeat this column selection and assignment process for all other biosensor types to be used in the experiment. The software will automatically update the number of biosensor trays needed and biosensor assignments in all trays according to the column assignments made in Tray 1.

In the example shown in Figure 6-39, Protein A and Protein G biosensor types are used for a multiple analyte experiment using two replicates. Three heterogeneous biosensor trays will be needed for the experiment.

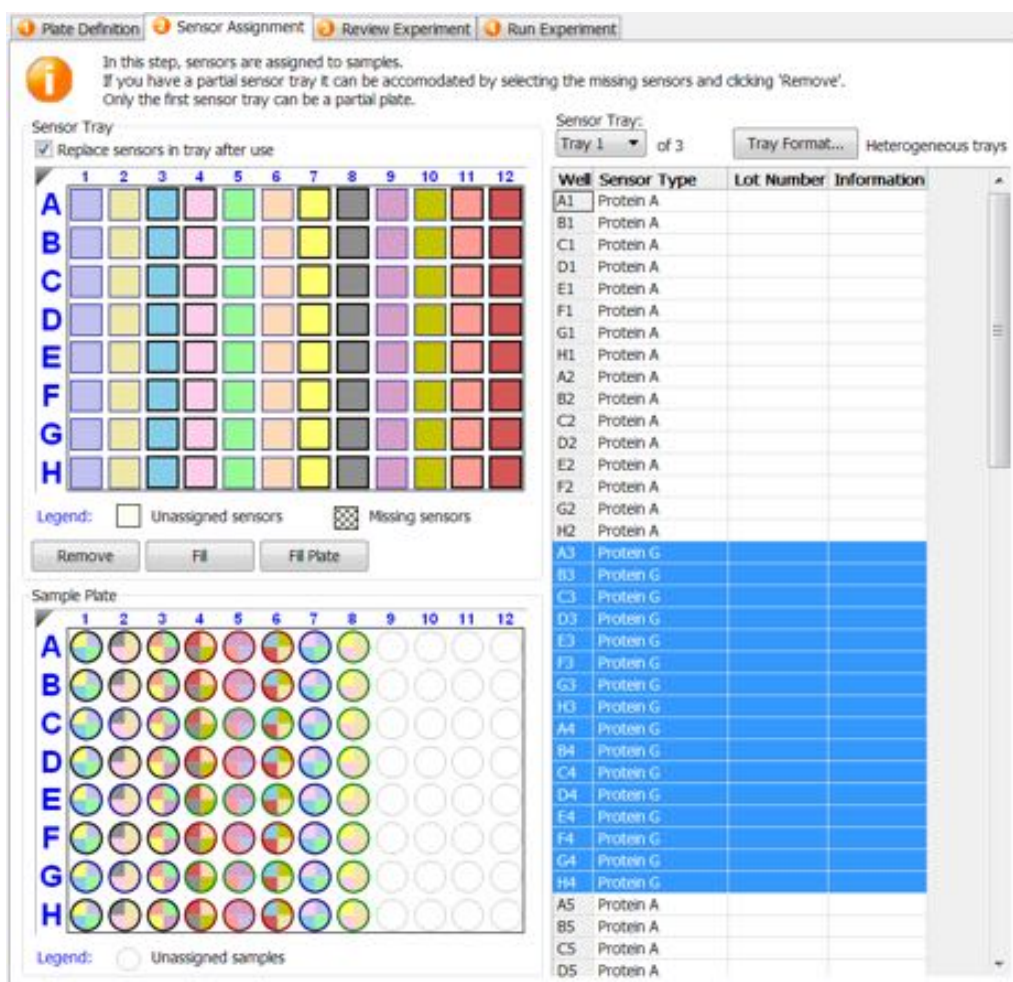


Figure 6-39: Biosensor Assignment using Heterogeneous Trays and Two Biosensor Types

- To view or change the biosensor assignments in another tray, click the **Sensor Tray** button and select a tray number from the drop down list.

The **Sensor Tray Map** and table for the tray selected will be shown and biosensor assignments can be changed as needed (see Figure 6-40).

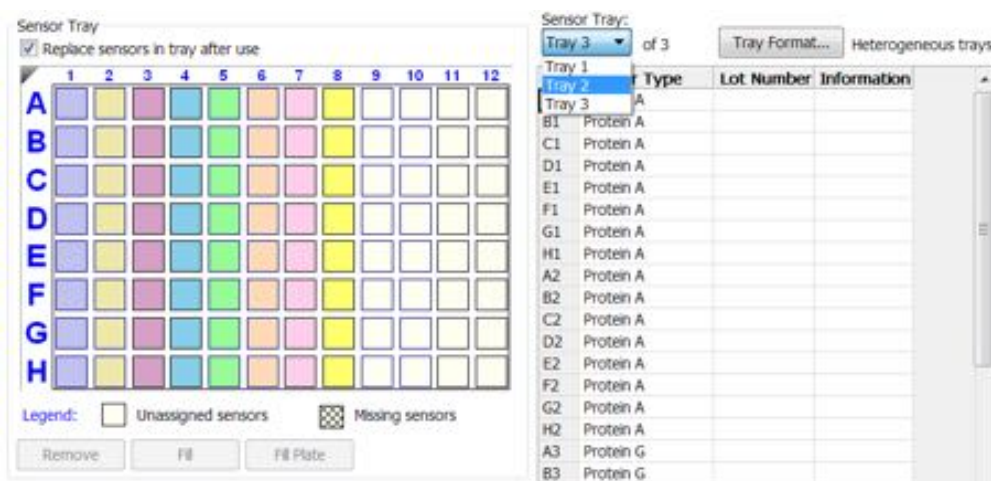


Figure 6-40: Tray Selection

7. To designate reference biosensors, select the desired biosensors in the **Sensor Tray Map**, right-click and select **Reference**.

The reference biosensors are marked with an **R**.



NOTE: Reference biosensors may also be designated in the **Runtime Binding Chart** during acquisition.

8. Optional: Double-click in any cell in the **Lot Number** column to enter a biosensor lot number. All wells in the **Lot Number** column for that biosensor type will automatically populate with the lot number entered.
9. Optional: Double-click in a cell in the **Information** column to enter biosensor information for a particular cell.



NOTE: Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut - Ctrl+x**, **Copy - Ctrl+c**, **Paste - Ctrl+v**, **Undo - Ctrl+z**) are available in the table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: For greater clarity, annotation text may be displayed as the legend of the **Runtime Binding Chart** during data acquisition but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it will not be available for display as a legend.

10. Optional: After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace sensors in tray after use** check box (see Figure 6-41).

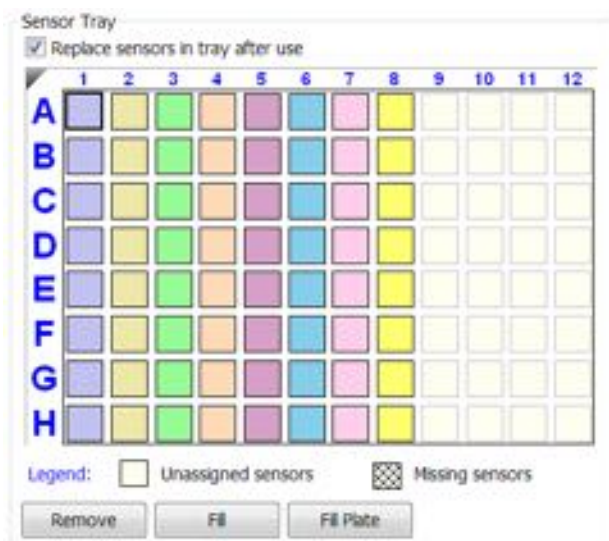


Figure 6-41: Replace Sensors in Tray After Use Check Box



NOTE: Biosensors can be regenerated up to a max of 11 times per experiment.

Biosensor Assignment Using Homogeneous Trays

Homogeneous biosensor trays contain only one biosensor type.



NOTE: Using the **Homogeneous** option will necessitate switching trays during the experiment.

1. Click **Tray Format**.

The **Tray Format** dialog box displays (see Figure 6-42) and the **Sensors** box will be populated with the default biosensor type.

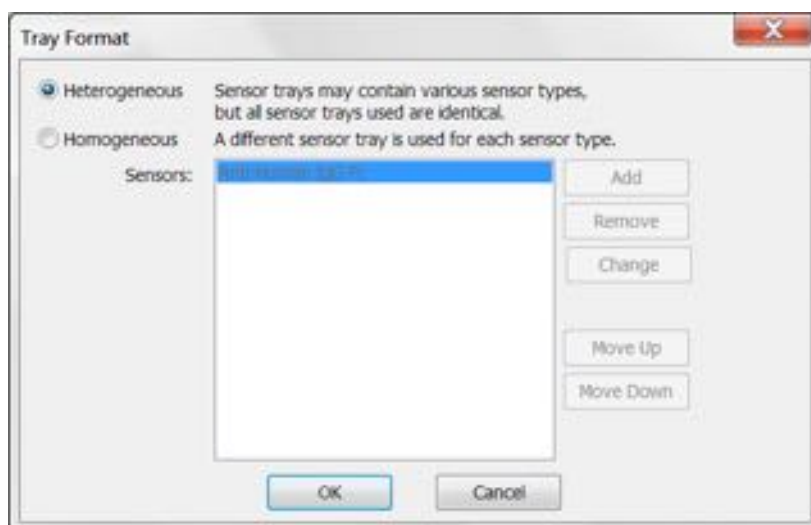


Figure 6-42: Tray Format Dialog Box

2. Select **Homogeneous**. Click **Add** to select the first biosensor type (see Figure 6-43).

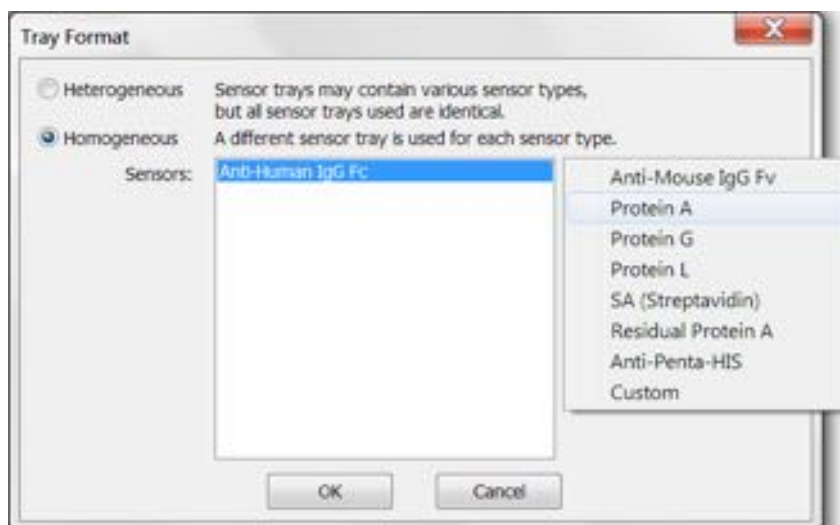


Figure 6-43: Selecting a Biosensor Type in the Tray Format Dialog Box

3. Repeat this step to add any additional biosensor types that will be used in the experiment. To remove a biosensor type, select a biosensor type in the **Sensor** box and click **Remove**.
4. Adjust the order of biosensor types as needed by selecting the biosensor type in the **Sensor** box and clicking **Move Up** or **Move Down**.

The order of biosensor types listed in the **Sensor** box will be used as the default tray assignment (see Figure 6-44).

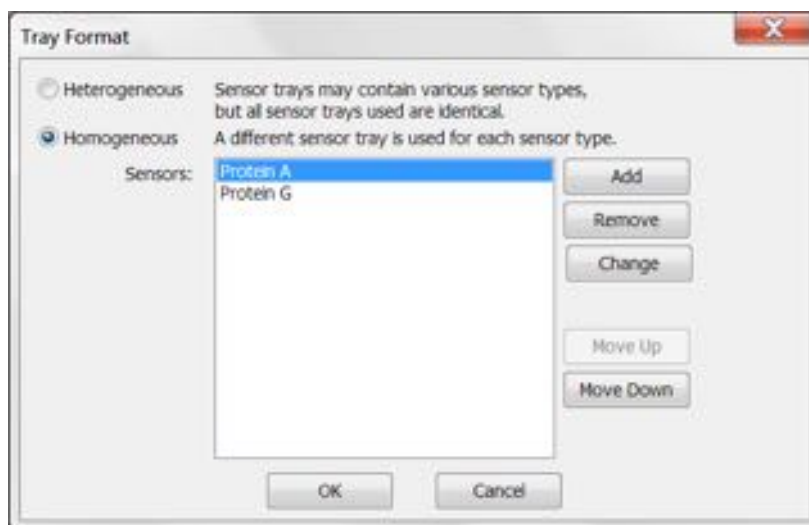


Figure 6-44: Biosensor Types List Order in Sensor Box

5. Click **OK**.

The software will automatically calculate the number of biosensor trays needed and assign biosensors types to each tray.

In the example shown in Figure 6-45, Protein A and Protein G biosensor types will be used for the multiple analyte experiment using two replicates. Four homogeneous biosensor trays (two for each biosensor type) will be needed for the experiment. The Tray 1 **Sensor Tray Map** will be displayed by default.

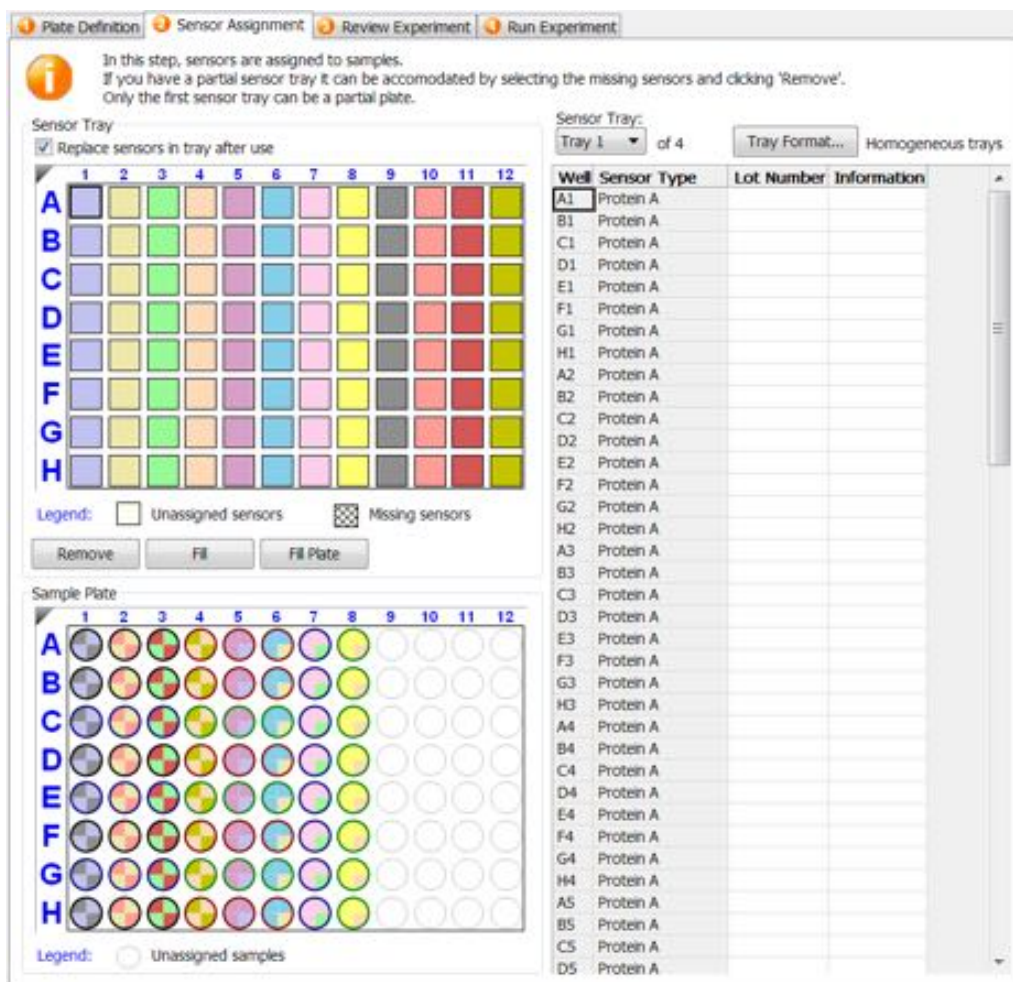


Figure 6-45: Biosensor Assignment using Homogeneous Trays and Two Biosensor Types

- To view the biosensor assignments in another tray, click the **Sensor Tray** button and select a tray number from the drop down list.

The **Sensor Tray Map** and table for the tray selected will be shown (see Figure 6-40).

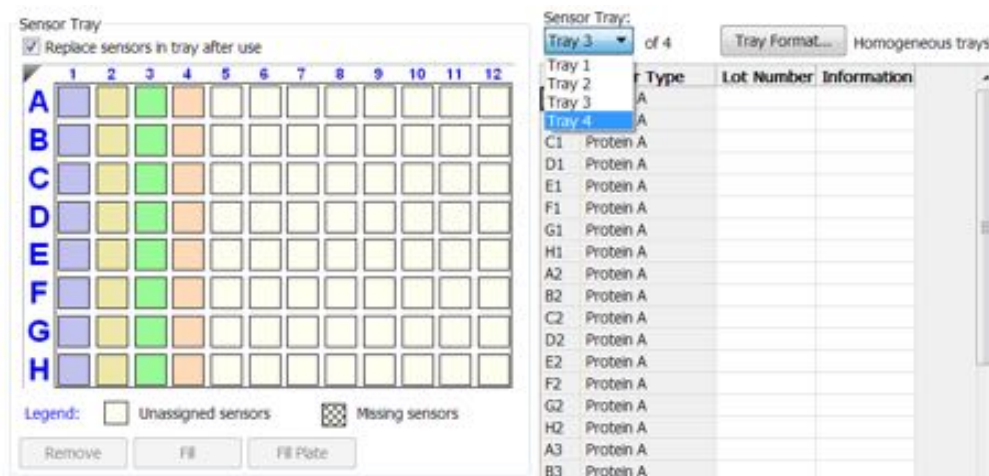


Figure 6-46: Tray Selection

- To designate reference biosensors, select the desired biosensors in the **Sensor Tray Map**, right-click and select **Reference**.

The reference biosensors are marked with an **R**.



NOTE: Reference biosensors may also be designated in the **Runtime Binding Chart** during acquisition.

- Optional: Double-click in any cell in the **Lot Number** column to enter a biosensor lot number.

All wells in the **Lot Number** column for the biosensor type selected will automatically populate with the lot number entered.

- Optional: Double-click in a cell in the **Information** column to enter biosensor information for a particular cell.



NOTE: Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: For greater clarity, annotation text may be displayed as the legend of the **Runtime Binding Chart** during data acquisition but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it will not be available for display as a legend.

10. Optional: After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace sensors in tray after use** check box (see Figure 6-47).

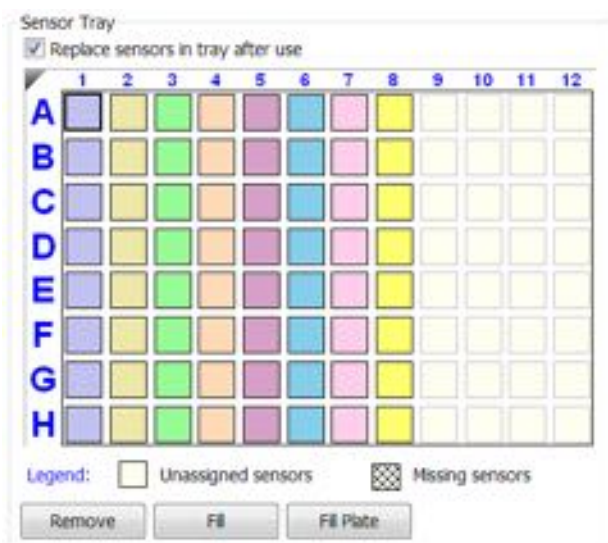


Figure 6-47: Replace Sensors in Tray After Use Check Box



NOTE: Biosensors can be regenerated up to a max of 11 times per experiment.

Biosensor Regeneration

For Basic Quantitation with Regeneration experiments only, the **Sensor Assignment** tab includes the **Regenerations** parameter, which specifies the maximum number of regeneration cycles for each column of biosensors. The specified number of regeneration cycles determines the minimum number of cycles required for each column of sensors. This calculation may result in non-equal regeneration cycles for columns of biosensors. The fractional use of the regeneration and neutralization wells by each column of sensors is represented by a pie chart (Figure 6-48).

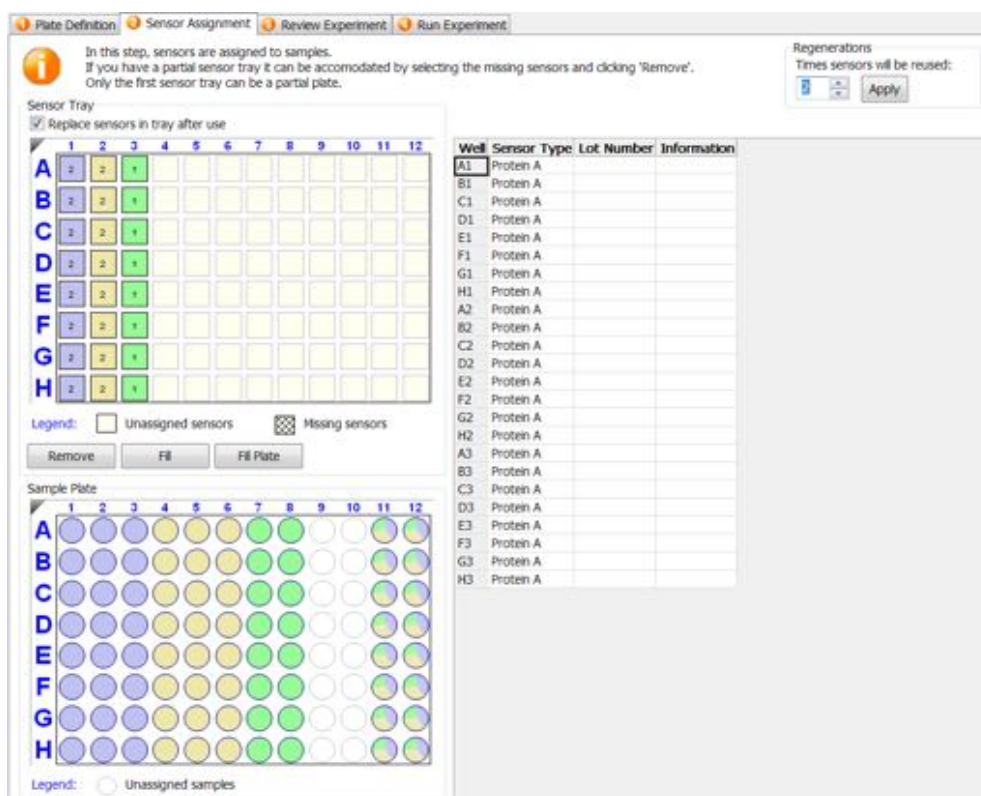


Figure 6-48: Fractional Use of Regeneration and Neutralization Wells

Using Partial Biosensor Trays

If you are using a partial tray of biosensors (some biosensors are missing), specify the missing columns in the **Sensor Tray Map**:

1. Select the column(s) without biosensors and click **Remove**, or right-click the selection and select **Remove**.

If the number of specified biosensors in the **Sensor Assignment** tab is less than the number required to perform the assay, the software automatically adds a second tray of biosensors and assigns the biosensors that are required for the assay.

2. To view the additional biosensor tray that is required for the assay, select Tray 2 from the **Sensor Tray** drop-down list (Figure 6-49). In the example shown, Tray 1 is a partial tray that does not contain enough biosensors for the assay. To designate a second tray, select Tray 2 from the **Sensor Tray** drop-down list (Figure 6-49 top). The **Sensor Tray Map** will then display the additional biosensors required for the assay (Figure 6-49 bottom).

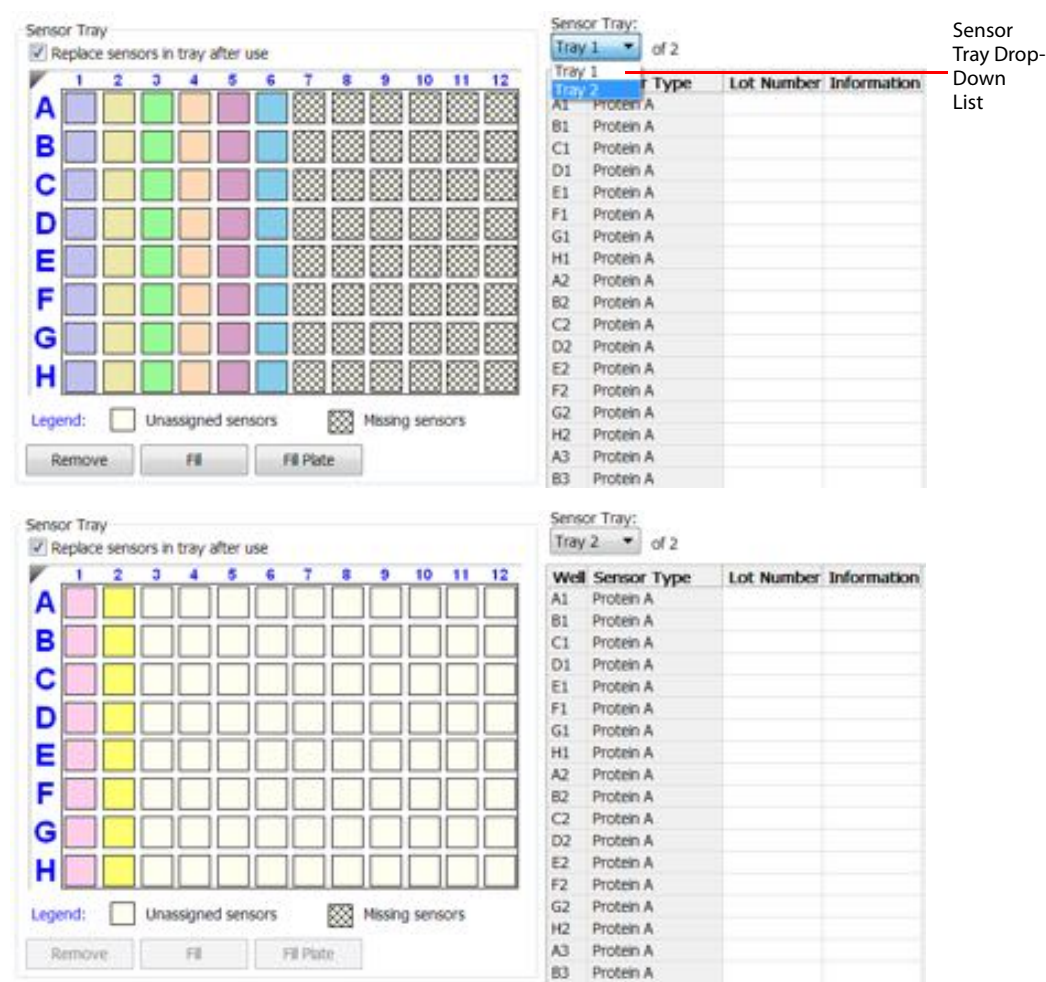


Figure 6-49: Example Assay Using One Partial Biosensor Tray and Biosensors from a Second Tray



To restore biosensors that have been removed, select the columns to restore and click **Fill**. To restore all sensors on the plate, click **Fill Plate**.



NOTE: If multiple biosensor trays are used, only the first biosensor tray can be a partial tray. During the experiment, the software prompts you to insert the appropriate tray in the Octet instrument.

REVIEWING EXPERIMENTS

Before running an experiment, you can review the sample plate layout and the biosensors assigned to each assay in the experiment.

In the **Review Experiment** window, move the slider left or right to highlight the biosensors and samples in an assay, or click the   arrows to select an assay.

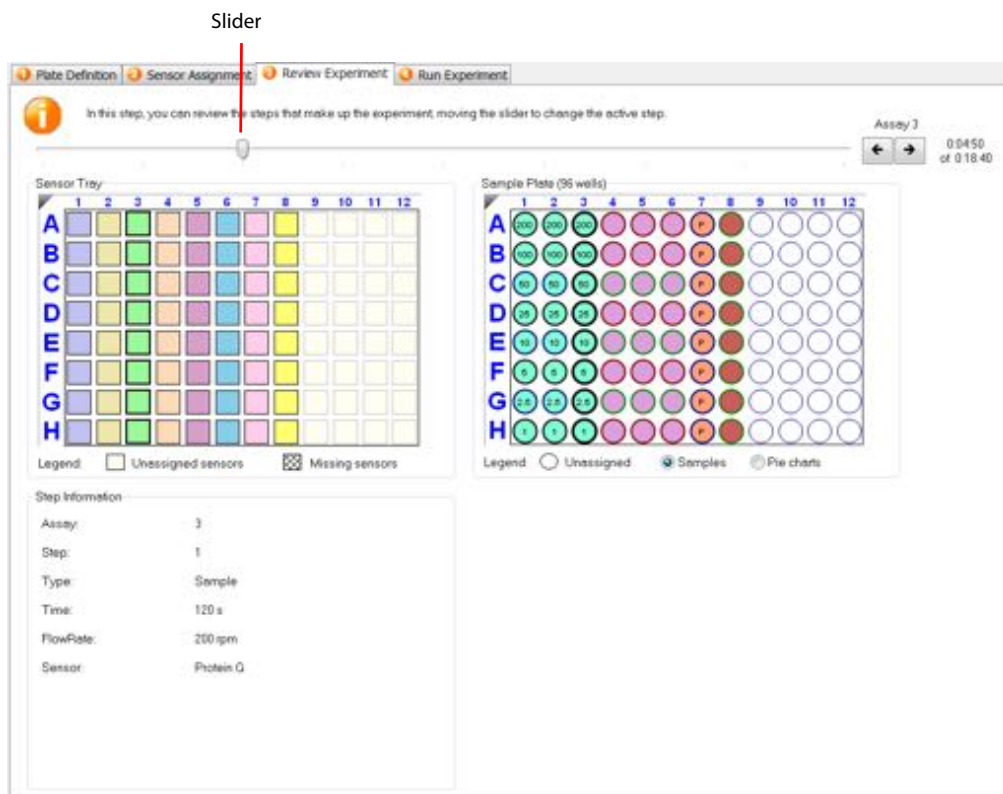




Figure 6-50: Review Experiment Window

SAVING EXPERIMENTS

After a run, the software automatically saves the experiment information that you specified (sample plate definition, biosensor assignment, assay settings) to an experiment method file (.fmf). If you set up an experiment, but do not start the run, you can manually save the experiment method.

To manually save an experiment method:

1. Click the **Save Method File** button , or on the main menu, click **File > Save Method File**. To save more than one open experiment, click the **Save All Methods Files** button .
2. In the **Save** dialog box, enter a name and location for the file, and click **Save**.



NOTE: If you edit a saved experiment and want to save it without overwriting the original file, select **File > Save Method File As** and enter a new name for the experiment.

Saving an Experiment to the Template Folder

If you save an experiment to the factory-installed Template folder, the experiment will be available on the menu bar. To view templates click **Experiment > Templates > Quantitation > Experiment Name** (see Figure 6-51).

Follow the steps above to save an experiment to the Template folder located at C:\Program Files\ForteBio\DataAcquisition\TemplateFiles.



IMPORTANT: Do not change the location of the Template folder. If the Template folder is not at the factory-set location, the software may not function properly.

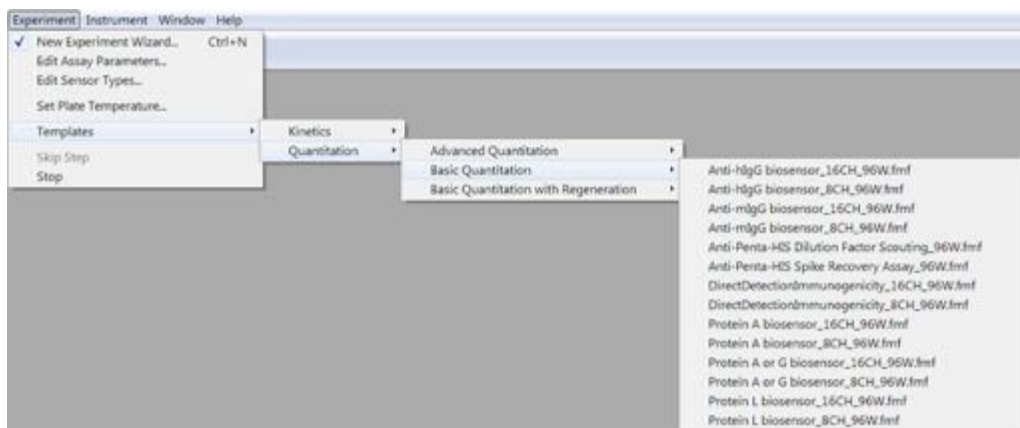


Figure 6-51: Experiments in the Template Folder

RUNNING A QUANTITATION EXPERIMENT



IMPORTANT: Before starting an experiment, ensure that the biosensors are properly rehydrated. For details on how to prepare the biosensors, see the appropriate biosensor product insert.

Loading the Biosensor Tray and Sample Plate

To load the biosensor tray and sample plate:

1. Open the Octet instrument door (lift the handle up).
2. Place the biosensor tray on the biosensor stage (left side) so that well A1 is located at the upper right corner (see Figure 6-52).
3. Place the sample plate on the sample stage (right side) so that well A1 is located at the upper right corner (see Figure 6-52).

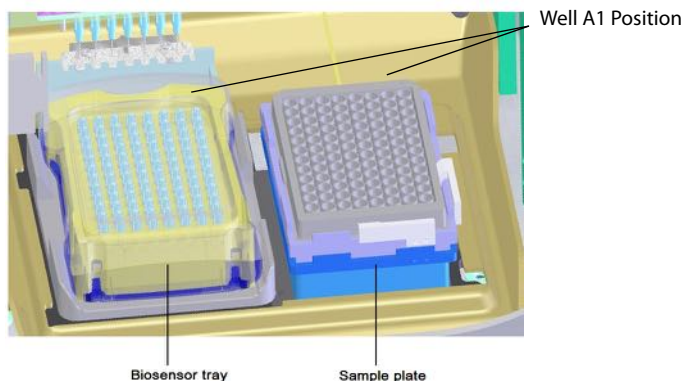


Figure 6-52: Biosensor Stage (left) and Sample Stage (right)



IMPORTANT: Ensure that the bottom of the sample plate and biosensor tray are flat on each stage.

4. **Octet RED96e only, optional.** Cover the microplate with the evaporation cover as recommended below to prevent evaporation from samples during analysis and lengthen the experiment time (only applies to RED96e instrument). For more information, see "Microplate Evaporation Cover" on page 51.




IMPORTANT: *Ensure the push bar is installed near the biosensor pickers in the Octet RED96e system prior to using the evaporation cover. The evaporation cover must be used with the push bar, otherwise the biosensors can crash into the cover.*

5. Close the Octet instrument door.
6. Allow the plate to equilibrate.

The time required for temperature equilibration depends on the temperature that your application requires and the initial temperature of the sample plate. For specific biosensor rehydration times, see the appropriate biosensor product insert. We recommend delaying the experiment time by 20 minutes to ensure the samples have equilibrated to the desired temperature, especially if you're cooling the samples to 15 °C or heating to 30 °C from an earlier experiment at 15 °C.

Starting an Experiment

To start the experiment:

1. Click the **Run Experiment** tab, or click the arrow  to access the Run Experiment window (see Figure 6-53).

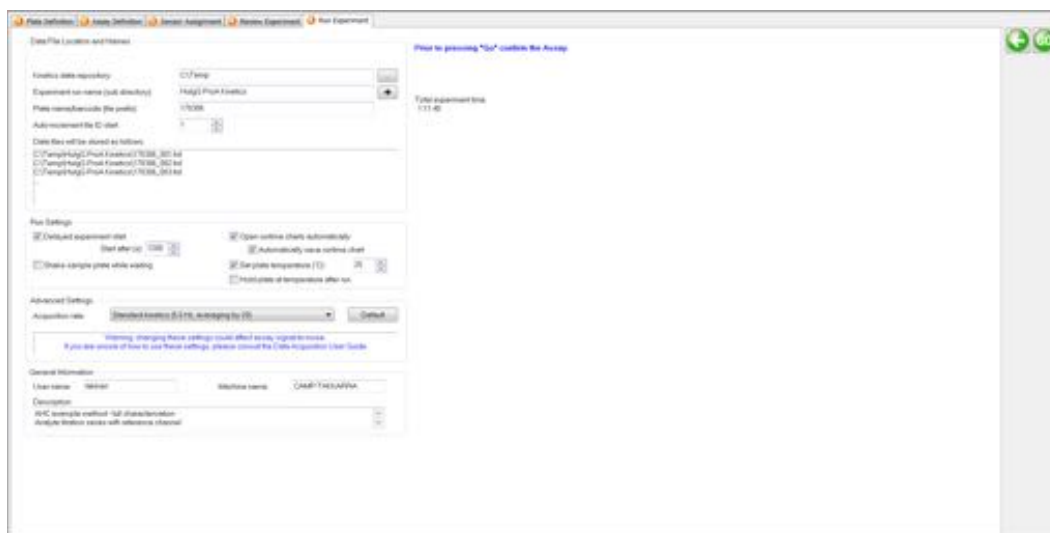



Figure 6-53: Run Experiment Window—Octet RED96

2. Confirm the defaults or enter new settings. See “Run Experiment Window Settings” on page 263 for more information on experimental settings.



NOTE: If you delay the experiment start, you have the option to shake the plate until the experiment starts. We recommend delaying the experiment time by 20 minutes to ensure the samples have equilibrated to the desired temperature, especially if you’re cooling the samples to 15 °C or heating to 30 °C from an earlier experiment at 15 °C.

3. **Optional if you are using a microplate evaporation cover.** Hold plate at temperature after run is pertinent when you are running very long experiments with the evaporation cover. If you are running a 10-12 hour assay and want to ensure that the plate temperature remains at the set plate temperature, then check **Hold plate at temperature after run**. If it is acceptable for the plate to go back to room temperature post-run, then leave that option unchecked.
4. To start the experiment, click .

If you specified a delayed experiment start, a message box displays the remaining time until the experiment starts.

If you selected the **Open runtime charts automatically** option, the **Runtime Binding Chart** window displays the binding data in real-time and the experiment progress (see Figure 6-54).



NOTE: For more details about the **Runtime Binding Chart**, see “Managing Runtime Binding Charts” on page 267.

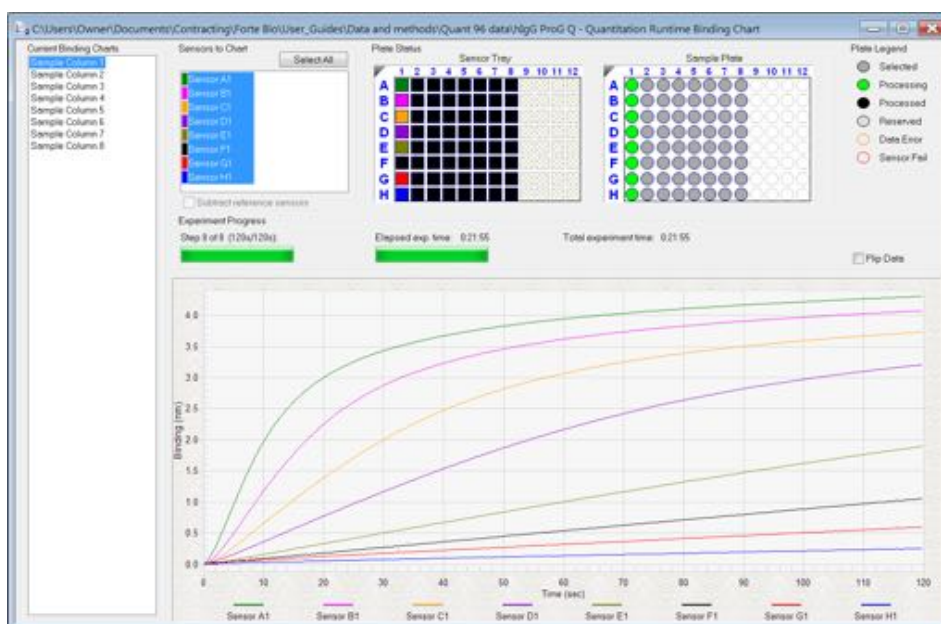


Figure 6-54: Runtime Binding Chart

5. Optional: Click **View > Instrument Status** to view the log file (see Figure 6-55).

The experiment temperature is recorded at the beginning of every experiment as well as each time the manifold picks up a new set of biosensors. Instrument events such as biosensor pick up, manifold movement, integration time, biosensor ejection and sample plate temperature are recorded in the log file.



WARNING: Do not open the Octet instrument door when an experiment is in progress. If the door is opened the data from the active acquisition step is lost. The data acquired in previous steps is saved, however the assay is aborted and cannot be restarted without ejecting the biosensors and starting from the beginning.



WARNING: N'ouvrez pas la porte de l'instrument Octet lorsqu'une analyse est en cours. En cas d'ouverture de la porte, les données issues de l'étape d'acquisition active seront perdues et cela entraînera l'échec de la procédure.



WARNING: Öffnen Sie die Instrumentenklappe des Octet-Systems nicht während eines laufenden Experiments. Wird die Klappe geöffnet, gehen die Daten des aktiven Erfassungsschritts verloren und das Experiment wird abgebrochen.

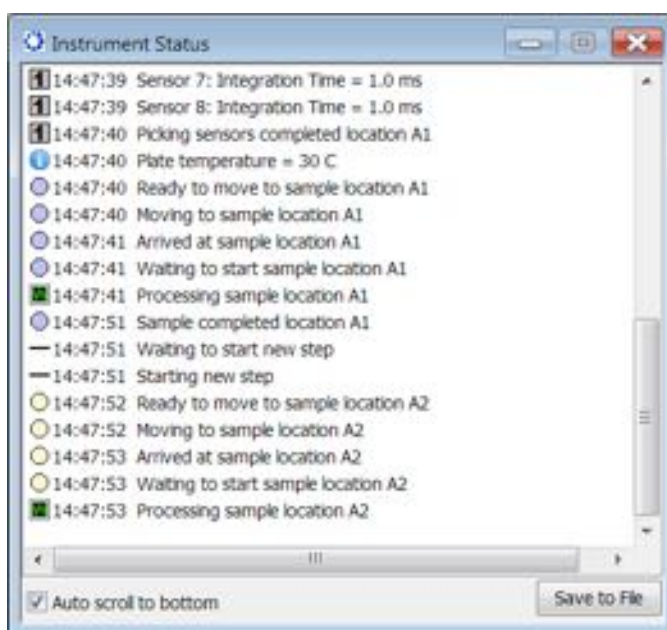


Figure 6-55: Instrument Status Log


Run Experiment Window Settings

The following **Data File Location and Name** settings are available on the **Run Experiment** Tab:

Table 6-9: Data File Location and Name

Item	Description
Assay type	The name of the selected assay.

Table 6-9: Data File Location and Name (Continued)

Item	Description
Quantitation data repository	The location where quantitation data files (.frd) are saved. Click Browse to select another data location.
	 NOTE: It is recommended that you save the data to the local machine first, then transfer to a network drive.
Experiment Run name (sub-directory)	Specifies a subdirectory name for the data files (.frd) that are created. The software generates one data file for each biosensor.
Plate name/barcode (file prefix)	A user-defined field where you can enter text or a barcode (barcode reader required).
2nd Plate name/barcode	A user-defined field where you can enter text or a barcode (barcode reader required) for a second plate.
Auto Increment File ID Start	Each file is saved with a number after the plate name. For example, if the Auto Increment File ID Start number is 1, the first file name is xxx_001.frd.

The following **Run Settings** are available on the **Run Experiment** Tab:

Table 6-10: Run Settings



Item	Description
Delayed experiment start	Specifies a time delay for the start of the experiment. Enter the number of seconds to wait before the experiment starts after you click  .
Start after	Enter the number of seconds to delay the start of the experiment.
Shake sample plate while waiting	If the experiment has a delayed start time, this setting shakes the plate until the experiment starts.
Open runtime charts automatically	Displays the Runtime Binding Chart for the current biosensor during data acquisition.
Automatically save runtime chart	Saves an image (.jpg) of the Runtime Binding Chart . The binding data (.frd) is saved as a text file, regardless of whether a chart image is created.



Table 6-10: Run Settings (Continued)

Item	Description
Set plate temperature (°C)	Specifies a plate temperature and enters the temperature in the dialog box. If not selected, the plate temperature is set to the default temperature specified in File > Options . The factory set default temperature is 30 °C.
<div> NOTE: If the actual plate temperature is not equal to the set plate temperature, a warning displays and the Octet System Data Acquisition software provides the option to wait until the set temperature is reached before proceeding with the run, continue without waiting until the set temperature is reached, or cancel the run.</div>	

Advanced settings are available for the Octet QK^e, Octet RED, Octet RED96 and Octet RED96e systems. The signal to noise ratio of the assay can be optimized by selecting different acquisition rates. The acquisition rate refers to the number of binding signal data points reported by the Octet system per second and is reported in Hertz (per second). A higher acquisition rate generates more data points per second and monitors faster binding events better than a slower acquisition rate. A lower acquisition rate allows the software enough time to perform more averages of the collected data. Typically, more averaging leads to

reduced noise and thus, better signal-to-noise ratios. Therefore, the frequency setting should be determined based on consideration of the binding rate, the amount of signal generated in your assay and some experimentation with the settings.

Table 6-11: Advanced Settings for Octet QK^e, Octet RED, Octet RED96 and Octet RED96e

Item	Description
Acquisition rate, Octet QK ^e	<ul style="list-style-type: none"> High sensitivity quantitation (0.3 Hz, averaging by 40)—The average of 40 data frames is reported as one data point. One data point is reported every 3.3 seconds. Standard quantitation (0.6 Hz, averaging by 5)—The average of five data frames is reported as one data point. One data point is reported every 1.6 seconds.
Acquisition rate, Octet RED, Octet RED96 and Octet RED96e	<div>  <p>NOTE: For the Octet RED, RED96 and RED96e systems, acquisition rate settings are available on the Plate Definition Tab.</p> </div> <ul style="list-style-type: none"> High concentration quantitation (10 Hz, averaging by 5) — The average of 5 data frames is reported as one data point. 10 data points are reported per second. High sensitivity quantitation (2 Hz, averaging by 50)—The average of 50 data frames is reported as one data point. Two data points are reported per second. Standard quantitation (5 Hz, averaging by 20)—The average of 20 data frames is reported as one data point. Five data points are reported per second.
Sensor offset (mm)	<p>Recommended sensor offset for quantitation—3 mm.</p> <div>  <p>NOTE: For more details on optimizing the sensor offset and acquisition rate please contact your local Pall ForteBio representative.</p> </div>
Default	Sets acquisition rate and sensor offset to the defaults.

The following **General Settings** are available on the **Run Experiment** Tab:


Table 6-12: General Settings

Item	Description
Machine name	The computer name that controls the Octet instrument and acquires the data.

Table 6-12: General Settings (Continued)

Item	Description
User name	The user logon name.
Description	A user-specified description of the assay or assay purpose. The description is saved with the method file (.fmf).

Stopping an Experiment

To stop an experiment in progress, click  or click **Experiment > Stop**.

The experiment is aborted. The data for the active biosensor is lost, the biosensor is ejected into the waste tray, and the event is recorded in the experimental log.



NOTE: After the experiment is run, the software automatically saves the experiment method (.fmf).

MANAGING RUNTIME BINDING CHARTS

If the **Open runtime charts automatically** check box is selected in the Run Experiment window, the Runtime Binding Charts are automatically displayed when data acquisition starts (see Figure 6-56). The **Runtime Binding Chart** window displays the current step number, time remaining for the current step, (total) elapsed experimental time, and total experiment time.

The **Runtime Binding Chart** is updated at the start of each experimental step. The active biosensor column is color-coded (A=green, B=magenta, C=orange, D=purple, E=olive, F=black, G=red, H=blue) within the **Sensor Tray Map**. Used sensor columns that are inactive are colored black. Active sample columns are colored green. Each data acquisition step is represented by **Sample Column X** in the **Current Binding Charts** box.

To selectively display acquisition data for a particular acquisition step:

1. Click the corresponding **Sample Column** number.
2. Select a sub-set of sensors for a displayed column under **Sensors to Chart** box (see Figure 6-56).



IMPORTANT: Do not close the **Runtime Binding Chart** window until the experiment is complete and all data is acquired. If the window is closed, the charts are not saved. To remove the chart from view, minimize the window. The Octet System Data Acquisition software saves the **Runtime Binding**

Chart as displayed at the end of the experiment. For example, modifying a chart by hiding the data for a particular biosensor will cause this data not to be included in the bitmap image generated at the end of the run.

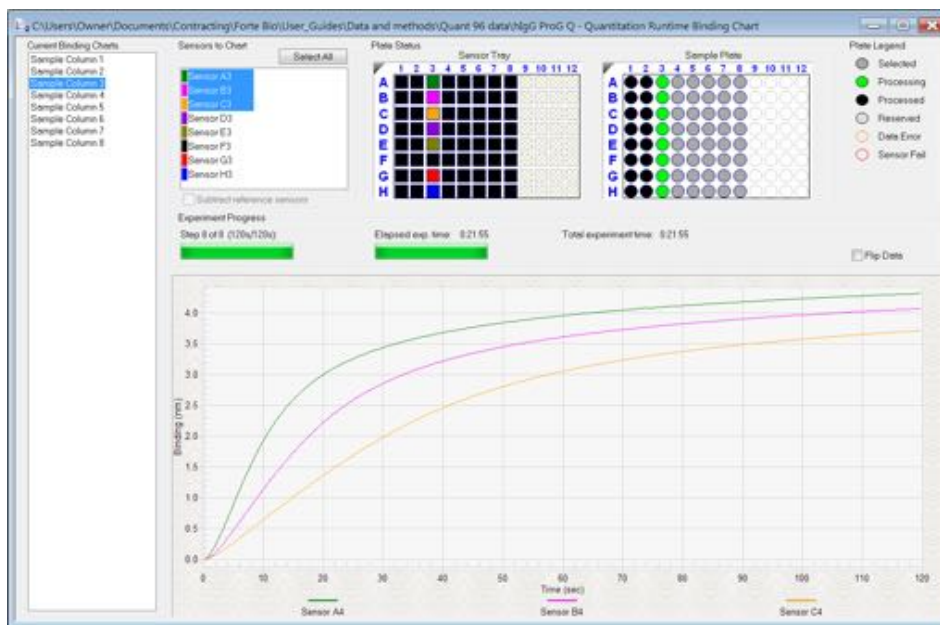


Figure 6-56: Runtime Binding Chart Window

Opening a Runtime Binding Chart

After an experiment is run, you can open and review the **Runtime Binding Chart** at any time:

1. Click **File > Open Experiment**.
2. In the dialog box that appears, select an experiment folder and click **Select**.

Viewing Reference-Subtracted Data

If the experiment includes reference biosensors, you can display reference-subtracted data during acquisition in the chart by clicking the **Subtract reference sensors** check box in the chart window. To view raw data, remove the check mark next to this option.

Reference biosensors can be designated:

- During experiment setup in the **Sensor Assignment** tab
- During acquisition in the Runtime Binding Chart **Sensors to Chart** box
- During analysis in the **Data Selection** tab

Designating a Reference Biosensor During Acquisition

To designate a reference biosensor during acquisition:

1. In the **Sensors to Chart** list or the **Sensor Tray**, right-click a biosensor and select **Reference** (see Figure 6-58).



Figure 6-57: Designating a Reference Biosensor in the Runtime Binding Chart

The selected biosensor will be shown with an **R** in the **Sensors to Chart** list and **Sensor Tray** (see Figure 6-58).

2. Click the **Subtract reference sensors** check box (see Figure 6-58).

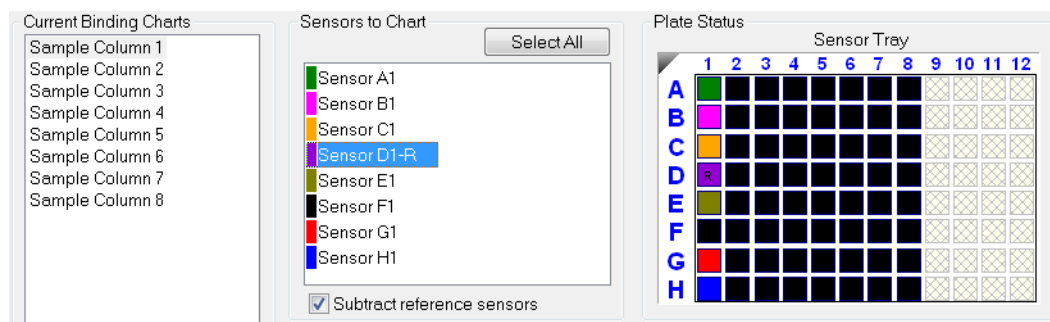


Figure 6-58: Subtract Reference Sensors check box in the Runtime Binding Chart



NOTE: Subtracting reference data in the **Runtime Binding Chart** only makes a visual change to the data on the screen. The actual raw data is unaffected and the reference subtraction must be re-done in data analysis if needed.

Viewing Inverted Data

The data displayed in the **Runtime Binding Chart** can be inverted during real-time data acquisition or data analysis after the experiment has completed. To invert data, select the **Flip Data** check box (see Figure 6-59). Uncheck the box to return to the default data display.

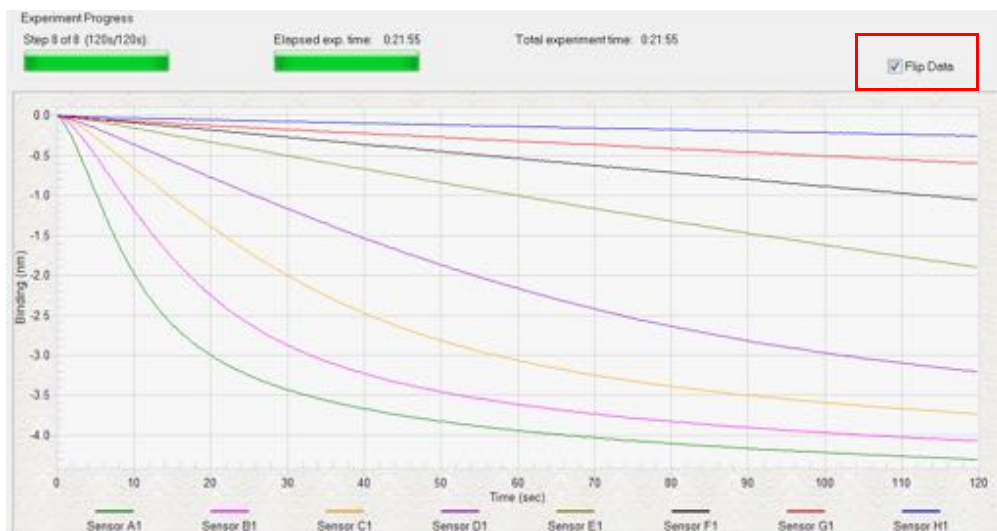


Figure 6-59: Data Inverted Using Flip Data Function

Magnifying the Runtime Binding Chart

To magnify the chart, press and hold the mouse button while you draw a box around the chart area to magnify.

To undo the magnification, right-click the chart and select **Undo Zoom**.

Scaling a Runtime Binding Chart

To scale the **Runtime Binding Chart**:

1. Right-click the chart and select **Properties**.
2. In the **Runtime Graph Properties** dialog box, select **Fullscale** or **Autoscale**.

Adding a Runtime Binding Chart Title

To add a **Runtime Binding Chart** title:

1. Right-click the chart and select **Properties**.
2. In the **Runtime Graph Properties** dialog box, enter a graph title or subtitle.

Selecting a Runtime Binding Chart Legend

To select a **Runtime Binding Chart** legend:

1. Right-click the chart and select **Properties**.
2. In the **Runtime Graph Properties** dialog box (see Figure 6-60), select one of the following legends:
 - Sensor Location
 - Sample ID
 - Sensor Information
 - Concentration/Dilution

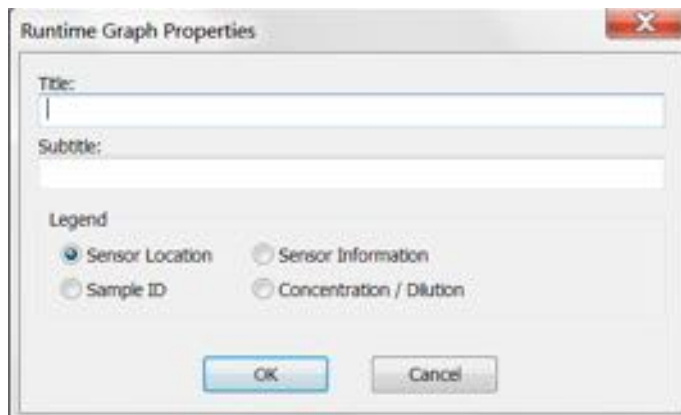


Figure 6-60: Selecting a Runtime Binding Chart Legend



NOTE: Text for **Sample ID**, **Sensor Information**, or **Concentration/Dilution** is taken from the **Plate Definition** and **Sensor Assignment** tabs, and must be entered before the experiment is started.

3. Click **OK**.

Viewing Multiple Runtime Binding Charts

To view multiple Runtime Binding Charts, click **Window > New Window**.

Exporting or Printing the Runtime Binding Chart

To export the **Runtime Binding Chart** as a graphic or data file:

1. Right-click the chart and select **Export Data**.
2. In the **Exporting** dialog box (see Figure 6-61), select the export options and click **Export**.

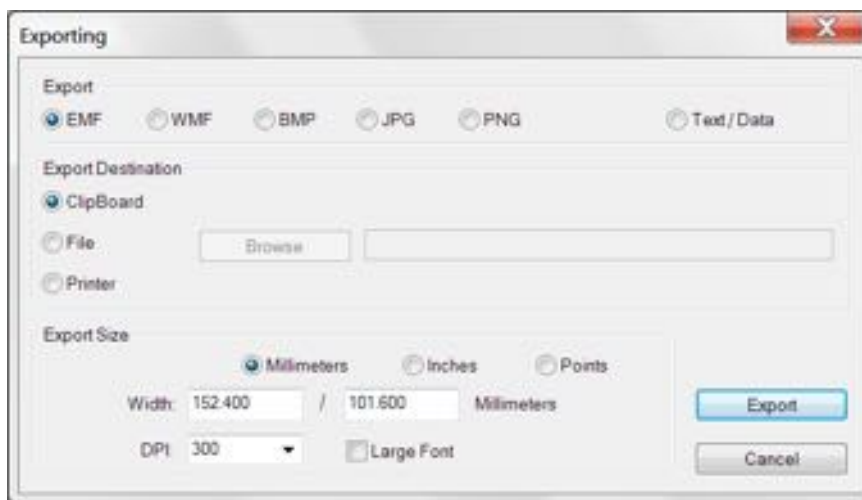


Figure 6-61: Exporting Dialog Box

Table 6-13: Runtime Binding Chart Export Options

Task	Export	Option	Export Destination	Result
	Text/ Data	EMF, WMF, BMP, JPG, or PNG		
Save the binding data	✓		Click File > Browse to select a folder and enter a file name.	Creates a tab-delimited text file of the numerical raw data from each biosensor. Open the file with a text editor such as Notepad.
Export the Runtime Binding Chart to a graphic file		✓	Click File > Browse to select a folder and enter a file name.	Creates a graphic image.

Table 6-13: Runtime Binding Chart Export Options (Continued)

Task	Export	Option	Export Destination	Result
Copy the Runtime Binding Chart		✓	Clipboard	Copies the chart to the system clipboard
Print the Runtime Binding Chart		✓	Printer	Opens the Print dialog box.

MANAGING EXPERIMENT METHOD FILES

After you run an experiment, the Octet System Data Acquisition software automatically saves the method file (.fmf), which includes the sample plate definition, biosensor assignment, and the run parameters. An experiment method file provides a convenient initial template for subsequent experiments. Open a method (.fmf) and edit it if necessary.



NOTE: When using the 21 CFR Part 11 version of the Octet System Data Acquisition software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.

Table 6-14: Managing Experiment Method Files

Menu Bar Command/ Toolbar Button	Description
File > Open Method File	Enables you to select and open a method file (.fmf)
File > Save Method File or	Saves one method file or all method files. Saves a method file before the experiment is run.
File > Save Method File As	Saves a method file to a new name so that the original file is not overwritten.

CUSTOM QUANTITATION ASSAYS

Defining a Custom Assay

To define a custom assay:

1. Click **Experiment > Edit Assay Parameters**.

The **Edit Assay Parameters** dialog box appears; see Figure 6-62.

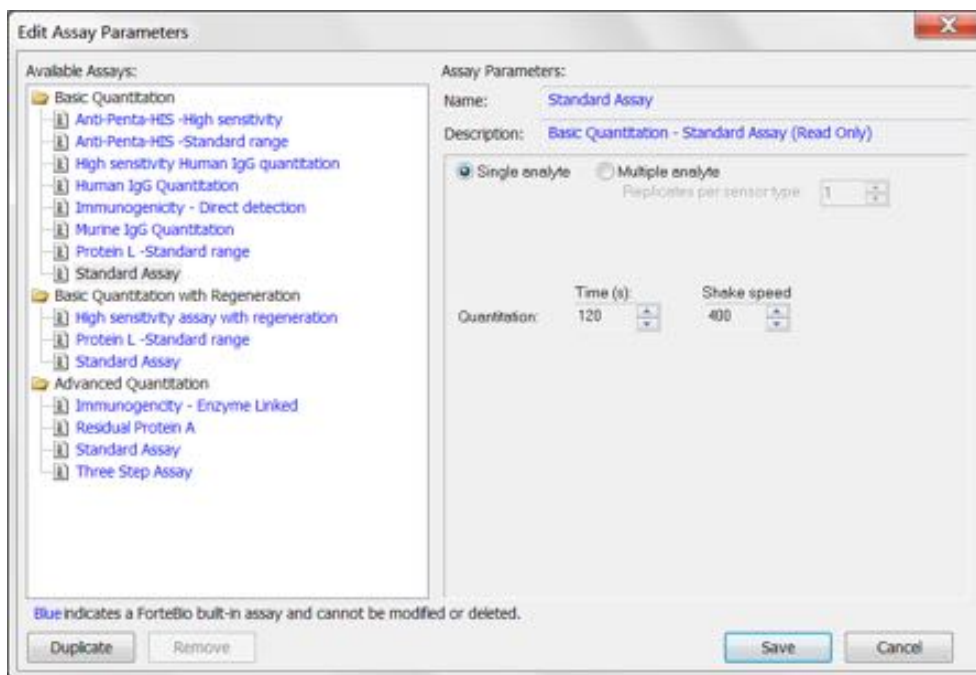


Figure 6-62: Edit Assay Parameters Dialog Box

2. In the directory tree of assays, select the type of standard assay to modify. For example, to define a new basic quantitation assay, in the Basic Quantitation folder, select **Standard Assay**.
3. Click **Duplicate**.
4. In the **New Assay** dialog box (see Figure 6-63 top), enter an **Assay name**.
5. Optional: In the **Assay Description**, enter information about the assay.
6. Click **Save**.

The new assay appears in the directory tree of available assays (see Figure 6-63 bottom).

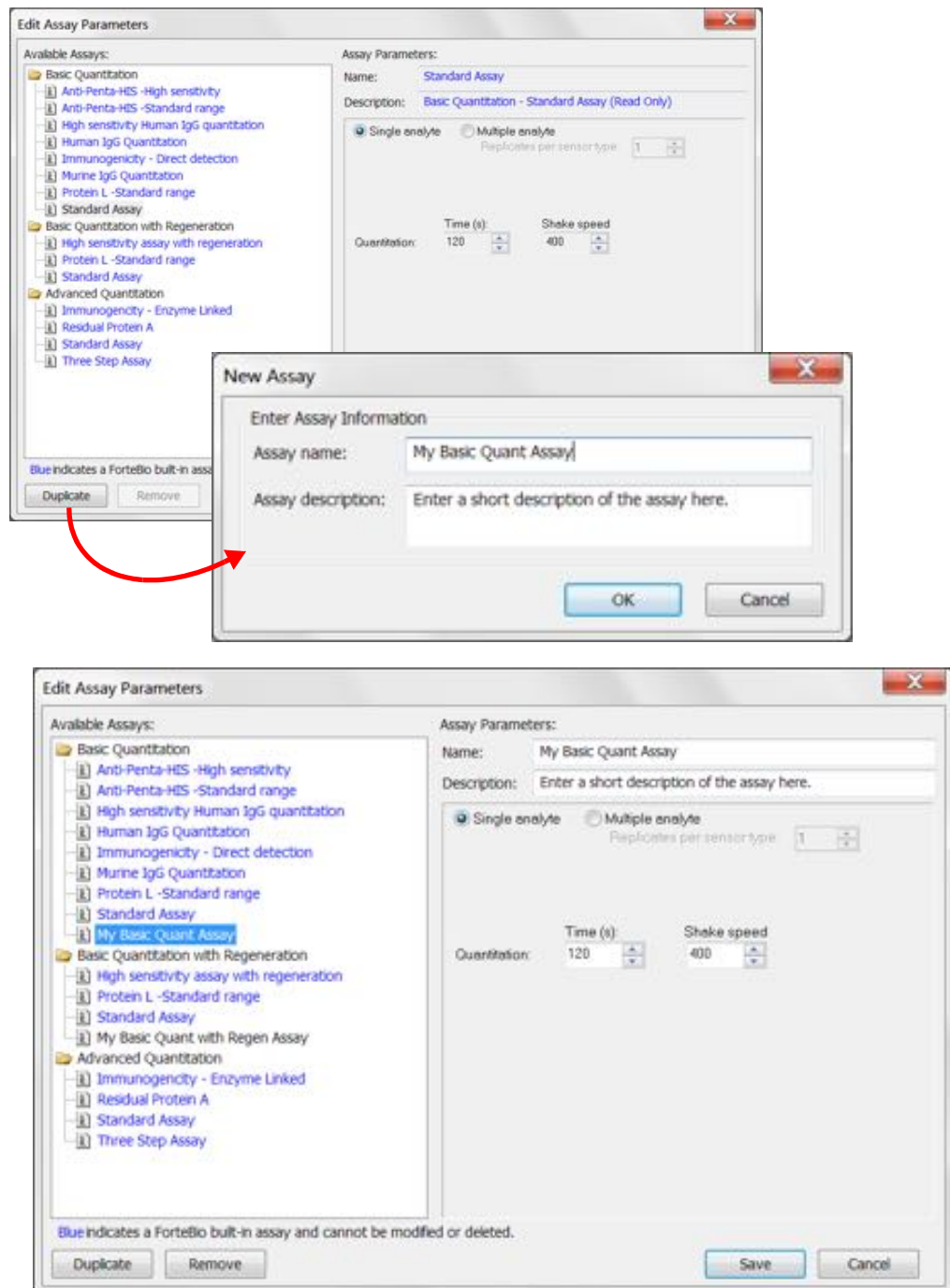


Figure 6-63: Defining a New Assay

Editing Assay Parameters

To edit assay parameters:

1. In the **Edit Assay Parameters** dialog box, confirm that the new assay is selected in **Available Assays** (see Figure 6-63 bottom).
2. Modify the assay parameters as needed. A complete list of parameters for each type of quantitation experiment follows this procedure.
3. Click **Save** to accept the new parameter values. The new assay is added to the system.



NOTE: Not all parameters are available for all of the assays.

Basic Quantitation Assay Parameters

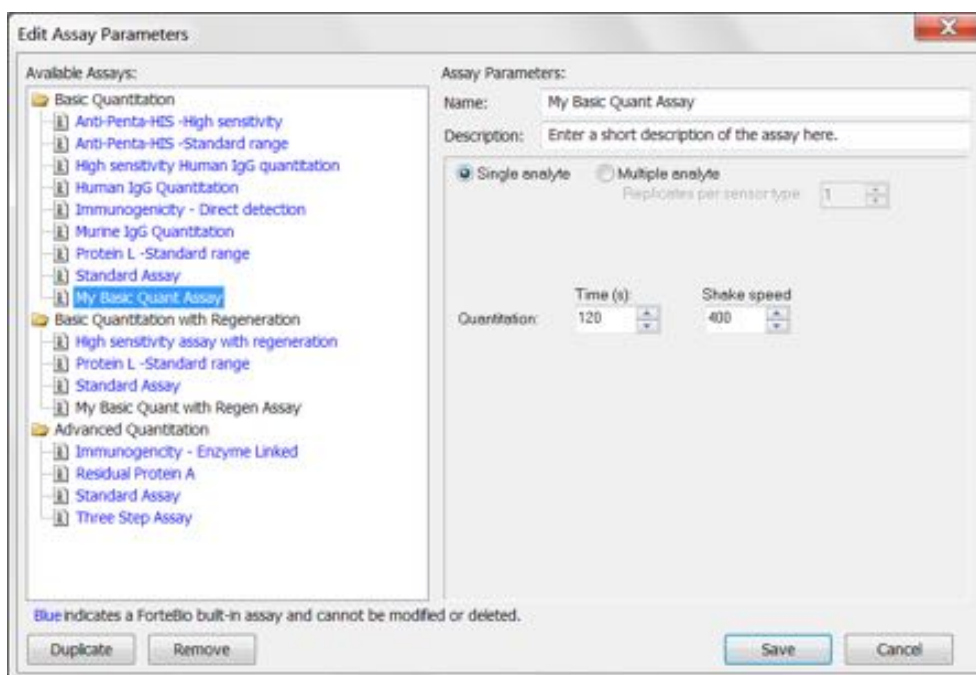



Figure 6-64: Assay Parameters—Basic Quantitation Assay

Table 6-15: Basic Quantitation Assay Parameters

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.

Table 6-15: Basic Quantitation Assay Parameters (Continued)

Parameter	Description
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time (s)	The duration of data acquisition seconds while the biosensor is incubated in sample.
	<div> NOTE: A subset of data points may be selected for processing during data analysis.</div>
Quantitation Shake speed (rpm)	The sample shaking speed (rotations per minute).

Basic Quantitation with Regeneration Assay Parameters

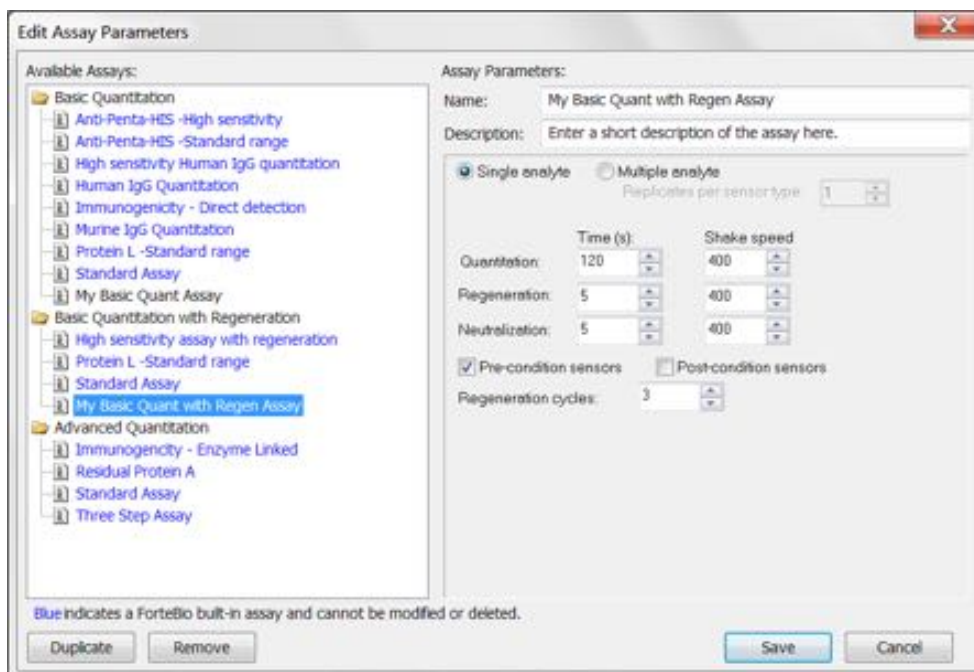


Figure 6-65: Assay Parameters—Basic Quantitation with Regeneration

Table 6-16: Assay Parameters—Basic Quantitation with Regeneration

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time(s) and Shake speed (rpm)	The duration of data acquisition in seconds while the biosensor is incubated in sample and the sample shaking speed (rotations per minute).
<div>➔</div> NOTE: A subset of data points may be selected for processing during data analysis.	
Regeneration Time(s) and Shake speed (rpm)	The duration time and shaking speed of the regeneration step where the biosensor is incubated in regeneration buffer to remove bound analyte.

Table 6-16: Assay Parameters—Basic Quantitation with Regeneration

Parameter	Description
Neutralization Time(s) and Shake speed (rpm)	The duration time and shaking speed of the neutralization step where the biosensor is incubated in neutralization buffer after the regeneration step.
Pre-condition sensors	Performs a set of regeneration/neutralization steps prior to the start of the experiment. The pre-conditioning settings are equivalent to the time and rpm settings for the regeneration in the assay. For example, an acidic pre-conditioning buffer maximizes the binding competence of Pro-A biosensors.
Post-condition sensors	Post-conditions biosensors, allowing re-racked biosensors to be stored in a regenerated state.
Regeneration cycles	The number of regeneration-neutralization cycles that a biosensor undergoes before reuse.

Advanced Quantitation Assay Parameters

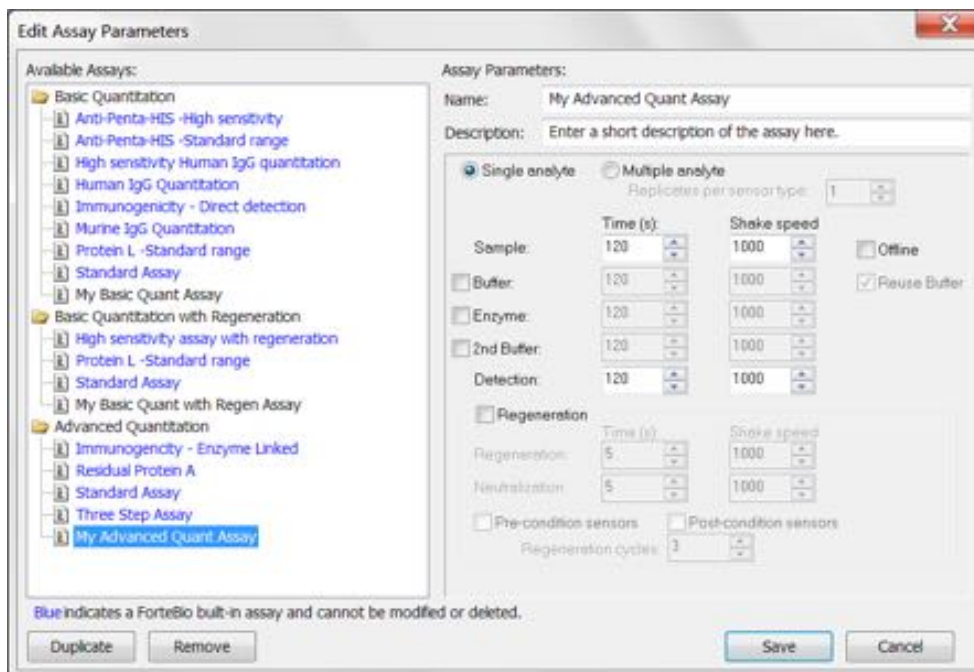


Figure 6-66: Assay Parameters—Advanced Quantitation

Table 6-17: Advanced Quantitation Assay Parameters

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Sample Time(s) and Shake speed (rpm)	The duration of data acquisition in seconds while the biosensor is incubated in sample and the sample shaking speed (rotations per minute).
<div>➔</div> NOTE: A subset of data points may be selected for processing during data analysis.	
Buffer Time(s) and Shake speed (rpm)	The duration of biosensor incubation in the first buffer in seconds and the sample shaking speed (rotations per minute).

Table 6-17: Advanced Quantitation Assay Parameters


Parameter	Description
Enzyme Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the enzyme solution and the sample shaking speed (rotations per minute).
2nd Buffer Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the second buffer solution and the sample shaking speed (rotations per minute).
Capture Antibody Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the first capture antibody solution and the shaking speed (rotations per minute).
2nd Antibody Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the secondary antibody solution and the shaking speed (rotations per minute).
Detection Time(s) & Shake speed (rpm)	The duration of data acquisition during the detection step in seconds in an advanced quantitation assay.
<div>  NOTE: A subset of data points may be selected for processing during data analysis. </div>	
Offline	Choose this option to incubate sample with biosensors outside the Octet system. Offline incubation is best performed on the Pall ForteBio Sidekick biosensor immobilization station.
Reuse Buffer	Allows buffer wells to be reused. If unselected, the number of buffer columns must equal the number of sample columns. If selected, the number of buffer columns may be less than the number of sample columns as the buffer columns are reused.
Regeneration Time(s) and Shake speed (rpm)	The duration time and shaking speed of the regeneration step where the biosensor is incubated in regeneration buffer to remove bound analyte.
Neutralization Time(s) and Shake speed (rpm)	The duration time and shaking speed of the neutralization step where the biosensor is incubated in neutralization buffer after the regeneration step.
Pre-condition sensors	Performs a set of regeneration/neutralization steps prior to the start of the experiment. The pre-conditioning settings are equivalent to the time and rpm settings for the regeneration in the assay. For example, an acidic pre-conditioning buffer maximizes the binding competence of Protein A biosensors.

Table 6-17: Advanced Quantitation Assay Parameters

Parameter	Description
Post-condition sensors	Post-conditions biosensors, allowing re-racked biosensors to be stored in a regenerated state.
Regeneration cycles	The number of regeneration-neutralization cycles that a biosensor undergoes before reuse.



NOTE: In an Advanced Quantitation experiment, this option is only available if the first step (biosensor incubation in sample) is performed online.

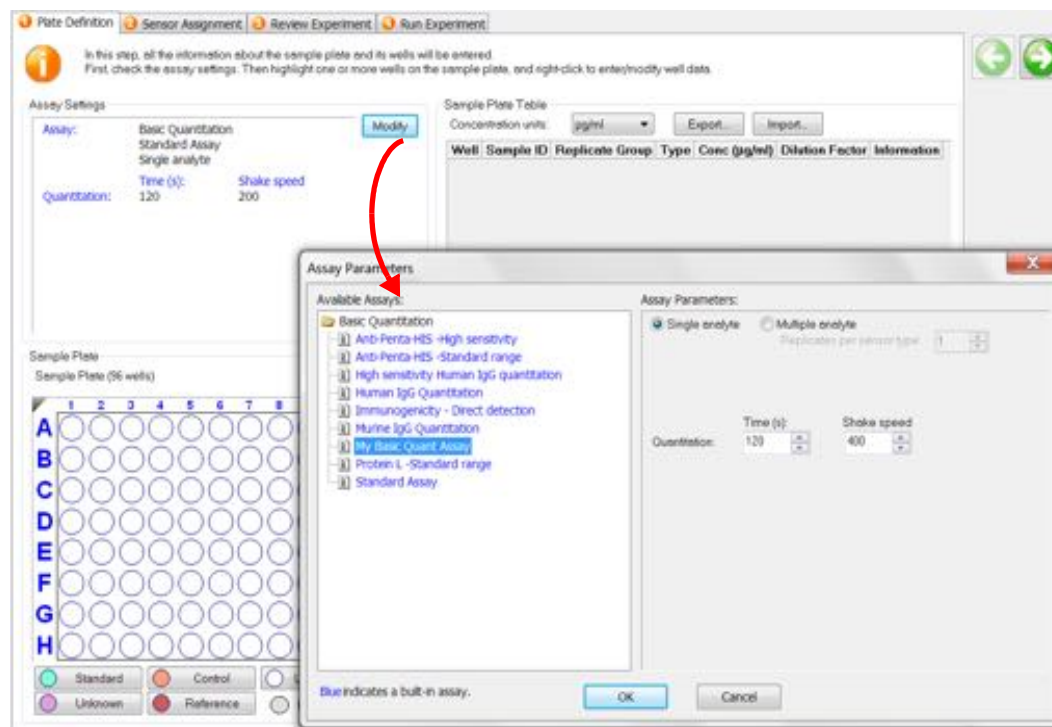
Selecting a Custom Assay

You can select a custom assay when you define a sample plate.

To select a custom assay:

1. In the **Plate Definition** tab, click **Modify** in the **Assay Settings** box.

The **Edit Assay Parameters** dialog box displays (see Figure 6-67).

**Figure 6-67: Selecting a Custom Assay**

2. Select the custom assay from the directory tree and click **OK**.

MULTI-STEP ADVANCED QUANTITATION EXPERIMENTS

The multi-step selection interface for Advanced Quantitation methods increases the flexibility to add more assay steps prior to the Sample or Detection steps. In addition, all steps in an Advanced Quantitation assay may be viewed and analyzed in the Octet Data Analysis software.

After starting the Octet system and the Octet Data Acquisition software, follow the steps below to set up and run an Advanced Quantitation experiment. You can start an Advanced Quantitation experiment using one of the following options:

- Launch the **Experiment Wizard**.
- Open a method file (.fmf) by clicking **File > Open Method File**. Method files may be saved and recalled using the **File** menu and are automatically saved when an experiment is run.
- On the menu bar, click **Experiment > Templates > Quantitation > Advanced Quantitation**.

These options are explained further in “Starting an Experiment Using the Experiment Wizard” on page 211.



NOTE: The Sample plate and the Reagent plate are now referred to as “Plate 1” and “Plate 2” in the software.

1. To add or edit assay steps in Tab 1 (Plate Definition), click **Modify** in Assay Settings to display the Assay Parameters window. Click on the **Step Type** drop-down list or highlight the parameter you want to change:

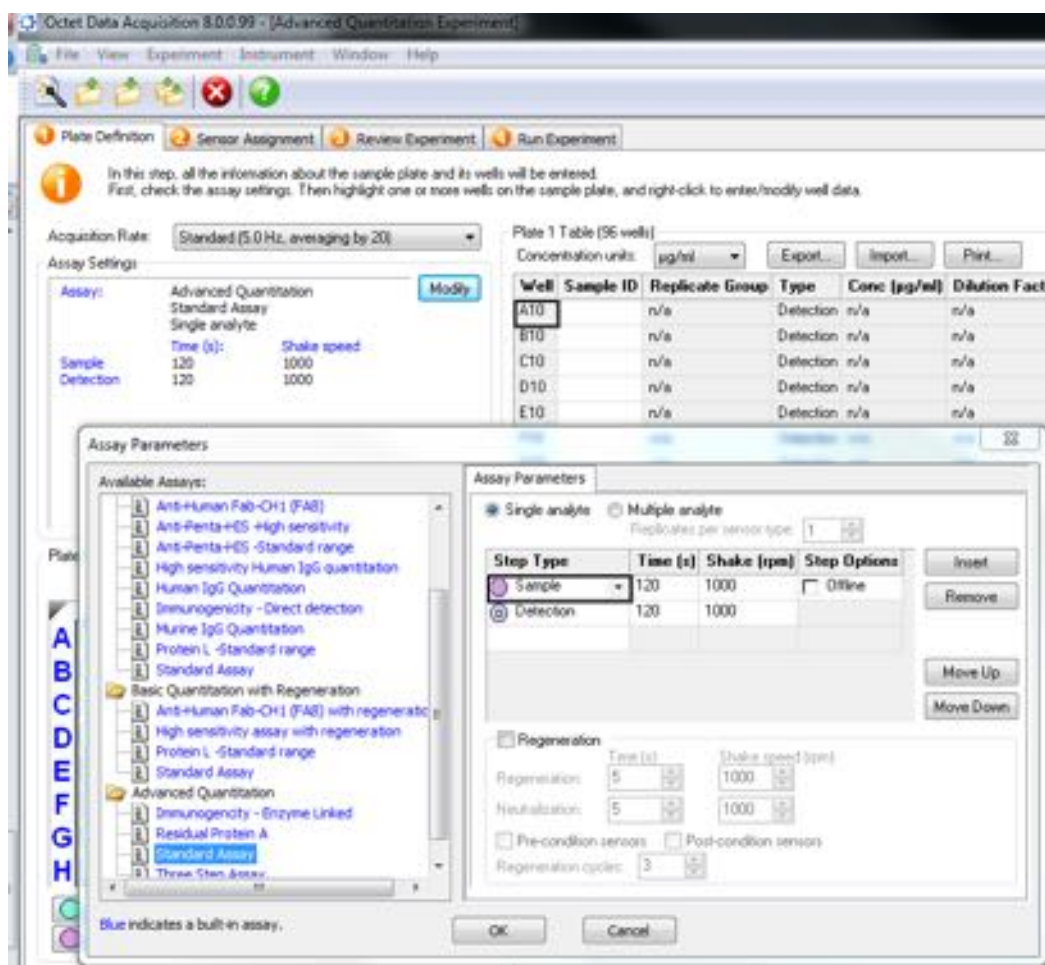


Figure 6-68: Assay Parameters Window.

To add or remove steps, click the **Insert** or **Remove** buttons. Individual steps may be re-organized using the **Move Up** or **Move Down** buttons. Click **OK** to save any changes.

2. Continue with the plate layout and sample well designation in Tab 1. For more details see “Defining the Sample Plate” on page 213, “Managing Sample Plate Definitions” on page 230 and “Managing Assay Parameter Settings” on page 234.
3. Proceed to Tab 2 (Sensor Assignment) and the remaining tabs as described starting with “Assigning Biosensors to Samples” on page 239 before running the Advanced Quantitation method.

CHAPTER 7:

Quantitation Experiments: Octet RED384, QK384 and HTX

Introduction	286
Starting a Quantitation Experiment	287
Defining the Sample Plate	289
Managing Sample Plate Definitions	313
Working with a Reagent Plate	316
Managing Assay Parameter Settings	319
Assigning Biosensors to Samples	325
Reviewing Experiments	343
Saving Experiments	343
Running a Quantitation Experiment	345
Managing Runtime Binding Charts	352
Managing Experiment Method Files	358
Custom Quantitation Assays	359
Multi-Step Advanced Quantitation Experiments	368




INTRODUCTION

A quantitation experiment enables you to determine analyte concentration within a sample using a reference set of standards. After starting the Octet system hardware and the Octet System Data Acquisition software, follow the steps (in Table 7-1) to set up and analyze a quantitation experiment.



NOTE: Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet Data Acquisition software versions 8.0 and higher.

Table 7-1: Setting Up and Analyzing a Quantitative Experiment

Software	Step	See
Data Acquisition 	1. Select a quantitation experiment in the Experiment Wizard or open a method file (.fmf).	"Starting a Quantitation Experiment" on page 287
	2. Define a sample plate or import a sample plate definition.	"Defining the Sample Plate" on page 289
	3. Define a or import a reagent plate (optional) for a Basic Quantitation with Regeneration experiment or an Advanced Quantitation experiment).	"Working with a Reagent Plate" on page 316
	4. Confirm or edit the assay settings.	"Modifying Assay Parameter Settings" on page 319
	5. Assign biosensors to samples.	"Assigning Biosensors to Samples" on page 325
	6. Run the experiment.	"Running a Quantitation Experiment" on page 345
Data Analysis  or Data Analysis HT 	7. Analyze the binding data.	<i>Octet System Data Analysis Software or Octet Data Analysis HT Software User Guide</i>
	8. Generate a report.	

STARTING A QUANTITATION EXPERIMENT



NOTE: Before starting an experiment, check the plate temperature displayed in the status bar. Confirm that the temperature is appropriate for your experiment and if not, set a new temperature. If the Octet System Data Acquisition software is closed, the plate temperature will reset to the default startup value specified in the **Options** dialog box when the software is relaunched.

You can start a quantitation experiment using one of the following options:

- Launch the **Experiment Wizard**.
- Open a method file (.fmf) by clicking **File > Open Method File**. Method files may be saved and recalled using the **File** menu and are automatically saved when an experiment is run. For more details on method files see “Managing Experiment Method Files” on page 358.
- On the menu bar, click **Experiment > Templates > Quantitation**.



NOTE: When using the 21 CFR Part 11 version of the Octet System Data Acquisition software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.

Starting an Experiment Using the Experiment Wizard

To start an experiment using the **Experiment Wizard**:


1. If the **Experiment Wizard** is not displayed when the software is launched, click the **Experiment Wizard** toolbar button  or click **Experiment > New Experiment Wizard (Ctrl+N)** from the **Main Menu**.
2. In the **Experiment Wizard**, select **New Quantitation Experiment** (see Figure 7-1, left).
3. Select a type of quantitation experiment (see Table 7-2 for options).

Table 7-2: Quantitation Experiment Selection

Quantitation Experiment	Description
Basic Quantitation	A standard quantitation assay.
Basic Quantitation with Regeneration	A standard quantitation assay that enables regeneration of biosensors.

Table 7-2: Quantitation Experiment Selection

Quantitation Experiment	Description
Advanced Quantitation	A standard two-or three-step quantitation assay that enables signal amplification for higher detection sensitivity.

- Optional: You can also click **Recent Methods** to display a list of recently used methods. You can open any method file from the list and use it with or without modifications to run a new experiment.

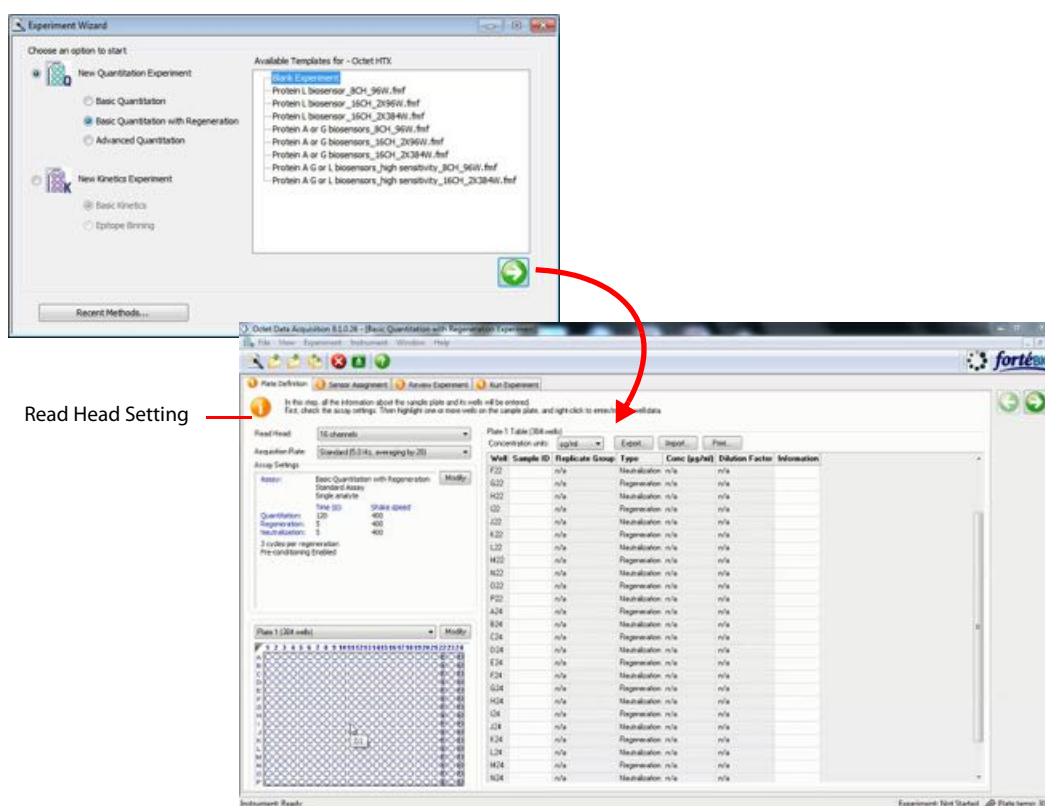


Figure 7-1: Selecting an Experiment Type in the Experiment Wizard (for Octet RED384)

- Click the  arrow.

The **Experiment** window displays (Figure 7-1, right).

- Octet HTX Only.** Open Tab 1 (Plate Definition) for Read Head configuration and plate(s) layout. The default Read Head setting is 96 channels, which dips 96 biosensors simultaneously for a given assay step.

- Click on the drop-down list for Read Head to select 96, 48, 32, 16 or 8 channels (Figure 7-2) as the new Read Head setting. An individual assay is defined as a series of steps or dips starting with pick up of the biosensors, followed by the assay steps, and ending with ejection of biosensors back into the biosensor tray or disposal chute. A Quantitation method file may contain multiple assays.

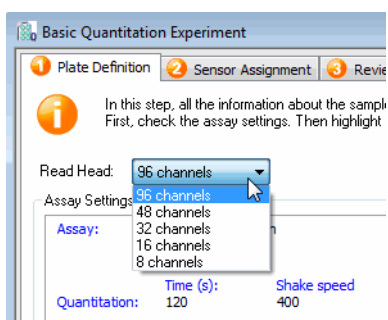


Figure 7-2: Selecting Read Head Channels

DEFINING THE SAMPLE PLATE



NOTE: Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet Data Acquisition software versions 8.0 and higher (Figure 7-3).

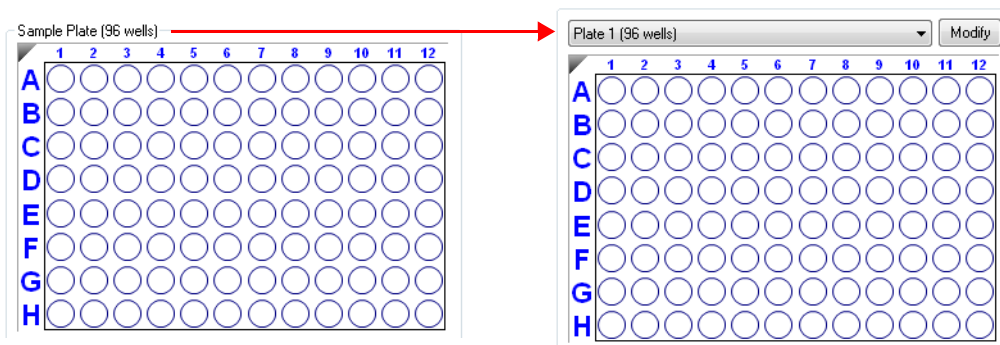


Figure 7-3: Sample Plate Renamed Plate 1 in Software Versions 8.0 and Higher

Table 7-3 lists the steps to define a sample plate.

Table 7-3: Defining a Sample Plate

Step	See Page
1. Select the instrument read head configuration (8 or 16 channels).	290
2. Select the sample plate format (96 or 384 wells).	291
3. Designate the samples.	295
4. Annotate the samples (optional).	307
5. Save the sample plate definition (optional).	313

Read Head Configuration and Plate Layout

Octet RED384 and QK384

The Octet read head contains the collection optics. If the read head is set to 8 channels, one column of 8 biosensors interrogate 8 plate wells. If the read head is set to 16 channels, two columns of biosensors interrogate 16 wells (Figure 7-1).

The read head configuration and the plate format (96 or 384 wells) determine the plate layout (Figure 7-4).

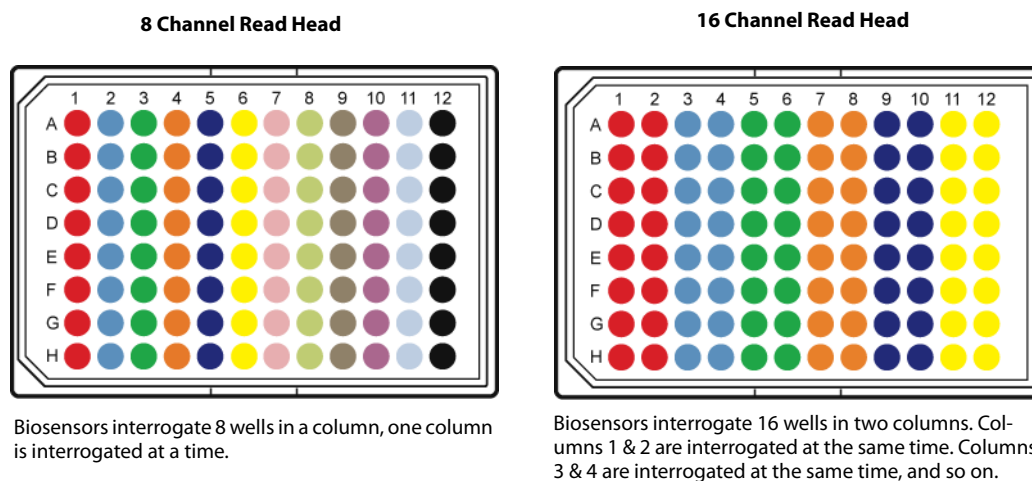


Figure 7-4: Color-Coded Wells Display How Biosensors Interrogate a 96-well Plate, 8 Channel or 16-Channel Read Head

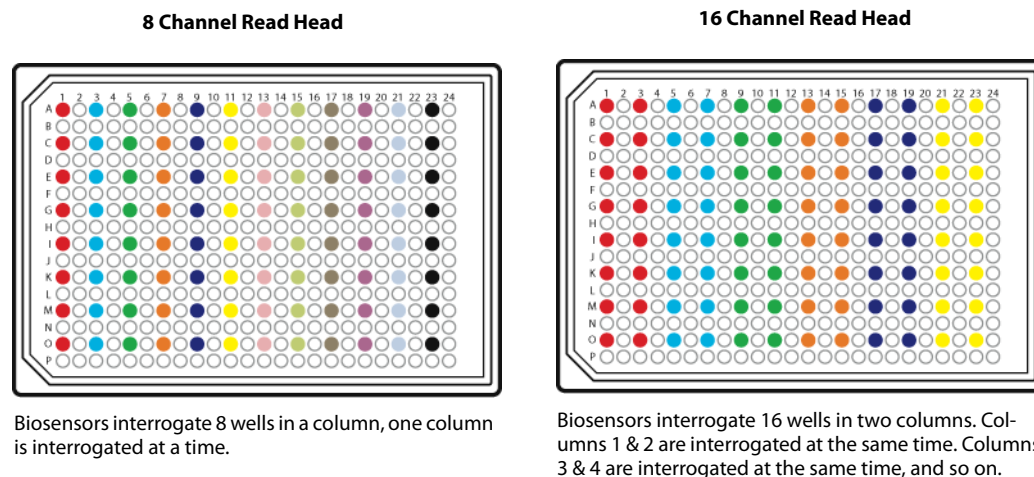


Figure 7-5: Color-Coded Wells Display How Biosensors Interrogate a 384-well Plate, 8 Channel or 16 Channel Read Head



NOTE: Keep the read head configuration in mind when laying out the sample plate. While reading a 384-well sample plate, both the 8 channel and 16 channel read heads can freely step through the plate by either moving left or right to step across columns or step one row up or down.

Octet HTX

The Octet HTX has a user-selectable Read Head for monitoring 8, 16, 32, 48, or 96 wells in parallel so you can tailor your assay design to maximize either throughput or detection sensitivity.

The 96 biosensor mode uses multiplexer switching to read 96 wells simultaneously either in a 96- or 384-well plate, with similar sensitivity as the Octet QK384 system. Large sample sets are analyzed in the shortest amount of time using this Read Head setting, which is also ideal for rapid, whole plate analysis and biosensor loading in multi-step assays.

Figure 7-6 shows the biosensor layout in a 96- and 384-well plate with the 96-channels Read Head setting. Biosensors interrogate 96 wells in 12 columns at the same time.

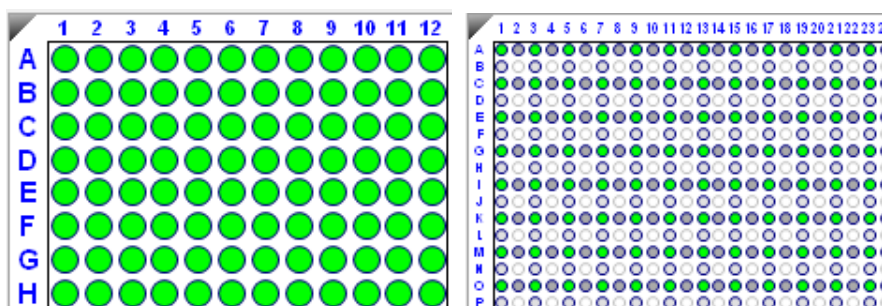


Figure 7-6: Biosensor Layout in 96- and 384-well Plates Using 96-channels Read Head Setting.



NOTE: A column of 16 wells is read in two sets of interrogations. Biosensors interrogate 8 wells in a column at a time: rows A, C, E, G, I, K, M and O are read first followed by rows B, D, F, H, J, L, N and P.

The 32 and 48 biosensor modes also use multiplexer switching to read 32 and 48 wells in parallel, with sensitivity equivalent to the Octet QK384 system. Cross-blocking experiments as large as 32 x 32 or larger may be accomplished with the 32 or 48 biosensor modes combined with 384-well tilted-bottom plates in a shorter amount of time compared to other Octet systems.

In Figure 7-7, biosensors interrogate 32 wells in 4 columns at a time or 48 wells in 6 columns at a time. Columns 1, 3, 5 and 7 are interrogated at the same time, and so on for the 32-channels setting. Columns 1, 3, 5, 7, 9 and 11 are interrogated at the same time, and so on for the 48-channel setting:



Figure 7-7: Biosensor Layout in 384-well Plates Using 32 (left) and 48 (right) Channels Read Head Setting.

The 8 and 16 biosensor modes provide high sensitivity for measuring small molecule binding interactions and protein quantitation down to 50 ng/mL, similar to the Octet RED96 and RED384 systems. These two modes are best for assays requiring a wide dynamic range or fine signal resolution, and may be combined with the other Read Head options in a single experiment.

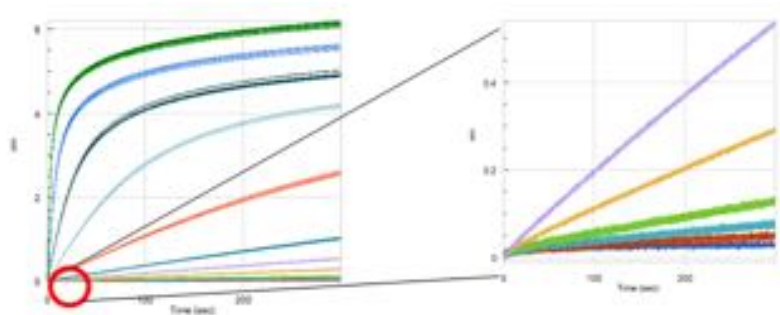


Figure 7-8: Zoomed View of Closely Overlaid Traces Shows Fine Signal Resolution for Human IgG Quantitation Assay with Protein A Biosensors

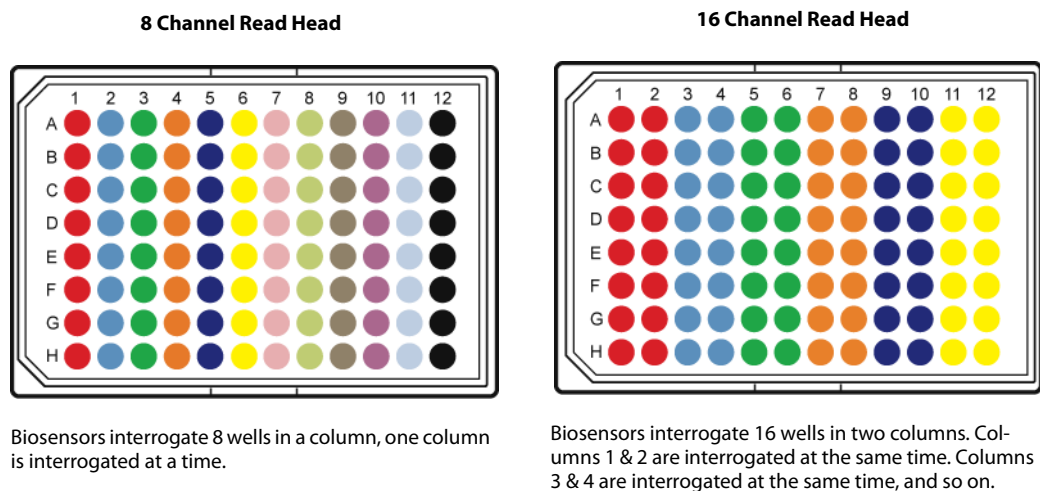


Figure 7-9: Color-Coded Wells Display How Biosensors Interrogate a 96-well Plate, 8 Channel or 16-Channel Read Head

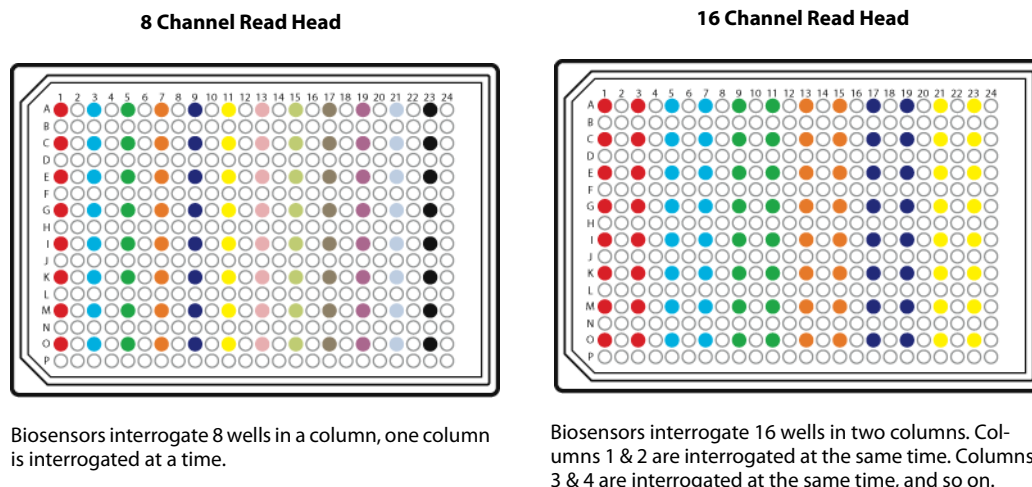


Figure 7-10: Color-Coded Wells Display How Biosensors Interrogate a 384-well Plate, 8 Channel or 16 Channel Read Head

Changing the Plate Format



NOTE: Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet Data Acquisition software versions 8.0 and higher.

To change the sample plate format:

1. Click the **Modify** button above the plate map.
2. In the **Modify Plates** box, select **96 Well** or **384 Well** format.

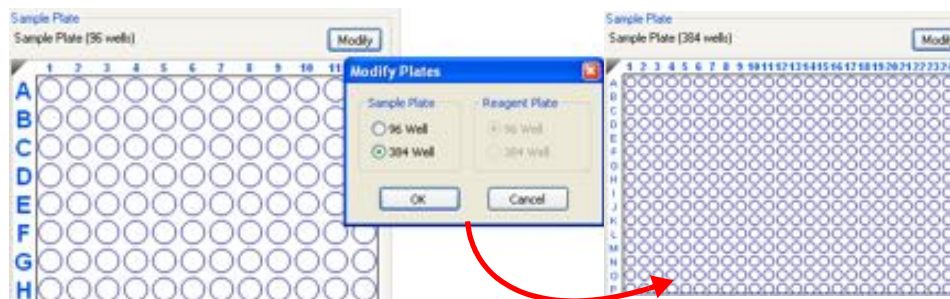


Figure 7-11: Changing the Sample Plate Format



NOTE: In Basic Quantitation with Regeneration and Advanced Quantitation experiments, a reagent plate format option is also available. Please refer to “Working with a Reagent Plate” on page 316 for more information.

Designating Samples









NOTE: Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet Data Acquisition software versions 8.0 and higher.

Each well may be designated as a **Standard**, **Unknown**, **Control**, or **Reference**. A well may also remain **Unassigned** or be designated as **Reserved** by the system for Basic Quantitation with Regeneration and Advanced Quantitation experiments.



NOTE: It is important to define all of the wells that will be used in the assay. Only wells that are selected and defined using one of the sample types in Table 7-4 will be included in the assay.

Table 7-4: Types of Sample Wells

Icon	Description
 Standard	Contains an analyte of known concentration. Data from the well is used to generate a standard curve during analysis.
 Unknown	Contains an analyte of unknown concentration. The concentration of the analyte is calculated from the well data and the standard curve.
 Control	A control sample, either positive or negative, of known analyte composition. Data from the well is not used to generate a standard curve during analysis. <ul style="list-style-type: none"> Positive Control: A control sample that contains analyte of known concentration Negative Control: A control sample known not to contain analyte
 Reference	Provides a baseline signal which serves as a reference signal for Unknowns , Controls , and Standards . The reference signal can be subtracted during data acquisition in the Runtime Binding Chart and during data analysis.
 Unassigned	Not used during the experiment.
 Reserved	Used by the system during Basic Quantitation with Regeneration experiments and Advanced Quantitation multi-step experiments for Regeneration (R) , Neutralization (N) , Detection (D) , or Capture Antibody (C) . Reserved wells are not available for use as Standards , Unknowns , Controls , or References .

Reserved Wells

In a Basic Quantitation with Regeneration or an Advanced Quantitation experiment, the **Sample Plate Map** includes gray wells. These wells are reserved by the system and specify the location of particular sample types. The default location of the reserved wells depends on the sample plate format (96 or 384-wells) and the Octet instrument read head configuration (8 or 16 channels).

Reserved samples cannot be removed from the sample plate, but you can change their column location. To change the location of a reserved column (R, N, D, or C) right-click a column header in the **Sample Plate Map** and select **Regeneration**, **Neutralization**, **Detection**, or **Capture Antibody**.

Table 7-5: Reserved Well Requirements

Reserved Well	Must Contain
R Regeneration	Regeneration buffer that is used to remove analyte from the biosensor (typically low pH, high pH, or high ionic strength).
N Neutralization	Neutralization buffer that is used to neutralize the biosensor after the regeneration step.
D Detection	Secondary antibody or precipitating substrate that is used with an enzyme-antibody conjugate to amplify the analyte signal. Sample concentrations are computed using the binding data from the detection wells.
C Capture Antibody	Capture antibody or molecule that is used to immobilize the specific molecule of interest onto the biosensor.

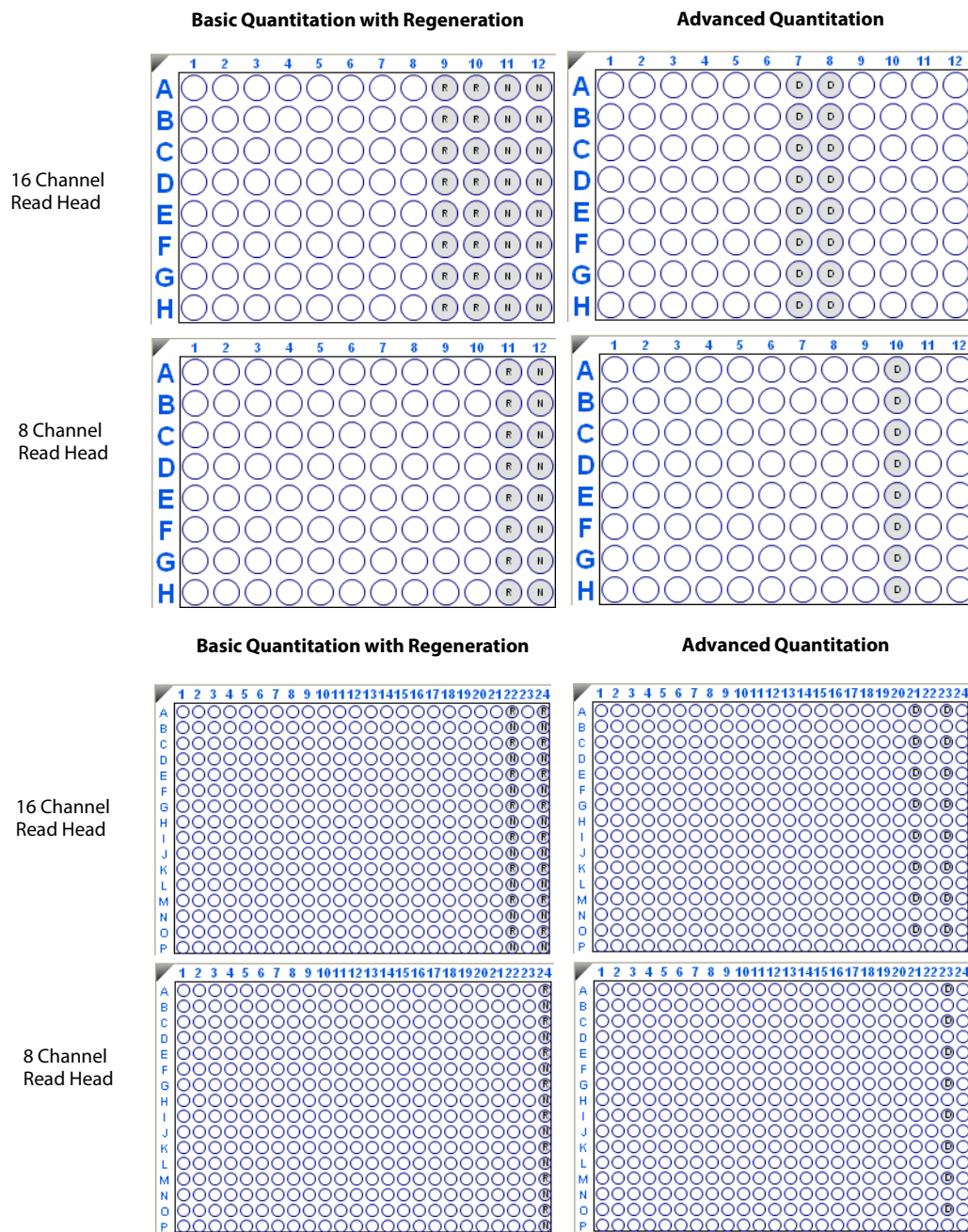


Figure 7-12: Default Locations for Reserved Wells in 96-well (top) and 384-well Sample Plate Maps (bottom)

Selecting Wells in the Sample Plate Map



NOTE: Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet Data Acquisition software versions 8.0 and higher.

There are several ways to select wells in the **Sample Plate Map**:

- Click a column header or select adjacent column headers by click-hold-drag (Figure 7-13 left). To select non-adjacent columns, hold the **Ctrl** key and click the column header.
- Click a row header or select adjacent row headers by click-hold-drag (Figure 7-13, center).
- Click a well or draw a box around a group of wells (Figure 7-13, right).

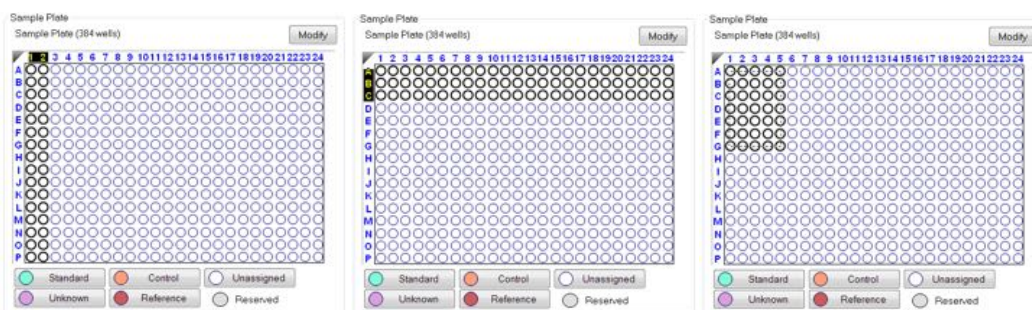


Figure 7-13: Selecting Wells in the Sample Plate Map



NOTE: Shift-clicking in the **Sample Plate Map** mimics the head of the instrument during the selection.

Designating Standards



NOTE: Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet Data Acquisition software versions 8.0 and higher.

To designate standards:

1. In the **Sample Plate Map**, select the wells to define as standards.
2. Click the **Standard** button below the **Sample Plate Map** (see Figure 7-14), or right-click and select **Standard**.

The standards are marked in the plate map and the **Sample Plate Table** is updated.

3. Select the concentration units for the standards using the **Concentration Units** drop-down list above the **Sample Plate Table**.

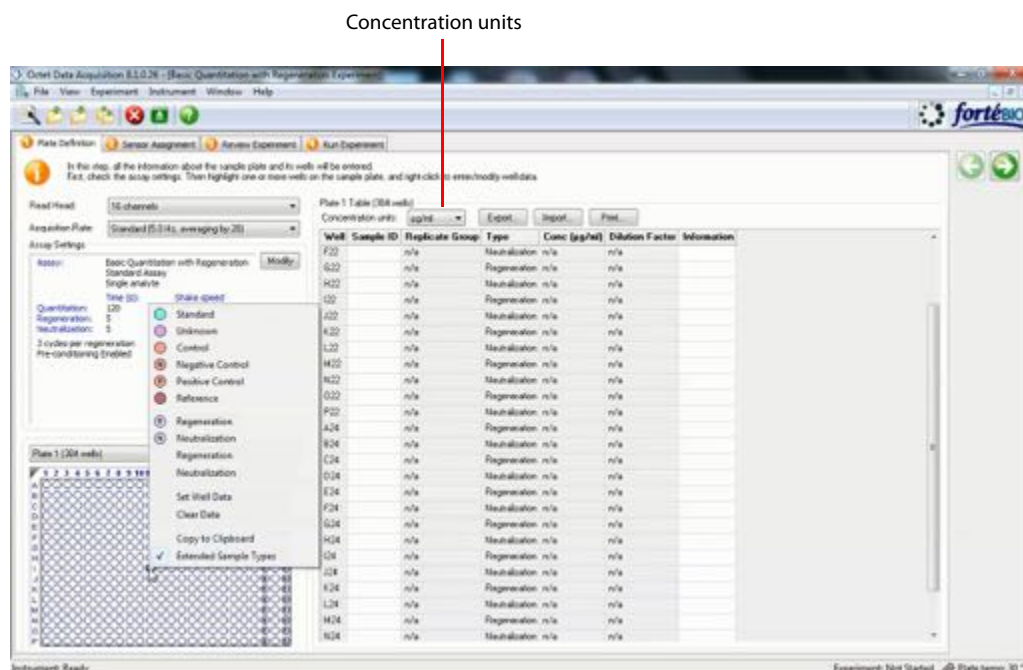


Figure 7-14: Plate Definition Window—Designating Standards

To remove a well designation, select the well(s) and click **Unassigned**. Or, right-click the well(s) and select **Clear Data**.

Assigning Standard Concentrations Using a Dilution Series

To assign standard concentrations using a dilution series:

1. In the **Sample Plate Map**, select the standard wells, right-click and select **Set Well Data**.

The **Set Well Data** dialog box displays (see Figure 7-15).

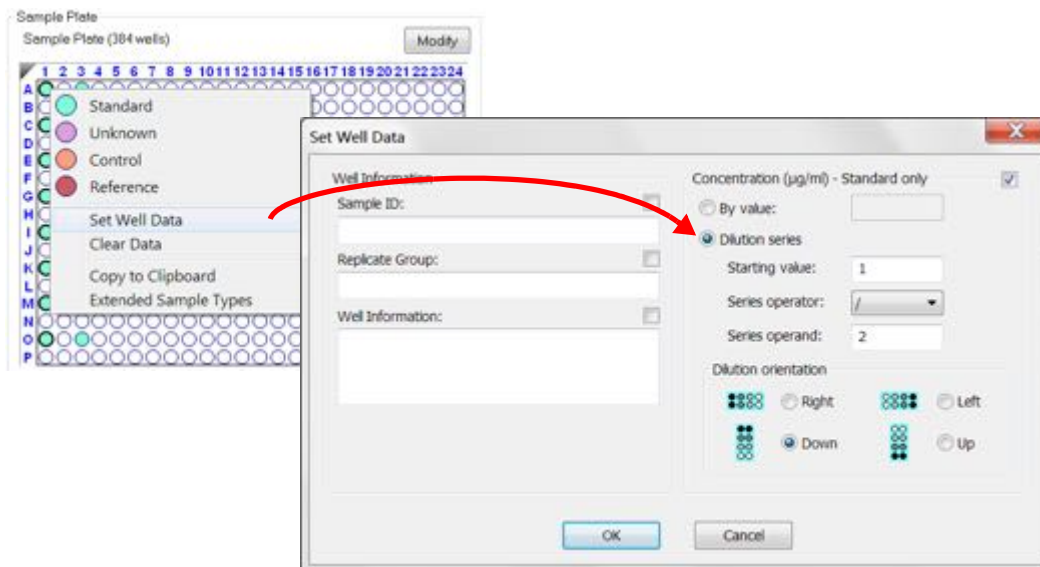


Figure 7-15: Sample Plate Map—Setting a Dilution Series

2. Select the **Dilution Series** option and enter the starting concentration value.
3. Select a series operator, enter an operand, and select the appropriate dilution orientation (see Figure 7-17).

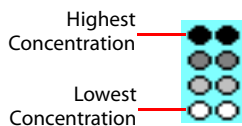


Figure 7-16: Concentration Representation in Dilution Series

4. Click **OK**.

The **Sample Plate Table** will display the standard concentrations entered.

Assigning a User-Specified Concentration to Standards

To assign a user-specified concentration to standards:

1. In the **Sample Plate Map**, select the standard wells, right-click and select **Set Well Data**.

The **Set Well Data** dialog box displays (see Figure 7-17).

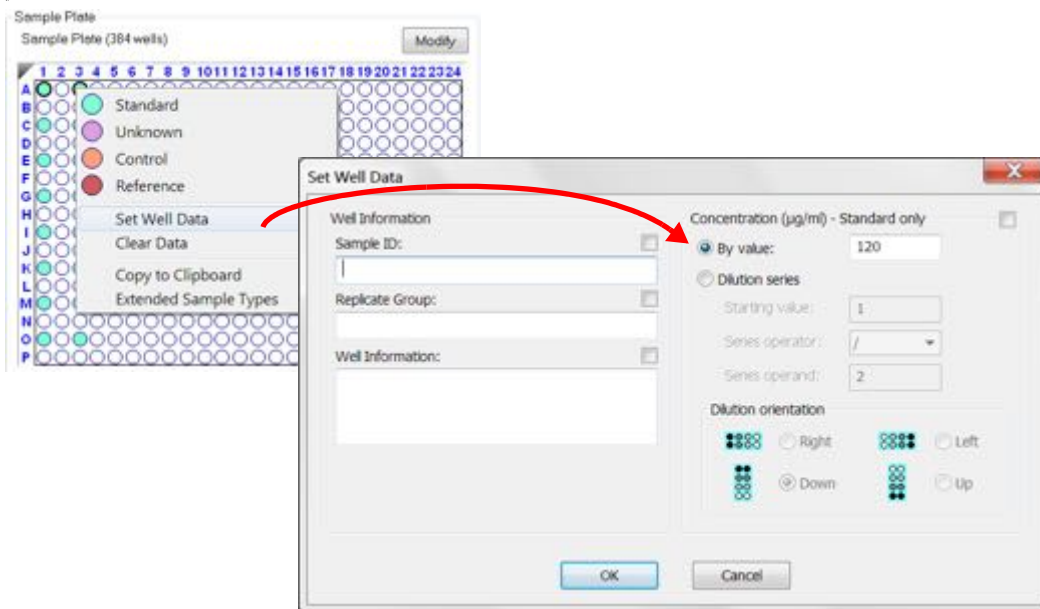


Figure 7-17: Sample Plate Map—Assigning a Standard Concentration

2. Select the **By value** option and enter the starting concentration value.
3. Click **OK**. The **Sample Plate Table** will display the standard concentrations entered.

Editing an Individual Standard Concentration

To enter or edit an individual standard concentration, in the **Conc** column of the **Sample Plate Table**, double-click the value and enter a new value (see Figure 7-18).

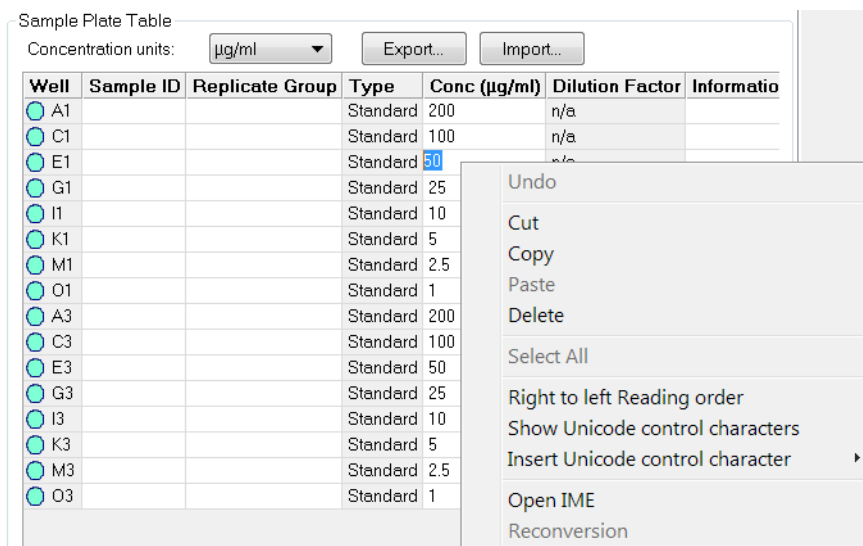


Figure 7-18: Sample Plate Table—Shortcut Menu of Edit Commands



NOTE: Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the **Sample Plate Table**. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the **Sample Plate Map** menu used to designate sample types.

Designating Unknowns



NOTE: Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet Data Acquisition software versions 8.0 and higher.

To designate unknowns in the **Sample Plate Map**, select the wells to define as unknown, right-click and select **Unknown**. The unknown wells are marked in the plate map and the **Sample Plate Table** is updated (see Figure 7-19).

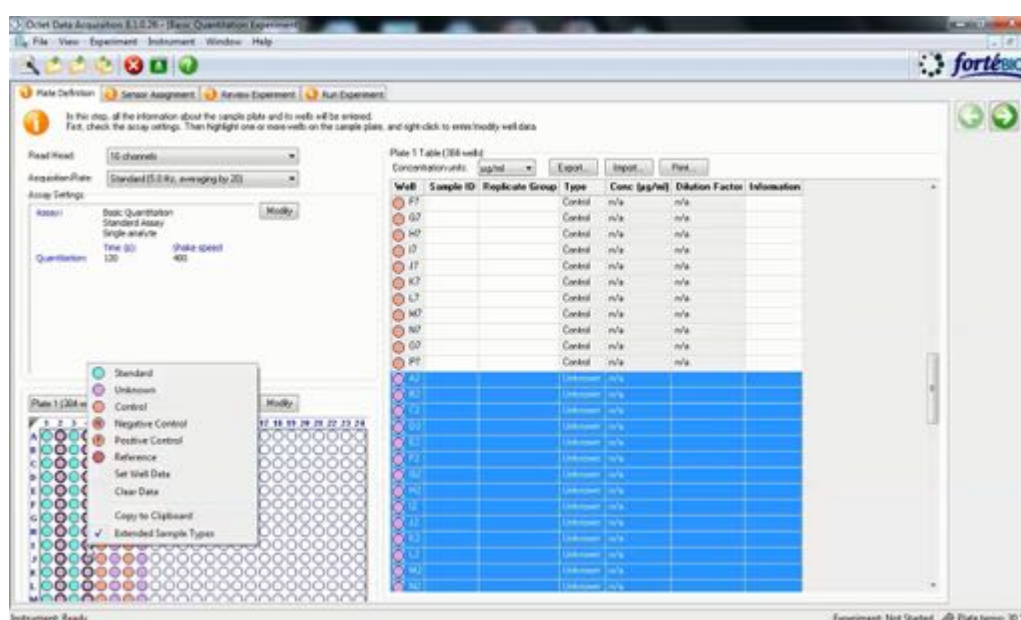


Figure 7-19: Plate Definition Window—Designate Unknown Wells

To remove a well designation, select the well(s) and click **Unassigned**. Or, right-click the well(s) and select **Clear Data**.

Assigning a Dilution Factor or Serial Dilution to Unknowns

To assign a dilution factor or serial dilution to unknowns:

1. In the **Sample Plate Map**, select the unknown wells (see Figure 7-19).
2. Right-click and select **Set Well Data**.

The **Set Well Data** dialog box displays (see Figure 7-20).

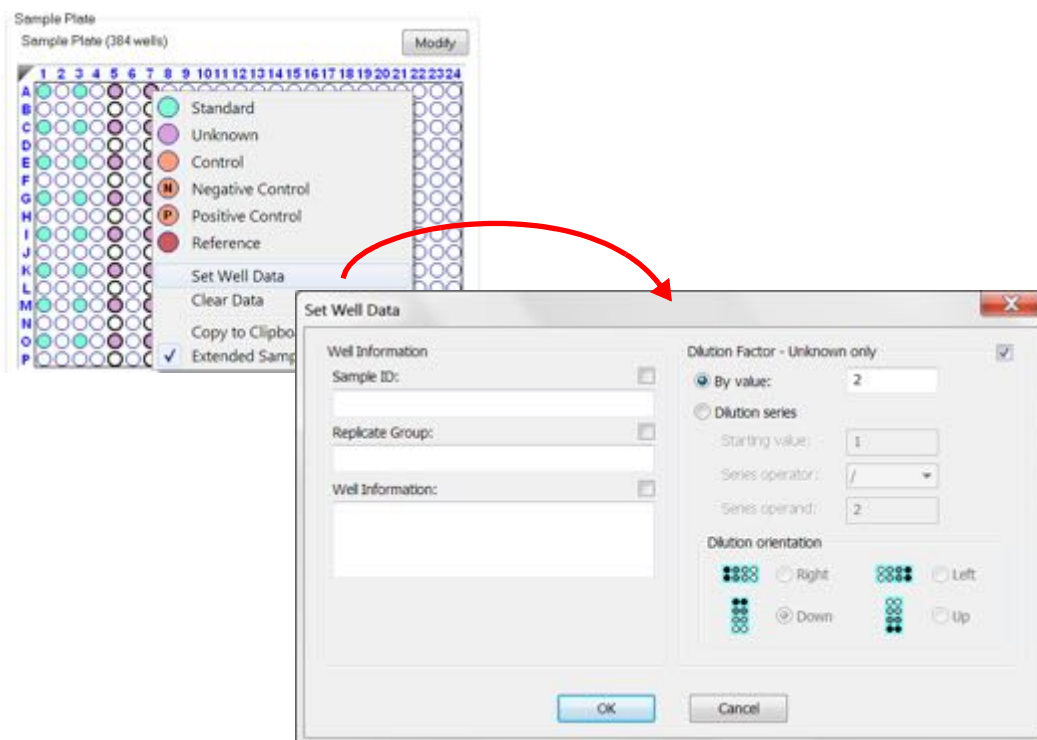


Figure 7-20: Sample Plate Map—Setting a Dilution Factor or a Serial Dilution

To assign a dilution factor to selected wells:

1. In the **Set Well Data** dialog box (see Figure 7-20), select the **By Value** option.
2. Enter the dilution factor value and click **OK**.

To assign a serial dilution to selected wells:

1. In the **Set Well Data** dialog box (see Figure 7-20), select the **Dilution series** option.
2. Enter the starting dilution, select a series operator, and enter a series operand.
3. Select the appropriate dilution orientation (see Figure 7-21).

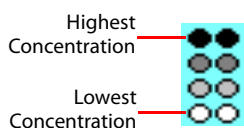


Figure 7-21: Concentration Representation in Dilution Series

4. Click **OK**.

The **Sample Plate Table** will display the dilution factors entered.

Editing a Dilution Factor in the Sample Plate Table

To edit a dilution factor in the **Sample Plate Table**:

1. In the **Sample Plate Table** (see Figure 7-22), double-click a cell in the **Dilution Factor** column for the desired unknown.
2. Enter the new value (the default dilution factor is 1).

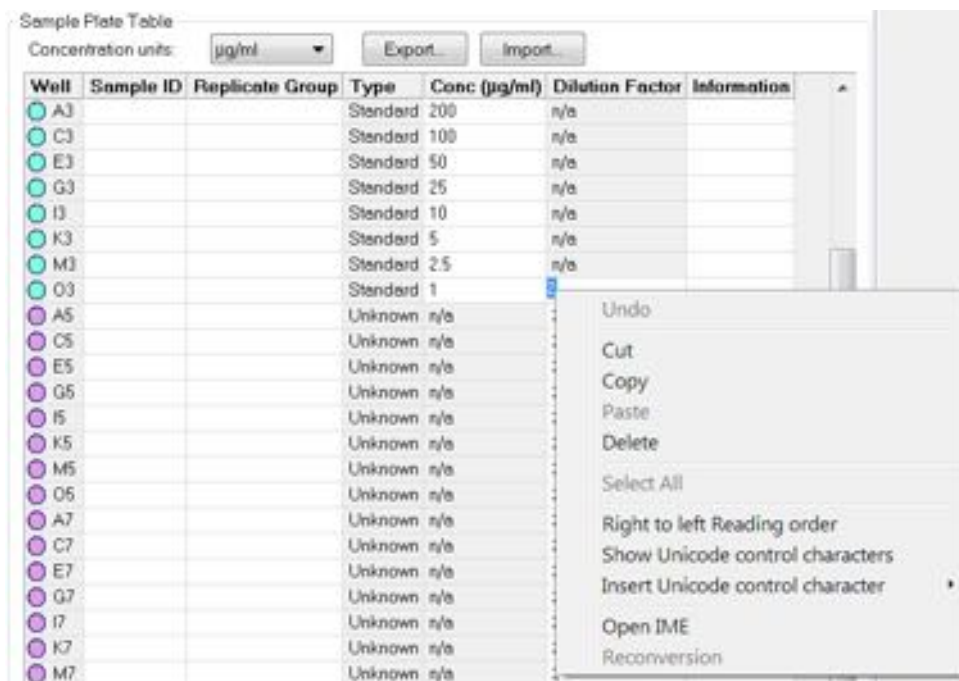


Figure 7-22: Sample Plate Table—Shortcut Menu of Edit Commands



NOTE: Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the **Sample Plate Table**. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the **Sample Plate Map** menu used to designate sample types.

Designating Controls or Reference Wells



NOTE: Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet Data Acquisition software versions 8.0 and higher.

Controls are samples of known concentration that are not used to generate a standard curve. A reference well contains sample matrix only, and is used to subtract non-specific binding of the sample matrix to the biosensor. During data analysis, data from reference wells can be subtracted from standards and unknowns to correct for background signal.

- To designate controls, select the control wells and click **Control** (below the **Sample Plate Map**), or right-click and select **Control**. Positive and Negative Control types can also be assigned using this menu.
- To designate reference wells, select the reference wells and click the **Reference** button below the **Sample Plate Map**, or right-click the selection and choose **Reference**.

The wells are marked in the **Sample Plate Map** and the **Sample Plate Table** is updated (see Figure 7-22).

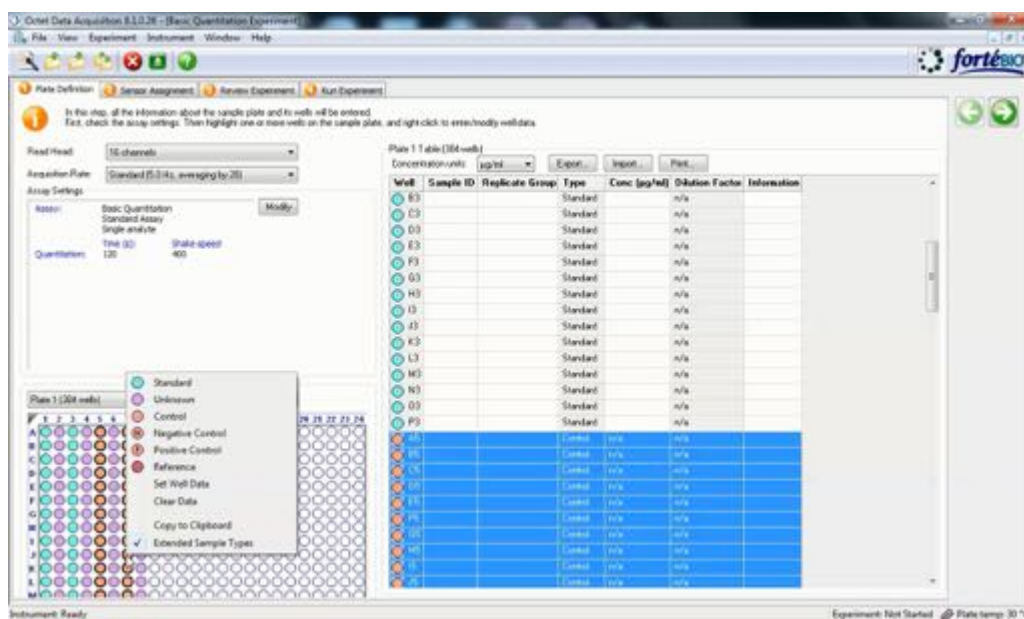


Figure 7-23: Designate Controls or Reference Wells



NOTE: Shift-clicking in the **Sample Plate Map** mimics the head of the instrument during the selection.

To remove a well designation, select the well(s) and click **Unassigned**. Or, right-click the well(s) and select **Clear Data**.

Annotating Samples

You can enter annotations (notes) for multiple samples in the **Sample Plate Map** or enter information for an individual sample in the **Sample Plate Table**. For greater clarity, annotation text may be displayed as the legend of the **Runtime Binding Chart** during data acquisition, but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it will not be available for display as a legend.

Annotating Wells in the Sample Plate Map

To annotate one or more wells:

1. In the **Sample Plate Map**, select the samples to annotate, right-click and select **Set Well Data**.
2. In the **Set Well Data** dialog box (see Figure 7-24), enter **Sample ID** and/or **Well Information** and click **OK**.

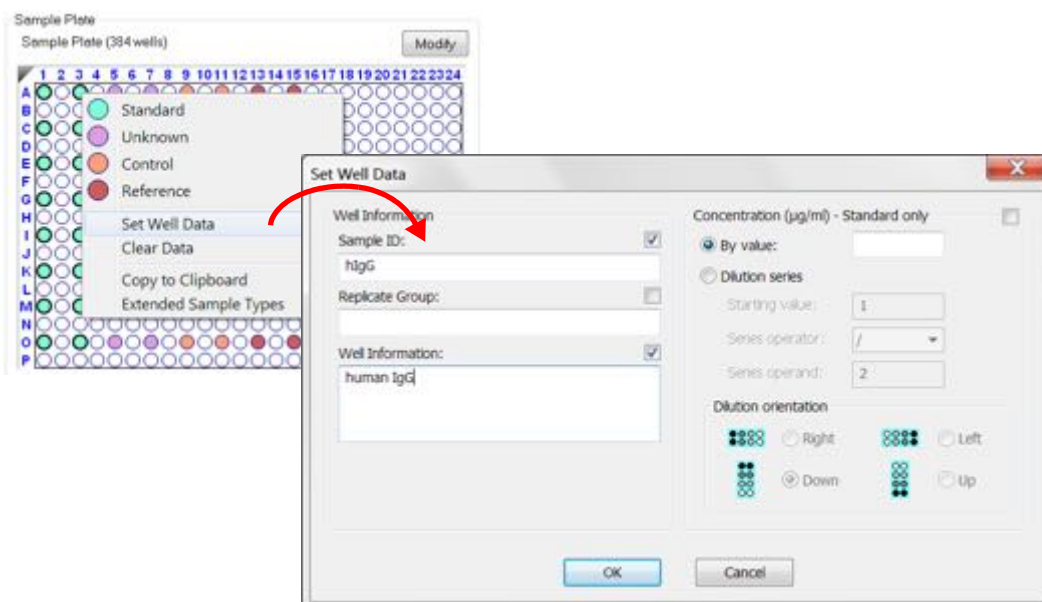


Figure 7-24: Adding Sample Annotations from the Sample Plate Map

Annotating Wells in the Sample Plate Table

To annotate an individual well in the **Sample Plate Table**:

1. Double-click the table cell for **Sample ID** or **Well Information**.
2. Enter the desired information in the respective field (see Figure 7-25).



NOTE: A series of Sample IDs may also be assembled in Excel and pasted into the **Sample Plate Table**.

Sample Plate Table

Concentration units: µg/ml Export... Import...

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	Dilution Factor	Information
A1	hlgG		Standard	200	n/a	human IgG
C1			Standard	100	n/a	
E1			Standard	50	n/a	
G1			Standard	25	n/a	
I1			Standard	10	n/a	
K1			Standard	5	n/a	

Figure 7-25: Adding Sample Annotations in the Sample Plate Table



NOTE: Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the **Sample Plate Table**. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the **Sample Plate Map** menu used to designate sample types.

Replicate Groups

When samples are assigned to a **Replicate Group**, the Octet System Data Analysis software will automatically calculate statistics for all samples in that group. The average binding rate, average concentration and corresponding standard deviation as well CV% are presented in the **Results** table for each group (see Figure 7-26).

Sensor...	Replicat...	BR Avg	BR SD	BR CV	Conc. Avg	Conc. SD	Conc. CV
Protein A	Group 1	0.66	0.01	1.5	604.5	17.8	2.9
Protein A	Group 1	0.66	0.01	1.5	604.5	17.8	2.9
Protein A	Group 1	0.66	0.01	1.5	604.5	17.8	2.9
Protein A	Group 1	0.66	0.01	1.5	604.5	17.8	2.9
Anti-Hu...	Group 2	0.6589	0.0052	0.8	602.5	9.15	1.5
Anti-Hu...	Group 2	0.6589	0.0052	0.8	602.5	9.15	1.5
Anti-Hu...	Group 2	0.6589	0.0052	0.8	602.5	9.15	1.5
Anti-Hu...	Group 2	0.6589	0.0052	0.8	602.5	9.15	1.5
Anti-Mo...	Group 3	0.6773	0.0087	1.3	635.3	15.4	2.4
Anti-Mo...	Group 3	0.6773	0.0087	1.3	635.3	15.4	2.4
Anti-Mo...	Group 3	0.6773	0.0087	1.3	635.3	15.4	2.4
Anti-Mo...	Group 3	0.6773	0.0087	1.3	635.3	15.4	2.4
Protein A	Group 4	0.6544	0.0073	1.1	594.6	12.9	2.2
Protein A	Group 4	0.6544	0.0073	1.1	594.6	12.9	2.2
Protein A	Group 4	0.6544	0.0073	1.1	594.6	12.9	2.2
Protein A	Group 4	0.6544	0.0073	1.1	594.6	12.9	2.2

Figure 7-26: Replicate Group Result Table Statistics



NOTE: Replicate Group information can also be entered in the Results table in the Octet System Data Analysis software.

Assigning Replicate Groups in the Sample Plate Map

To assign **Replicate Groups** in the **Sample Plate Map**:

1. Select the samples to group, right-click and select **Set Well Data**.
2. In the **Set Well Data** dialog box (see Figure 7-27), enter a name in the **Replicate Group** box and click **OK**.

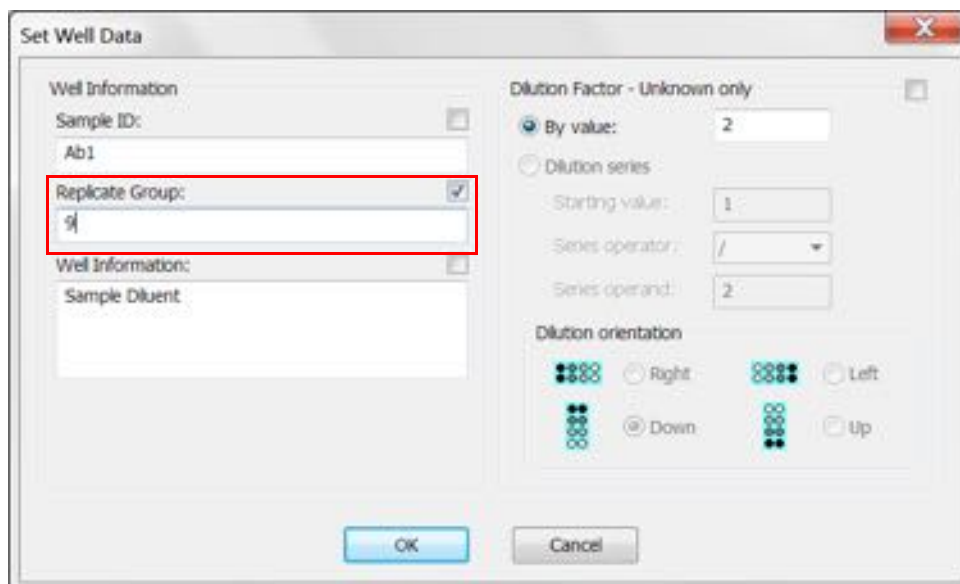


Figure 7-27: Add Replicate Group from the Sample Plate Map

3. Repeat the previous steps to assign new samples to the existing **Replicate Group**, or to designate another set of samples to a new **Replicate Group**. Multiple groups can be used in an experiment.



IMPORTANT: The Octet System Data Analysis software will only recognize and calculate statistics for samples that use the same **Replicate Group** names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.



NOTE: When performing a Multiple Analyte experiment, if the same **Replicate Group** name is used with different biosensor types, they will be treated as separate groups. Statistics for these groups will be calculated separately for each biosensor type.

Wells in the **Sample Plate Map** will show color-coded outlines as a visual indication of which wells are in the same group (see Figure 7-28).



Figure 7-28: Replicate Groups Displayed in Sample Plate Map

The **Sample Plate Table** will update with the **Replicate Group** names entered (see Figure 7-29).

Sample Plate Table

Concentration units:

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	Dilution Factor	Information
A1	hlgG	1	Standard	200	n/a	human IgG
C1	hlgG	2	Standard	100	n/a	human IgG
E1	hlgG	3	Standard	50	n/a	human IgG
G1	hlgG	4	Standard	25	n/a	human IgG
I1	hlgG	5	Standard	10	n/a	human IgG
K1	hlgG	6	Standard	5	n/a	human IgG
M1	hlgG	7	Standard	2.5	n/a	human IgG
O1	hlgG	8	Standard	1	n/a	human IgG
A3	hlgG	1	Standard	200	n/a	human IgG
C3	hlgG	2	Standard	100	n/a	human IgG
E3	hlgG	3	Standard	50	n/a	human IgG
G3	hlgG	4	Standard	25	n/a	human IgG
I3	hlgG	5	Standard	10	n/a	human IgG
K3	hlgG	6	Standard	5	n/a	human IgG
M3	hlgG	7	Standard	2.5	n/a	human IgG
O3	hlgG	8	Standard	1	n/a	human IgG
A5	Ab1	9	Unknown	n/a	2	Sample Diluent
C5	Ab2	10	Unknown	n/a	2	Sample Diluent
E5	Ab3	11	Unknown	n/a	2	Sample Diluent
G5	Ab4	12	Unknown	n/a	2	Sample Diluent
I5	Ab5	13	Unknown	n/a	2	Sample Diluent
K5	Ab6	14	Unknown	n/a	2	Sample Diluent
M5	Ab7	15	Unknown	n/a	2	Sample Diluent
O5	Ab8	16	Unknown	n/a	2	Sample Diluent
A7	Ab9	9	Unknown	n/a	2	Sample Diluent
C7	Ab10	10	Unknown	n/a	2	Sample Diluent
E7	Ab11	11	Unknown	n/a	2	Sample Diluent
G7	Ab12	12	Unknown	n/a	2	Sample Diluent

Figure 7-29: Replicate Groups in Sample Plate Table

Assigning Replicate Groups in the Sample Plate Table

To assign **Replicate Groups** in the **Sample Plate Table**:

1. Double-click the desired cell in the **Replicate Group** table column.
2. Enter a group name (see Figure 7-30).

Sample Plate Table

Concentration units: µg/ml Export... Import...

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	Dilution Factor	Information
K3	hlgG		Standard	5	n/a	human IgG
M3	hlgG		Standard	2.5	n/a	human IgG
O3	hlgG		Standard	1	n/a	human IgG
A5	Ab1	3	Unknown	n/a	2	Sample Diluent
C5	Ab2		Unknown	n/a	2	Sample Diluent
E5	Ab3		Unknown	n/a	2	Sample Diluent
G5	Ab4		Unknown	n/a	2	Sample Diluent

Figure 7-30: Add Replicate Group from the Sample Plate Table



NOTE: Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the **Sample Plate Table**. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the **Sample Plate Map** menu used to designate sample types.

3. Repeat the previous steps to assign new samples to the existing **Replicate Group**, or to designate another set of samples to a new **Replicate Group**. Multiple groups can be used in an experiment.



IMPORTANT: The Octet System Data Analysis software will only recognize and calculate statistics for samples that use the same **Replicate Group** names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.



NOTE: When performing a Multiple Analyte experiment, if the same Replicate Group name is used with different biosensor types, they will be treated as separate groups. Statistics for these groups will be calculated separately for each biosensor type.

MANAGING SAMPLE PLATE DEFINITIONS



NOTE: After you define a sample plate, you can export and save the plate definition for future use.



NOTE: Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet Data Acquisition software versions 8.0 and higher.

Exporting a Plate Definition

To export a plate definition:

1. In the **Sample Plate Table** (see Figure 7-31), click **Export**.

Sample Plate Table

Concentration units: µg/ml Export... Import...

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	Dilution Factor	Information
A1	hlgG	1	Standard	200	n/a	human IgG
C1	hlgG	2	Standard	100	n/a	human IgG
E1	hlgG	3	Standard	50	n/a	human IgG
G1	hlgG	4	Standard	25	n/a	human IgG
I1	hlgG	5	Standard	10	n/a	human IgG
K1	hlgG	6	Standard	5	n/a	human IgG

Figure 7-31: Export Button in Sample Plate Table

2. In the **Export Plate Definition** window (see Figure 7-31), select a folder, enter a name for the plate (.csv), and click **Save**.

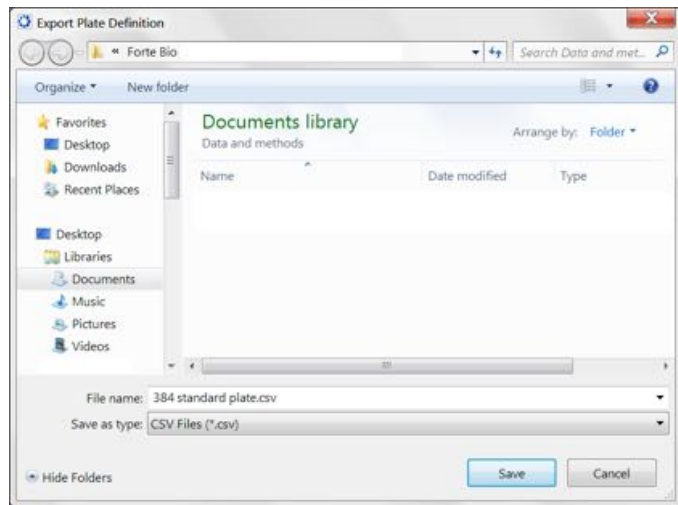


Figure 7-32: Export Plate Definition Window

Importing a Plate Definition

To import a plate definition:

- 1. In the **Sample Plate Table** (see Figure 7-33), click **Import**.

Sample Plate Table							
Concentration units:		<div>µg/ml</div>	Export...		Import...		
Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	Dilution Factor	Information	
A1	hlgG	1	Standard	200	n/a	human IgG	
C1	hlgG	2	Standard	100	n/a	human IgG	
E1	hlgG	3	Standard	50	n/a	human IgG	
G1	hlgG	4	Standard	25	n/a	human IgG	
I1	hlgG	5	Standard	10	n/a	human IgG	
K1	hlgG	6	Standard	5	n/a	human IgG	

Figure 7-33: Import Button in Sample Plate Table

- 2. In the **Import Plate Definition** window (see Figure 7-35), select the plate definition (.csv), and click **Open**.

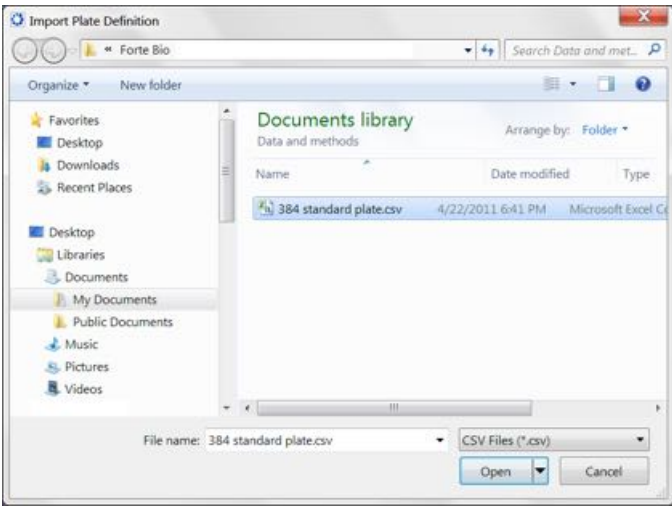


Figure 7-34: Import Plate Definition Window



NOTE: You can also create a .csv file for import. Figure 7-35 shows the appropriate column information layout.

	A	B	C	D	E	F	G
1	PlateWells	384					
2	Well	ID	Replicate Group	Group	Concentration (µg/ml)	Dilution	Information
3	A1	hlgG		1 Standard	200		human IgG
4	C1	hlgG		2 Standard	100		human IgG
5	E1	hlgG		3 Standard	50		human IgG
6	G1	hlgG		4 Standard	25		human IgG
7	I1	hlgG		5 Standard	10		human IgG
8	K1	hlgG		6 Standard	5		human IgG
9	M1	hlgG		7 Standard	2.5		human IgG
10	O1	hlgG		8 Standard	1		human IgG

Figure 7-35: Example Sample Plate Definition File (.csv)

Printing a Sample Plate Definition

To print a plate definition:

1. In the **Sample Plate/Plate 1 Map** (see Figure 7-36), click **Print**.

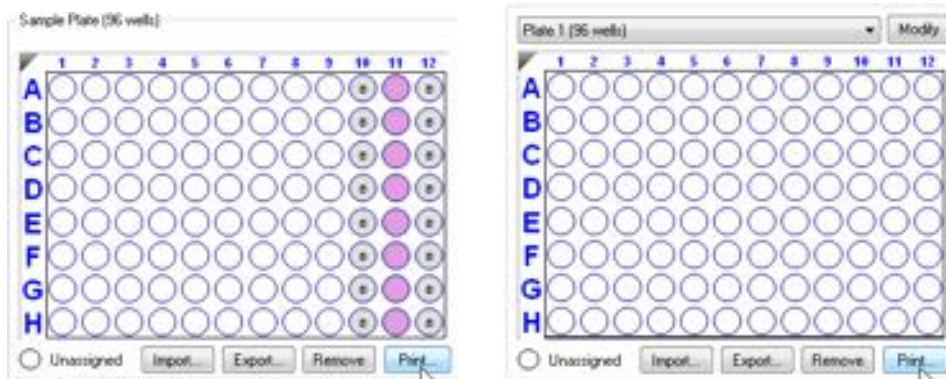


Figure 7-36: Sample Plate/Plate 1 Print Button

The associated **Sample Plate Table** information will print.

WORKING WITH A REAGENT PLATE



NOTE: Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet Data Acquisition software versions 8.0 and higher (Figure 7-37).

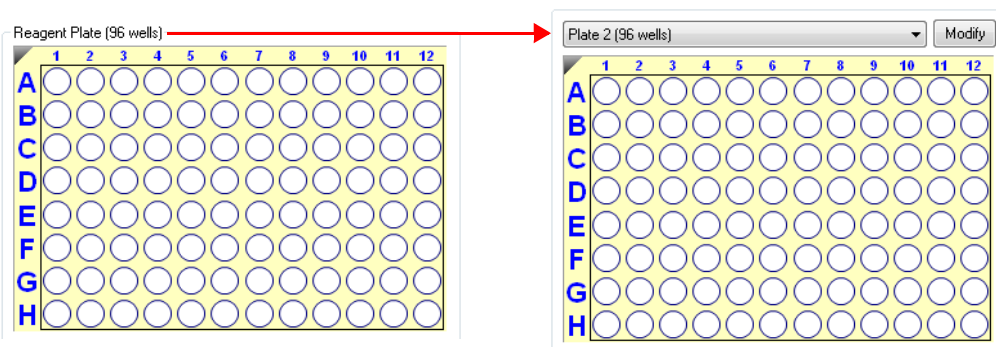


Figure 7-37: Reagent Plate Renamed Plate 2 in Software Versions 8.0 and Higher

You can include an optional reagent plate in a Basic Quantitation with Regeneration or Advanced Quantitation experiment. Using a reagent plate enables higher sample throughput since no reagents are included in the sample plate. A reagent plate can contain:

- Regeneration and neutralization reagents for Basic Quantitation with Regeneration experiments

- Buffers, enzyme solutions, and detection reagents for Advanced Quantitation experiments

An experiment can include any combination of sample and reagent plate formats (96- or 384-well). However, a reagent plate can include only reagent wells (regeneration, neutralization, detection). Wells for standards, unknowns, controls and references can not be assigned to the reagent plate.



NOTE: Reagent plates can only contain reagents. Standards, unknown samples, controls and references must be assigned to the sample plate.



NOTE: The reagent plate format (96- or 384-well) and the read head configuration (8 or 16 channels) determine the reagent plate layout. For more details, see "Read Head Configuration and Plate Layout" on page 290.

To define a reagent plate:

1. Select the **Reagent Plate** radio button above the plate map to display the **Reagent Plate Map** (Figure 7-38).
2. Click **Modify** to display the **Modify Plates** dialog box.

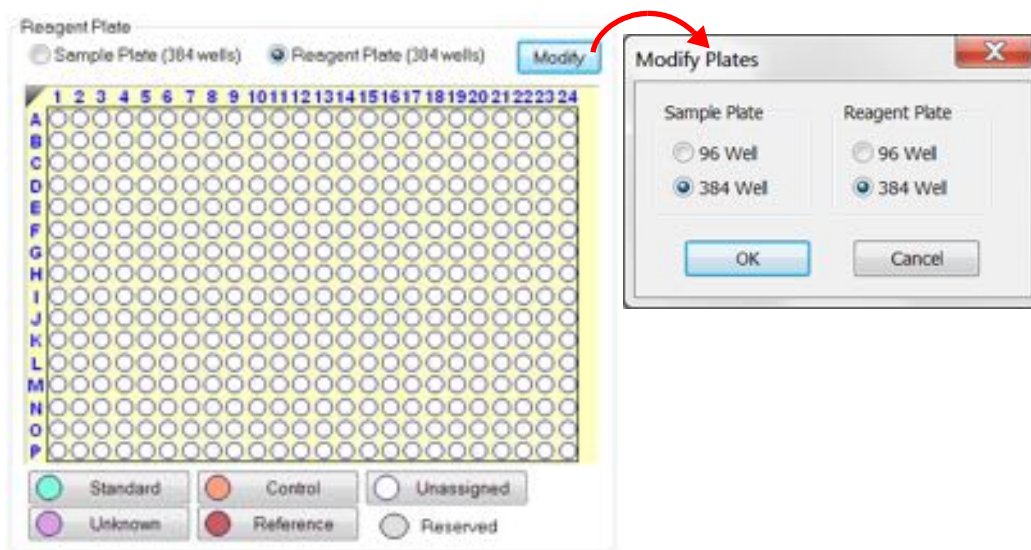


Figure 7-38: Modifying the Reagent Plate

3. Select a reagent plate format (**96 Well** or **384 Well**) and click **OK**.

4. In the **Reagent Plate Map**, right-click a column to use and make a selection on the shortcut menu that appears:
 - **Advanced Quantitation**—Select **Detection**.
 - **Basic Quantitation with Regeneration**—Select **Regeneration** or **Neutralization**. Repeat this step to set both the regeneration and neutralization reagent columns.

The **Reagent Plate Map** then shows where to dispense the reagents in the plate (Figure 7-39).

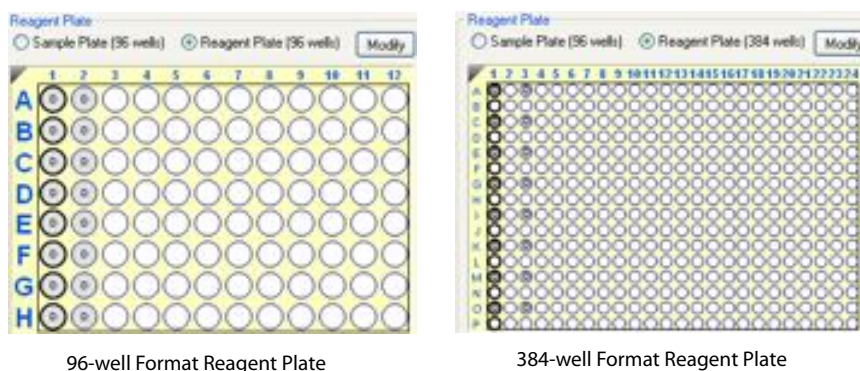


Figure 7-39: Example Reagent Plate Layouts for an Advanced Quantitation Experiment—16 Channel Read Head

To remove well designations, select the column(s) and click **Unassigned**, or right-click and choose **Clear Data**.

Saving a Reagent Plate Definition

Exporting and saving a reagent plate definition is done in the same manner as you would for sample plates. For details “Managing Sample Plate Definitions” on page 313.

Printing a Reagent Plate Definition

To print a plate definition:

1. In the **Reagent Plate/Plate 2 Map** (see Figure 7-40), click **Print**.

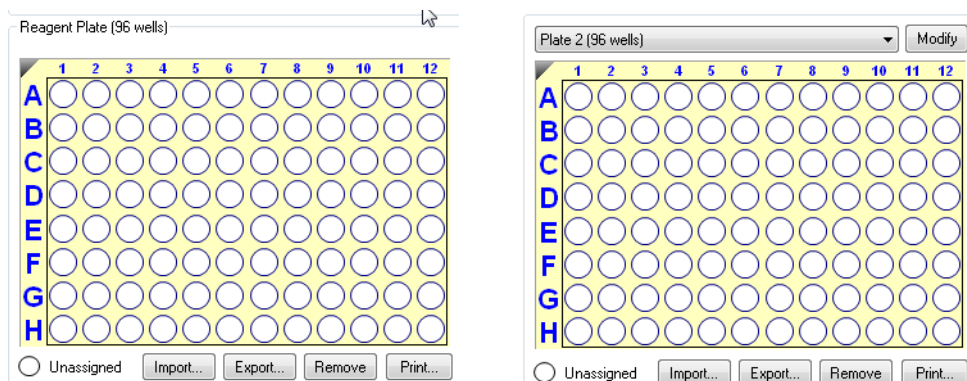


Figure 7-40: Reagent Plate/Plate 2 Print Button

The associated **Reagent Plate Table** information will print.

MANAGING ASSAY PARAMETER SETTINGS

Modifying Assay Parameter Settings

You can modify the assay parameter settings during sample plate definition. However, the changes are only applied to the current experiment. To save modified parameter settings, you must define a new assay. For details on creating a new assay, see “Custom Quantitation Assays” on page 359.

Viewing User-Modifiable Assay Parameter Settings

To view the user-modifiable settings for an assay, click **Modify** in the **Assay Settings** box. The **Assay Parameters** box will display (Figure 7-41). The settings available are experiment-dependent.

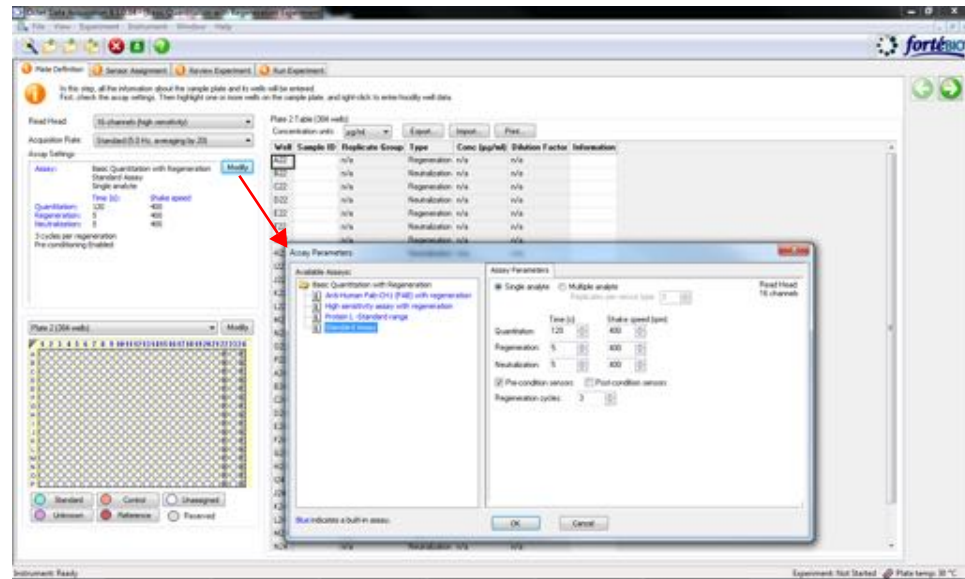


Figure 7-41: Modifying Assay Parameters.

Basic Quantitation Assay Parameters

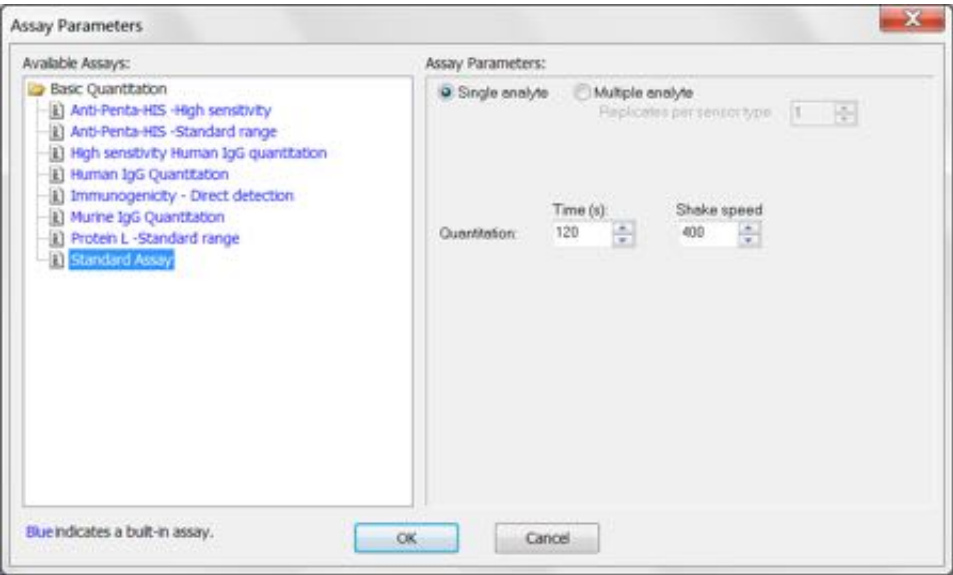



Figure 7-42: Assay Parameters—Basic Quantitation Assay

Table 7-6: Basic Quantitation Assay Parameters

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time (s)	The duration of data acquisition seconds while the biosensor is incubated in sample.
<div> NOTE: A subset of data points may be selected for processing during data analysis.</div>	
Quantitation Shake speed (rpm)	The sample shaking speed (rotations per minute).

Basic Quantitation with Regeneration Assay Parameters

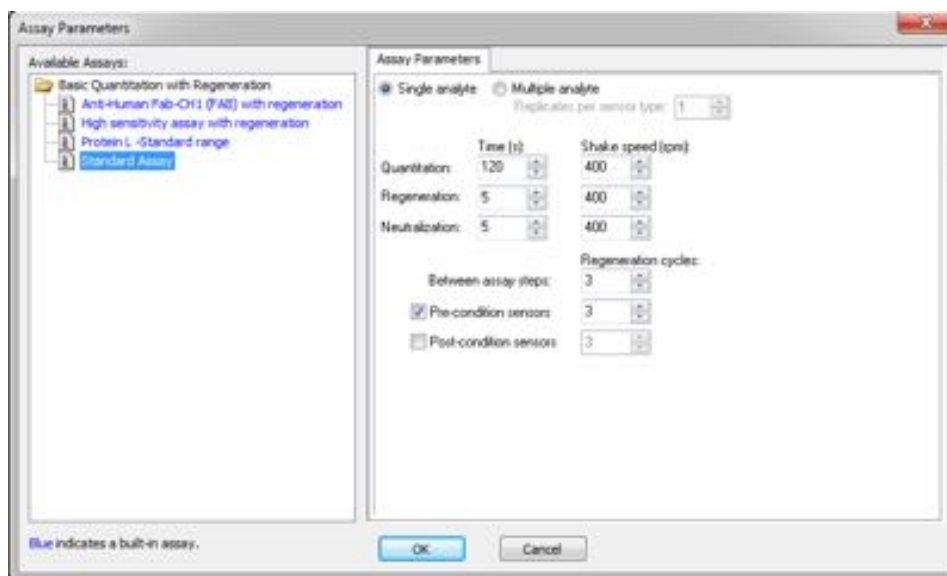


Figure 7-43: Assay Parameters—Basic Quantitation with Regeneration

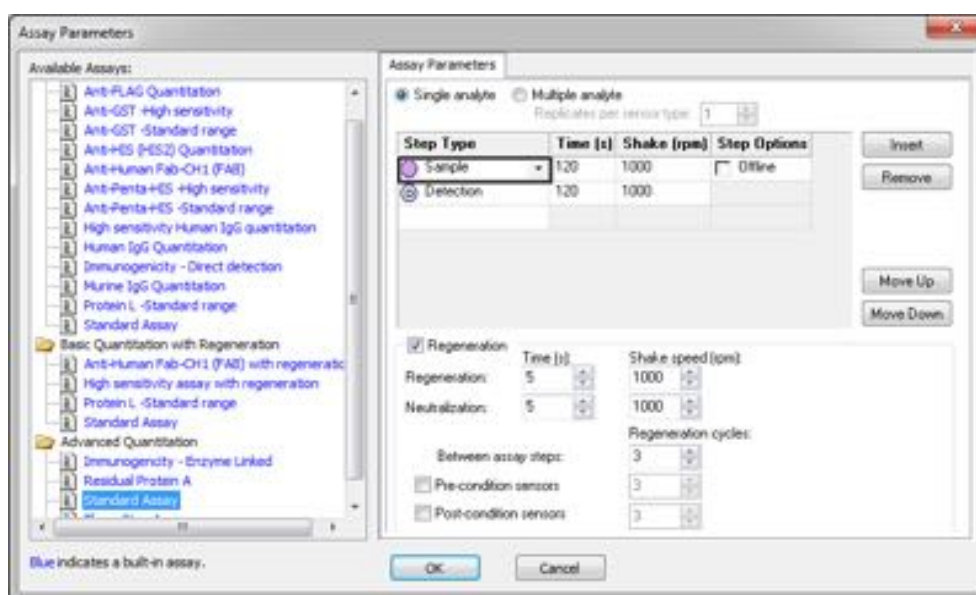
Table 7-7: Assay Parameters—Basic Quantitation with Regeneration

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time(s) and Shake speed (rpm)	The duration of data acquisition in seconds while the biosensor is incubated in sample and the sample shaking speed (rotations per minute).
<div>➔</div> NOTE: A subset of data points may be selected for processing during data analysis.	
Regeneration Time(s) and Shake speed (rpm)	The duration time and shaking speed of the regeneration step where the biosensor is incubated in regeneration buffer to remove bound analyte.
Neutralization Time(s) and Shake speed (rpm)	The duration time and shaking speed of the neutralization step where the biosensor is incubated in neutralization buffer after the regeneration step.

Table 7-7: Assay Parameters—Basic Quantitation with Regeneration

Parameter	Description
Pre-condition sensors	Performs a set of regeneration/neutralization steps prior to the start of the experiment. The pre-conditioning settings are equivalent to the time and rpm settings for the regeneration in the assay. For example, an acidic pre-conditioning buffer maximizes the binding competence of Pro-A biosensors.
Post-condition sensors	Post-conditions biosensors, allowing re-racked biosensors to be stored in a regenerated state.
Regeneration cycles	The number of regeneration-neutralization cycles that a biosensor undergoes before reuse.

Advanced Quantitation Assay Parameters

**Figure 7-44:** Assay Parameters—Advanced Quantitation**Table 7-8:** Advanced Quantitation Assay Parameters

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.

Table 7-8: Advanced Quantitation Assay Parameters



Parameter	Description
Sample Time(s) and Shake speed (rpm)	The duration of data acquisition in seconds while the biosensor is incubated in sample and the sample shaking speed (rotations per minute).
 NOTE: A subset of data points may be selected for processing during data analysis.	
Buffer Time(s) and Shake speed (rpm)	The duration of biosensor incubation in the first buffer in seconds and the sample shaking speed (rotations per minute).
Enzyme Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the enzyme solution and the sample shaking speed (rotations per minute).
2nd Buffer Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the second buffer solution and the sample shaking speed (rotations per minute).
Capture Antibody Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the first capture antibody solution and the shaking speed (rotations per minute).
2nd Antibody Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the secondary antibody solution and the shaking speed (rotations per minute).
Detection Time(s) & Shake speed (rpm)	The duration of data acquisition during the detection step in seconds in an advanced quantitation assay.
 NOTE: A subset of data points may be selected for processing during data analysis.	
Offline	Choose this option to incubate sample with biosensors outside the Octet system. Offline incubation is best performed on the Pall ForteBio Sidekick biosensor immobilization station.
Reuse Buffer	Allows buffer wells to be reused. If unselected, the number of buffer columns must equal the number of sample columns. If selected, the number of buffer columns may be less than the number of sample columns as the buffer columns are reused.
Regeneration Time(s) and Shake speed (rpm)	The duration time and shaking speed of the regeneration step where the biosensor is incubated in regeneration buffer to remove bound analyte.

Table 7-8: Advanced Quantitation Assay Parameters

Parameter	Description
Neutralization Time(s) and Shake speed (rpm)	The duration time and shaking speed of the neutralization step where the biosensor is incubated in neutralization buffer after the regeneration step.
Pre-condition sensors	Performs a set of regeneration/neutralization steps prior to the start of the experiment. The pre-conditioning settings are equivalent to the time and rpm settings for the regeneration in the assay. For example, an acidic pre-conditioning buffer maximizes the binding competence of Protein A biosensors.
Post-condition sensors	Post-conditions biosensors, allowing re-racked biosensors to be stored in a regenerated state.
Regeneration cycles	The number of regeneration-neutralization cycles that a biosensor undergoes before reuse.



NOTE: In an Advanced Quantitation experiment, this option is only available if the first step (biosensor incubation in sample) is performed online.

ASSIGNING BIOSENSORS TO SAMPLES

After the sample plate is defined, biosensors must be assigned to the samples.



NOTE: When using a 96-well plate with the 8 channel read head, do not put biosensors in columns 2, 4, 6, 8, 10, and 12 if the biosensors will be returned to the biosensor tray and not discarded. If the biosensors will be ejected, biosensors can be placed in all columns.

Biosensor Assignment in Single-Analyte Experiments

In a single analyte experiment, only one biosensor type is assigned to each sample and only one analyte is analyzed per experiment.



NOTE: For single analyte experiments, the **Single Analyte** option must be selected in the **Assay Parameters** dialog box. For more information, please see “Managing Assay Parameter Settings” on page 319.

Click the **Sensor Assignment** tab, or click the  arrow to access the Sensor Assignment window (see Figure 7-45).

The software generates a color-coded **Sensor Tray Map** and **Sample Plate Map** that shows how the biosensors are assigned to the samples by default.

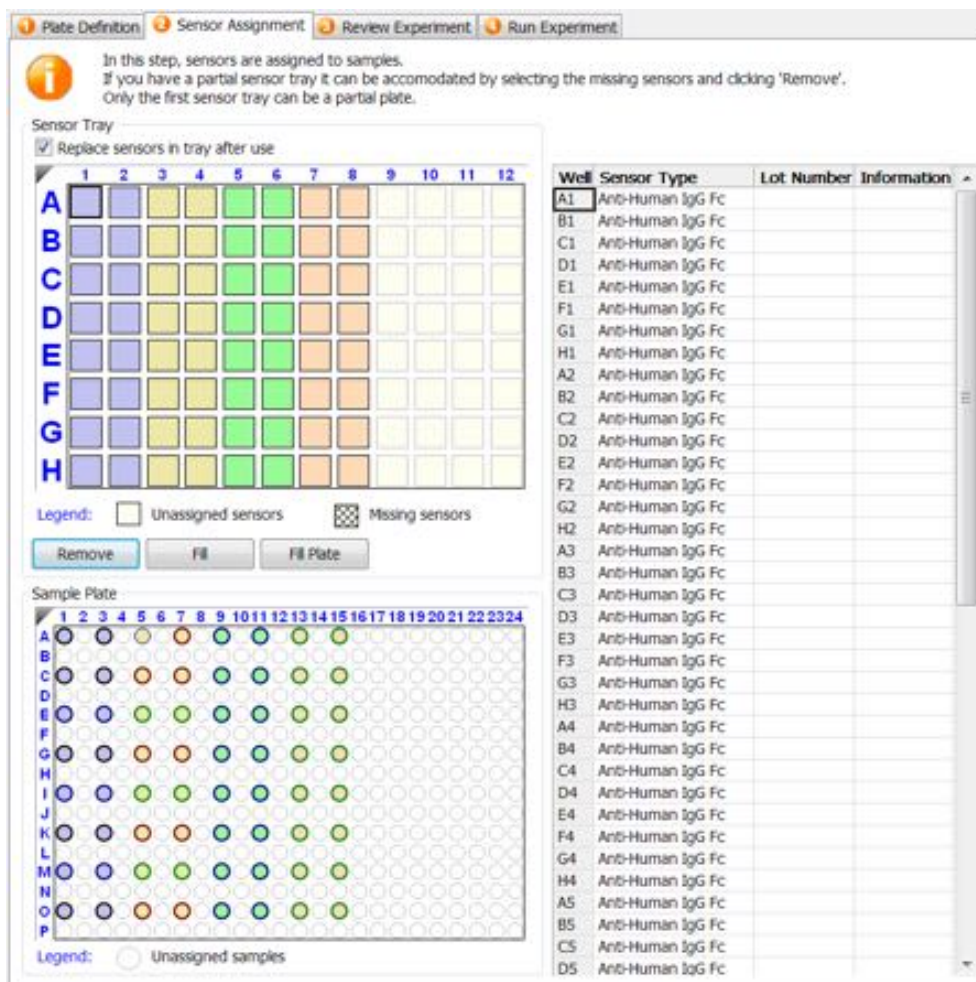


Figure 7-45: Sensor Assignment Window for Basic Quantitation without Regeneration

- Assign biosensors in one of two ways:
 - Select column(s) in the **Sensor Tray Map**, right-click and select a biosensor type from the drop-down list.
 - Select a cell in the **Sensor Type** table column, click the down arrow and select a biosensor type from the drop-down list (see Figure 7-45).

All wells in the **Sensor Type** column will automatically populate with the biosensor type selected.

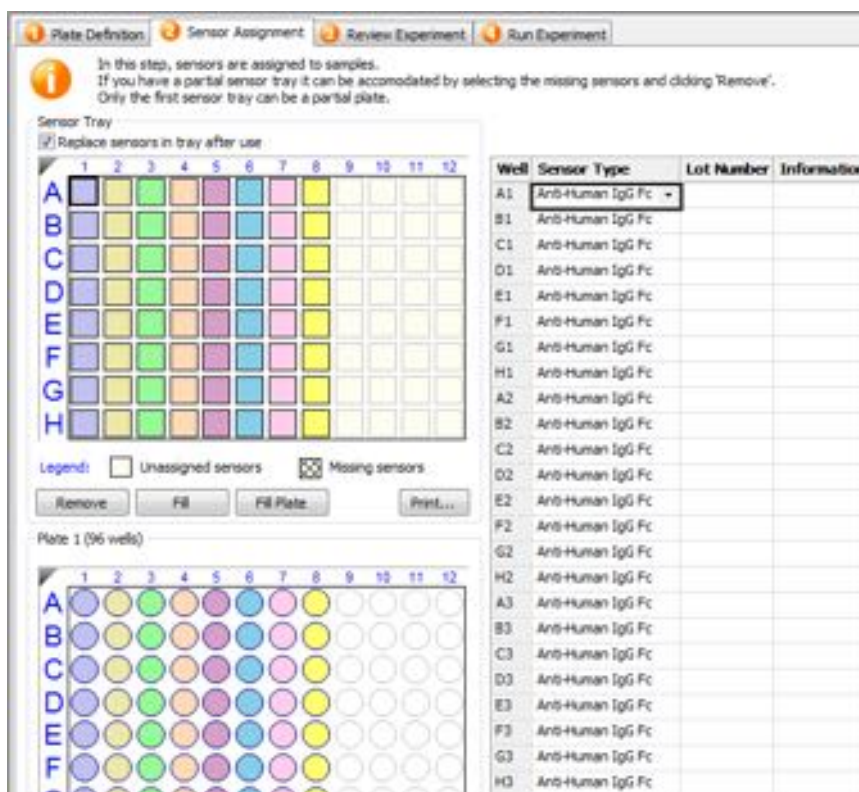


Figure 7-46: Changing Biosensor Types

- To designate reference biosensors, select the desired biosensors in the **Sensor Tray Map**, right-click and select **Reference**. The reference biosensors are marked with an **R**.



NOTE: Reference biosensors may also be designated in the **Runtime Binding Chart** during acquisition.

- Optional: Double-click in any cell in the **Lot Number** column to enter the biosensor lot number. All wells in the **Lot Number** column will automatically populate with the lot number entered.
- Optional: Double-click in a cell in the **Information** column to enter biosensor information for a particular cell.



NOTE: Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: For greater clarity, annotation text may be displayed as the legend of the **Runtime Binding Chart** during data acquisition, but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it will not be available for display as a legend.

5. Optional: After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace sensors in tray after use** check box (see Figure 7-47).

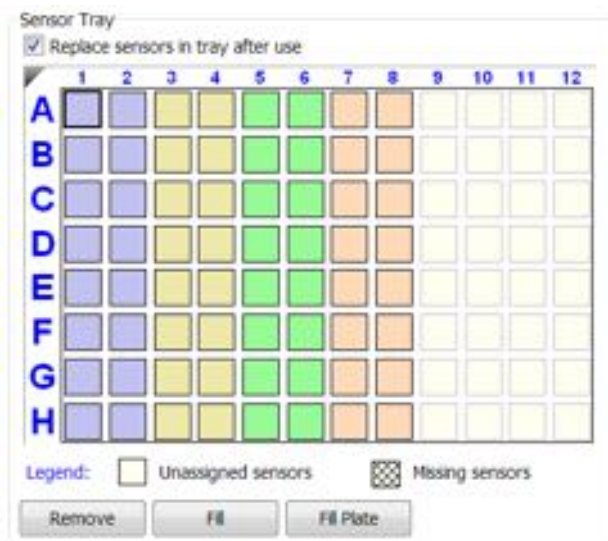


Figure 7-47: Replace Sensors in Tray After Use Check Box




NOTE: Biosensors can be regenerated up to a max of 11 times per experiment.

Biosensor Assignment in Multiple Analyte Experiments

In a multiple analyte experiment, more than one biosensor type is assigned to the same sample, allowing multiple analytes to be analyzed in a single experiment.



NOTE: For multiple analyte experiments, the **Multiple Analyte** option must be selected in the **Assay Parameters** dialog box. For more information, please see “Managing Assay Parameter Settings” on page 319.

Click the **Sensor Assignment** tab, or click the  arrow to access the Sensor Assignment window (see Figure 7-45).

The software generates a color-coded **Sensor Tray Map** and **Sample Plate Map** that shows how the biosensors are assigned to the samples by default. In the example shown in Figure 7-45, **one** replicate had been previously selected with the **Multiple Analyte** assay parameter option.

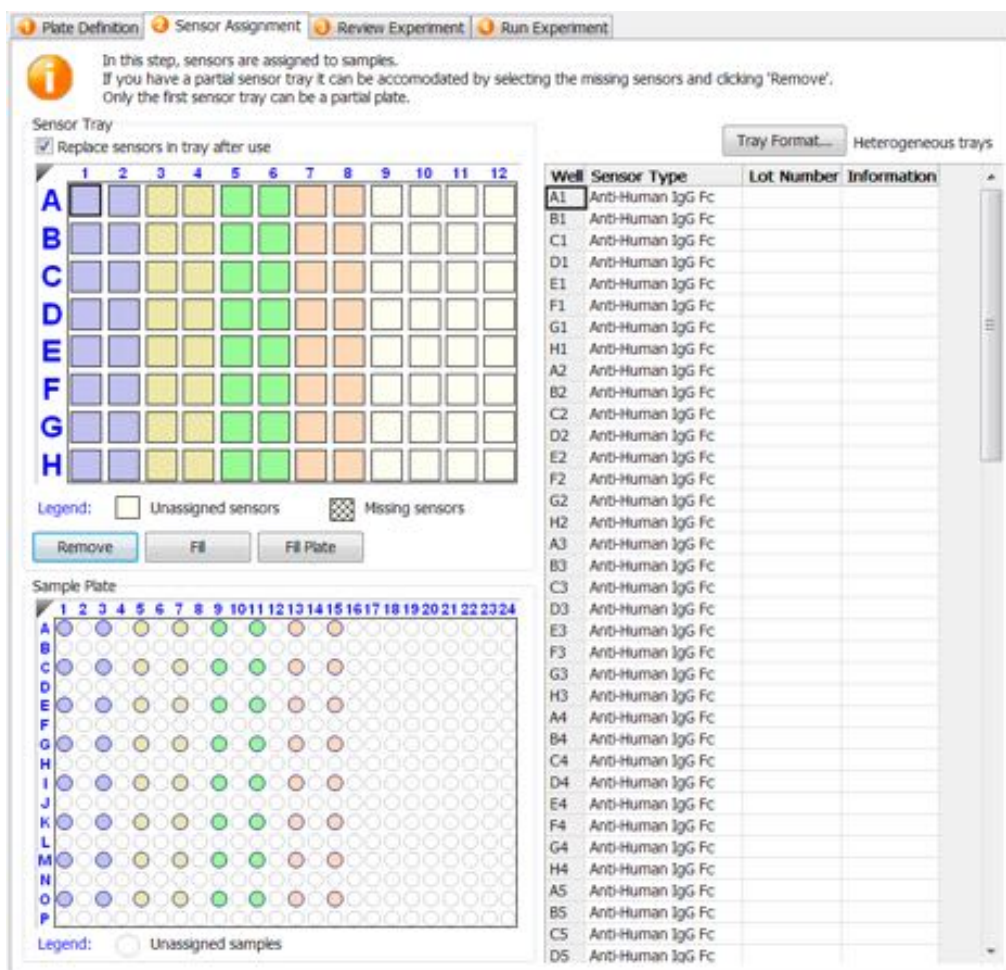


Figure 7-48: Sensor Assignment Window for Basic Quantitation Using the Multiple Analyte Option

There are two ways to assign biosensors:

- Select a column in the **Sensor Tray Map**, right-click and select a biosensor type from the drop-down list.
- Select a cell in the **Sensor Type** table column, click the down arrow and select a biosensor type from the drop-down list (see Figure 7-49).

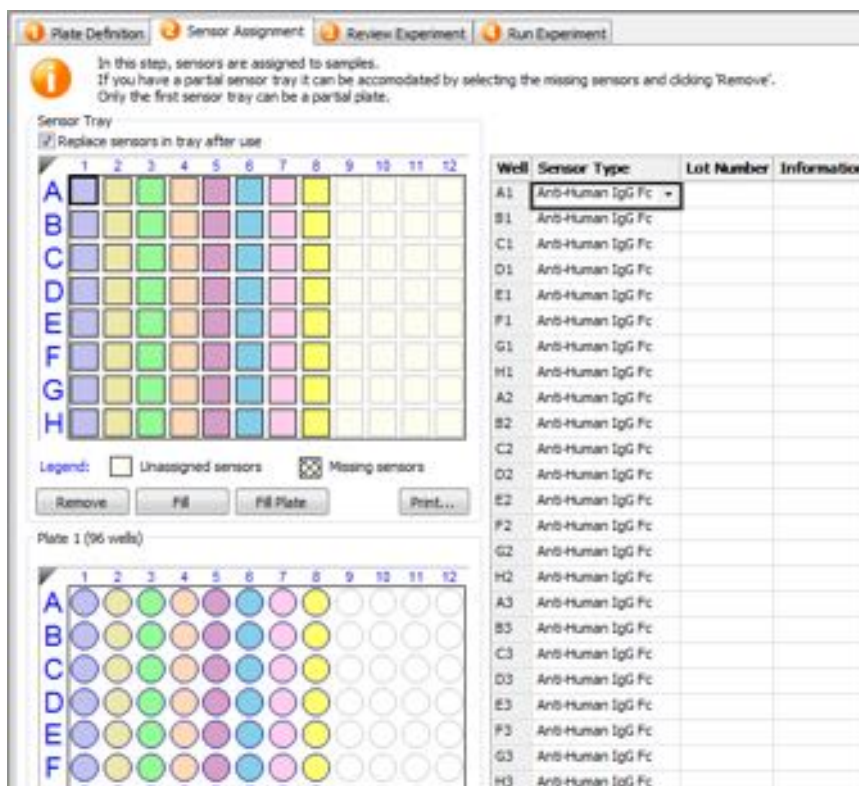


Figure 7-49: Changing Biosensor Types

Biosensor Assignment Using Heterogeneous Biosensor Trays

The default **Tray Format** is **Heterogeneous**. Heterogeneous biosensor trays contain a mixture of biosensor types.



NOTE: When using this **Heterogeneous** option, the order of biosensor types in each tray must be identical.

1. If Heterogeneous Trays is not displayed next to the **Tray Format** button, click the button.

The **Tray Format** dialog box displays (see Figure 7-50).

2. Select **Heterogeneous** and click **OK**.

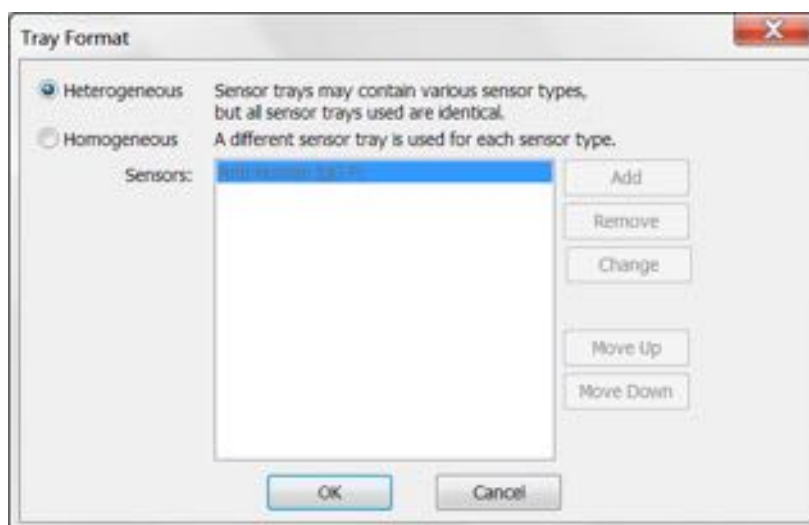


Figure 7-50: Tray Format Dialog Box

The Tray 1 **Sensor Tray Map** will be displayed by default.

3. Select **all** columns with default biosensor assignments in the **Sensor Tray Map**, right-click and select the first biosensor type to be used (see Figure 7-51).

The **Sensor Type** column will update accordingly.



Figure 7-51: Populating the Sensor Tray Map with First Biosensor Type

4. Select the columns in the **Sensor Tray Map** that should contain the second biosensor type, right-click and select the second biosensor type (see Figure 7-53).

The **Sensor Type** column will update accordingly.



Figure 7-52: Populating the Sensor Tray Map with Second Biosensor Type

5. Repeat this column selection and assignment process for all other biosensor types to be used in the experiment. The software will automatically update the number of biosensor trays needed and biosensor assignments in all trays according to the column assignments made in Tray 1.

In the example shown in Figure 7-53, Protein A and Protein G biosensor types are used for a multiple analyte experiment using two replicates. Three heterogeneous biosensor trays will be needed for the experiment.

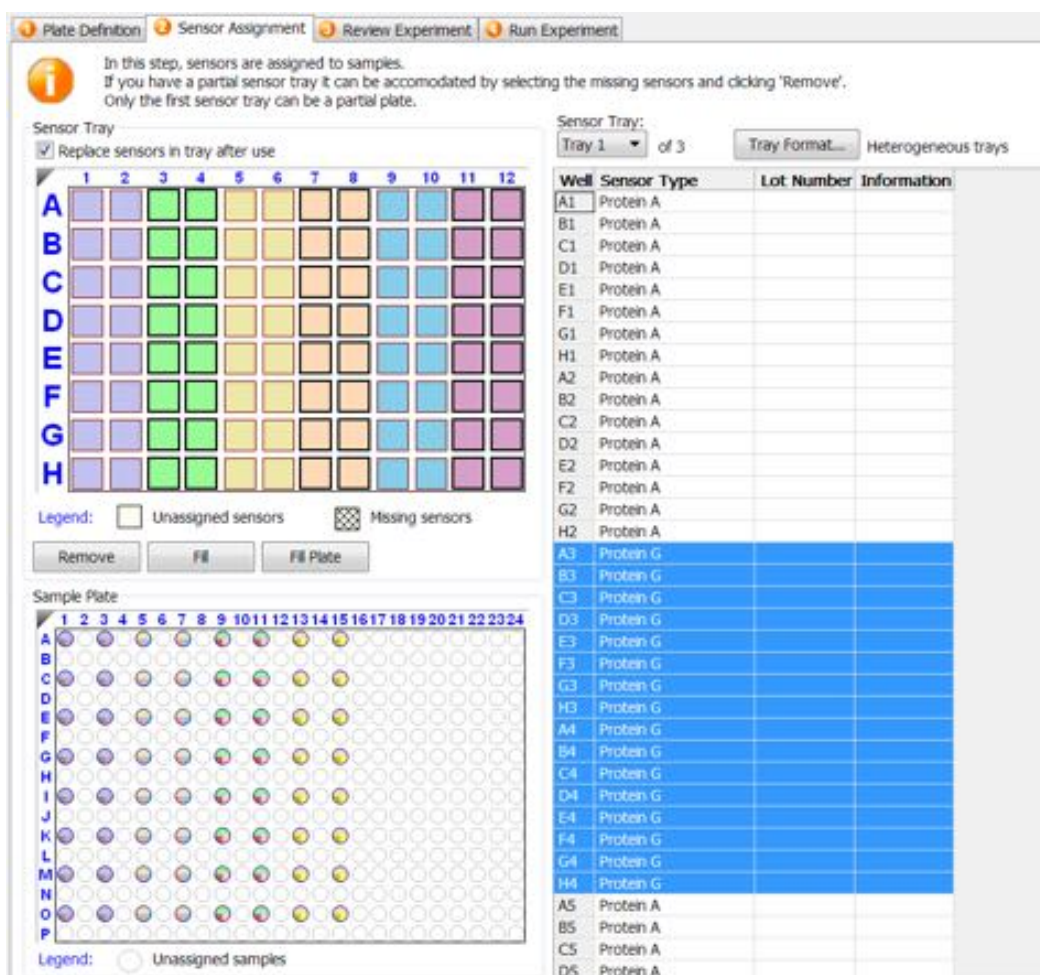


Figure 7-53: Biosensor Assignment using Heterogeneous Trays and Two Biosensor Types

- To view or change the biosensor assignments in another tray, click the **Sensor Tray** button and select a tray number from the drop down list.

The **Sensor Tray Map** and table for the tray selected will be shown and biosensor assignments can be changed as needed (see Figure 7-54).

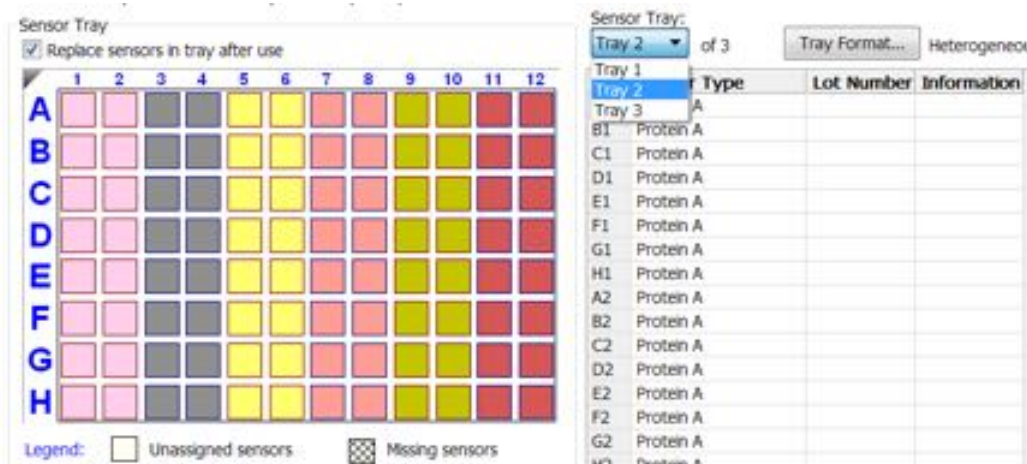


Figure 7-54: Tray Selection

7. To designate reference biosensors, select the desired biosensors in the **Sensor Tray Map**, right-click and select **Reference**.

The reference biosensors are marked with an R.



NOTE: Reference biosensors may also be designated in the **Runtime Binding Chart** during acquisition.

8. Optional: Double-click in any cell in the **Lot Number** column to enter a biosensor lot number. All wells in the **Lot Number** column for that biosensor type will automatically populate with the lot number entered.
9. Optional: Double-click in a cell in the **Information** column to enter biosensor information for a particular cell.



NOTE: Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut - Ctrl+x**, **Copy - Ctrl+c**, **Paste - Ctrl+v**, **Undo - Ctrl+z**) are available in the table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: For greater clarity, annotation text may be displayed as the legend of the **Runtime Binding Chart** during data acquisition but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it will not be available for display as a legend.

10. Optional: After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace sensors in tray after use** check box (see Figure 7-47).

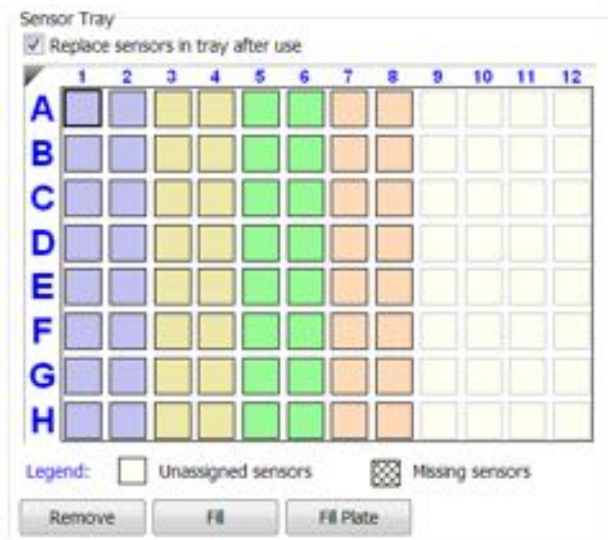


Figure 7-55: Replace Sensors in Tray After Use Check Box



NOTE: Biosensors can be regenerated up to a max of 11 times per experiment.

Biosensor Assignment Using Homogeneous Trays

Homogeneous biosensor trays contain only one biosensor type.



NOTE: Using the **Homogeneous** option will necessitate switching trays during the experiment.

1. Click **Tray Format**.

The **Tray Format** dialog box displays (see Figure 7-56) and the **Sensors** box will be populated with the default biosensor type.

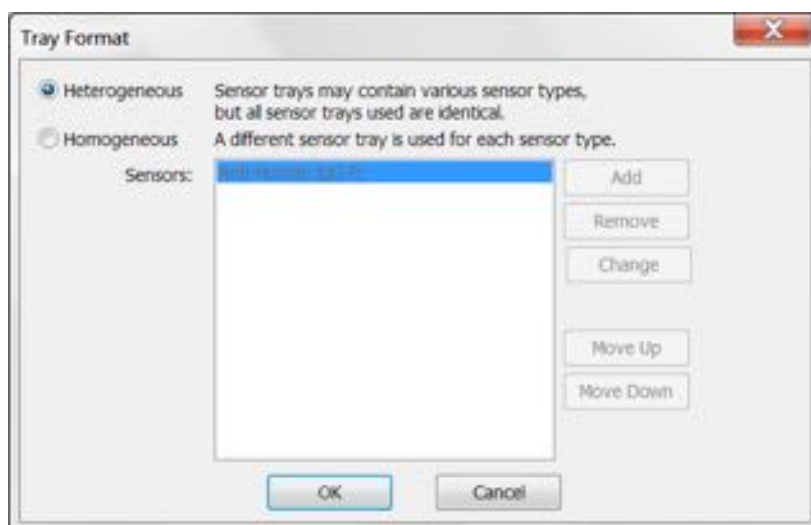


Figure 7-56: Tray Format Dialog Box

2. Select **Homogeneous**. Click **Add** to select the first biosensor type (see Figure 7-57).

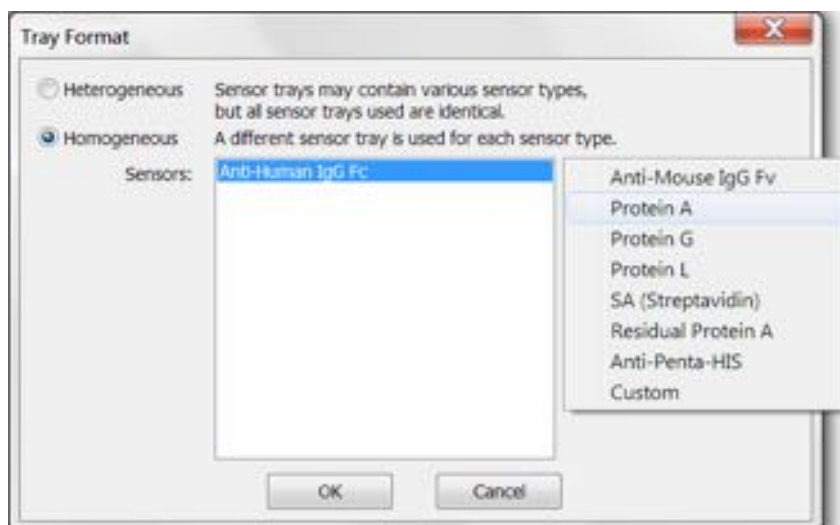


Figure 7-57: Selecting a Biosensor Type in the Tray Format Dialog Box

3. Repeat this step to add any additional biosensor types that will be used in the experiment. To remove a biosensor type, select a biosensor type in the **Sensor** box and click **Remove**.
4. Adjust the order of biosensor types as needed by selecting the biosensor type in the **Sensor** box and clicking **Move Up** or **Move Down**.

The order of biosensor types listed in the **Sensor** box will be used as the default tray assignment (see Figure 7-58).

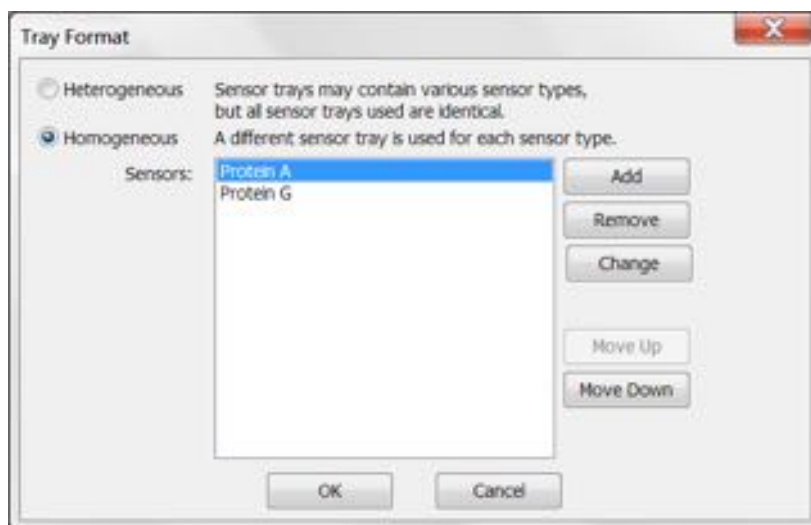


Figure 7-58: Biosensor Types List Order in Sensor Box

5. Click **OK**.

The software will automatically calculate the number of biosensor trays needed and assign biosensors types to each tray.

In the example shown in Figure 7-59, Protein A and Protein G biosensor types will be used for the multiple analyte experiment using two replicates. Four homogeneous biosensor trays (two for each biosensor type) will be needed for the experiment. The Tray 1 **Sensor Tray Map** will be displayed by default.

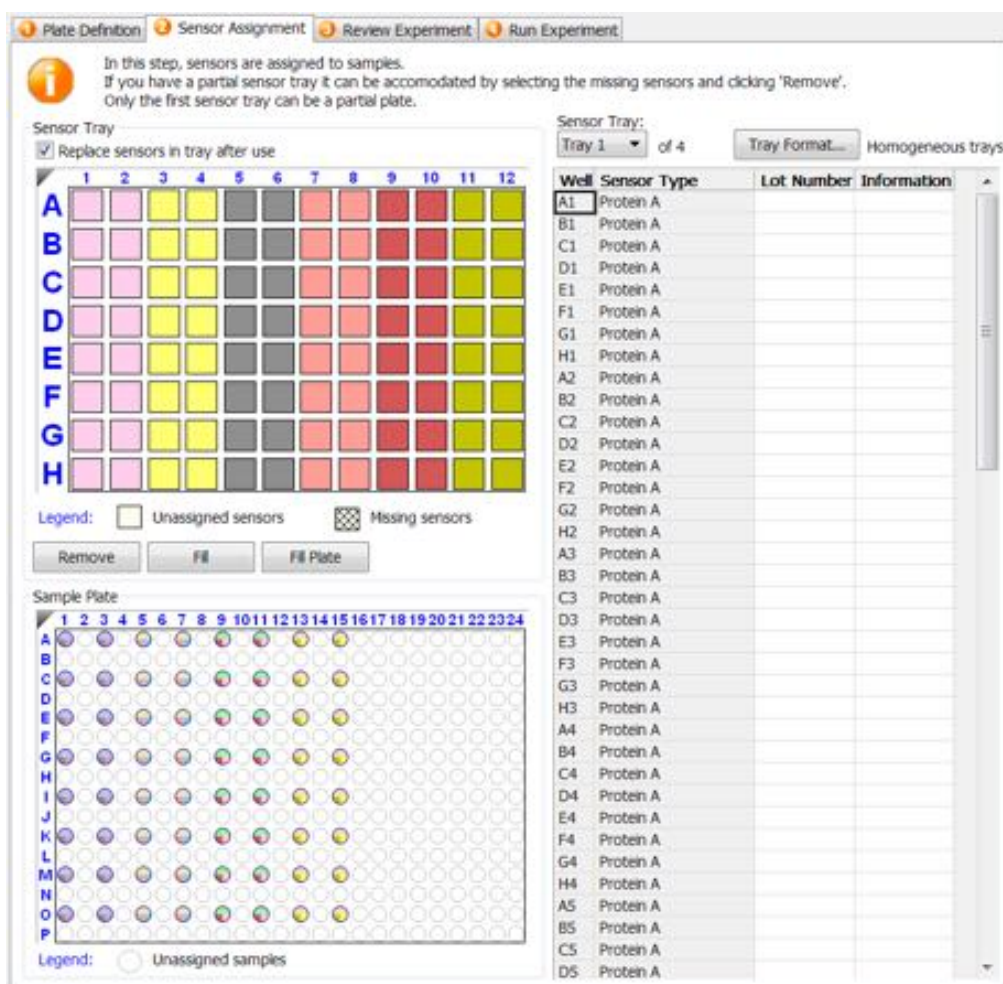


Figure 7-59: Biosensor Assignment using Homogeneous Trays and Two Biosensor Types

- To view the biosensor assignments in another tray, click the **Sensor Tray** button and select a tray number from the drop down list.

The **Sensor Tray Map** and table for the tray selected will be shown (see Figure 7-54).

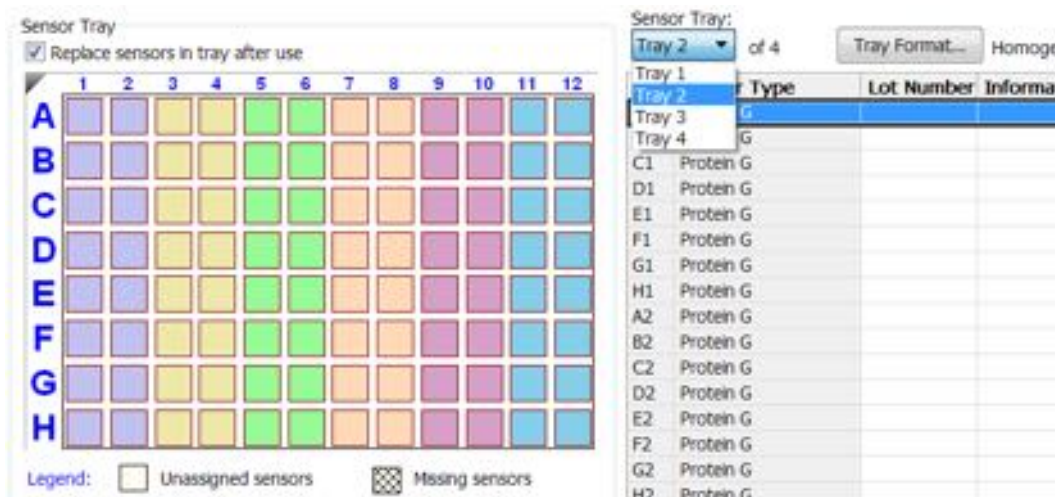


Figure 7-60: Tray Selection

7. To designate reference biosensors, select the desired biosensors in the **Sensor Tray Map**, right-click and select **Reference**.

The reference biosensors are marked with an **R**.



NOTE: Reference biosensors may also be designated in the **Runtime Binding Chart** during acquisition.

8. Optional: Double-click in any cell in the **Lot Number** column to enter a biosensor lot number. All wells in the **Lot Number** column for the biosensor type selected will automatically populate with the lot number entered.
9. Optional: Double-click in a cell in the **Information** column to enter biosensor information for particular cell.



NOTE: Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut - Ctrl+x**, **Copy - Ctrl+c**, **Paste - Ctrl+v**, **Undo - Ctrl+z**) are available in the table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: For greater clarity, annotation text may be displayed as the legend of the **Runtime Binding Chart** during data acquisition but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it will not be available for display as a legend.

10. Optional: After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace sensors in tray after use** check box (see Figure 7-47).

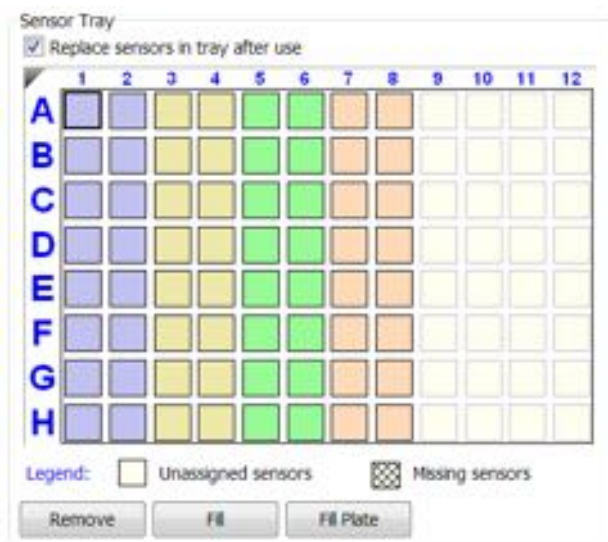


Figure 7-61: Replace Sensors in Tray After Use Check Box



NOTE: Biosensors can be regenerated up to a max of 11 times per experiment.

Biosensor Regeneration

For Basic Quantitation with Regeneration experiments only, the **Sensor Assignment** tab includes the **Regenerations** parameter, which specifies the maximum number of regeneration cycles for each column of biosensors. The specified number of regeneration cycles determines the minimum number of cycles required for each column of sensors. This calculation may result in non-equal regeneration cycles for columns of biosensors. The fractional use of the regeneration and neutralization wells by each column of sensors is represented by a pie chart (Figure 7-62).

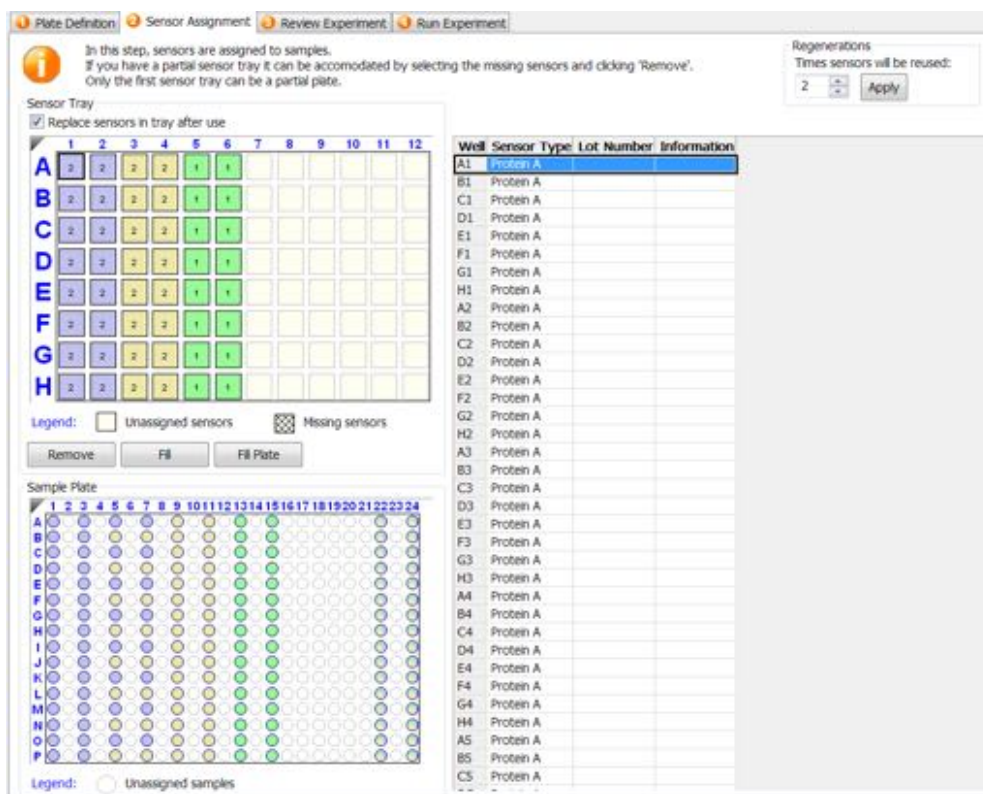


Figure 7-62: Fractional Use of Regeneration and Neutralization Wells

Using Partial Biosensor Trays

If you are using a partial tray of biosensors (some biosensors are missing), specify the missing columns in the **Sensor Tray Map**:

1. Select the column(s) without biosensors and click **Remove**, or right-click the selection and select **Remove**.

If the number of specified biosensors in the **Sensor Assignment** tab is less than the number required to perform the assay, the software automatically adds a second tray of biosensors and assigns the biosensors that are required for the assay.

2. To view the additional biosensor tray that is required for the assay, select Tray 2 from the **Sensor Tray** drop-down list (Figure 7-63). In the example shown, Tray 1 is a partial tray that does not contain enough biosensors for the assay. To designate a second tray, select Tray 2 from the **Sensor Tray** drop-down list (Figure 7-63 top). The **Sensor Tray Map** will then display the additional biosensors required for the assay (Figure 7-63 bottom).

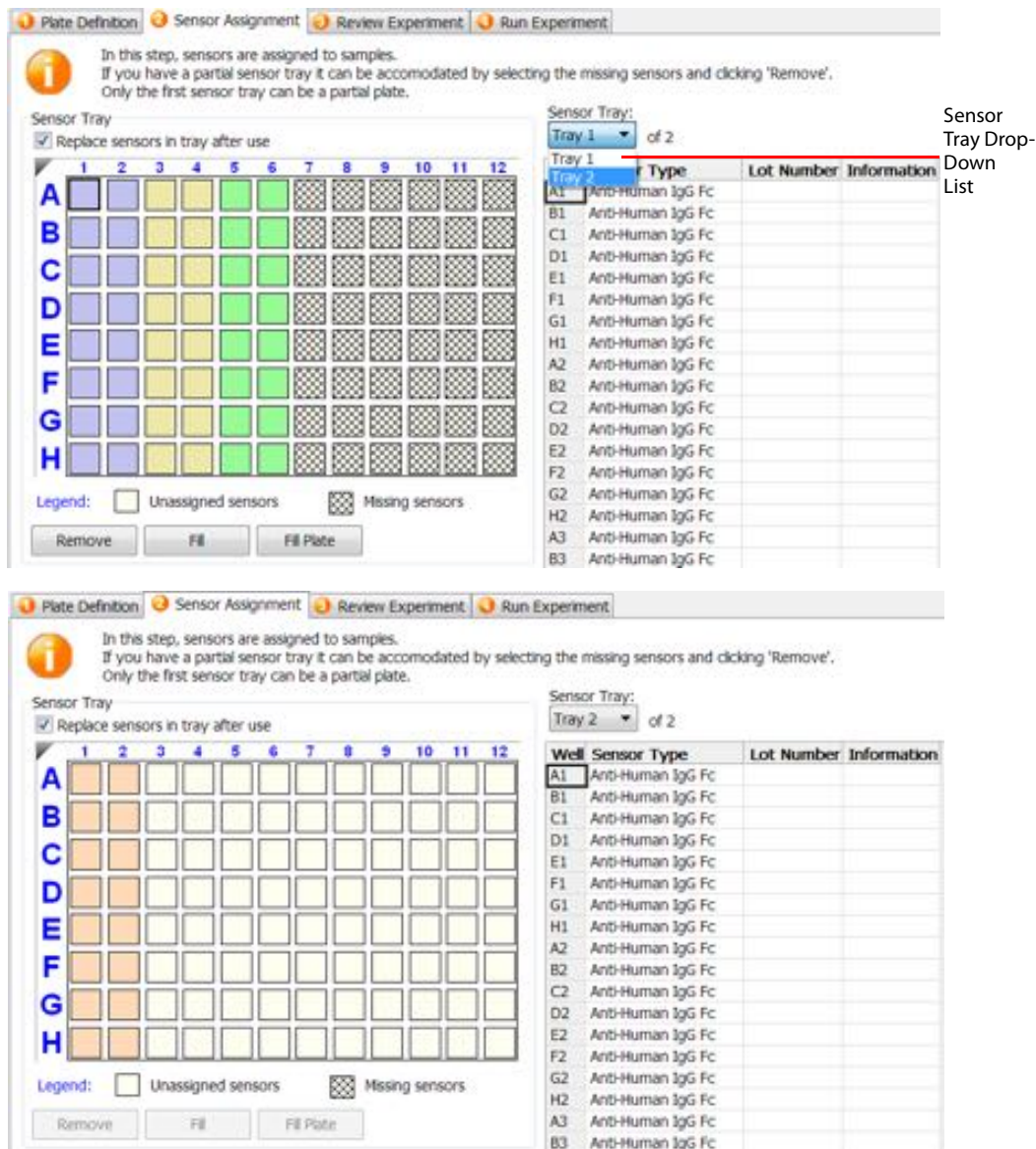


Figure 7-63: Example Assay Using One Partial Biosensor Tray and Biosensors from a Second Tray



To restore biosensors that have been removed, select the columns to restore and click **Fill**. To restore all sensors on the plate, click **Fill Plate**.



NOTE: If multiple biosensor trays are used, only the first biosensor tray can be a partial tray. During the experiment, the software prompts you to insert the appropriate tray in the Octet instrument.

REVIEWING EXPERIMENTS

Before running an experiment, you can review the sample plate layout and the biosensors assigned to each assay in the experiment.

In the **Review Experiment** window, move the slider left or right to highlight the biosensors and samples in an assay, or click the   arrows to select an assay.

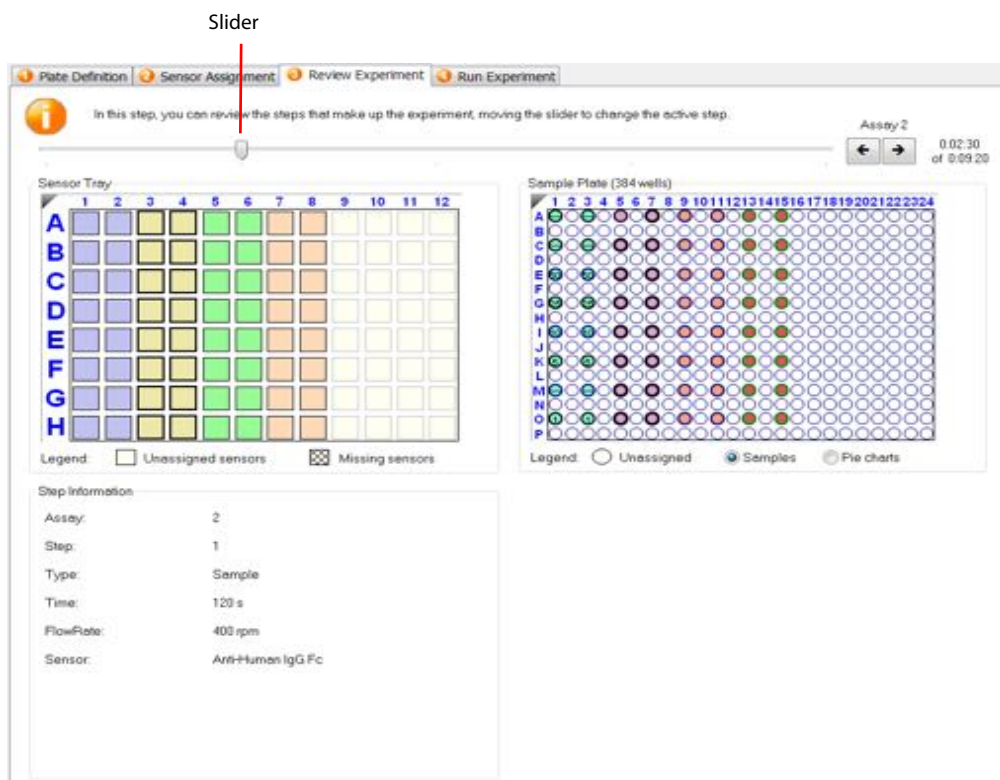




Figure 7-64: Review Experiment Window

SAVING EXPERIMENTS

After a run, the software automatically saves a read-only copy of the experiment information that you specified (sample plate definition, biosensor assignment, assay settings) to an experiment method file (.fmf). If you set up an experiment, but do not start the run, you can manually save the experiment method.

To manually save an experiment method:

1. Click the **Save Method File** button , or on the main menu, click **File > Save Method File**. To save more than one open experiment, click the **Save All Methods Files** button .
2. In the **Save** dialog box, enter a name and location for the file, and click **Save**.



NOTE: If you edit a saved experiment and want to save it without overwriting the original file, select **File > Save Method File As** and enter a new name for the experiment.

Saving an Experiment to the Template Folder

If you save an experiment to the factory-installed Template folder, the experiment will be available for selection. To view templates, click **Experiment > Templates > Quantitation > Experiment Name** (see Figure 7-65).

Follow the steps above to save an experiment to the Template folder located at C:\Program Files\ForteBio\DataAcquisition\TemplateFiles.



IMPORTANT: Do not change the location of the Template folder. If the Template folder is not at the factory-set location, the software may not function properly.

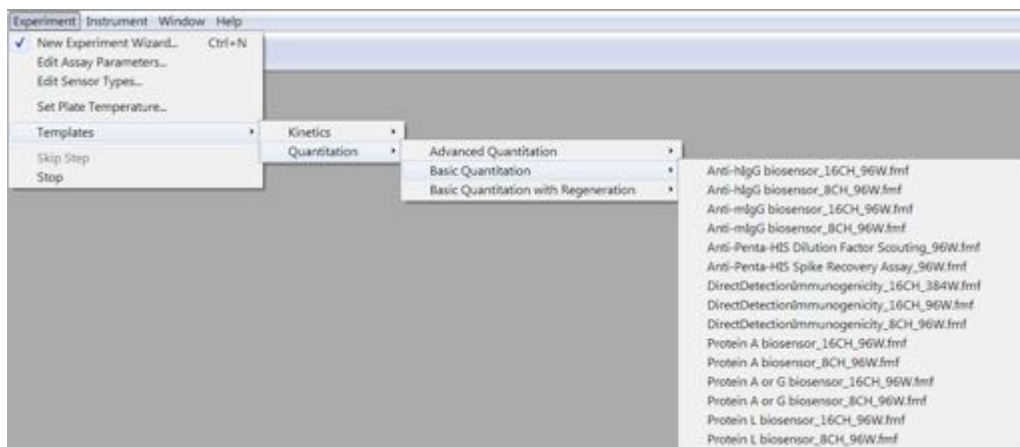


Figure 7-65: Experiments in the Template Folder

RUNNING A QUANTITATION EXPERIMENT



IMPORTANT: Before starting an experiment, ensure that the biosensors are properly rehydrated. For details on how to prepare the biosensors, see the appropriate biosensor product insert.

Loading the Biosensor Tray, Sample and Reagent Plates

To load the biosensor tray, sample plate, and reagent plate:

1. Open the Octet instrument door (lift the handle up) and present the instrument stage (click the **Present Stage** button).
2. Place the biosensor tray, sample plate, and reagent plate on the appropriate stage so that well A1 is located at the upper right corner (see Figure 7-66):
 - a. Place the rehydration plate and biosensor tray on the biosensor stage (left platform).
 - b. Place the sample plate on the sample stage (middle platform).
 - c. Optional: Place the reagent plate on the reagent stage (right platform) if you are using a reagent plate.

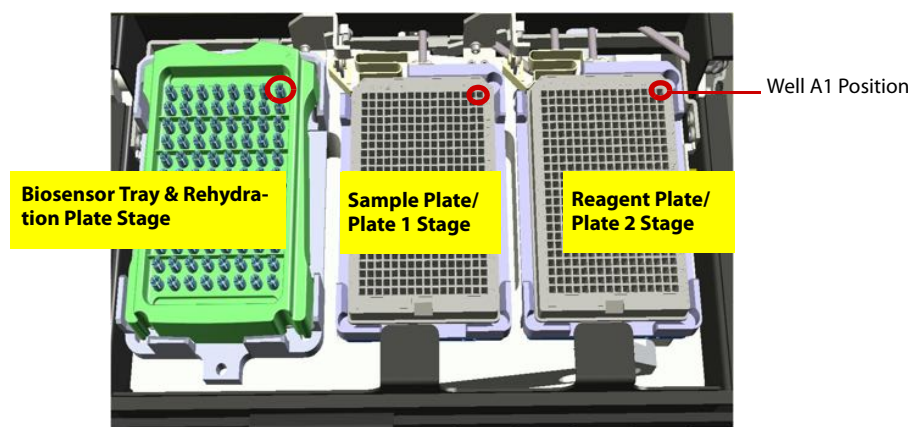


Figure 7-66: Octet Instrument Stage Platform




IMPORTANT: Ensure that the bottom of the sample plate, reagent plate, biosensor tray and rehydration plate are flat on each stage.

3. Click to close the Octet instrument door.
4. Allow the plate to equilibrate.

The time required for temperature equilibration depends on the temperature that your application requires and the initial temperature of the sample plate. For specific biosensor rehydration times, see the appropriate biosensor product insert.

Starting an Experiment

To start the experiment:

1. Click the **Run Experiment** tab, or click the arrow  to access the Run Experiment window (see Figure 7-67).

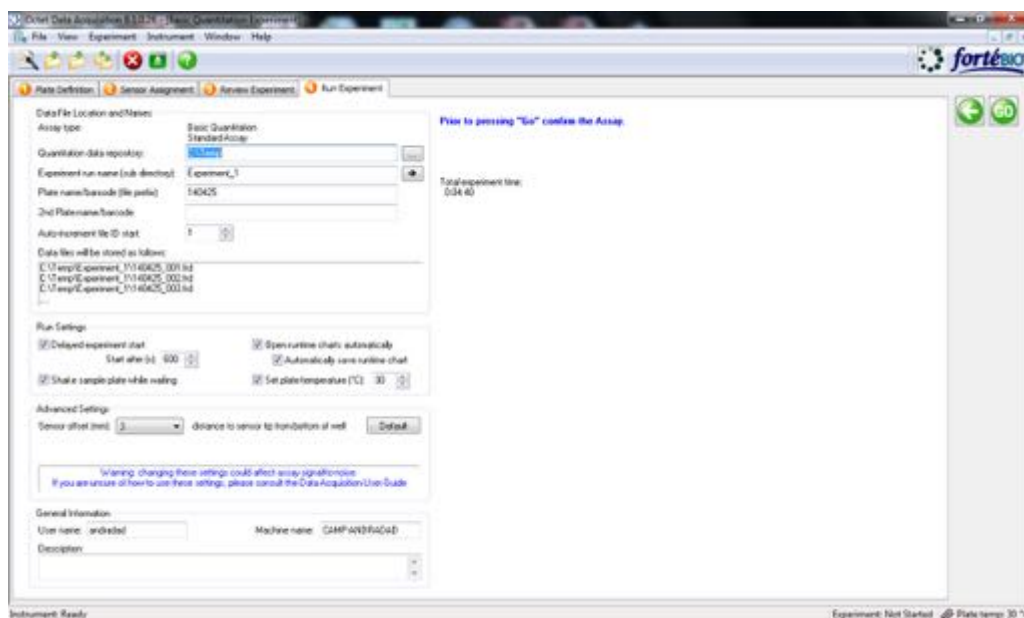


Figure 7-67: Run Experiment Window—Octet RED384

2. Confirm the defaults or enter new settings. See “Run Experiment Window Settings” on page 348 for more information on experimental settings.



NOTE: If you delay the experiment start, you have the option to shake the plate until the experiment starts.

3. To start the experiment, click .

If you specified a delayed experiment start, a message box displays the remaining time until the experiment starts.

If you selected the **Open runtime charts automatically** option, the **Runtime Binding Chart** window displays the binding data in real-time and the experiment progress (see Figure 7-68).



NOTE: For more details about the **Runtime Binding Chart**, see “Managing Runtime Binding Charts” on page 352.

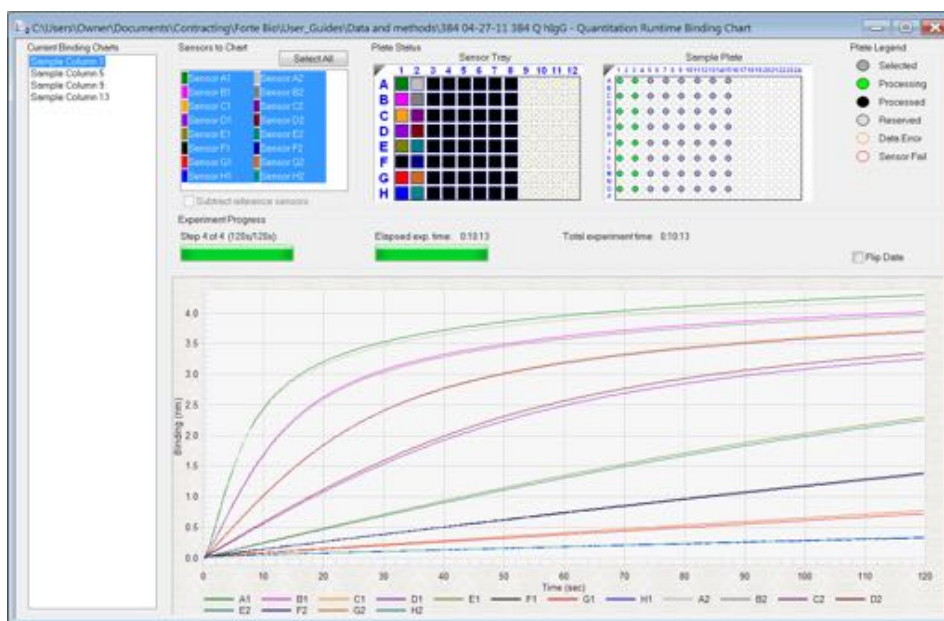


Figure 7-68: Runtime Binding Chart

4. Optional: Click **View > Instrument Status** to view the log file (see Figure 7-69).

The experiment temperature is recorded at the beginning of every experiment as well as each time the manifold picks up a new set of biosensors. Instrument events such as biosensor pick up, manifold movement, integration time, biosensor ejection and sample plate temperature are recorded in the log file.



WARNING: Do not open the Octet instrument door when an experiment is in progress. If the door is opened the data from the active acquisition step is lost. The data acquired in previous steps is saved, however the assay is aborted and cannot be restarted without ejecting the biosensors and starting from the beginning.



WARNING: N'ouvrez pas la porte de l'instrument Octet lorsqu'une analyse est en cours. En cas d'ouverture de la porte, les données issues de l'étape d'acquisition active seront perdues et cela entraînera l'échec de la procédure.



WARNING: Öffnen Sie die Instrumentenklappe des Octet-Systems nicht während eines laufenden Experiments. Wird die Klappe geöffnet, gehen die Daten des aktiven Erfassungsschritts verloren und das Experiment wird abgebrochen.

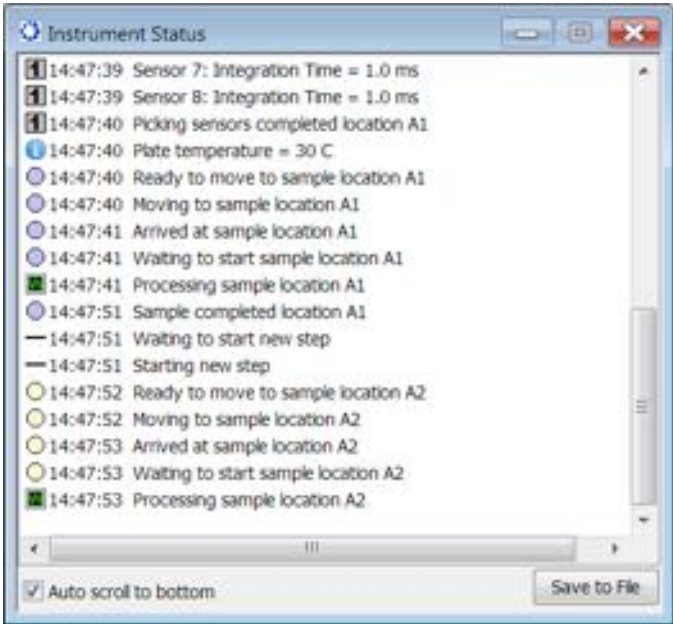


Figure 7-69: Instrument Status Log

Run Experiment Window Settings

The following **Data File Location and Name** settings are available on the **Run Experiment** Tab:

Table 7-9: Data File Location and Name

Item	Description
Assay type	The name of the selected assay.
Quantitation data repository	The location where quantitation data files (.frd) are saved. Click Browse to select another data location.



NOTE: It is recommended that you save the data to the local machine first, then transfer to a network drive.

Table 7-9: Data File Location and Name (Continued)

Item	Description
Experiment Run Name (sub-directory)	Specifies a subdirectory name for the data files (.frd) that are created. The software generates one data file for each biosensor.
Plate name/ barcode (file prefix)	A user-defined field where you can enter text or a barcode (barcode reader required).
2nd Plate name/barcode	A user-defined field where you can enter text or a barcode (barcode reader required) for a second plate.
Auto Increment File ID Start	Each file is saved with a number after the plate name. For example, if the Auto Increment File ID Start number is 1, the first file name is xxx_001.frd.

The following **Run Settings** are available on the **Run Experiment** Tab:

Table 7-10: Run Settings



Item	Description
Delayed experiment start	Specifies a time delay for the start of the experiment. Enter the number of seconds to wait before the experiment starts after you click  .
Start after	Enter the number of seconds to delay the start of the experiment.
Shake sample plate while waiting	If the experiment has a delayed start time, this setting shakes the plate until the experiment starts.
Open runtime charts automatically	Displays the Runtime Binding Chart for the current biosensor during data acquisition.
Automatically save runtime chart	Saves an image (.jpg) of the Runtime Binding Chart . The binding data (.frd) is saved as a text file, regardless of whether a chart image is created.


Table 7-10: Run Settings (Continued)

Item	Description
Set plate temperature (°C)	Specifies a plate temperature and enters the temperature in the dialog box. If not selected, the plate temperature is set to the default temperature specified in File > Options . The factory set default temperature is 30 °C.
<div><div></div><div>NOTE: If the actual plate temperature is not equal to the set plate temperature, a warning displays and the Octet System Data Acquisition software provides the option to wait until the set temperature is reached before proceeding with the run, continue without waiting until the set temperature is reached, or cancel the run.</div></div>	

Advanced settings are available for Octet RED384 and Octet QK384 systems. The signal to noise ratio of the assay can be optimized by selecting different acquisition rates. The acquisition rate refers to the number of binding signal data points reported by the Octet system per second and is reported in Hertz (per second). A higher acquisition rate generates more data points per second and monitors faster binding events better than a slower acquisition rate. A lower acquisition rate allows the software enough time to perform more averages of the collected data. Typically, more averaging leads to reduced noise and thus, better signal-to-noise ratios. Therefore, the frequency setting should be determined based on consideration of the binding rate, the amount of signal generated in your assay and some experimentation with the settings.

The following **Advanced Settings** are available for the Octet384 system:

Table 7-11: Advanced Settings Octet RED384

Item	Description
Acquisition rate	<div>  NOTE: For the Octet RED384 system, acquisition rate settings are available on the Plate Definition Tab. </div> <ul style="list-style-type: none"> High concentration quantitation (10 Hz, averaging by 5) — The average of 5 data frames is reported as one data point. 10 data points are reported per second. High sensitivity quantitation (2.0 Hz, averaging by 50)—The average of 50 data frames is reported as one data point. Two data points are reported per second. Standard quantitation (5.0 Hz, averaging by 20)—The average of 50 data frames is reported as one data point. Five data points are reported per second.
Sensor off set (mm)	Recommended sensor offset: Quantitation—3 mm
Default	Sets the acquisition speed and sensor offset at the default settings.

The following **Advanced Settings** are available for the OctetQK384 system:

Table 7-12: Advanced Settings Octet QK384


Item	Description
Acquisition rate	<ul style="list-style-type: none"> High sensitivity quantitation (0.3 Hz, averaging by 40)—The average of 40 data frames is reported as one data point. One data point is reported every 3.3 seconds. Standard quantitation (0.6 Hz, averaging by 5)—The average of 5 data frames is reported as one data point. One data point is reported every 1.6 seconds.
Sensor off set (mm)	Recommended sensor offset: Quantitation—3 mm
Default	Sets the acquisition speed and sensor offset at the default settings.

The following **General Settings** are available on the **Run Experiment** Tab:

Table 7-13: General Settings

Item	Description
Machine name	The computer name that controls the Octet instrument and acquires the data.
User name	The user logon name.
Description	A user-specified description of the assay or assay purpose. The description is saved with the method file (.fmf).

Stopping an Experiment

To stop an experiment in progress, click  or click **Experiment > Stop**.

The experiment is aborted. The data for the active biosensor is lost, the biosensor is ejected into the waste tray, and the event is recorded in the experimental log.



NOTE: After the experiment is run, the software automatically saves the experiment method (.fmf).

MANAGING RUNTIME BINDING CHARTS

If the **Open runtime charts automatically** check box is selected in the Run Experiment window, the Runtime Binding Charts are automatically displayed when data acquisition starts (see Figure 7-70). The **Runtime Binding Chart** window displays the current step number, time remaining for the current step, (total) elapsed experimental time, and total experiment time.

The **Runtime Binding Chart** is updated at the start of each experimental step. The active biosensor column is color-coded (A=green, B=magenta, C=orange, D=purple, E=olive, F=black, G=red, H=blue) within the **Sensor Tray Map**. Used sensor columns that are inactive are colored black. Active sample columns are colored green. Each data acquisition step is represented by **Sample Column X** in the **Current Binding Charts** box.

To selectively display acquisition data for a particular acquisition step:

1. Click the corresponding **Sample Column** number.
2. Select a sub-set of sensors for a displayed column in the **Sensors to Chart** box (see Figure 7-70).



IMPORTANT: Do not close the **Runtime Binding Chart** window until the experiment is complete and all data is acquired. If the window is closed, the charts are not saved. To remove the chart from view, minimize the window. The Octet System Data Acquisition software saves the **Runtime Binding Chart** as displayed at the end of the experiment. For example, modifying a chart by hiding the data for a particular biosensor will cause this data not to be included in the bitmap image generated at the end of the run.

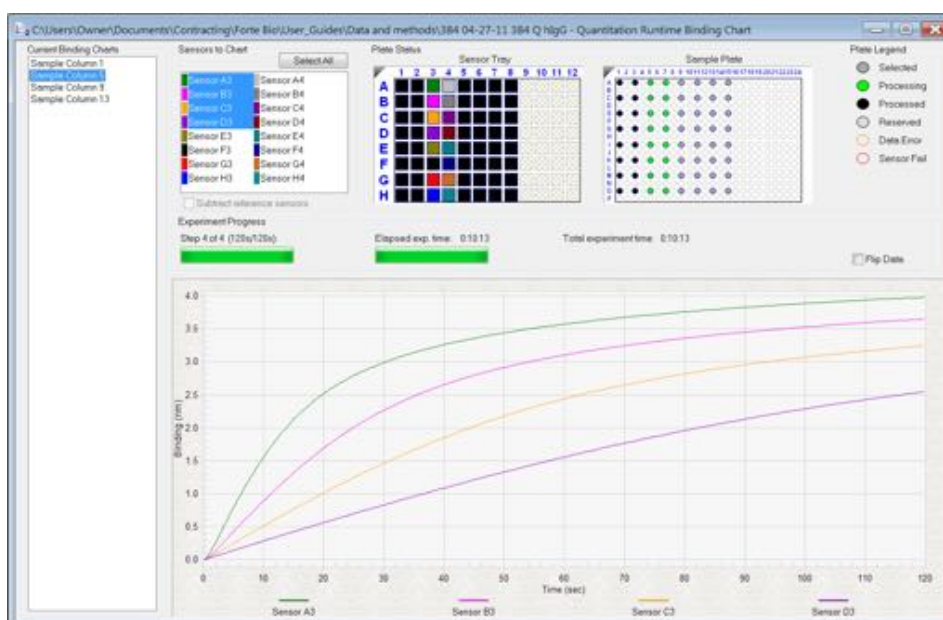


Figure 7-70: Runtime Binding Chart Window

Opening a Runtime Binding Chart

After an experiment is run, you can open and review the **Runtime Binding Chart** at any time:

1. Click **File > Open Experiment**.
2. In the dialog box that appears, select an experiment folder and click **Select**.

Viewing Reference-Subtracted Data

If the experiment includes reference biosensors, you can display reference-subtracted data during acquisition in the chart by clicking the **Subtract reference sensors** check box in the chart window. To view raw data, remove the check mark next to this option.

Reference biosensors can be designated:

- During experiment setup in the **Sensor Assignment** tab
- During acquisition in the Runtime Binding Chart **Sensors to Chart** box
- During analysis in the **Data Selection** tab

Designating a Reference Biosensor During Acquisition

To designate a reference biosensor during acquisition:

1. In the **Sensors to Chart** list or the **Sensor Tray**, right-click a biosensor and select **Reference** (see Figure 7-71).

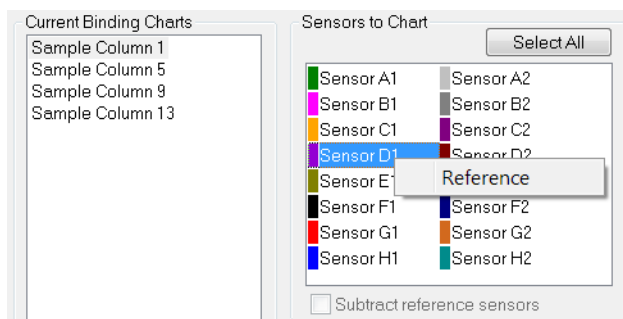


Figure 7-71: Designating a Reference Biosensor in the Runtime Binding Chart

The selected biosensor will be shown with an **R** in the **Sensors to Chart** list and **Sensor Tray** (see Figure 7-74).

2. Click the **Subtract reference sensors** check box (see Figure 7-74).

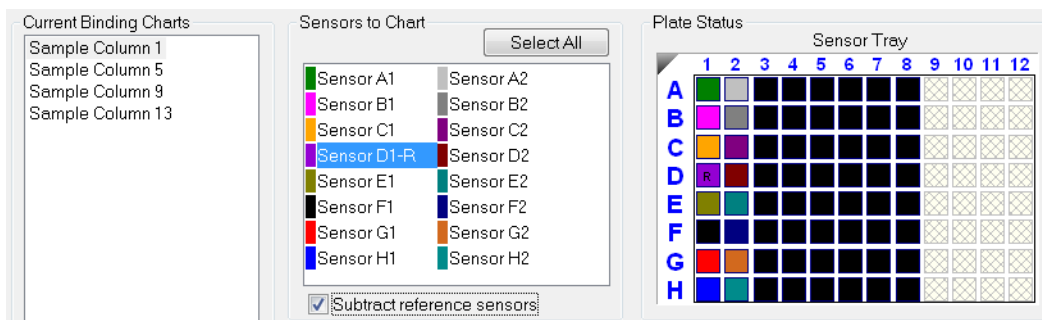


Figure 7-72: Subtract Reference Sensors check box in the Runtime Binding Chart



NOTE: Subtracting reference data in the **Runtime Binding Chart** only makes a visual change to the data on the screen. The actual raw data is unaffected and the reference subtraction must be re-done in data analysis if needed.

Viewing Inverted Data

The data displayed in the **Runtime Binding Chart** can be inverted during real-time data acquisition or data analysis after the experiment has completed. To invert data, select the **Flip Data** check box (see Figure 7-73). Uncheck the box to return to the default data display.

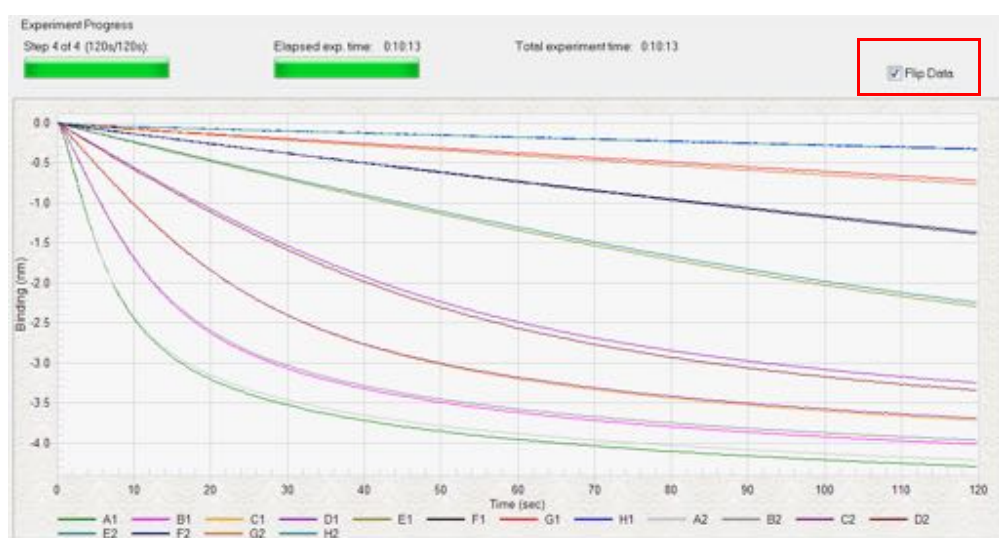


Figure 7-73: Data Inverted Using Flip Data Function

Magnifying the Runtime Binding Chart

To magnify the chart, press and hold the mouse button while you draw a box around the chart area to magnify.

To undo the magnification, right-click the chart and select **Undo Zoom**.

Scaling a Runtime Binding Chart

To scale the **Runtime Binding Chart**:

1. Right-click the chart and select **Properties**.
2. In the **Runtime Graph Properties** dialog box, select **Fullscale** or **Autoscale**.

Adding a Runtime Binding Chart Title

To add a **Runtime Binding Chart** title:

1. Right-click the chart and select **Properties**.
2. In the **Runtime Graph Properties** dialog box, enter a graph title or subtitle.

Selecting a Runtime Binding Chart Legend

To select a **Runtime Binding Chart** legend:

1. Right-click the chart and select **Properties**.
2. In the **Runtime Graph Properties** dialog box (see Figure 7-74), select one of the following legends:
 - Sensor Location
 - Sample ID
 - Sensor Information
 - Concentration/Dilution

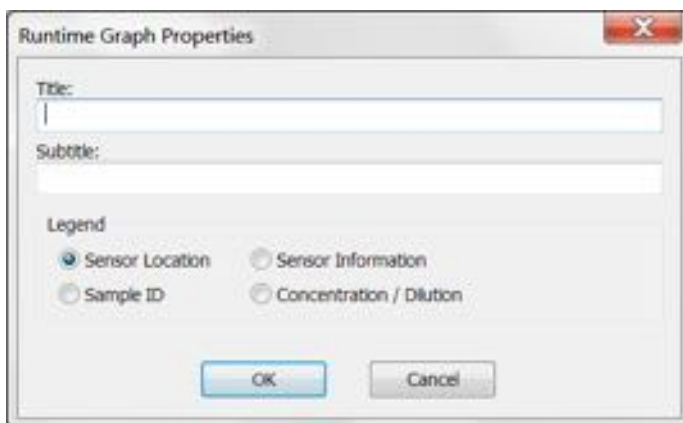


Figure 7-74: Selecting a Runtime Binding Chart Legend



NOTE: Text for **Sample ID**, **Sensor Information**, or **Concentration/Dilution** is taken from the **Plate Definition** and **Sensor Assignment** tabs, and must be entered before the experiment is started.

3. Click **OK**.

Viewing Multiple Runtime Binding Charts

To view multiple Runtime Binding Charts, click **Window > New Window**.

Exporting or Printing the Runtime Binding Chart

To export the **Runtime Binding Chart** as a graphic or data file:

1. Right-click the chart and select **Export Data**.
2. In the **Exporting** dialog box (see Figure 7-75), select the export options and click **Export**.

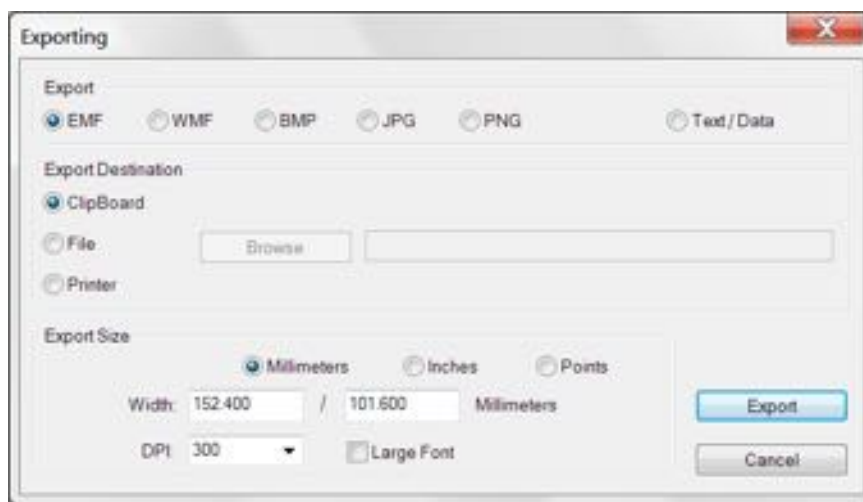


Figure 7-75: Exporting Dialog Box

Table 7-14: Runtime Binding Chart Export Options

Task	Export	Option	Export Destination	Result
	Text/ Data	EMF, WMF, BMP, JPG, or PNG		
Save the binding data	✓		Click File > Browse to select a folder and enter a file name.	Creates a tab-delimited text file of the numerical raw data from each biosensor. Open the file with a text editor such as Notepad.
Export the Runtime Binding Chart to a graphic file		✓	Click File > Browse to select a folder and enter a file name.	Creates a graphic image.

Table 7-14: Runtime Binding Chart Export Options (Continued)

Task	Export	Option	Export Destination	Result
Copy the Runtime Binding Chart		✓	Clipboard	Copies the chart to the system clipboard
Print the Runtime Binding Chart		✓	Printer	Opens the Print dialog box.




MANAGING EXPERIMENT METHOD FILES

After you run an experiment, the Octet System Data Acquisition software automatically saves the method file (.fmf), which includes the sample plate definition, biosensor assignment, and the run parameters. An experiment method file provides a convenient initial template for subsequent experiments. Open a method (.fmf) and edit it if necessary.



NOTE: When using the 21 CFR Part 11 version of the Octet System Data Acquisition software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.

Table 7-15: Managing Experiment Method Files

Menu Bar Command/ Toolbar Button	Description
File > Open Method File 	Enables you to select and open a method file (.fmf)
File > Save Method File  or 	Saves one method file or all method files. Saves a method file before the experiment is run.
File > Save Method File As	Saves a method file to a new name so that the original file is not overwritten.

CUSTOM QUANTITATION ASSAYS

Defining a Custom Assay

To define a custom assay:

1. Click **Experiment > Edit Assay Parameters**.

The **Edit Assay Parameters** dialog box appears (see Figure 7-76).

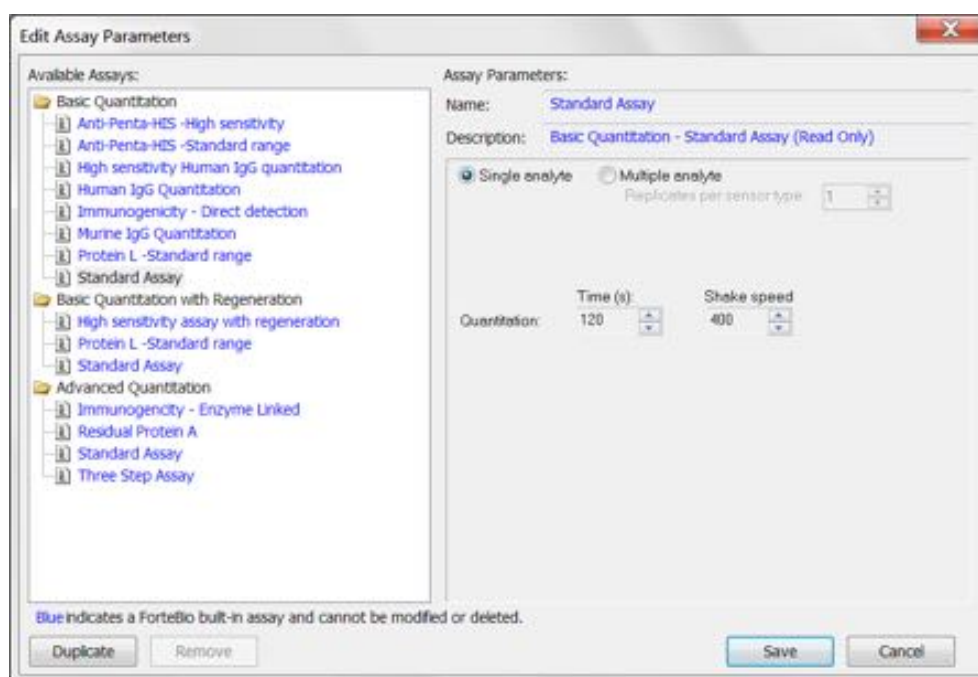


Figure 7-76: Edit Assay Parameters Dialog Box

2. In the directory tree of assays, select the type of standard assay to modify. For example, to define a new basic quantitation assay, in the Basic Quantitation folder, select **Standard Assay**.
3. Click **Duplicate**.
4. In the **New Assay** dialog box (see Figure 7-77 top), enter an **Assay name**.
5. Optional: In the **Assay Description**, enter information about the assay.
6. Click **Save**.

The new assay appears in the directory tree of available assays (see Figure 7-77 bottom).

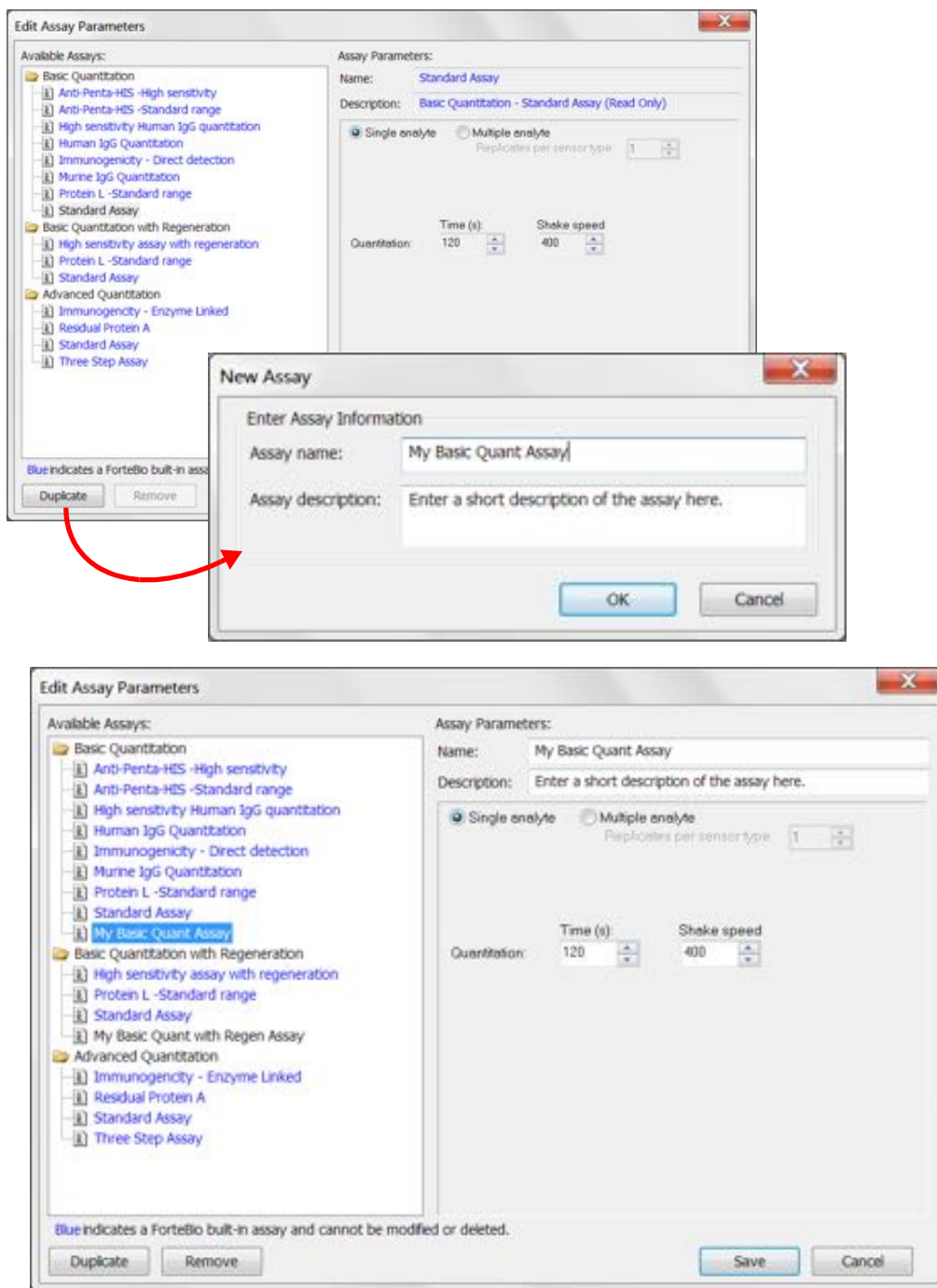


Figure 7-77: Defining a New Assay

Editing Assay Parameters

To edit assay parameters:

1. In the **Edit Assay Parameters** dialog box, confirm that the new assay is selected in **Available Assays** (see Figure 7-77 bottom).
2. Modify the assay parameters as needed. A complete list of parameters for each type of quantitation experiment follows this procedure.
3. Click **Save** to accept the new parameter values. The new assay is added to the system.



NOTE: Not all parameters are available for all of the assays.

Basic Quantitation Assay Parameters

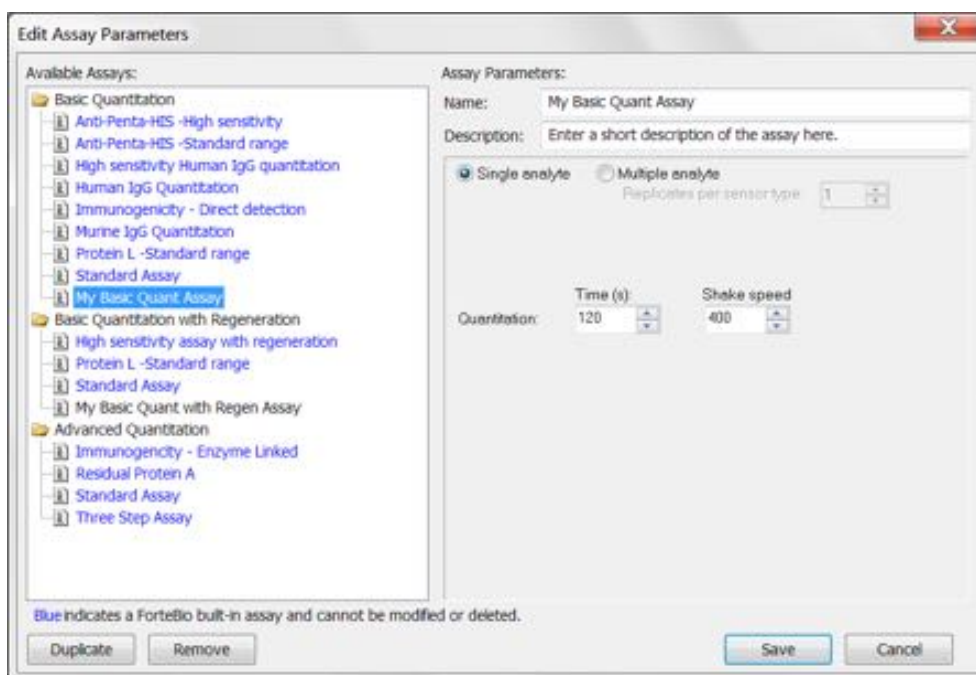



Figure 7-78: Assay Parameters—Basic Quantitation Assay

Table 7-16: Basic Quantitation Assay Parameters

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.

Table 7-16: Basic Quantitation Assay Parameters (Continued)

Parameter	Description
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time (s)	The duration of data acquisition seconds while the biosensor is incubated in sample. <div><div></div><div>NOTE: A subset of data points may be selected for processing during data analysis.</div></div>
Quantitation Shake speed (rpm)	The sample shaking speed (rotations per minute).

Basic Quantitation with Regeneration Assay Parameters

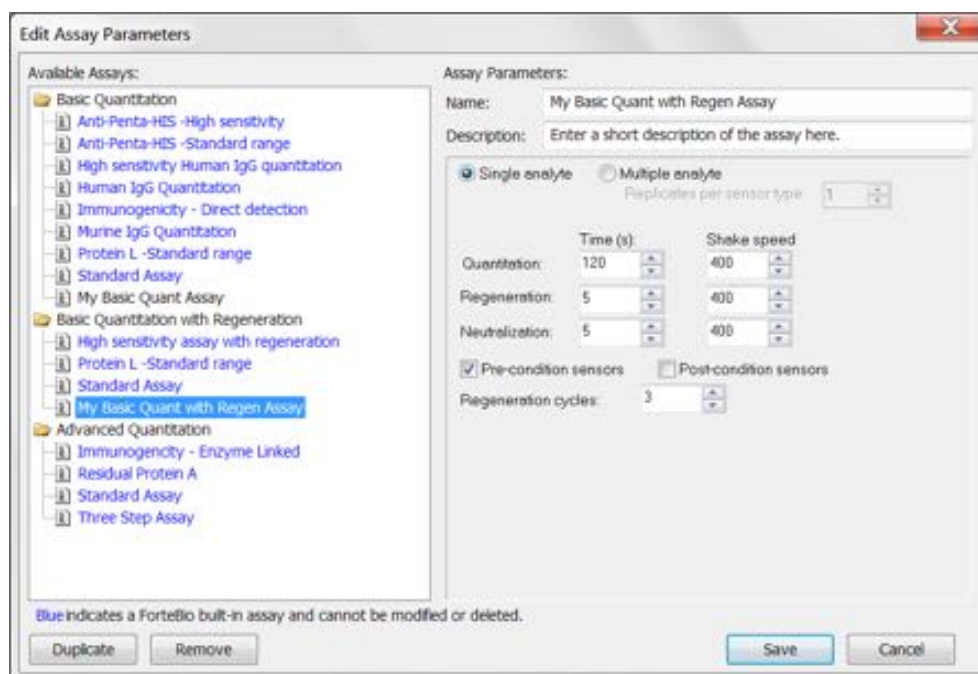


Figure 7-79: Assay Parameters—Basic Quantitation with Regeneration

Table 7-17: Assay Parameters—Basic Quantitation with Regeneration

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Quantitation Time(s) and Shake speed (rpm)	The duration of data acquisition in seconds while the biosensor is incubated in sample and the sample shaking speed (rotations per minute).
<div>➔</div> NOTE: A subset of data points may be selected for processing during data analysis.	
Regeneration Time(s) and Shake speed (rpm)	The duration time and shaking speed of the regeneration step where the biosensor is incubated in regeneration buffer to remove bound analyte.

Table 7-17: *Assay Parameters—Basic Quantitation with Regeneration*

Parameter	Description
Neutralization Time(s) and Shake speed (rpm)	The duration time and shaking speed of the neutralization step where the biosensor is incubated in neutralization buffer after the regeneration step.
Pre-condition sensors	Performs a set of regeneration/neutralization steps prior to the start of the experiment. The pre-conditioning settings are equivalent to the time and rpm settings for the regeneration in the assay. For example, an acidic pre-conditioning buffer maximizes the binding competence of Pro-A biosensors.
Post-condition sensors	Post-conditions biosensors, allowing re-racked biosensors to be stored in a regenerated state.
Regeneration cycles	The number of regeneration-neutralization cycles that a biosensor undergoes before reuse.

Advanced Quantitation Assay Parameters

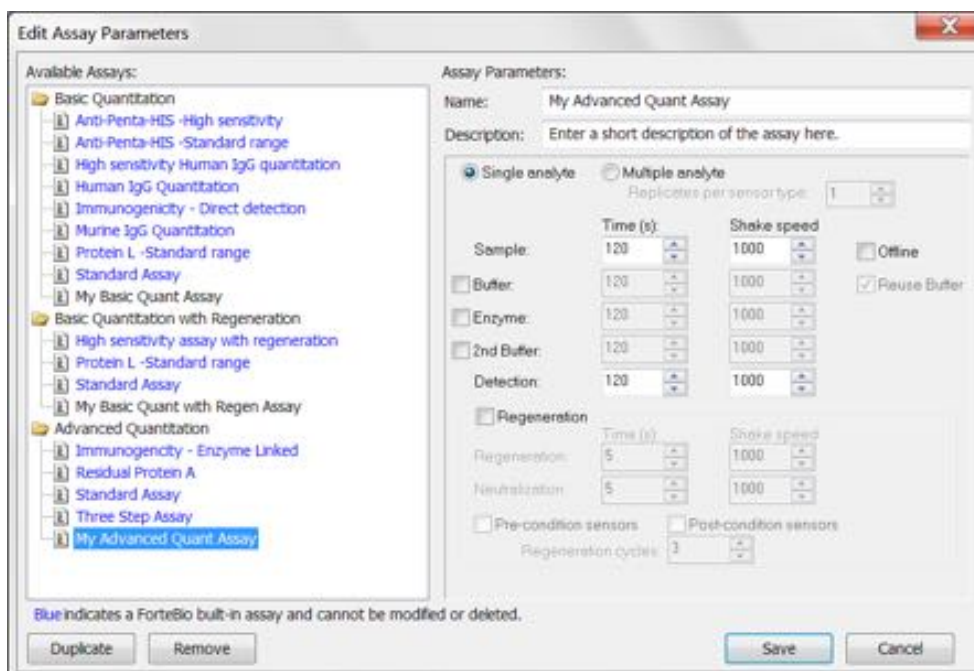


Figure 7-80: Assay Parameters—Advanced Quantitation

Table 7-18: Advanced Quantitation Assay Parameters

Parameter	Description
Single analyte	For single-analyte experiments using only one biosensor type per sample well.
Multiple analyte and Replicates per sensor type	For multi-analyte experiments using multiple biosensor types per sample well, and the number of replicate assays in each well per biosensor type.
Sample Time(s) and Shake speed (rpm)	The duration of data acquisition in seconds while the biosensor is incubated in sample and the sample shaking speed (rotations per minute).
<div>➔</div> NOTE: A subset of data points may be selected for processing during data analysis.	
Buffer Time(s) and Shake speed (rpm)	The duration of biosensor incubation in the first buffer in seconds and the sample shaking speed (rotations per minute).

Table 7-18: Advanced Quantitation Assay Parameters


Parameter	Description
Enzyme Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the enzyme solution and the sample shaking speed (rotations per minute).
2nd Buffer Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the second buffer solution and the sample shaking speed (rotations per minute).
Capture Antibody Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the first capture antibody solution and the shaking speed (rotations per minute).
2nd Antibody Time(s) and Shake speed (rpm)	The duration of biosensor incubation in seconds in the secondary antibody solution and the shaking speed (rotations per minute).
Detection Time(s) & Shake speed (rpm)	The duration of data acquisition during the detection step in seconds in an advanced quantitation assay.
<div>  NOTE: A subset of data points may be selected for processing during data analysis. </div>	
Offline	Choose this option to incubate sample with biosensors outside the Octet system. Offline incubation is best performed on the Pall ForteBio Sidekick biosensor immobilization station.
Reuse Buffer	Allows buffer wells to be reused. If unselected, the number of buffer columns must equal the number of sample columns. If selected, the number of buffer columns may be less than the number of sample columns as the buffer columns are reused.
Regeneration Time(s) and Shake speed (rpm)	The duration time and shaking speed of the regeneration step where the biosensor is incubated in regeneration buffer to remove bound analyte.
Neutralization Time(s) and Shake speed (rpm)	The duration time and shaking speed of the neutralization step where the biosensor is incubated in neutralization buffer after the regeneration step.
Pre-condition sensors	Performs a set of regeneration/neutralization steps prior to the start of the experiment. The pre-conditioning settings are equivalent to the time and rpm settings for the regeneration in the assay. For example, an acidic pre-conditioning buffer maximizes the binding competence of Protein A biosensors.

Table 7-18: Advanced Quantitation Assay Parameters

Parameter	Description
Post-condition sensors	Post-conditions biosensors, allowing re-racked biosensors to be stored in a regenerated state.
Regeneration cycles	The number of regeneration-neutralization cycles that a biosensor undergoes before reuse.



NOTE: In an Advanced Quantitation experiment, this option is only available if the first step (biosensor incubation in sample) is performed online.

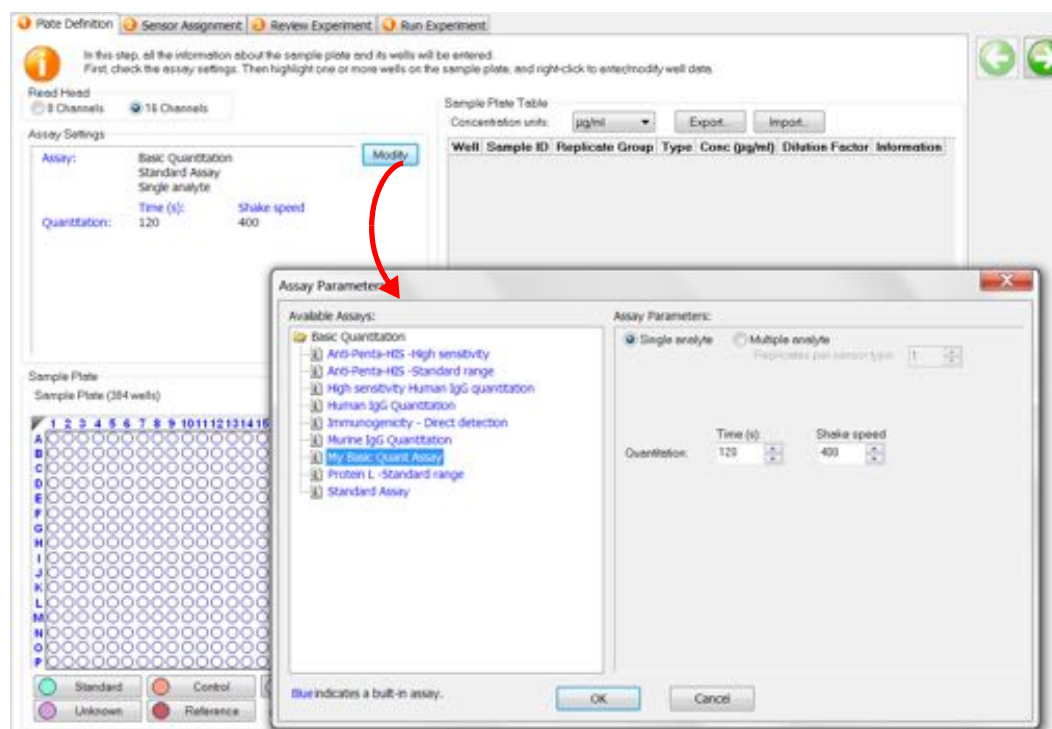
Selecting a Custom Assay

You can select a custom assay when you define a sample plate.

To select a custom assay:

1. In the **Plate Definition** tab, click **Modify** in the **Assay Settings** box.

The **Edit Assay Parameters** dialog box displays (see Figure 7-81).

**Figure 7-81: Selecting a Custom Assay**

2. Select the custom assay from the directory tree and click **OK**.

MULTI-STEP ADVANCED QUANTITATION EXPERIMENTS

Octet RED384 and QK384

The multi-step selection interface for Advanced Quantitation methods increases the flexibility to add more assay steps prior to the Sample or Detection steps. In addition, all steps in an Advanced Quantitation assay may be viewed and analyzed in the Octet Data Analysis software.

After starting the Octet system and the Octet Data Acquisition software, follow the steps below to set up and run an Advanced Quantitation experiment. You can start an Advanced Quantitation experiment using one of the following options:

- Launch the **Experiment Wizard**.
- Open a method file (.fmf) by clicking **File > Open Method File**. Method files may be saved and recalled using the **File** menu and are automatically saved when an experiment is run.
- On the menu bar, click **Experiment > Templates > Quantitation > Advanced Quantitation**.

These options are explained further in “Starting an Experiment Using the Experiment Wizard” on page 287.



NOTE: The Sample plate and the Reagent plate are now referred to as “Plate 1” and “Plate 2” in the software.

1. To add or edit assay steps in Tab 1 (Plate Definition), click **Modify** in Assay Settings to display the Assay Parameters window. Click on the **Step Type** drop-down list or highlight the parameter you want to change:

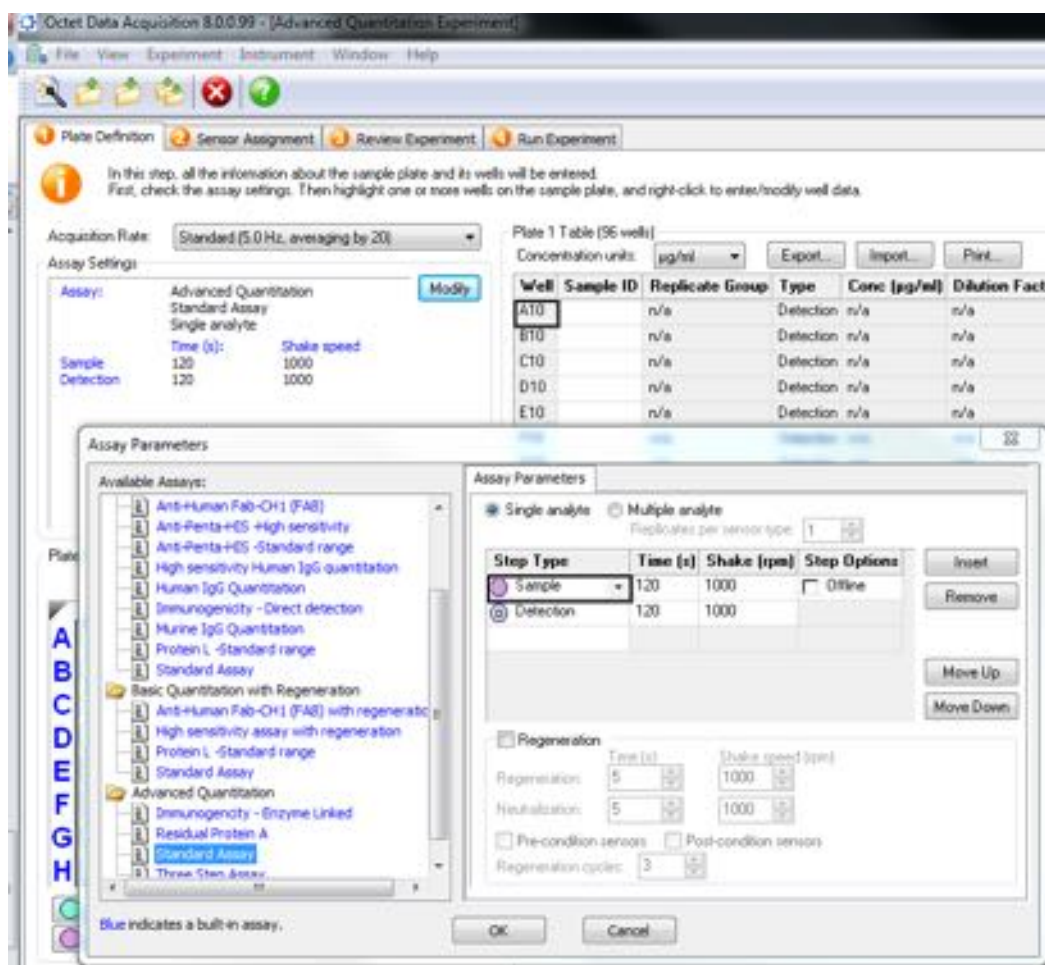


Figure 7-82: Assay Parameters Window.

To add or remove steps, click the **Insert** or **Remove** buttons. Individual steps may be re-organized using the **Move Up** or **Move Down** buttons. Click **OK** to save any changes.

2. Continue with the plate layout and sample well designation in Tab 1. For more details see “Defining the Sample Plate” on page 289, “Managing Sample Plate Definitions” on page 313 and “Managing Assay Parameter Settings” on page 319.
3. Proceed to Tab 2 (Sensor Assignment) and the remaining tabs as described starting with “Assigning Biosensors to Samples” on page 325 before running the Advanced Quantitation method.

Octet HTX

The Advanced Quantitation application combines the flexibility of the user-selectable Read Head with easier visualization of all the steps in a quantitation assay, including multiple steps preceding the Detection or Sample step. Users can configure the initial assay steps

with a Read Head of 8, 16, 32, 48 or 96 biosensors, separately from the later detection steps. Analysis from 8 or 16 biosensors provides the greatest sensitivity and finer signal resolution whereas data acquisition from 32, 48 or 96 biosensors provides higher throughput.

Two new tabs, Sensor Loading and Plate Definition, provide individual control for preliminary assay steps, apart from the detection steps. An Advanced Quantitation Method file (*.fmf) may contain assays with two different Read Head configurations. An example of this would be to immobilize 96 biosensors all at once, re-rack all 96 biosensors, and then analyze 16 biosensors at a time for the entire biosensor tray. Quantitation analysis will be performed on the default Detection step type, typically the last assay step in Plate Definition.

After starting the Octet HTX system and the Octet Data Acquisition software, follow the steps below to set up and run an Advanced Quantitation experiment with user-selectable Read Head configurations. For information on how to connect the Octet instrument to the computer and starting the software, please refer to Chapter 3, "Getting Started" on page 55.

You can start an Advanced Quantitation experiment using one of the following options:

- Launch the **Experiment Wizard**.
- Open a method file (.fmf) by clicking **File > Open Method File**. Method files may be saved and recalled using the File menu and are automatically saved when an experiment is run.
- On the menu bar, click **Experiment > Templates > Quantitation > Advanced Quantitation**.

These options are explained further in "Starting an Experiment Using the Experiment Wizard" on page 287.

1. Open Tab 1 (Sensor Loading) to configure the Read Head for the preliminary assay steps that will have a different setting from the later steps or Detection step. The default Read Head configuration is 96 channels which dips 96 biosensors simultaneously for the Sensor Loading steps.
2. Click on the drop-down list for Read Head to select 96, 48, 32, 16 or 8 channels as the new Read Head setting for all of these early assay steps (Figure 7-83).

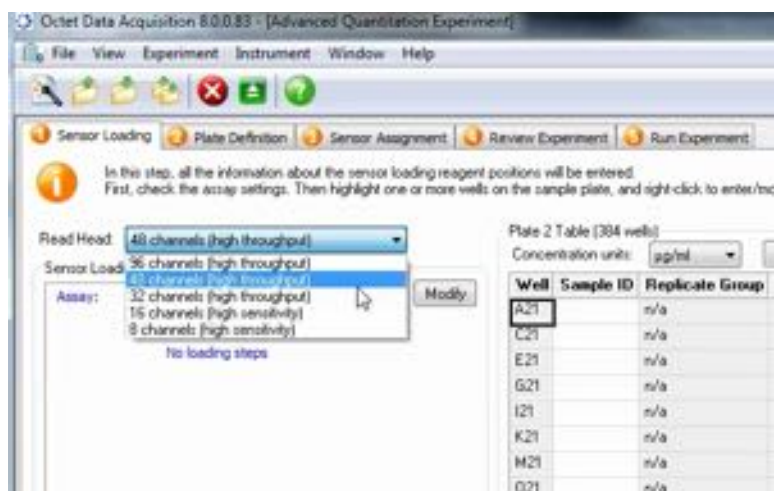


Figure 7-83: Selecting a New Read Head Setting

- To add or edit the Sensor Loading steps, click **Modify** in Sensor Loading Settings to bring up the Sensor Loading tab in the Assay Parameters window. Click on the drop-down list for Step Type or highlight the parameter you want to change. Click **OK** to complete the changes (Figure 7-84).

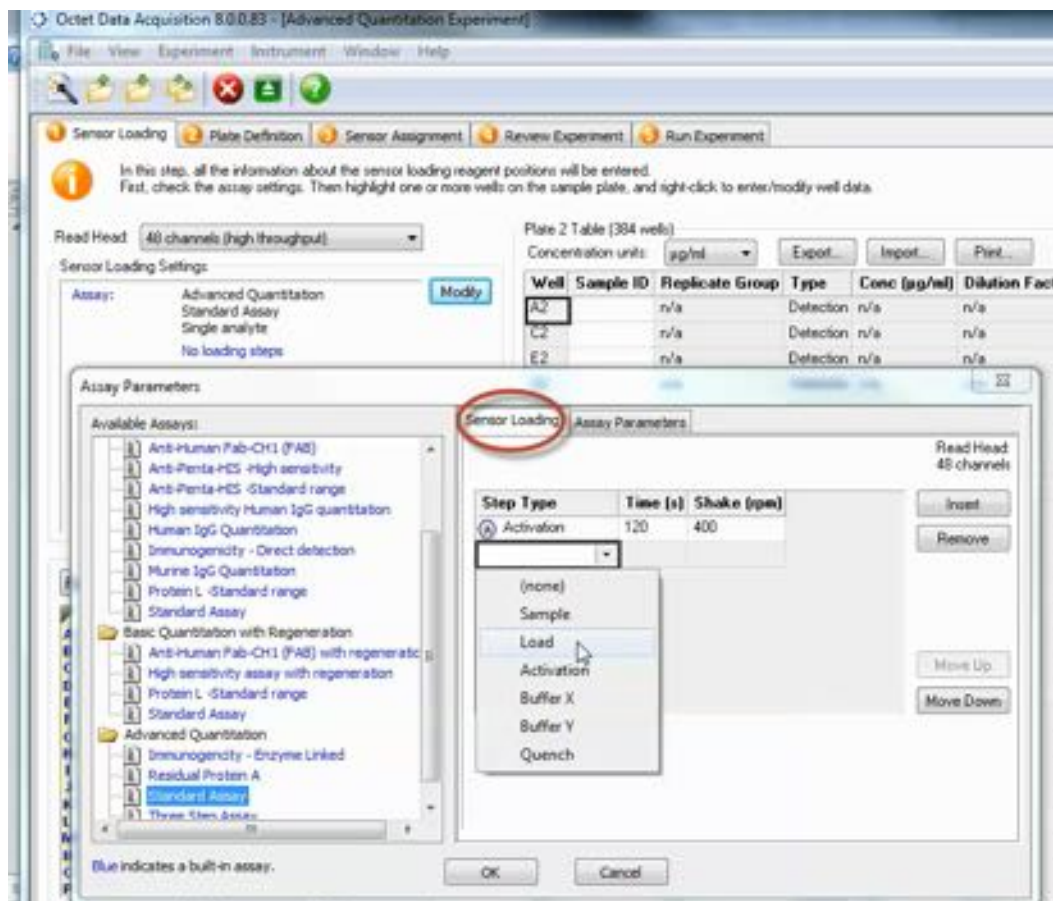


Figure 7-84: Modifying Sensor Loading Parameters

4. Continue with the plate layout and sample well designation for the Sensor Loading steps.



NOTE: All sample types such as Standards, Unknowns, Controls and References can now be loaded in either plate positions 1 or 2, or both.

5. Proceed to Tab 2 (Plate Definition) to configure the Read Head for the later steps or Detection step that will have a different setting from the preliminary Sensor Loading step(s). The default Read Head configuration will be the same setting previously selected in Tab 1 (Sensor Loading).
6. Click on the drop-down list for Read Head to select 96, 48, 32, 16 or 8 channels as the new Read Head setting for all of these later assay steps:

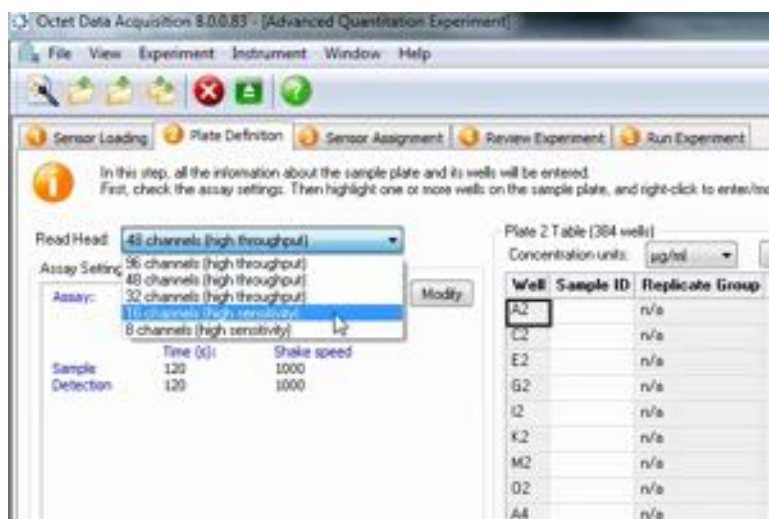


Figure 7-85: Selecting a New Read Head Setting

- To add or edit the later steps or detection step, click **Modify** in Assay Settings to bring up the Assay Parameters tab in the Assay Parameters window. Click on the drop-down list for Step Type or highlight the parameter you want to change. Click **OK** to complete the changes:

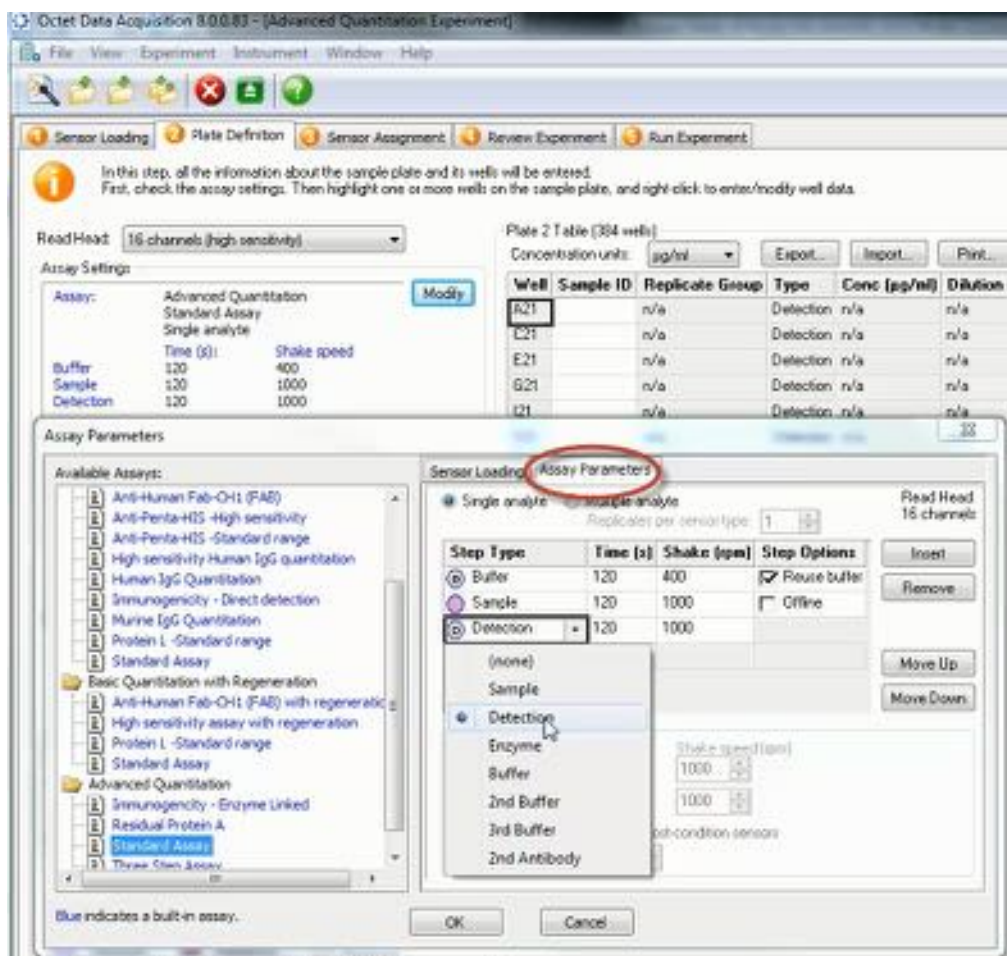


Figure 7-86: Modifying Assay Parameters



NOTE: Quantitation analysis will be performed on the default Detection step type, typically the last assay step in Plate Definition.

8. Continue with the plate layout and sample well designation for the Plate Definition assay steps. For more details see “Defining the Sample Plate” on page 289, “Managing Sample Plate Definitions” on page 313 and “Managing Assay Parameter Settings” on page 319.
9. Proceed to Tab 2 (Sensor Assignment) and the remaining tabs as described starting with “Assigning Biosensors to Samples” on page 325 before running the Advanced Quantitation method.

CHAPTER 8:

Kinetics Experiments: Octet K2 System

Introduction	376
Starting a Basic Kinetics Experiment	377
Defining the Sample Plate	378
Managing Sample Plate Definitions	393
Defining a Kinetic Assay	397
Assigning Biosensors to Samples	411
Reviewing Experiments	418
Saving Experiments	419
Running a Kinetics Experiment	420
Managing the Runtime Binding Chart	427
Managing Experiment Method Files	434
Epitope Binning	435




INTRODUCTION

A basic kinetics experiment enables you to determine the association and dissociation rate of a molecular interaction. After starting the Octet system hardware and the Octet System Data Acquisition software, follow the steps (in Table 8-1) to set up and analyze a kinetics experiment.



NOTE: It is highly recommended that you use the kinetics templates in the experiment wizard rather than creating a custom experiment to avoid acquiring data that cannot be analyzed by the Octet Data Analysis Software.

Table 8-1: Setting Up and Analyzing a Kinetic Experiment

Software	Step	See
Data Acquisition 	1. Select a kinetics experiment in the Experiment Wizard or open a method file (.fmf).	"Starting a Basic Kinetics Experiment" on page 377
	2. Define a sample plate or import a sample plate definition.	"Defining the Sample Plate" on page 378
	3. Specify assay steps.	"Defining a Kinetic Assay" on page 397
	4. Assign biosensors to samples.	"Assigning Biosensors to Samples" on page 411
	5. Run the experiment.	"Running a Kinetics Experiment" on page 420
Data Analysis  or Data Analysis HT 	6. View and process the raw data.	Octet System Data Analysis Software or Octet Data Analysis HT Software User Guide
	7. Analyze the data.	



NOTE: Before starting an experiment, check the sample plate temperature displayed in the status bar. Confirm that the temperature is appropriate for your experiment and if not set a new temperature. If the Octet System Data Acquisition software is closed, the plate temperature will reset to the default startup value specified in the **Options** window when the software is relaunched.

STARTING A BASIC KINETICS EXPERIMENT



IMPORTANT: Using 96-well half-area plates on the Octet K2 system will result in non-optimal system performance. Pall ForteBio cannot guarantee results within the optimal performance specifications of the system when these plates are used.


You can start a kinetics experiment using one of the following options:

- Launch the **Experiment Wizard**.
- Open a method file (.fmf) by clicking **File > Open Method File**. Method files may be saved and recalled using the **File** menu and are automatically saved when an experiment is run. For more details on method files see “Managing Experiment Method Files” on page 434.
- On the menu bar, click **Experiment > Templates > Kinetics**.



NOTE: When using the 21 CFR Part 11 version of the Octet System Data Acquisition software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.

Starting an Experiment Using the Experiment Wizard

1. If the **Experiment Wizard** is not displayed when the software is launched, click the **Experiment Wizard** toolbar button , or click **Experiment > New Experiment Wizard (Ctrl+N)** from the **Main Menu**.
2. In the **Experiment Wizard**, click **New Kinetics Experiment** (Figure 8-1, left).



NOTES:

Octet K2 method templates are not compatible with other Octet instruments.

It is highly recommended that you use the kinetics templates in the experiment wizard rather than creating a custom experiment to avoid acquiring data that cannot be analyzed by the Octet Data Analysis Software.

3. All available Kinetics templates for the Octet K2 system are displayed. You can:

- Click **Recent Methods** to display a list of recently used methods. You can open any method file from the list and use it with or without modifications to run a new experiment.
 - Select a template.
 - If none of the templates are suitable for your experiment, select **Blank Experiment** to create a custom one.
4. Click the arrow button(➡). The Basic Kinetics Experiment window displays (Figure 8-1, right).

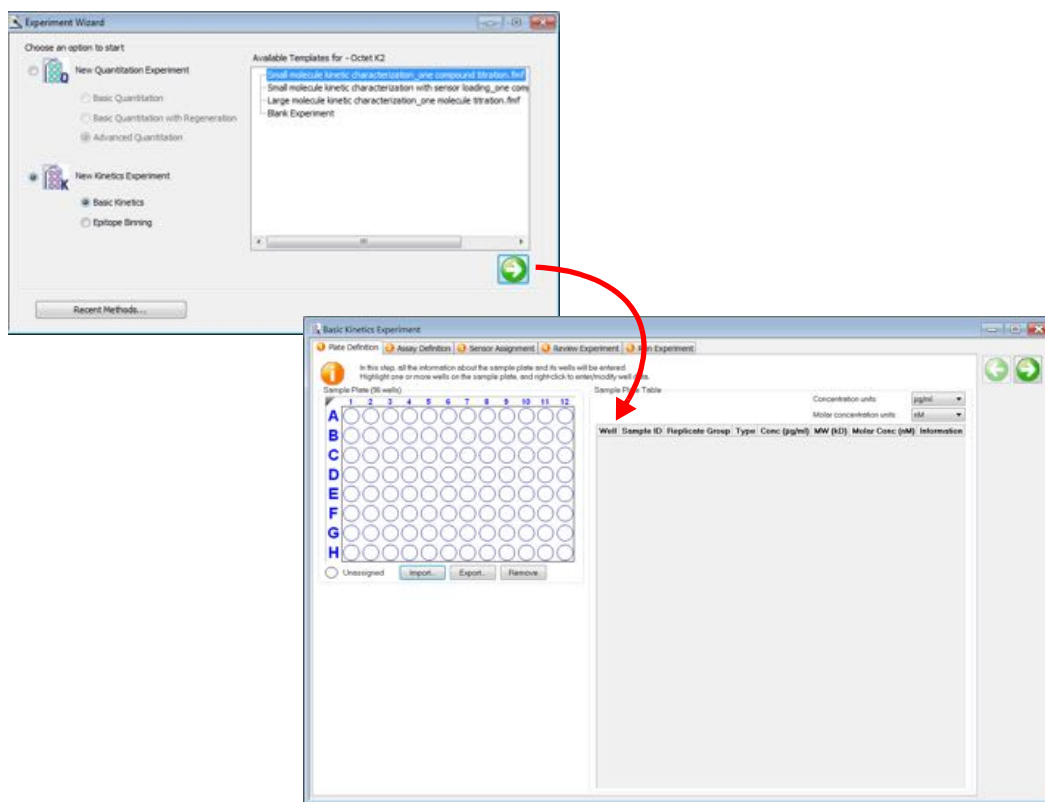


Figure 8-1: Starting a Kinetics Experiment with the Experiment Wizard

DEFINING THE SAMPLE PLATE

The steps to define a sample plate include:

Step	See Page
5. Designate the samples.	379
6. Save the sample plate definition (optional).	393

Designating Samples



NOTE: It is important to define all of the wells that will be used in the assay. Only wells that are selected and defined using one of the sample types in Table 8-2 will be included in the assay.

Table 8-2 displays the well types that can be assigned to a plate map.

Table 8-2: Types of Sample Wells

Icon	Description
Sample	Any type of sample. For example, an analyte.
Reference	Reference sample. For example, a buffer-only control biosensor that is used to correct for system drift.
Controls	A control sample, either positive or negative, of known analyte composition. <ul style="list-style-type: none"> Positive Control: A control sample that contains analyte of known concentration Negative Control: A control sample known not to contain analyte
Buffer	Any type of buffer. For example, the buffer in a baseline, association, or dissociation step.
Activation	Activation reagent. Makes the biosensor competent for binding.
Quench	Quenching reagent. Blocks unreacted immobilization sites on the biosensor surface.
Load	Ligand to be immobilized (loaded) on the biosensor surface.
Wash	Wash buffer.
Regeneration	Regeneration reagents dissociate the analyte from the ligand.
Neutralization	Neutralization buffer that is used to neutralize the biosensor after the regeneration step.

Selecting Wells in the Sample Plate Map



NOTE: For the Octet K2 system, wells in sample plate are restricted to rows AB, CD, EF and GH. Sample wells cannot be designated in row pairs BC, DE and FG.

There are several ways to select wells in the **Sample Plate Map**:

- Click a column header or select adjacent column headers by click-hold-drag. To select non-adjacent columns, hold the **Ctrl** key and click the column header (Figure 8-2 left).
- Click a row header or select adjacent row headers by click-hold-drag (Figure 8-2, center).
- Click a well or draw a box around a group of wells (Figure 8-2, right).

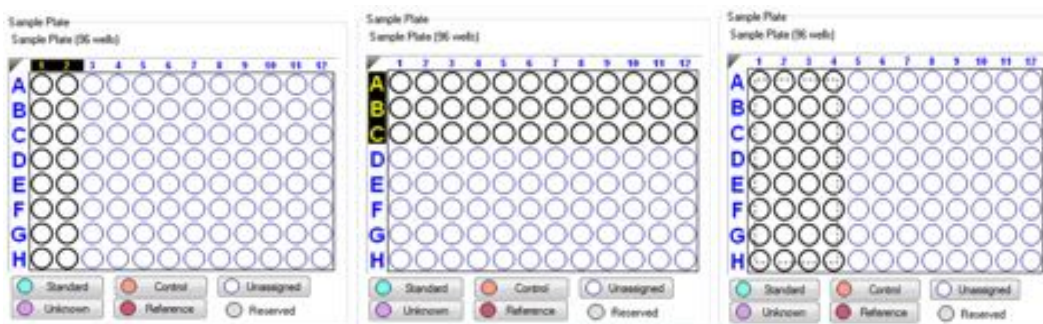


Figure 8-2: Selecting Wells in the Sample Plate Map



NOTE: Shift-clicking in the **Sample Plate Map** mimics the head of the instrument during the selection.

Designating Well Types

In the **Sample Plate Map**, select the wells, right-click and select a sample type (see Figure 8-25).

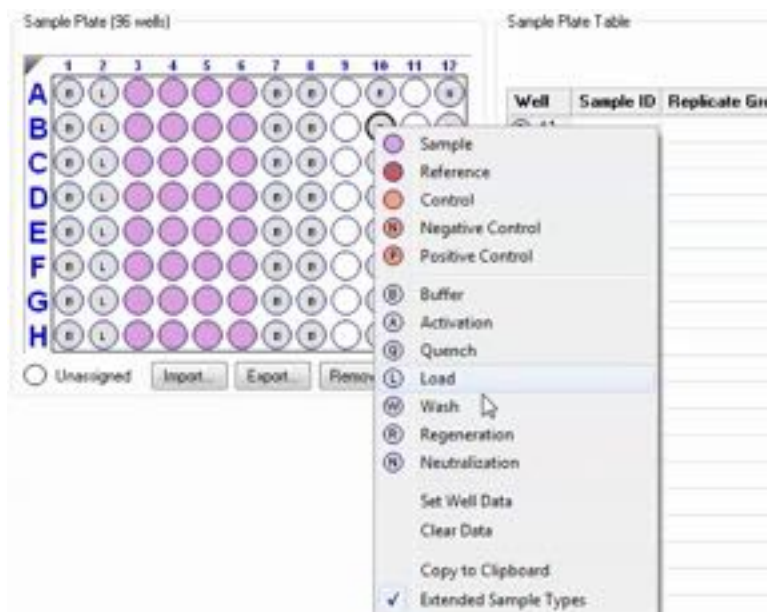


Figure 8-3: Designating a Well Type in the Plate Definition Window

To remove a well designation, in the **Sample Plate Map**, select the well(s) and click **Remove**. Or, right-click the well(s) and select **Clear Data** (see Figure 8-4).

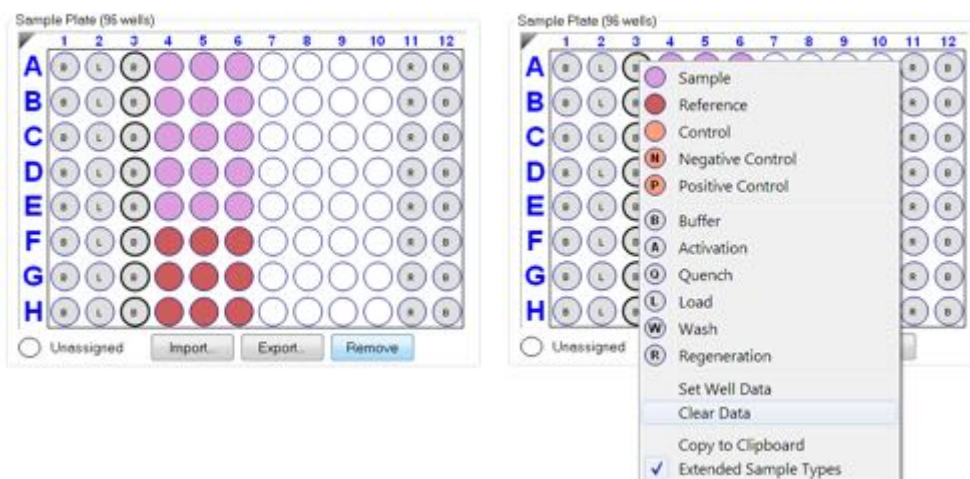


Figure 8-4: Clearing Sample Data from a Sample Plate

Entering Sample Information



NOTE: You must specify sample (analyte) concentration and molecular weight, otherwise the Octet System Data Acquisition software cannot compute a K_D value. If the sample concentration is not specified, only k_d and k_{obs} are calculated. You can also annotate any well with **Sample ID** or **Well Information**, and assign **Replicate Groups**.

Assigning Molecular Weight and Molar Concentration

1. In the **Sample Plate Map**, select the sample wells, right-click and select **Set Well Data**.
2. In the **Set Well Data** dialog box, enter the analyte molecular and molar concentration (Figure 8-5).

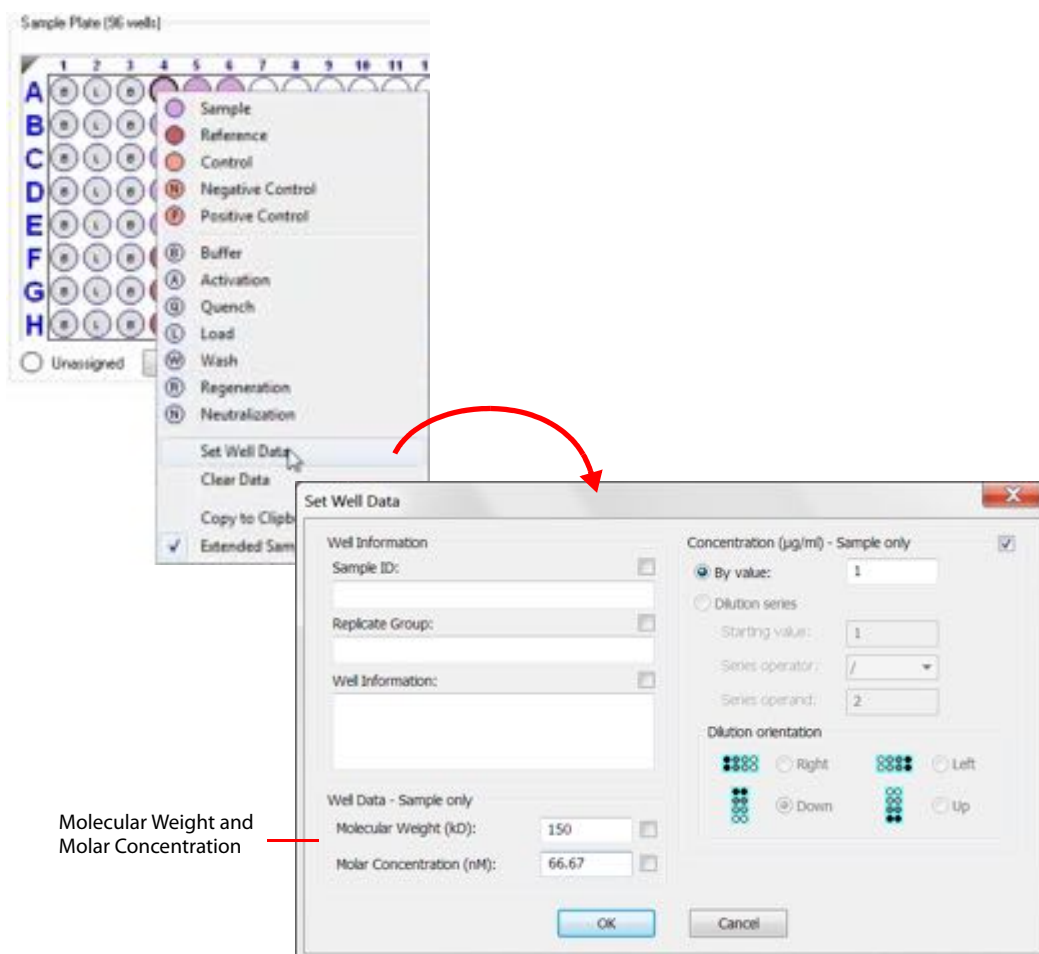


Figure 8-5: Entering Molecular Weight and Molar Concentration from the Sample Plate Map

The information displays in the **Sample Plate Table** (see Figure 8-6).

3. In the **Sample Plate Table**, select the sample concentration units and the molar concentration units.

Concentration units:

Molar concentration units:

Concentration units

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information
F3			Buffer				
G3			Buffer				
H3			Buffer				
A4			Sample	150	66.67		
B4			Sample	150	33.33		
C4			Sample	150	16.67		
D4			Sample	150	8.333		
E4			Sample	150	4.167		
F4			Reference				
G4			Reference				
H4			Reference				
A5			Sample	150	66.67		
B5			Sample	150	33.33		
C5			Sample	150	16.67		
D5			Sample	150	8.333		
E5			Sample	150	4.167		
F5			Reference				
G5			Reference				
H5			Reference				
A6			Sample	150	66.67		
B6			Sample	150	33.33		
C6			Sample	150	16.67		
D6			Sample	150	8.333		
E6			Sample	150	4.167		
F6			Reference				

Figure 8-6: Entering Molecular Weight and Molar Concentration from the Plate Table

Assigning User Specified Sample Concentrations

To assign sample concentrations using a dilution series:

1. In the **Sample Plate Map**, select the desired wells, right-click and select **Set Well Data**. The **Set Well Data** dialog box displays (see Figure 8-7).
2. Select the **By value** option and enter the starting concentration value.

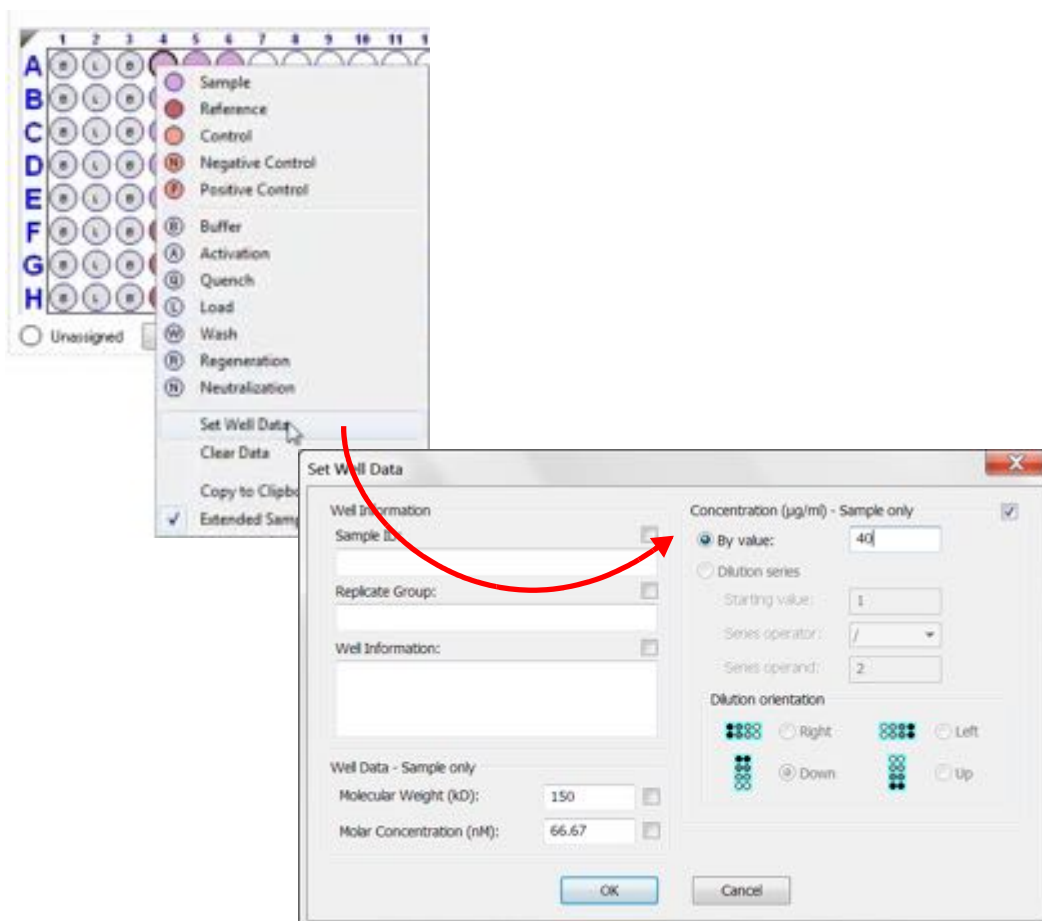


Figure 8-7: Sample Plate Map—Assigning Sample Concentrations by Value

3. Click **OK**. The **Sample Plate Table** will display the entered concentration.

Assigning Concentrations Using a Dilution Series

To assign sample concentrations using a dilution series:

1. In the **Sample Plate Map**, select the wells, right-click, and select **Set Well Data**.
The **Set Well Data** dialog box displays (see Figure 8-8)
2. Select the **Dilution Series** option and enter the starting concentration value.

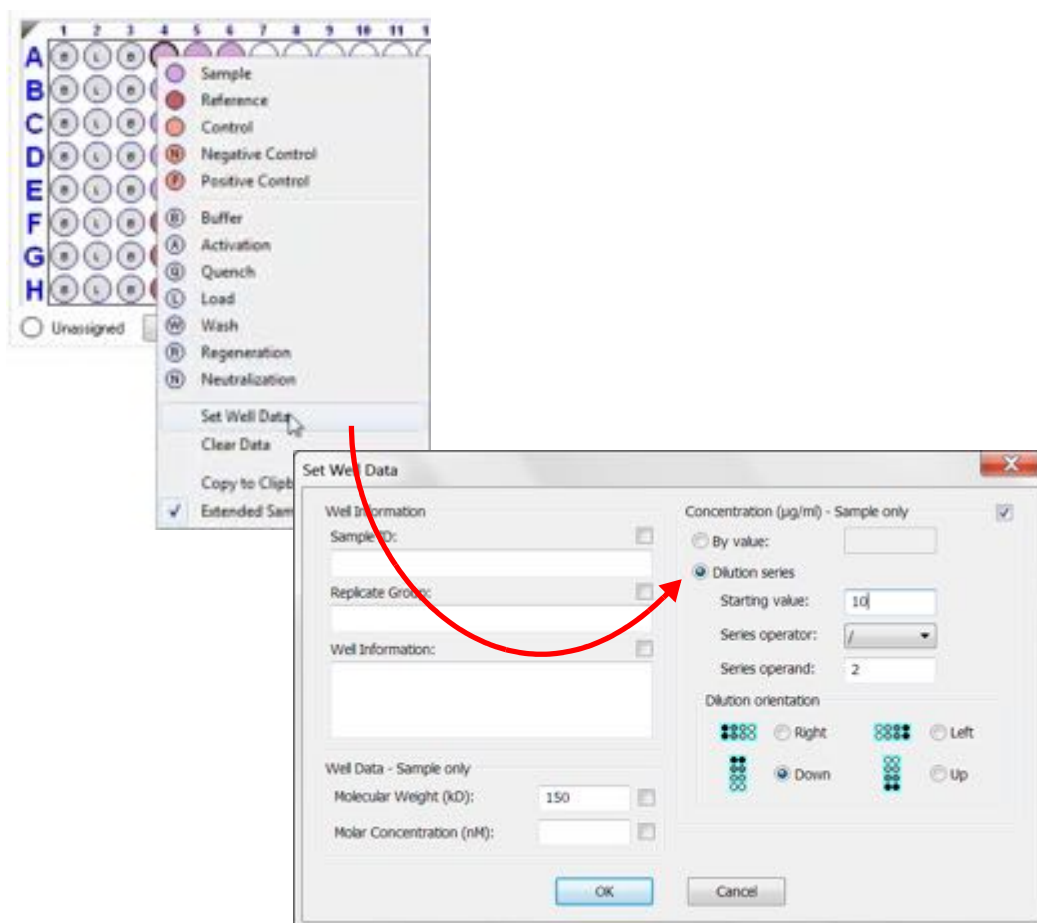


Figure 8-8: Sample Plate Map—Assigning Sample Concentrations Using Dilution Series

3. Select a series operator, enter an operand, and select the appropriate dilution orientation (see Figure 8-9).

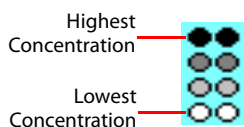


Figure 8-9: Concentration Representation in Dilution Series

4. Click **OK**.

The **Sample Plate Table** displays the standard concentrations.

Annotating Samples

You can enter annotations (notes) for multiple samples in the **Sample Plate Map** or enter information for an individual sample in the **Sample Plate Table**. For greater clarity, annotation text may be displayed as the legend of the **Runtime Binding Chart** during data acquisition, but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it will not be available for display as a legend.

Annotating Wells in the Sample Plate Map

To annotate one or more wells:

1. In the **Sample Plate Map**, select the samples to annotate, right-click and select **Set Well Data**.
2. In the **Set Well Data** dialog box (see Figure 8-10), enter the **Sample ID** and/or **Well Information** and click **OK**.

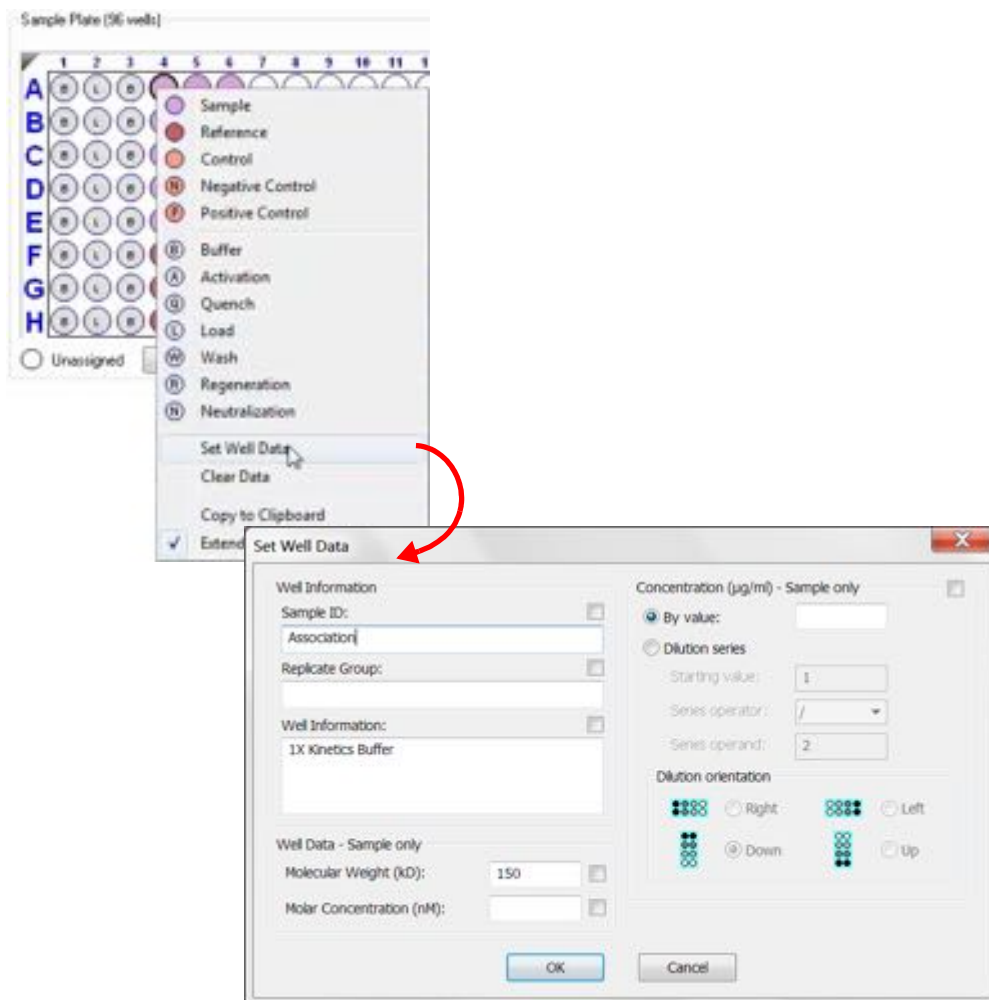


Figure 8-10: Add Sample Annotations from the Sample Plate Map

Annotating Wells in the Sample Plate Table

To annotate an individual well in the **Sample Plate Table**:

1. Double-click the table cell for **Sample ID** or **Well Information**.
2. Enter the desired information in the respective field (see Figure 8-11).



NOTE: A series of Sample IDs may also be assembled in Excel and pasted into the **Sample Plate Table**.

Sample Plate Table

Concentration units: µg/ml

Molar concentration units: nM

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information
G3	Dissociation		Buffer				1X Kinetics Buffer
H3	Dissociation		Buffer				1X Kinetics Buffer
A4	Association		Sample	10	150	66.67	1X Kinetics Buffer
B4	Association		Sample	5	150	33.33	1X Kinetics Buffer
C4	Association		Sample	2.5	150	16.67	1X Kinetics Buffer
D4	Association		Sample	1.25	150	8.333	1X Kinetics Buffer
E4	Association		Sample	0.625	150	4.167	1X Kinetics Buffer
F4	Association		Reference				1X Kinetics Buffer
G4	Association		Reference				1X Kinetics Buffer
H4	Association		Reference				1X Kinetics Buffer

Figure 8-11: Add Sample Annotations in the Sample Plate Table



NOTE: Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the **Sample Plate Table**. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the **Sample Plate Map** menu used to designate sample types.

Replicate Groups

Replicate Groups enable data to be organized into custom groups during data analysis (see Figure 8-12).

Index	Include	Color	Sensor Location	Sensor Type	Sensor Info	Replicate Group	Baseline Loc.
20	x		C2	SA (Streptavidin)	3		C3
21	x		C2	SA (Streptavidin)	3		C3
22	x		D2	SA (Streptavidin)	4		D3
23	x		D2	SA (Streptavidin)	4		D3
24	x		E2	SA (Streptavidin)	5		E3
25	x		E2	SA (Streptavidin)	5		E3
26	x		F2	SA (Streptavidin)	6		F3
27	x		F2	SA (Streptavidin)	6		F3
28	x		G2	SA (Streptavidin)	6		G3
29	x		G2	SA (Streptavidin)	6		G3
30	x		H2	SA (Streptavidin)	6		H3
31	x		H2	SA (Streptavidin)	6		H3
32	x		A3	SA (Streptavidin)	1		A3
33	x		A3	SA (Streptavidin)	1		A3
34	x		B3	SA (Streptavidin)	2		B3
35	x		B3	SA (Streptavidin)	2		B3
36	x		C3	SA (Streptavidin)	3		C3
37	x		C3	SA (Streptavidin)	3		C3
38	x		D3	SA (Streptavidin)	4		D3
39	x		D3	SA (Streptavidin)	4		D3

Figure 8-12: Replicate Group Color-Coding



NOTE: Replicate Group information can also be entered in the Octet System Data Analysis software.

Assigning Replicate Groups in the Sample Plate Map

To assign **Replicate Groups** in the **Sample Plate Map**:

1. Select the samples you wish to group, right-click and select **Set Well Data**.
2. In the **Set Well Data** dialog box (see Figure 8-13), enter a name in the **Replicate Group** box and click **OK**.

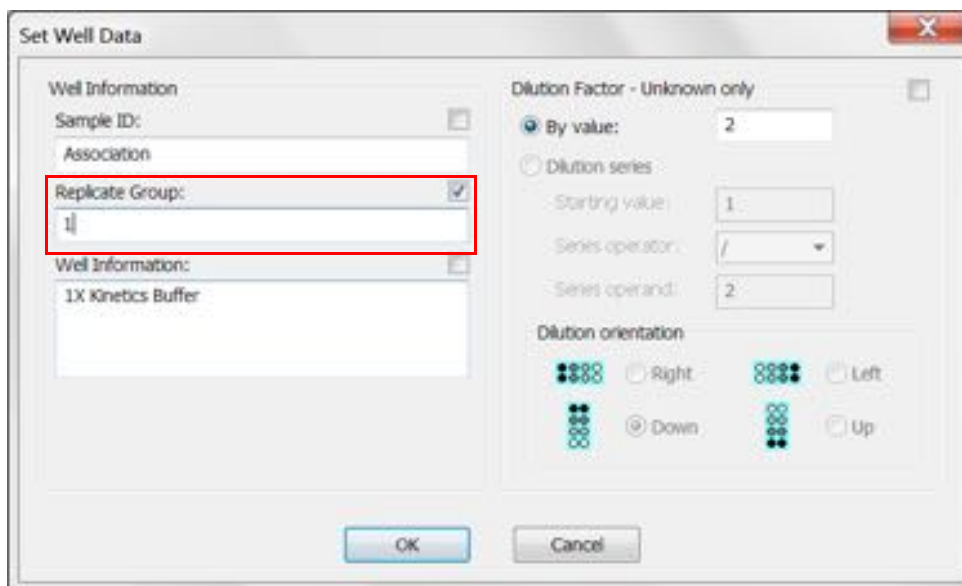


Figure 8-13: Add Replicate Group from the Sample Plate Map

3. Repeat the previous steps to assign new samples to the existing **Replicate Group**, or to designate another set of samples to a new **Replicate Group**. Multiple groups can be used in an experiment.



IMPORTANT: The Octet System Data Analysis software will only recognize and group samples that use the same Replicate Group names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.

Wells in the **Sample Plate Map** will show color-coded outlines as a visual indication of which wells are in the same group (see Figure 8-14).

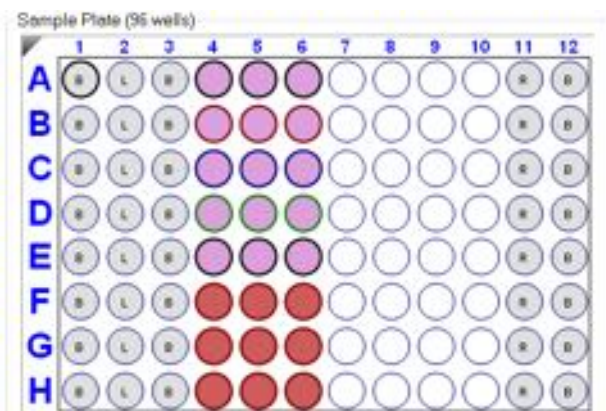


Figure 8-14: Replicate Groups Displayed in Sample Plate Map

The **Sample Plate Table** will update with the **Replicate Group** names entered (see Figure 8-15)

Sample Plate Table

Concentration units:

Molar concentration units:

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information
A4	Association	1	Sample	10	150	66.67	1X Kinetics Buffer
B4	Association	2	Sample	5	150	33.33	1X Kinetics Buffer
C4	Association	3	Sample	2.5	150	16.67	1X Kinetics Buffer
D4	Association	4	Sample	1.25	150	8.333	1X Kinetics Buffer
E4	Association	5	Sample	0.625	150	4.167	1X Kinetics Buffer
F4	Association	6	Reference				1X Kinetics Buffer
G4	Association	6	Reference				1X Kinetics Buffer
H4	Association	6	Reference				1X Kinetics Buffer
A5	Association	1	Sample	10	150	66.67	1X Kinetics Buffer
B5	Association	2	Sample	5	150	33.33	1X Kinetics Buffer
C5	Association	3	Sample	2.5	150	16.67	1X Kinetics Buffer
D5	Association	4	Sample	1.25	150	8.333	1X Kinetics Buffer
E5	Association	5	Sample	0.625	150	4.167	1X Kinetics Buffer
F5	Association	6	Reference				1X Kinetics Buffer
G5	Association	6	Reference				1X Kinetics Buffer
H5	Association	6	Reference				1X Kinetics Buffer

Figure 8-15: Replicate Groups in Sample Plate Table

Assigning Replicate Groups in the Sample Plate Table

To assign **Replicate Groups** in the **Sample Plate Table**:

1. Double-click the desired cell in the **Replicate Group** table column.
2. Enter a group name (see Figure 8-16).

Sample Plate Table

Concentration units:

Molar concentration units:

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information
A4	Association	1	Sample	10	150	66.67	1X Kinetics Buffer
B4	Association	2	Sample	5	150	33.33	1X Kinetics Buffer
C4	Association	3	Sample	2.5	150	16.67	1X Kinetics Buffer
D4	Association	4	Sample	1.25	150	8.333	1X Kinetics Buffer
E4	Association	5	Sample	0.625	150	4.167	1X Kinetics Buffer
F4	Association	6	Reference				1X Kinetics Buffer
G4	Association	6	Reference				1X Kinetics Buffer
H4	Association	6	Reference				1X Kinetics Buffer

Figure 8-16: Add Replicate Group from the Sample Plate Table

Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the **Sample Plate Table**. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the **Sample Plate Map** menu used to designate sample types.

- Repeat the previous steps to assign new samples to the existing **Replicate Group**, or to designate another set of samples to a new **Replicate Group**. Multiple groups can be used in an experiment.



IMPORTANT: The Octet System Data Analysis software will only recognize and group samples that use the same **Replicate Group** names, spacing and capitalization must be identical. For example, samples assigned to **Group 2** and **group2** are treated as two groups.

Editing the Sample Table

Changing Sample Well Designations

To change a well designation, right-click the well in the **Sample Plate Table** and make a new selection (see Figure 8-17).

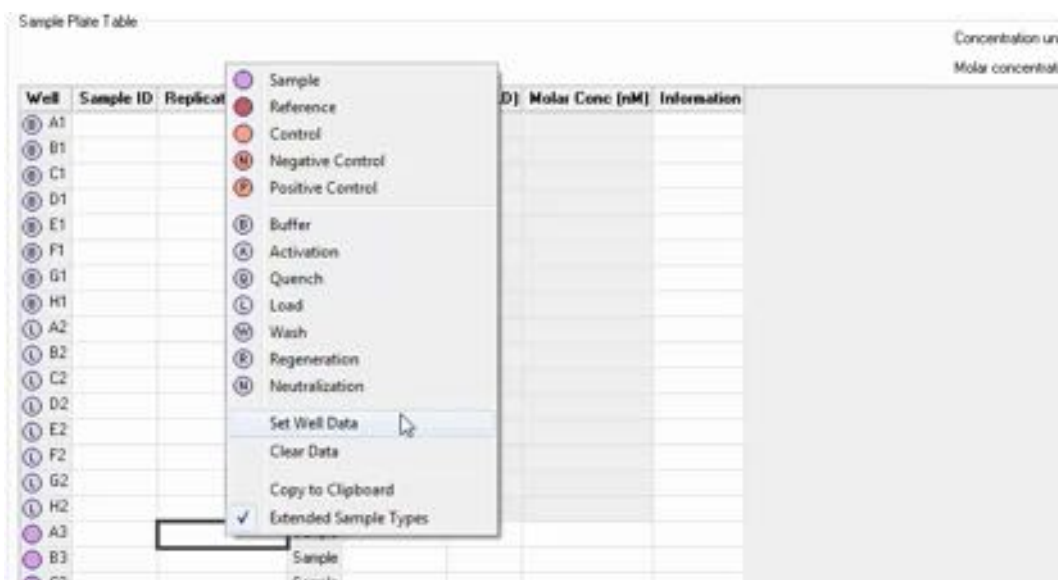


Figure 8-17: Sample Plate Table—Well Designation

Editing Sample Information

To edit sample data in the **Sample Plate Table**, double-click a value and enter a new value (see Figure 8-18).

Sample Plate Table

Concentration units: µg/ml

Molar concentration units: nM

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information
H3	Dissociation		Buffer				1X Kinetics Buffer
A4	Association	1	Sample	10	150	66.67	1X Kinetics Buffer
B4	Association	2	Sample	5	150		
C4	Association	3	Sample	2.5	150		
D4	Association	4	Sample	1.25	150		
E4	Association	5	Sample	0.625	150		
F4	Association	6	Reference				
G4	Association	6	Reference				
H4	Association	6	Reference				
A5	Association	1	Sample	10	150		
B5	Association	2	Sample	5	150		
C5	Association	3	Sample	2.5	150		
D5	Association	4	Sample	1.25	150		
E5	Association	5	Sample	0.625	150		
F5	Association	6	Reference				
G5	Association	6	Reference				
H5	Association	6	Reference				

Undo
Cut
Copy
Paste
Delete
Select All
Right to left Reading order
Show Unicode control characters
Insert Unicode control character
Open IME
Reconversion

Figure 8-18: Sample Plate Table—Editing Sample Data

Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the **Sample Plate Table**. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the right-click menu used to designate sample types.

MANAGING SAMPLE PLATE DEFINITIONS



NOTE: After you define a sample plate, you can export and save the plate definition for future use.

Exporting a Plate Definition

To export a plate definition:

1. In the **Sample Plate Map**, click **Export** (see Figure 8-19).

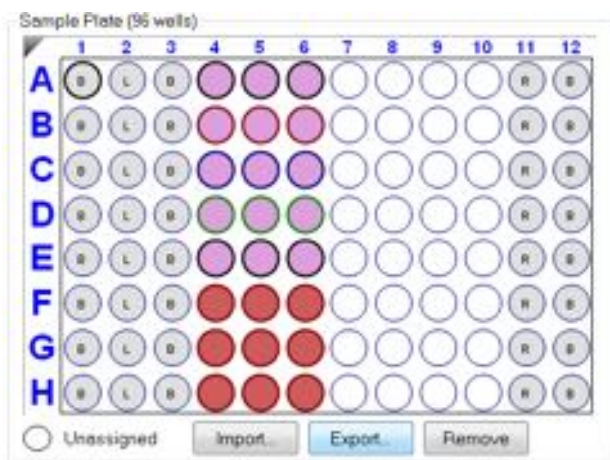


Figure 8-19: Sample Plate Map—Export Button

2. In the **Export Plate Definition** window (see Figure 8-20), select a folder, enter a name for the plate (.csv), and click **Save**.

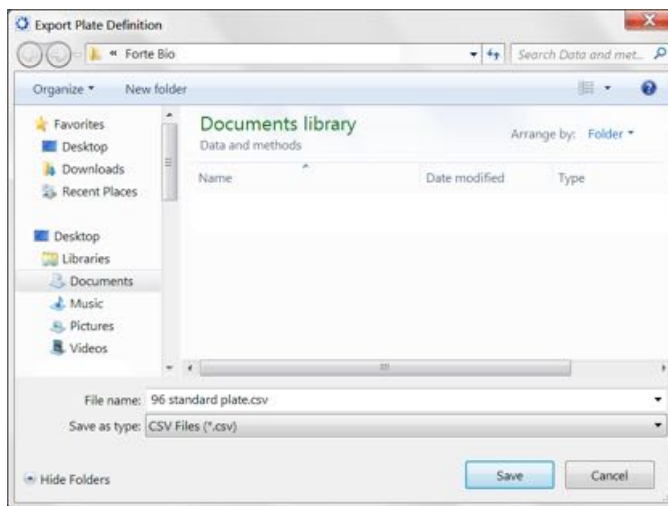


Figure 8-20: Export Plate Definition Window

Importing a Plate Definition

To import a plate definition:

1. In the Sample Plate Definition window (see Figure 8-19: on page 394), click **Import**.

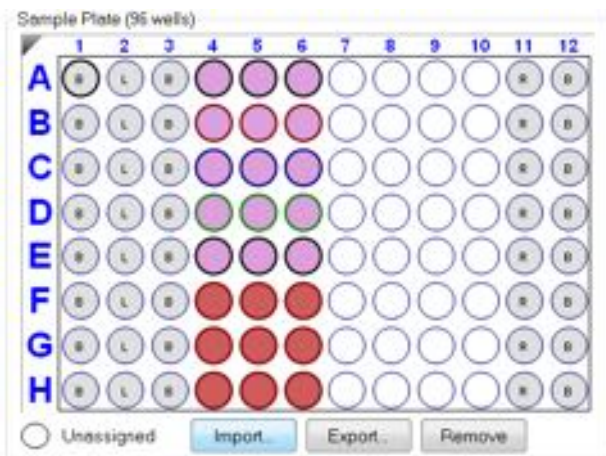


Figure 8-21: Sample Plate Map—**Import** Button

2. In the **Import Plate Definition** window (see Figure 8-22), select the plate definition (.csv), and click **Open**.

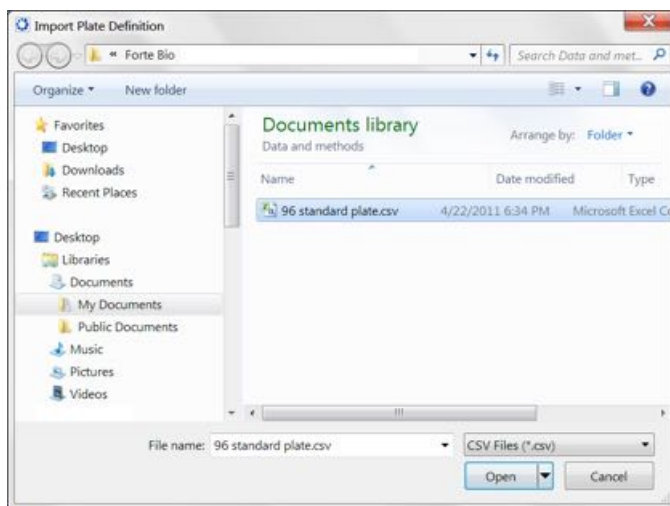


Figure 8-22: Import Plate Definition Window



NOTE: You can also create a .csv file for import. Figure 8-23 shows the appropriate column information layout.

	A	B	C	D	E	F	G	H
1	PlateWells	96						
2	Well	ID	Replicate Group	Group	Concentration (µg/ml)	Molecular Weight (kD)	Molar Concentration (M)	Information
3	A1	Kinetics Buffer		Buffer				1X Kinetics Buffer
4	B1	Kinetics Buffer		Buffer				1X Kinetics Buffer
5	C1	Kinetics Buffer		Buffer				1X Kinetics Buffer
6	D1	Kinetics Buffer		Buffer				1X Kinetics Buffer
7	E1	Kinetics Buffer		Buffer				1X Kinetics Buffer
8	F1	Kinetics Buffer		Buffer				1X Kinetics Buffer
9	G1	Kinetics Buffer		Buffer				1X Kinetics Buffer
10	H1	Kinetics Buffer		Buffer				1X Kinetics Buffer
11	A2	Loading		Load				12.5 ug/ml ProA
12	B2	Loading		Load				12.5 ug/ml ProA

Figure 8-23: Example Plate Definition File (.csv)

Printing a Sample Plate Definition

To print a plate definition:

- 1. In the **Sample Plate Map** (see Figure 8-24), click **Print**.

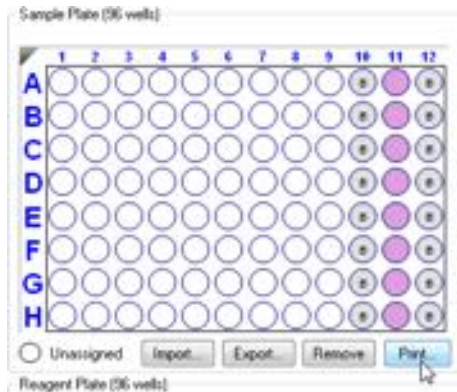


Figure 8-24: Sample Plate Print Button

The associated **Sample Plate Table** information will print.

DEFINING A KINETIC ASSAY

After the sample plate is defined, the assay must be defined. The steps to define a kinetic assay include:

Step	See Page
1. Define the step types.	397
2. Build the assay by assigning a step type to a column(s) in the sample plate.	401
3. Save the sample plate definition (optional).	393

Defining Step Types

Table 8-3 lists the example step types to define a kinetic assay. Use these examples as a starting point to create your own step types.

Table 8-3: Sample Step Types for Kinetic Assays




Step Type	Step Description
Association	Calculates the k_{obs} . Select this step type when binding the second protein of interest (analyte) to the biosensor. This step should be performed at 1,000 rpm.
Dissociation	Calculates the k_d . Select this step type when monitoring the dissociation of the protein complex. This step should be performed at 1,000 rpm.
Baseline	Can be used to align the data. Select this step type when establishing the biosensor baseline in the presence of buffer. This step can be performed with no flow (0 rpm). However, if the baseline step directly precedes an association step, perform the baseline step at 1,000 rpm.
<div>  IMPORTANT: An assay must include a baseline step followed by a set of association/dissociation steps to be analyzed. The Octet System Data Analysis software recognizes the baseline/association/dissociation step series during processing. Data cannot be processed if this sequence is not included in the assay setup. </div>	
Loading	Not used in data analysis. Select this step type when binding the first protein of interest (ligand) to the biosensor.
<div>  NOTE: This step may be performed offline (outside the Octet instrument). </div>	

Table 8-3: Sample Step Types for Kinetic Assays (Continued)

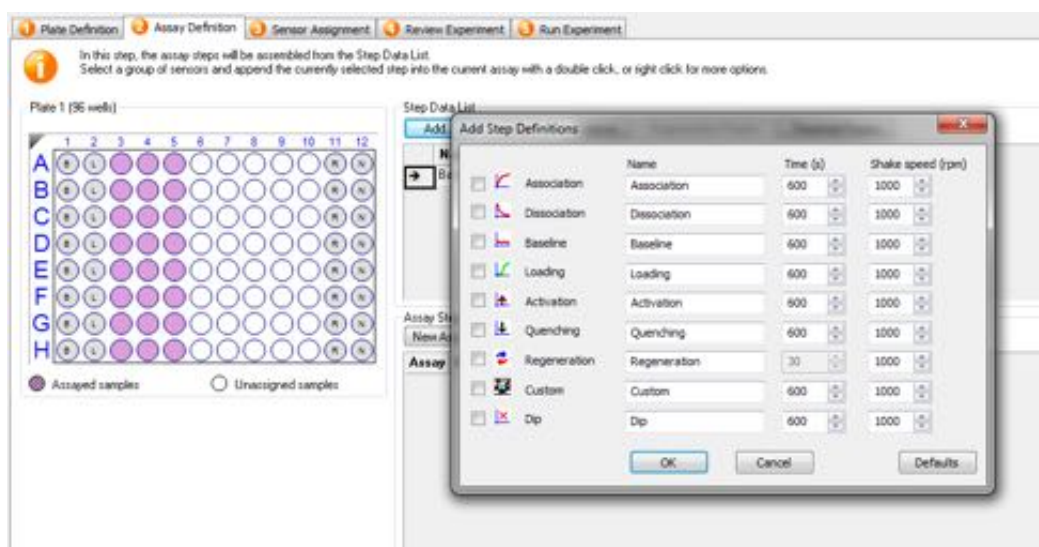
Step Type	Step Description
Activation	Used when employing a reagent to chemically prepare the biosensor for loading.
Quenching	Used to render unreacted immobilization sites on the biosensor inactive.
Regeneration	Used when employing a reagent to chemically regenerate biosensors and remove bound analyte.
Custom	Can be used for an activity not included in any of the above step types.

Creating Step Types

Click the **Assay Definition** tab, or click the  arrow to access the Assay Definition window (see Figure 8-25). The **Step Data List** shows the types of assay steps that are available to build an assay. By default, the list includes a baseline step.

To create different types of assay steps:

1. Click **Add**.
2. In the **Assay Step Definition** dialog box (Figure 8-25), multiple assay steps can be added at the same time. For each step, specify the step information:
 - a. Choose a step type.
 - b. Set the step time and shake speed (**Time** range: 2 to 48,000 seconds, **Shake speed** range: 100 to 1,500 rpm or 0).
3. The step name can be edited after it has been added to the Step Data List table. Edit the step name by double clicking the table cell.

**Figure 8-25:** Creating an Assay Step Type

4. Apply a threshold to the step:
 - a. In the **Step Data List**, click the **Threshold** check box.
The **Threshold Parameters** dialog box displays (see Figure 8-26).
 - b. Set the threshold parameters (refer to Table 8-4 for the parameter definitions).

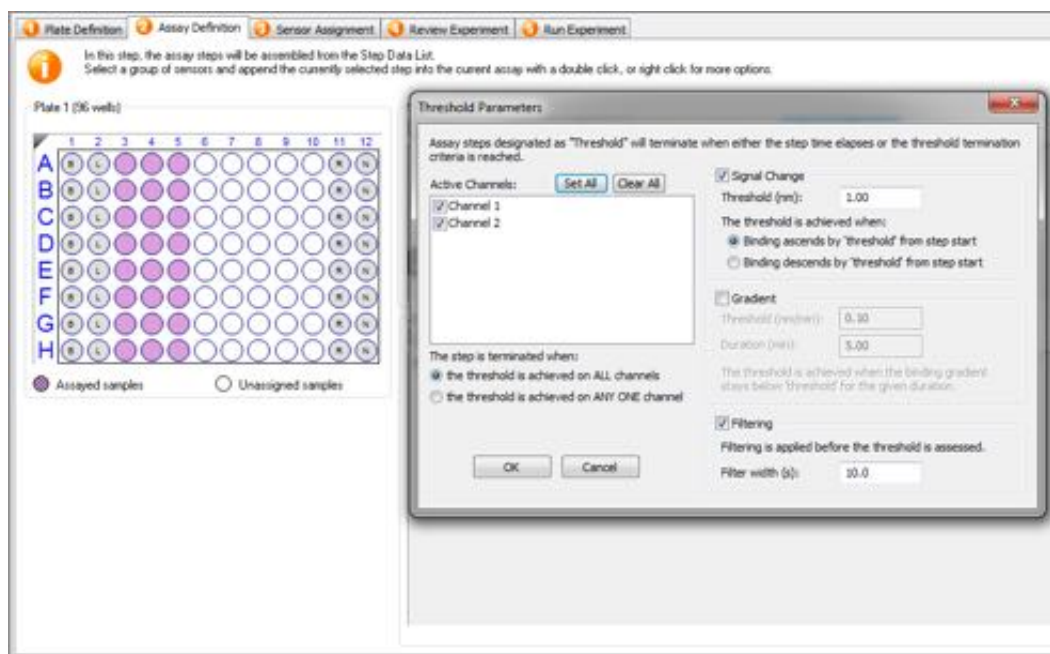


Figure 8-26: Setting Assay Step Threshold Parameters



NOTE: If thresholds are applied, the step is terminated when either the step time elapses or the threshold termination criteria is reached.

Table 8-4: Threshold Parameters

Item	Description
Active Channels	Specifies the instrument channels that monitor the threshold criteria for the assay step. Select an option for terminating the step: <ul style="list-style-type: none"> • The threshold is achieved on ALL channels • The threshold is achieved on ANY ONE channel
Signal Change	The threshold is a user-specified amount of ascending or descending signal change (nm).
Gradient	The threshold is a binding gradient (nm/min) for a user-specified time (min).

Table 8-4: Threshold Parameters (Continued)

Item	Description
Filtering	The amount of data (seconds) to average when computing the signal change or gradient threshold.

- Click **OK** to save the newly-defined step. The new step type appears in the **Step Data List**.
- Repeat the previous steps for each step type to create until all the desired steps are added (see Figure 8-27).

Step Data List









	Name	Time	Shake speed	Type	Threshold
	Baseline	10	1000	 Baseline	<input type="checkbox"/>
	Loading	20	1000	 Loading	<input type="checkbox"/>
	Wash	15	1000	 Custom	<input type="checkbox"/>
	Association	30	1000	 Association	<input type="checkbox"/>
	Long Dissociation	2000	1000	 Dissociation	<input type="checkbox"/>
	Regeneration	24	1000	 Regeneration	<input type="checkbox"/>
	Activation	25	1000	 Activation	<input type="checkbox"/>

Figure 8-27: Step Data List—Displaying Step Types

- To delete a step type from the list, click the corresponding row in the **Step Data List** and click **Remove**, or press the **Delete** key.

Copying and Editing Step Types

To define a step type by copying an existing one, click the step type (row) in the **Step Data List** and click **Copy**. The copied step type appears at the end of the **Step Data List**.

To define a step type by editing an existing one:

- Double-click the cell in the step's **Name**, **Time** or **Shake speed** column and then enter a new value. Or, right-click the cell to display a shortcut menu of editing commands (see Figure 8-28, left).



NOTE: Keyboard commands can also be used (**Ctrl+x**=cut, **Ctrl+c**=copy, **Ctrl+v**=paste, **Ctrl+z**=undo).

- Click the cell in the step's **Type** column, then select another name from the drop-down list (see Figure 8-28, right).

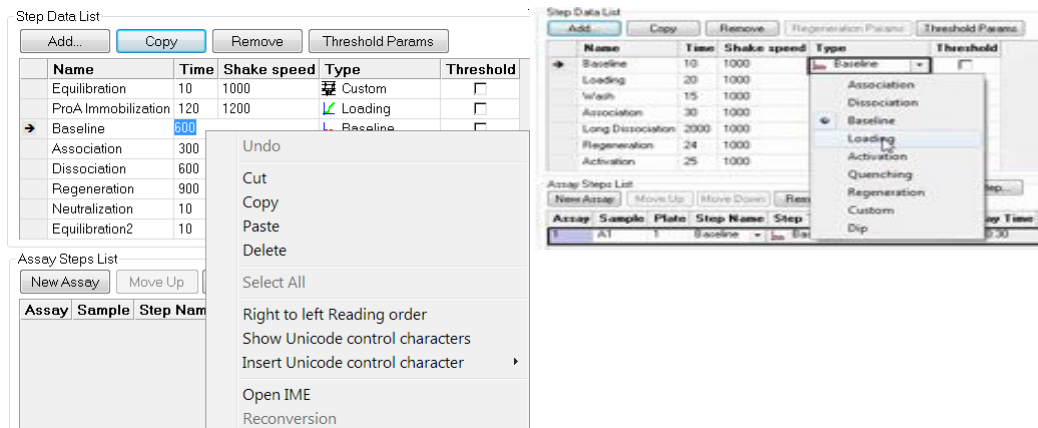


Figure 8-28: Editing a Step Value (left) or Step Type (right)

Building an Assay

After creating the different step types that the assay will use, step types are assigned to sets of wells in the Sample Plate or Reagent Plate maps.

To build an assay:

1. Select a step type in the **Step Data List**.
2. In the **Sample Plate Map**, double-click the set of wells associated with the selected step type. For information about sample plate wells, mouse over a well to view a tool tip (see Figure 8-29).

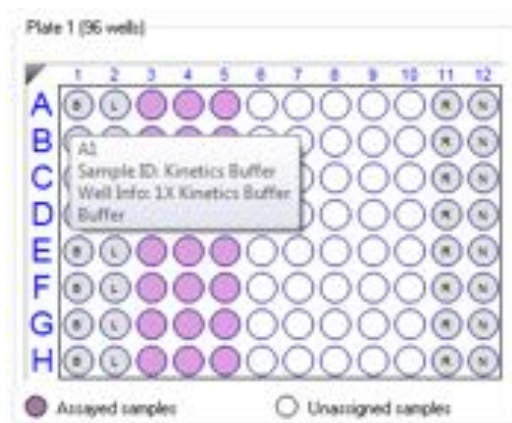



Figure 8-29: Tool Tip of Well Information

The selected wells are marked with hatching (for example, ) and the step appears in the **Assay Steps List** (see Figure 8-30) with an associated **Assay Time**.

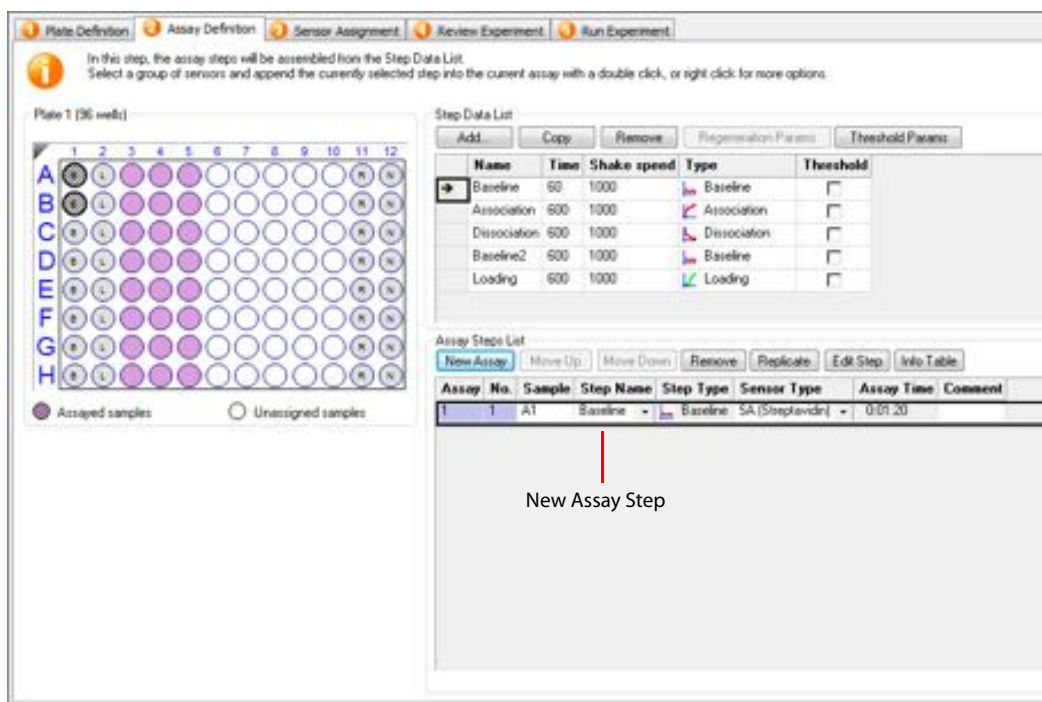


Figure 8-30: Assigning a Step Type to a Column in the Sample Plate

- Repeat the previous steps to define each step in the assay. As each step is added, the total **Experiment** and **Assay Time** update (see Figure 8-32).



NOTE: All assay steps, within an assay or in a different assay, are restricted within row pairs AB, CD, EF and GH. Steps within an assay are restricted to the same row pair. If the selected step is outside the row, then it will be added as a new assay (see Figure 8-31).

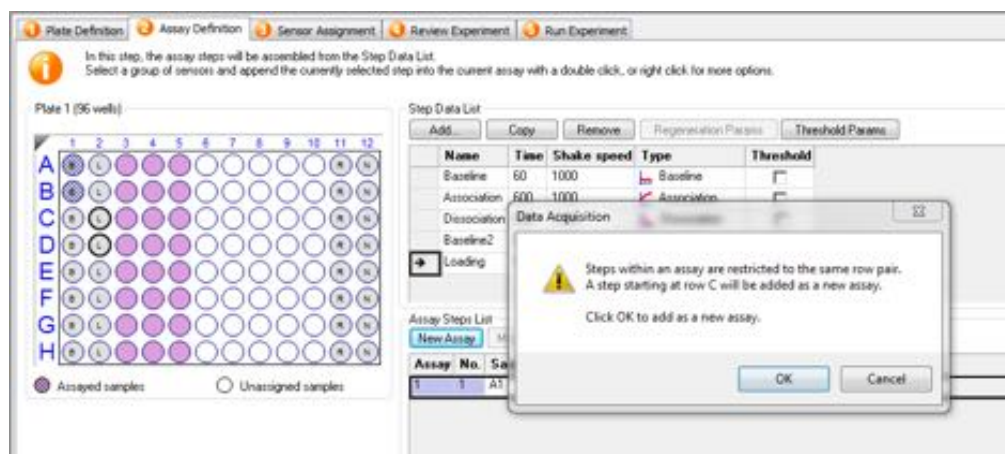


Figure 8-31: Adding a Step Outside a Pair Adds it as a New Assay

Assay Steps List

New Assay Move Up Move Down Remove Replicate... Edit Step...

Assay	Sample	Step Name	Step Type	Sensor Type	Assay Time
1	1	Baseline	Baseline	SA (Streptavidin)	
1	2	Loading	Loading	SA (Streptavidin)	
1	7	Wash	Custom	SA (Streptavidin)	
1	3	Association	Association	SA (Streptavidin)	
1	8	Long Dissociation	Dissociation	SA (Streptavidin)	
1	10	Regeneration	Regeneration	SA (Streptavidin)	0:35:23
2	1	Baseline	Baseline	SA (Streptavidin)	
2	2	Loading	Loading	SA (Streptavidin)	
2	7	Wash	Custom	SA (Streptavidin)	
2	4	Association	Association	SA (Streptavidin)	
2	8	Long Dissociation	Dissociation	SA (Streptavidin)	0:35:15
3	1	Baseline	Baseline	SA (Streptavidin)	
3	2	Loading	Loading	SA (Streptavidin)	
3	7	Wash	Custom	SA (Streptavidin)	
3	5	Association	Association	SA (Streptavidin)	
3	8	Long Dissociation	Dissociation	SA (Streptavidin)	
3	10	Regeneration	Regeneration	SA (Streptavidin)	0:35:23

Total Assay Time

Figure 8-32: Experiment and Assay Time Updates as Steps Are Added to the Assay



IMPORTANT: If you intend to analyze the data from a sample using the **Inter-step correction** feature in the Octet System Data Acquisition software, the assay must use the same well to perform baseline and dissociation for the sample.

Adding a Regeneration Step

1. In the **Sample Plate Map**, assign wells as **Regeneration** or **Neutralization** (Figure 8-33).



Figure 8-33: Regeneration Step

2. Click **Add** (Figure 8-34) to display the Add Step Definition dialog box (Figure 8-35).

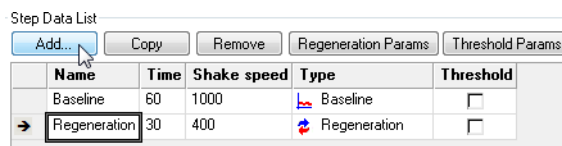


Figure 8-34: Add Button

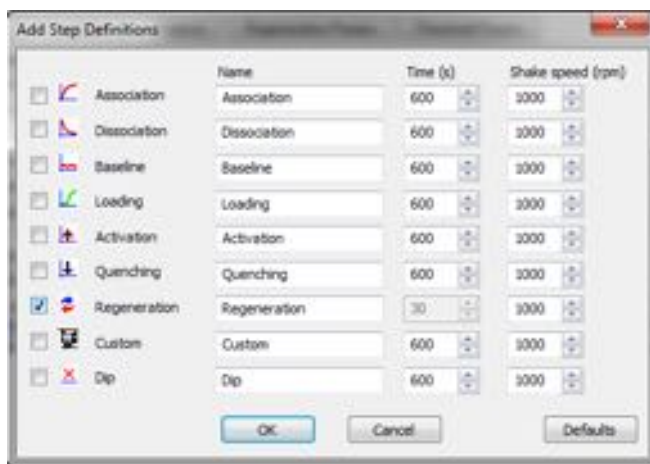


Figure 8-35: Add Step Definition Dialog Box

3. Select **Regeneration** and click **OK**.
4. Click **Regeneration Params** (Figure 8-36).

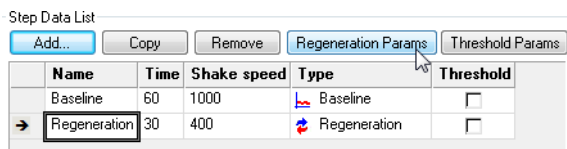


Figure 8-36: Regeneration Params Button

The **Regeneration Parameters** dialog box (Figure 8-37) displays, where you can edit Regeneration parameters as necessary.

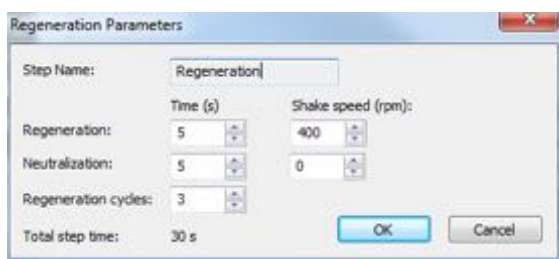


Figure 8-37: Regeneration Parameters Dialog Box

Replicating Steps within an Assay

To copy steps and add them to an assay:

- In the **Assay Steps List**, select the step(s) to copy and click **Replicate** (for example, in Figure 8-38, step rows 1–4 are selected).
 - To select adjacent steps, press and hold the **Shift** key while you click the first and last step in the selection.
 - To select non-adjacent steps, press and hold the **Ctrl** key while you click the desired steps.
- In the **Replicate Steps** dialog box (see Figure 8-38):
 - If you select **Append to current assay**: The Offset Steps option is not automatically selected. If you select it, only the horizontal option is available. The vertical option is not available as Octet K2 kinetic assays are restricted to a row pair. If a vertical offset is required, then replicate the steps as a new assay instead.
 - If you select **Add as a new assay**: The Offset Steps options is not automatically selected. If you select it, both vertical and horizontal offsets are allowed.
- Select and set the options in the Offset steps box as appropriate. (For more details on offset options, see Table 8-5.)

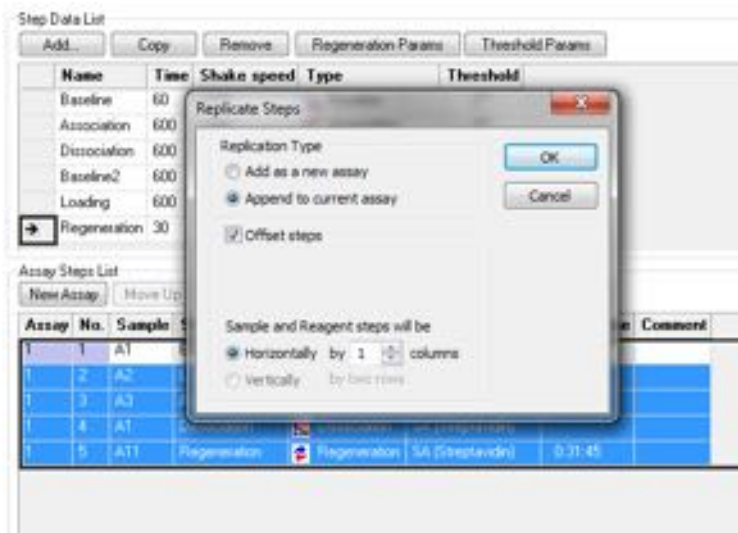


Figure 8-38: Replicating Assay Steps by Appending

- Click **OK**. The step(s) appear at the end of the assay in the **Assay Steps List**.

Table 8-5: Replicate Steps Options .

Item	Description
Add as a new assay	Adds the replicate step(s) as a new assay to the Assay Steps List .
Append to current assay	Adds the replicate step(s) to the end of the current assay.
Offset steps	Assigns the replicate steps to different columns in the sample plate.
All steps	Applies the offset to the sample and reagent steps in the plate.
Sample and Reagent steps will be adjusted horizontally by X columns	Specifies the column in which to add the new step(s). For example, if a step in column 11 is copied and the replicate step should begin in column 12, enter 1 . Enter 0 to apply the step(s) to the same columns.
Sample and Reagent steps will be adjusted vertically for two rows	Applies only to the Octet K2 instrument.

Starting a New Assay

A new assay will utilize a new set of biosensors. To start a new assay using the next available sensors:

- Select two wells in the **Sample Plate Map**.

2. Right-click to view the shortcut menu and select **Start New Assay** (see Figure 8-39).
3. Add steps to the assay as described earlier.



Figure 8-39: Start New Assay

Inserting or Adding an Assay Step

To insert an assay step:

1. Select a step in the **Step Data List**.
2. In the **Assay Steps List**, select the row above where you want to insert the step.
3. In the **Sample Plate Map**, right-click the column to which the step will be applied and select **Insert Assay Step**.

The step is inserted into the **Assay Steps List**.

To add an assay step:

1. Select a step type in the **Step Data List**.
2. In the **Sample Plate Map**, right-click the column to which the step will be applied, and select **Add Assay Step**.

The step is added to the end of the **Assay Steps List**.

Selecting a Biosensor for the Assay

To select the biosensor type associated with the assay, click the **Sensor Type** arrow (▼) for any step in the assay and select a sensor type from the drop-down list (Figure 8-40). The biosensor type will automatically update for every assay step.

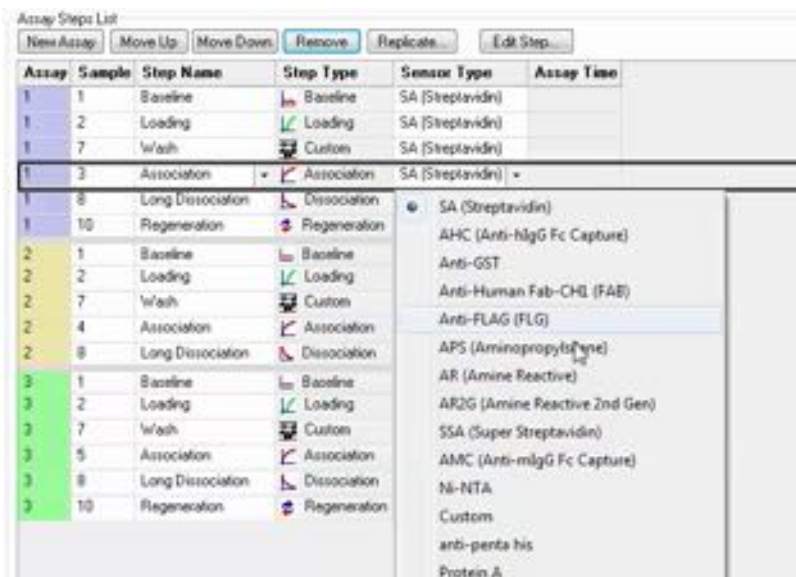


Figure 8-40: Selecting an Assay Sensor Type



NOTE: The **Sensor Type** for the assay must be selected or changed from the **Assay Steps List**. Changing the **Sensor Type** from the **Sensor Assignment Tab** will not update the assay.

Editing an Assay

To edit the step type or the biosensor type:

1. In the **Assay Steps List**:

- To change the step type, click the **Step Name** arrow (▼) and select a step name from the drop-down list (Figure 8-41, top).
- To change the biosensor type, click the **Sensor Type** arrow (▼) for any step in the assay and select a sensor type from the drop-down list (Figure 8-41, bottom). The biosensor type will automatically update for every assay step.



NOTE: The **Step Name** drop-down list includes only the step types defined in the **Step Data List**.

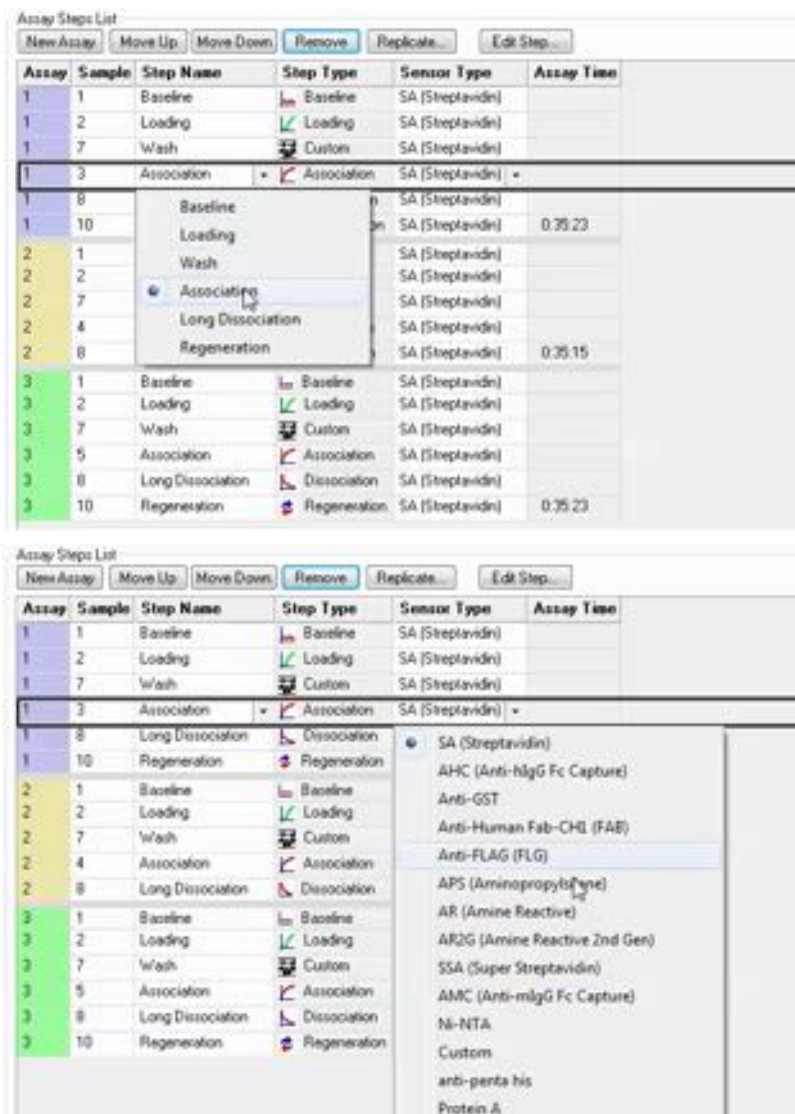


Figure 8-41: Editing an Assay Step Name (top) or Sensor Type (bottom) in the Assay Steps List

To reorder or remove an assay step:

1. Select a step (row) in the **Assay Steps List**.
2. Click the **Move Up**, **Move Down**, or **Remove** button located above the list.



NOTE With the Octet K2 system, steps can only be moved up and down within an assay.



IMPORTANT: An assay must have a baseline step followed by a set of association/dissociation steps to be analyzed. Octet System Data Analysis software recognizes the baseline/association/dissociation set of steps.

Adding an Assay Through Replication

A sample plate can include multiple assays that are the same (replicates) or different. Each assay utilizes a new set of biosensors. Replicates within a single assay will therefore use the same biosensor and replicates in different assays will use different biosensors.

To add a replicate assay to a plate:

1. In the **Assay Steps List**, select the steps to copy and click **Replicate**.
 - To select adjacent steps, press and hold the **Shift** key while you click the first and last step in the selection.
 - To select non-adjacent steps, press and hold the **Ctrl** key while you click the steps.
2. In the **Replicate Steps** dialog box, click the **Add as a new assay** option (Figure 8-42).

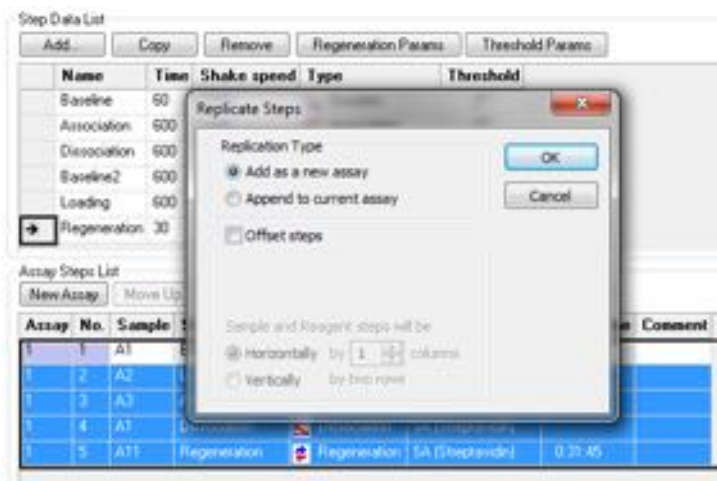



Figure 8-42: Adding a Replicate Assay to a Plate

3. Click the **Offset steps** check box and set the options as appropriate (see Table 8-5 on page 406 for more information). If the replicate assay uses the same sample columns as the original assay, do not choose the **Offset steps** option. If the replicate assay uses a different sample column, select **Offset steps** and the appropriate options.
 - **Sample and Reagent Steps** offsets all wells in the assay by the value specified.
4. Click **OK**. The new assay appears in the **Assay Steps List**.
5. Continue to add assay steps as needed.

ASSIGNING BIOSENSORS TO SAMPLES

After you define the sample plate and assay(s), click the **Sensor Assignment** tab, or click the arrow  to access the Sensor Assignment window. The color-coded **Sensor Tray** and **Sample Plate Map** show the locations of the biosensors associated with the samples (Figure 8-43).



NOTE: If an experiment includes more than one type of biosensor, the software automatically creates a separate sensor tray for each type of biosensor. If the different types of biosensors are in the same tray, change the biosensor type as appropriate.

The biosensor types shown in the **Sensor Type** table column are those designated during the kinetics assay definition. In the example shown in Figure 8-43, the experiment includes three assays in the same wells. The use of those wells by three different biosensors is indicated by the pie chart colors.



NOTE: The **Sensor Type** for the assay must be first be defined in the **Assay Steps List** on the **Assay Definition Tab**. Changing the **Sensor Type** from the **Sensor Assignment Tab** will not update the assay.

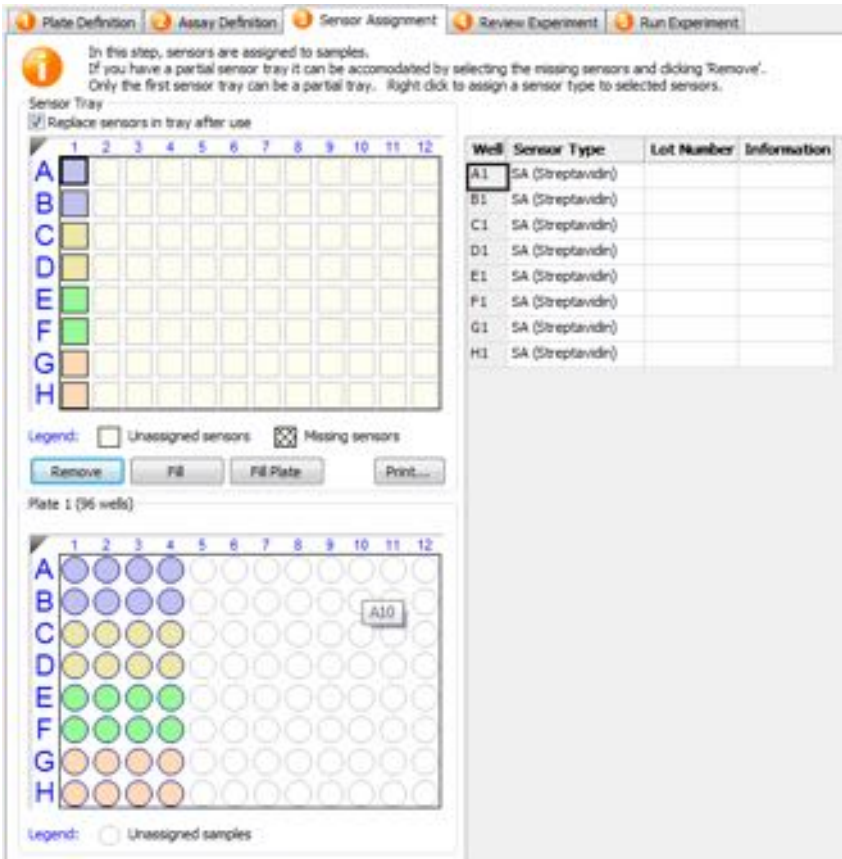


Figure 8-43: Sensor Assignment Window

Hover the cursor over a well in the **Sensor Tray Map** or **Sample Plate Map** to display a tool tip with sample or biosensor information (see Figure 8-44).

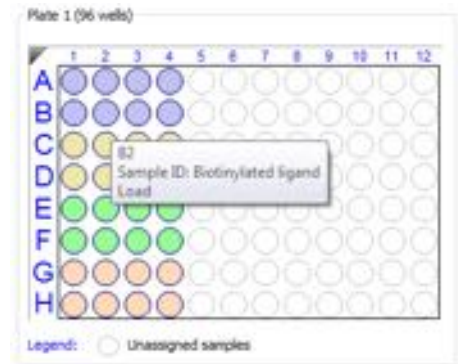


Figure 8-44: Tool Tip of Well Information

Replacing the Biosensors in the Biosensor Tray

After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace sensors in tray after use** check box (see Figure 8-45).



Figure 8-45: Replace Sensors in Tray After Use Check Box



NOTE: Biosensors can be regenerated up to a max of 11 times per experiment.

Entering Biosensor Information

To enter information about a biosensor:

1. Optional: Double-click in any cell in the **Lot Number** column to enter the biosensor lot number. All wells in the **Lot Number** column for that biosensor type will automatically populate with the lot number entered (see Figure 8-46).
2. Optional: Double-click a cell in the **Information** table column. Enter or edit the biosensor information as appropriate (see Figure 8-46).



NOTE: Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

Well	Sensor Type	Lot Number	Information
A1	SA (Streptavidin)	10102020	Default
B1	SA (Streptavidin)	10102020	
C1	SA (Streptavidin)	10102020	
D1	SA (Streptavidin)	10102020	
E1	SA (Streptavidin)	10102020	
F1	SA (Streptavidin)	10102020	
G1	SA (Streptavidin)	10102020	
H1	SA (Streptavidin)	10102020	
A2	SA (Streptavidin)	10102020	
B2	SA (Streptavidin)	10102020	
C2	SA (Streptavidin)	10102020	
D2	SA (Streptavidin)	10102020	
E2	SA (Streptavidin)	10102020	
F2	SA (Streptavidin)	10102020	
G2	SA (Streptavidin)	10102020	
H2	SA (Streptavidin)	10102020	

Undo
 Cut
 Copy
 Paste
 Delete
 Select All
 Right to left Reading order
 Show Unicode control characters
 Insert Unicode control character
 Open IME
 Reconversion

Figure 8-46: Entering or Editing Biosensor Information

Changing the Biosensor Location

If you prefer to not use the default biosensor locations, you can select other locations to use. There are two ways to do this:

- **Method 1**—In the **Sensor Tray Map**, **Remove** the sensor locations you do not want to use. The software automatically selects the next available location(s).
- **Method 2**—Remove all sensor locations from the **Sensor Tray Map**, then select the locations you want to use.

Method 1

1. In the **Sensor Tray Map** (see Figure 8-47), select the locations you do not want to use and click **Remove**. Or, right-click the selection and select **Remove** (Figure 8-47 left). The software automatically selects the next available biosensor locations in the tray (Figure 8-47 right).
2. Click **Fill Plate** to return the **Sensor Tray Map** to the default layout.

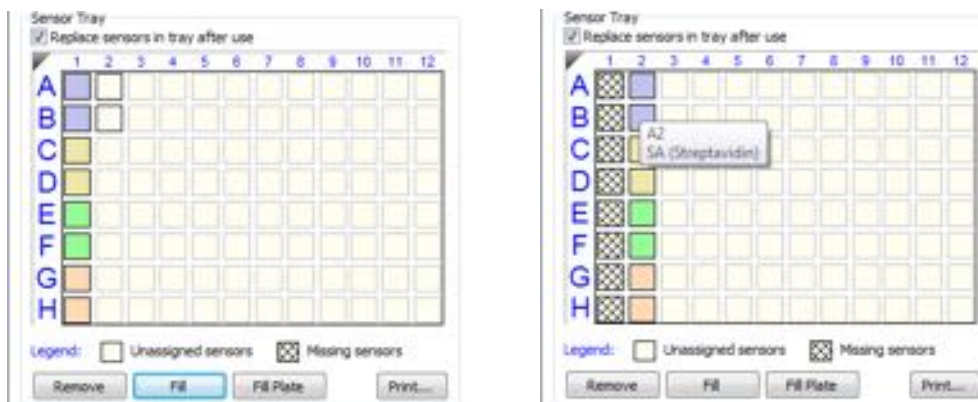


Figure 8-47: Changing Biosensor Location (Method 1)

Method 2

1. In the **Sensor Tray Map**, select all of the columns and click **Remove** (Figure 8-48 top left). Or, right-click the selection and select **Remove**. All columns will be shown as **Missing** (Figure 8-48 top right).
2. Select the sensor locations to use and click **Fill**. Or, right-click the selection and select **Fill** (Figure 8-48 bottom left). The software fills the selected columns in the tray (Figure 8-48 bottom right).

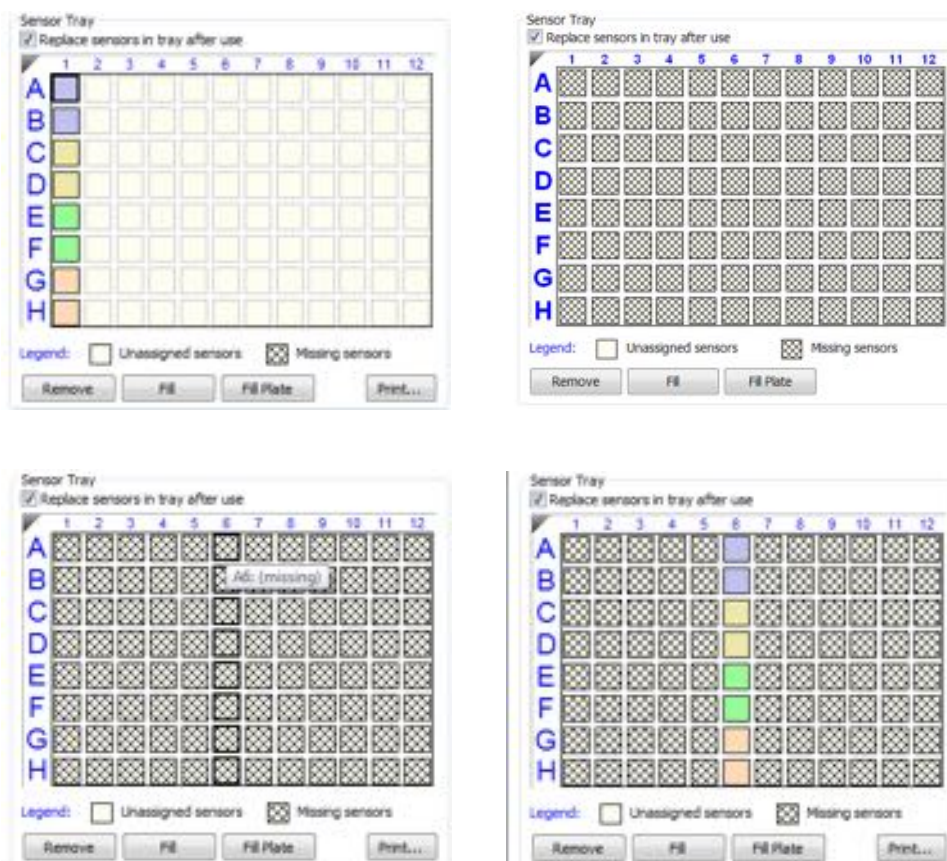


Figure 8-48: Changing Biosensor Location (Method 2)

Click **Fill Plate** to return the **Sensor Tray Map** to the default layout.

Using Heterogeneous Trays

If heterogeneous biosensor trays will be used, the well location of each biosensor type in the tray can be identified in the **Assay Definition Tab**. Assignment of biosensors that will not be used in the assay enables the software to auto-assign the biosensors that will be used in the assay by biosensor type.

The biosensor type can be changed per assay by selecting the desired biosensor type in the drop down list under sensor type in the Assay Steps List in the **Assay Definition Tab** (Figure 8-49).

Assay Steps List

New Assay Move Up Move Down Remove Replicate Edit Step Info Table

Assay	No.	Sample	Step Name	Step Type	Sensor Type	Assay Time	Comment
1	1	A1	Baseline	Baseline	Anti-mIgG1 Fc		
1	2	A2	Loading	Loading	SA (Streptavidin)		
1	3	A1	Baseline	Baseline	SAX (High Precision Streptavidin)		
1	4	A3	Baseline	Baseline	AHC (Anti-mIgG Fc Capture)		
1	5	A4	Association	Association	Anti-GST		
1	6	A3	Dissociation	Dissociation	Anti-Human Fab-CHL (FAB)		
2	1	C1	Baseline	Baseline	Anti-FLAG (FLAG)		
2	2	C2	Loading	Loading	APS (Aminopropylsilane)		
2	3	C1	Baseline	Baseline	AR (Amine Reactive)		
2	4	C3	Baseline	Baseline	AR2G (Amine Reactive 2nd Gen)		
2	5	C4	Association	Association	SSA (Super Streptavidin)		
2	6	C3	Dissociation	Dissociation	AMC (Anti-mIgG Fc Capture)		
3	1	E1	Baseline	Baseline	Ni-NTA		
3	2	E2	Loading	Loading	Custom		
3	3	E1	Baseline	Baseline	AHQ		
3	4	E3	Baseline	Baseline	Protein A		
3	5	E4	Association	Association			
3	6	E3	Dissociation	Dissociation			
4	1	G1	Baseline	Baseline	SA (Streptavidin)		
4	2	G2	Loading	Loading	SA (Streptavidin)		
4	3	G1	Baseline	Baseline	SA (Streptavidin)		
4	4	G3	Baseline	Baseline	SA (Streptavidin)		
4	5	G4	Association	Association	SA (Streptavidin)		
4	6	G3	Dissociation	Dissociation	SA (Streptavidin)	0:36:45	

Figure 8-49: Assay Steps List — Changing the Biosensor Type

Changing the Biosensor Type

The biosensor type used in each assay can be modified and must be selected in the **Assay Definition** window. To change the biosensor type:

1. Click the **Assay Definition Tab**.
2. In the **Assay Steps List**, click the cell in the **Sensor Type** column to change.
3. Select from the drop-down list (see Figure 8-49).



IMPORTANT: Ensure that the biosensor types selected in the **Assay Definition** window have been correctly assigned in the **Sensor Assignment** window or the experiment cannot be run.

Using Partial Biosensor Trays

If you remove biosensors from the **Sensor Tray Map** and there are not enough remaining biosensors for the experiment, the software automatically adds a second tray of biosensors and assigns the biosensors that are required for the assay(s).

The experiment in the example shown in (Figure 8-50) includes three assays, and Tray 1 does not include enough biosensors for the experiment. To view the additional biosensor tray that is required for the assay, select Tray 2 from the **Sensor Tray** drop-down list (Figure 8-50 top). The **Sensor Tray Map** will then display the additional biosensors required for the assay (Figure 8-50 bottom). If necessary, change the location of these biosensors.

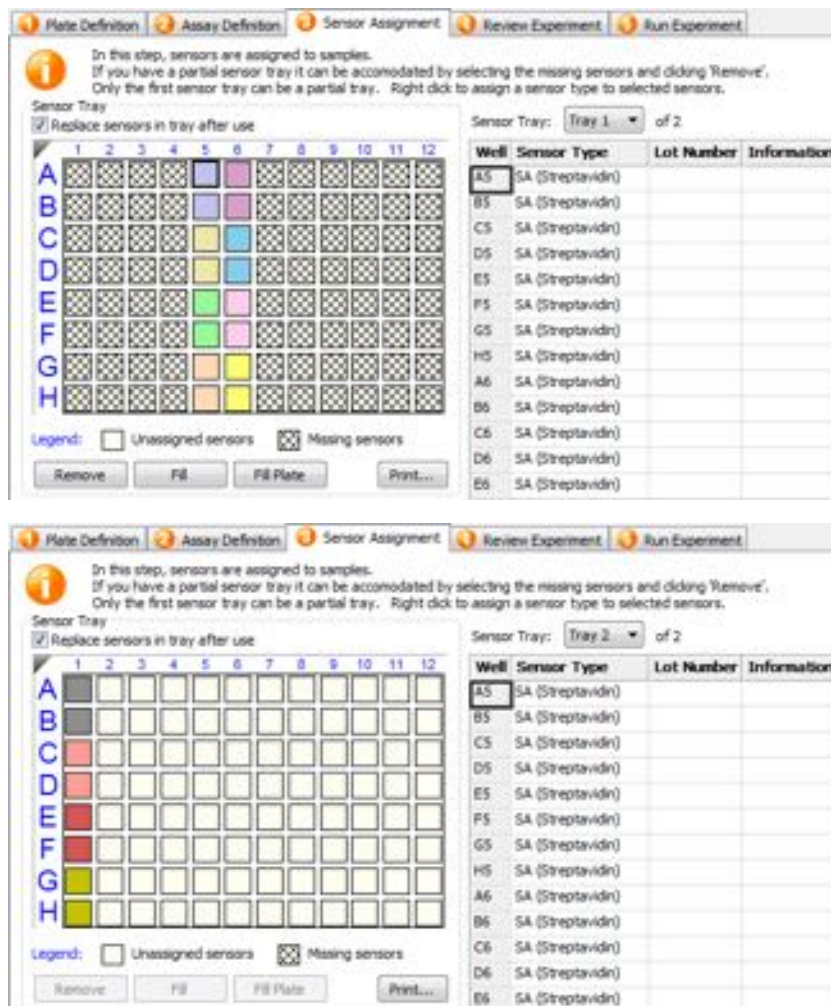


Figure 8-50: Example Experiment Using Two Biosensor Trays



NOTE: Up to two trays may be used per assay, but only the first biosensor tray can be a partial tray. During the experiment run, the software prompts you to insert the appropriate tray in the Octet instrument.

Reference Biosensors

To designate reference biosensors, select the desired biosensors in the **Sensor Tray Map**, right-click and select **Reference**. The reference biosensors are marked with an **R**.





NOTE: Reference biosensors may also be designated in the **Runtime Binding Chart** during acquisition.

REVIEWING EXPERIMENTS



NOTE: For optimal results, ensure total assay time is less than 3 hours.

Before running an experiment, you can review the sample plate layout, assays and assay steps as well as the biosensors assigned to each assay in the experiment.

In the **Review Experiment** window (Figure 8-51), move the slider left or right to highlight the biosensors and samples associated with an assay step, or click the   arrows. Alternatively, select an assay step to view the biosensors and samples associated with it.

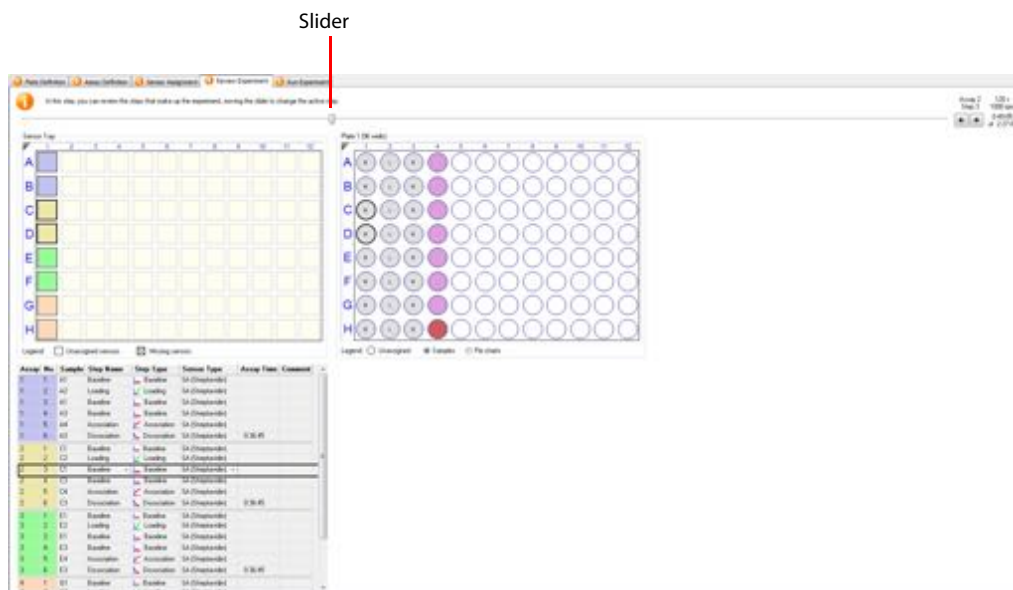


Figure 8-51: Review Experiment Window

SAVING EXPERIMENTS

After an experiment is run, the software automatically saves a read-only copy of the experiment information that you specified (sample plate definition, biosensor assignment, assay settings) to an experiment method file (.fmf). If you set up an experiment, but do not start the run, you can manually save the experiment method.

To manually save an experiment:

1. Click **Save Method File** (📁), or on the main menu, click **File > Save Method File**.

If there is more than one open experiment and you want to save all of them, click **Save All Methods Files** (📁).

2. In the **Save** dialog box, enter a name and location for the file, and click **Save**.



NOTE: If you edit a saved experiment and want to save it without overwriting the original file, click **File > Save Method File As** and enter a new name for the experiment.

Saving an Experiment to the Template Folder

If you save an experiment to the factory-installed Template folder, the experiment will be available for selection. To view templates, select **Experiment > Templates > Kinetics > Experiment Name** (Figure 8-52).

Follow the steps above to save an experiment to the Template folder located at C:\Program Files\ForteBio\DataAcquisition\TemplateFiles.



IMPORTANT: Do not change the location of the Template folder. If the Template folder is moved from the factory-set location, the software may not function properly.

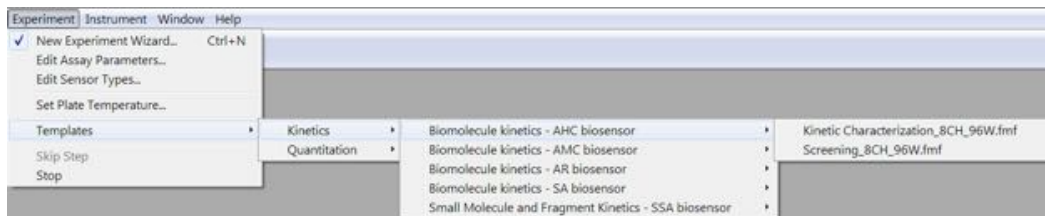


Figure 8-52: Saved Experiments in the Template Folder

RUNNING A KINETICS EXPERIMENT



IMPORTANT: Before starting an experiment, ensure that the biosensors are properly rehydrated. For details on how to prepare biosensors, see the appropriate biosensor product insert.

Loading the Biosensor Tray and Sample Plate

To load the biosensor tray and sample plate:

1. Open the Octet instrument door (lift the handle up).
2. Place the biosensor tray on the biosensor stage (left side) so that well A1 is located at the upper right corner (see Figure 8-53).
3. Place the sample plate on the sample stage (right side) so that well A1 is located at the upper right corner (see Figure 8-53).

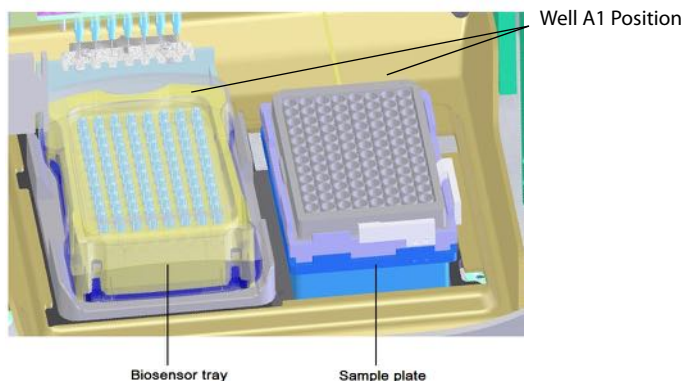


Figure 8-53: Biosensor Stage (left) and Sample Stage (right)



IMPORTANT: Make sure that the bottom of the sample plate and biosensor tray are flat on the stages.

4. Close the Octet instrument door.
5. Allow the plate to equilibrate.

The time required for temperature equilibration depends on the temperature that your application requires and the initial temperature of the sample plate. For specific biosensor rehydration times, see the appropriate biosensor product insert.

Starting the Experiment

To start the experiment:

1. Click the **Run Experiment** tab, or click the arrow (➡) to access the Run Experiment window (see Figure 8-54).

The screenshot shows the 'Run Experiment' window with the following details:

- Data File Location and Names:**
 - Kinetics data repository: C:\Users\Owner\Documents\ForteBio
 - Experiment run name (sub directory): HgG ProA Kinetics
 - Plate name/barcode (file prefix): 110424
 - Auto-increment file ID start: 1
 - Data files will be stored as follows:
 - Documents\ForteBio\HgG ProA Kinetics\110424_001.txt
 - Documents\ForteBio\HgG ProA Kinetics\110424_002.txt
 - Documents\ForteBio\HgG ProA Kinetics\110424_003.txt
- Run Settings:**
 - ☒ Delayed experiment start (Start after (s): 600)
 - ☒ Open runtime charts automatically
 - ☒ Shake sample plate while waiting
 - ☒ Automatically save runtime chart
 - ☐ Set plate temperature (°C): 30
- Advanced Settings:**
 - Acquisition rate: Standard kinetics (5.0 Hz, averaging by 20)
 - Warning: changing these settings could affect assay signal-to-noise. If you are unsure of how to use these settings, please consult the Data Acquisition User Guide.
- General Information:**
 - User name: Owner
 - Machine name: J.RICHARDS
 - Description:

Figure 8-54: Run Experiment Window

2. Confirm the default settings or enter new settings. See “Run Experiment Window Settings” on page 423 for more information on experimental settings.



NOTE: If you delay the experiment start, you have the option to shake the plate until the experiment starts.

3. To start the experiment, click **GO**.

If you specified a delayed experiment start, a message box displays the remaining time until the experiment starts.

If you select the **Open runtime charts automatically** option, the **Runtime Binding Chart** window displays the binding data in real-time, as well as the experiment progress (Figure 8-55).



NOTE: For more details about the **Runtime Binding Chart**, see “Managing the Runtime Binding Chart” on page 427.

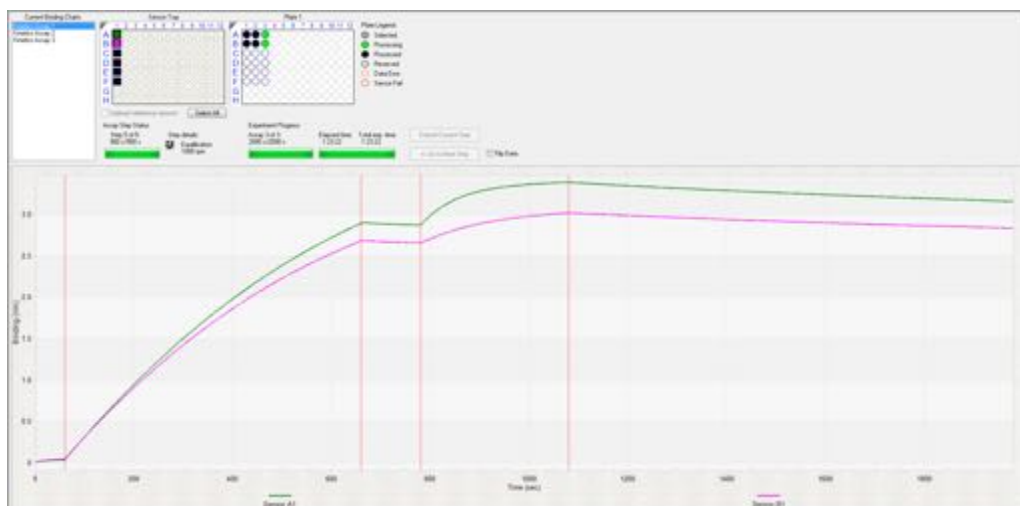


Figure 8-55: Runtime Binding Chart

4. Optional: Click **View > Instrument Status** to view the log file (see Figure 8-56).

The experiment temperature is recorded at the beginning of every experiment as well as each time the manifold picks up a new set of biosensors. Instrument events such as biosensor pick up, manifold movement, integration time, biosensor ejection and sample plate temperature are recorded in the log file.

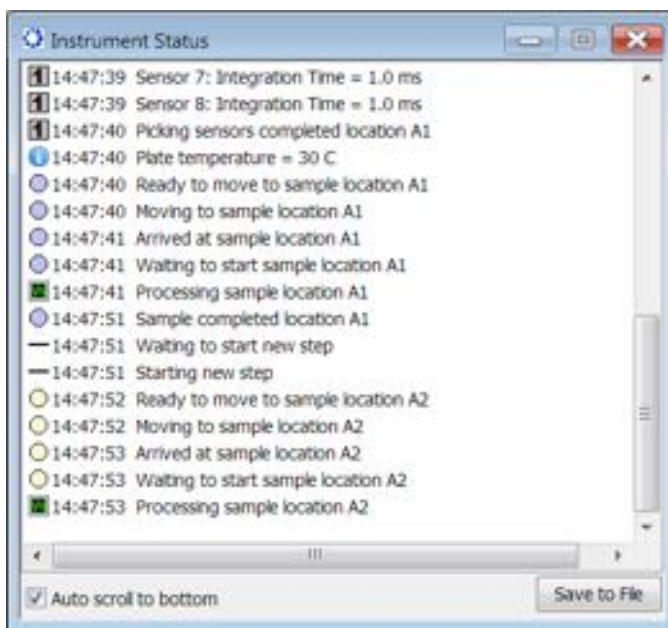


Figure 8-56: Instrument Status Log



WARNING: Do not open the Octet instrument door when an experiment is in progress. If the door is opened, the data from the active biosensors is lost. The data already acquired is saved, however the assay is aborted and cannot be restarted without ejecting the biosensors and starting from the beginning.



WARNING: N'ouvrez pas la porte de l'instrument Octet lorsqu'une analyse est en cours. En cas d'ouverture de la porte, les données issues de l'étape d'acquisition active seront perdues et cela entraînera l'échec de la procédure.



WARNING: Öffnen Sie die Instrumentenklappe des Octet-Systems nicht während eines laufenden Experiments. Wird die Klappe geöffnet, gehen die Daten des aktiven Erfassungsschritts verloren und das Experiment wird abgebrochen.

Run Experiment Window Settings

The following **Data File Location and Name** settings are available on the **Run Experiment** Tab:

Table 8-6: Data File Location and Name



Item	Description
Assay type	The name of the selected assay.
Kinetics data repository	The location where the subdirectory will be created. The subdirectory contains the data (.frd) files. Click Browse to select another data location.
<div>  NOTE: It is recommended that you save the data to the local machine first, then transfer to a network drive. </div>	
Experiment Run Name (sub-directory)	Specifies a subdirectory name for the data files (.frd). The software generates one data file for each biosensor that includes the data from all steps the biosensor performs.
Plate name/ barcode (file prefix)	A user-defined field where you can enter text or a barcode (barcode reader required).

Table 8-6: Data File Location and Name (Continued)

<i>Item</i>	<i>Description</i>
2nd Plate name/barcode	A user-defined field where you can enter text or a barcode (barcode reader required) for a second plate. This field is also used to generate the path of the saved directory.
Auto Increment File ID Start	Each file is saved with a number after the plate name. For example, if the Auto Increment File ID Start number is 1, the first file name is xxx_001.frd.

The following **Run Settings** are available on the **Run Experiment** Tab:

Table 8-7: Run Settings

<i>Item</i>	<i>Description</i>
Delayed experiment start	Specifies a time delay for the start of the experiment. Enter the number of seconds to wait before the experiment starts after you click  .
Start after	Enter the number of seconds to delay the start of the experiment.
Shake sample plate while waiting	If the experiment has a delayed start time, this setting shakes the plate until the experiment starts.
Open runtime charts automatically	Displays the Runtime Binding Chart for the current biosensor during data acquisition.
Automatically save runtime chart	Saves an image (.jpg) of the Runtime Binding Chart . The binding data (.frd) is saved as a text file, regardless of whether a chart image is created.
Set plate temperature (°C)	Specifies a plate temperature and enters the temperature in the dialog box. If not selected, the plate temperature is set to the default temperature specified in File > Options . The factory set default temperature is 30 °C.



NOTE: If the actual plate temperature is not equal to the set plate temperature, a warning displays and the Octet System Data Acquisition software provides the option to wait until the set temperature is reached before proceeding with the run, continue without waiting until the set temperature is reached, or cancel the run.


Advanced settings are available for the Octet K2 system. The signal to noise ratio of the assay can be optimized by selecting different acquisition rates. The acquisition rate refers to the number of binding signal data points reported by the Octet system per minute and is reported in Hertz (per second). A higher acquisition rate generates more data points per second and monitors faster binding events better than a slower acquisition rate. A lower acquisition rate allows the software enough time to perform more averages of the collected data. Typically, more averaging leads to reduced noise and thus, better signal-to-noise

ratios. The choice of a setting should be determined based upon consideration of the binding rate and the amount of signal generated in your assay, and some experimentation with the settings.

Table 8-8: Advanced Settings Octet K2 System

Item	Description
Acquisition rate	<ul style="list-style-type: none">High sensitivity kinetics (2 Hz, averaging by 50): - The average of 50 data frames is reported as one data point. Two data points are reported per second.Standard kinetics (5 Hz, averaging by 20 - The average of 20 data frames is reported as one data point.
Default	Sets acquisition rate and sensor offset to the defaults.

Stopping an Experiment

To stop an experiment in progress, click  or click **Experiment > Stop**.

The experiment is aborted. The data for the active biosensor is lost, the biosensor is ejected into the waste tray, and the event is recorded in the experimental log.



NOTE: After the experiment is run, the software automatically saves the experiment method (.fmf).

MANAGING THE RUNTIME BINDING CHART

If the **Open runtime charts automatically** check box is selected in the Run Experiment window (Figure 8-57), the Runtime Binding Charts are automatically displayed when data acquisition starts. The **Runtime Binding Chart** window displays the assay step status, experiment progress, and the elapsed experiment time.

The **Runtime Binding Chart** is updated at the start of each experimental step. The active set of biosensors is color-coded (A=green, B=magenta, C=orange, D=purple, E=olive, F=black, G=red, H=blue) within the **Sensor Tray Map**. Used sets of sensor columns that are inactive are colored black. Active sample columns are colored green. Each assay in the experiment is represented by **Assay X** in the **Current Binding Charts** box.

To selectively display data for particular assay:

1. Click the corresponding **Assay** number.
2. Select a subset of sensors for a displayed column under **Sensors to Chart** box (see Figure 8-57).



IMPORTANT: Do not close the **Runtime Binding Chart** window until the experiment is complete and all data is acquired. If the window is closed, the charts are not saved. To remove the chart from view, minimize the window. The Octet System Data Acquisition software saves the **Runtime Binding Chart** as displayed at the end of the experiment. For example, modifying a chart by hiding the data for a particular biosensor will cause this data not to be included in the bitmap image generated at the end of the run.

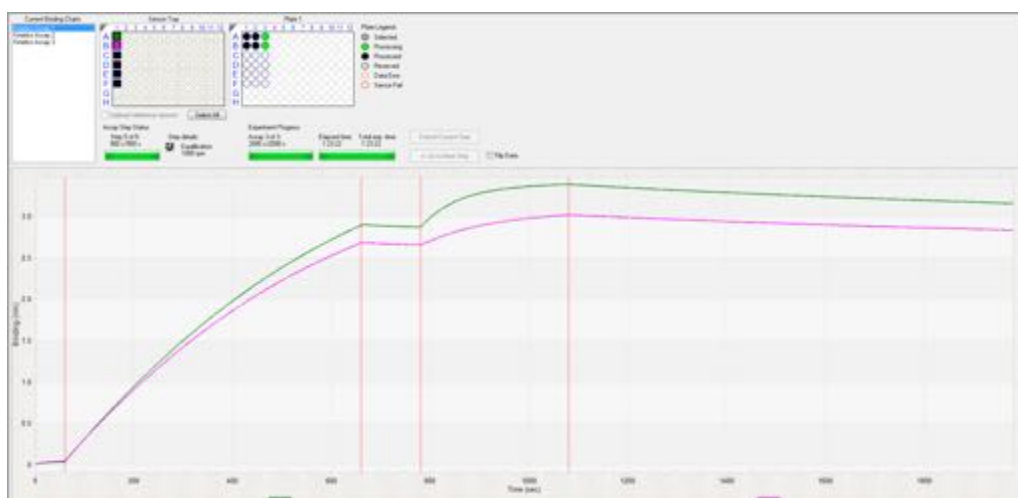


Figure 8-57: Runtime Binding Chart Window

Opening the Runtime Binding Chart

After an experiment is run, you can open and review the **Runtime Binding Chart** at any time:

1. Click **File > Open Experiment**.
2. In the dialog box that appears, select an experiment folder and click **Select**.

Viewing Reference-Subtracted Data

If the experiment includes reference biosensors, you can display reference-subtracted data in the chart by clicking the **Subtract Reference Biosensor** check box in the chart window. To view raw data, remove the check mark next to this option.

Reference biosensors can be designated:

- During experiment setup in the **Sensor Assignment** tab
- During acquisition in the Runtime Binding Chart **Sensors to Chart** box
- During analysis in the **Data Selection** tab

Designating a Reference Biosensor During Acquisition

To designate a reference biosensor during acquisition:

1. In the **Sensors to Chart** list or the **Sensor Tray**, right-click a biosensor and select **Reference** (see Figure 8-58).

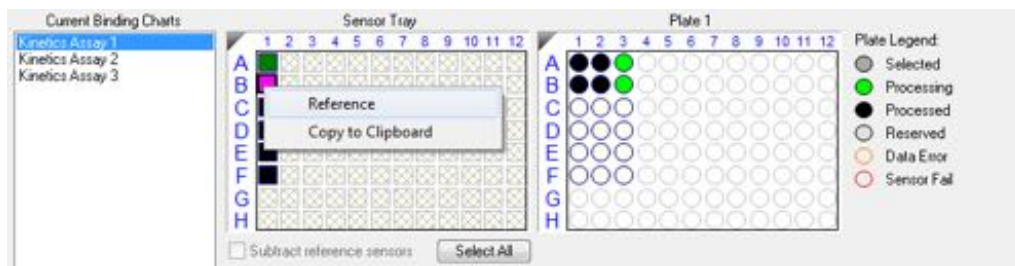


Figure 8-58: Designating a Reference Biosensor in the Runtime Binding Chart

The selected biosensor will be shown with an **R** in the **Sensors to Chart** list and **Sensor Tray** (see Figure 8-59).

2. Click the **Subtract reference sensors** check box (see Figure 8-59).

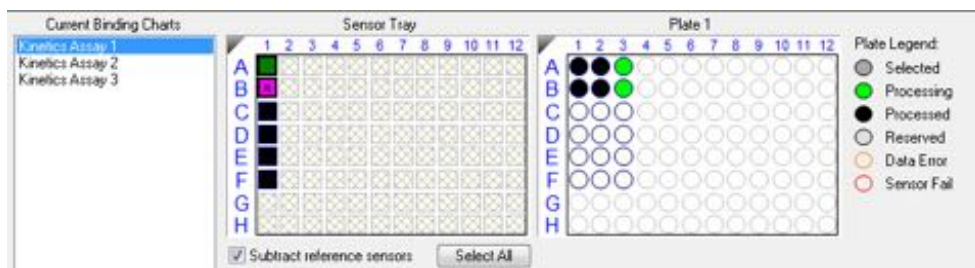


Figure 8-59: Subtract Reference Sensors check box in the Runtime Binding Chart



NOTE: Subtracting reference data in the **Runtime Binding Chart** only makes a visual change to the data on the screen. The actual raw data is unaffected and the reference subtraction must be repeated during data analysis if needed.

Viewing Inverted Data

The data displayed in the **Runtime Binding Chart** can be inverted during real-time data acquisition or data analysis after the experiment has completed. To invert data, select the **Flip Data** check box (see Figure 8-60). Uncheck the box to return to the default data display.

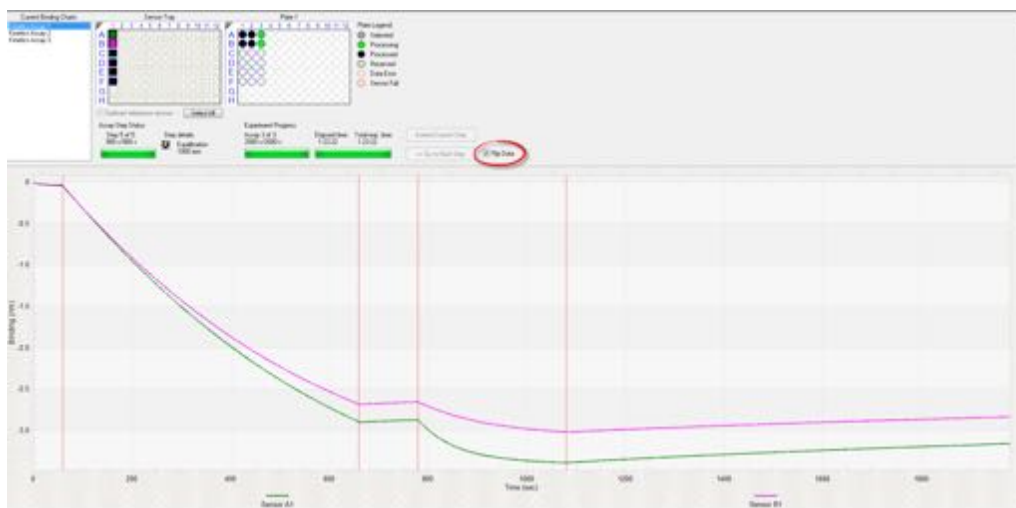


Figure 8-60: Data Inverted Using Flip Data Function

Aligning Data by a Selected Step

To align the binding data to the beginning of a user-selected step, in the **Runtime Binding Chart** (see Figure 8-61), right-click a step and select **Align to Step <number>**.

To remove the step alignment, right-click the step and select **Unaligned**.

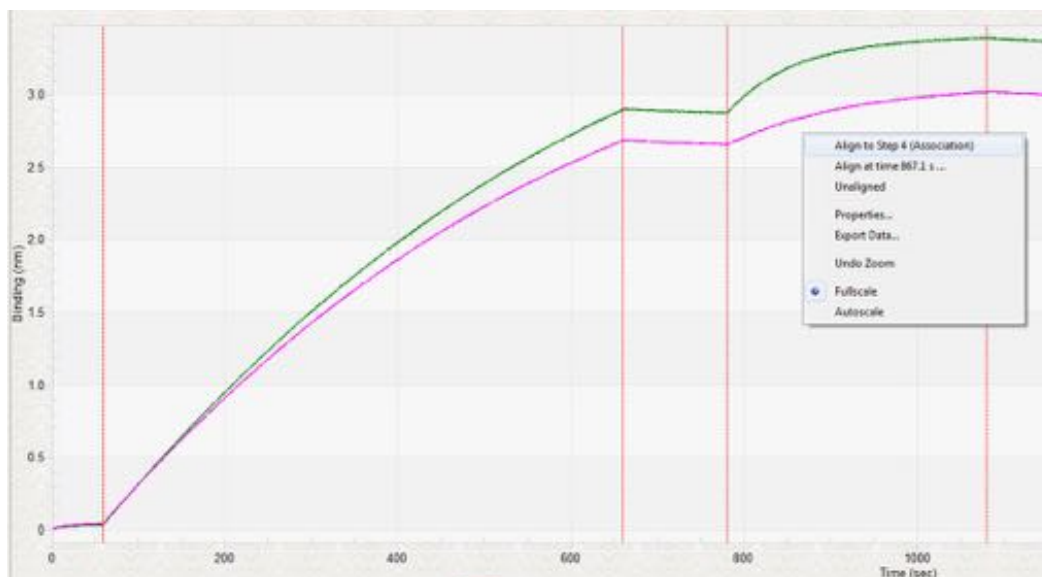


Figure 8-61: Runtime Binding Chart—Aligning the Data to a User-Selected Step

Aligning Data to a Specific Time

1. To align the binding data to a specific time, in the **Runtime Binding Chart** (see Figure 8-62), right-click and select **Align at time**.

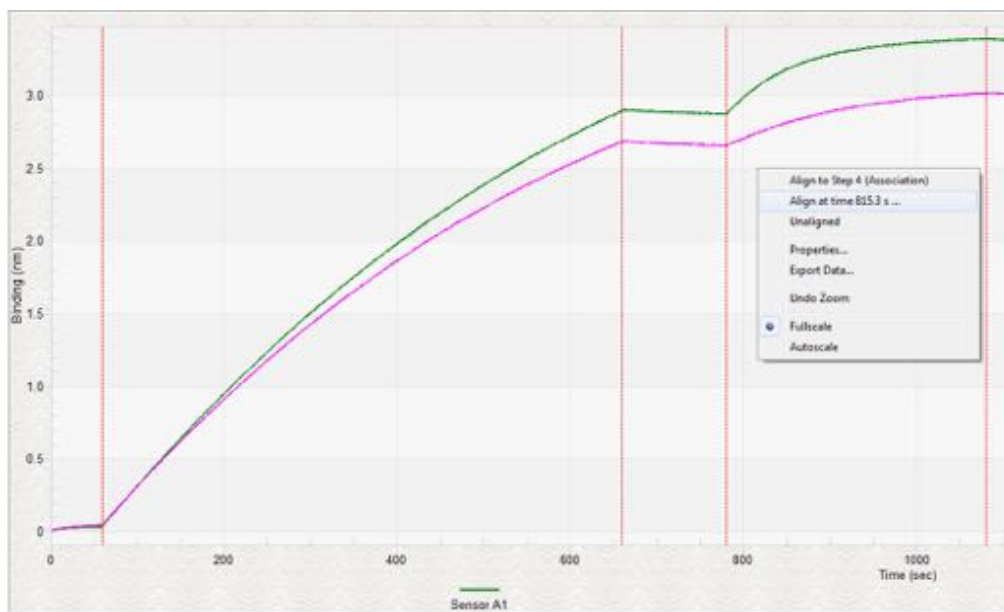


Figure 8-62: Runtime Binding Chart—Aligning the Data to a User-Specified Time

The Align at Time dialog box displays (Figure 8-63).

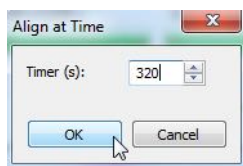


Figure 8-63: Align at Time Dialog Box

2. Enter the time point you want to align to and click **OK**. The binding chart will then align to the time point specified.

To remove the time alignment, right-click and select **Unaligned**.

Extending or Skipping an Assay Step

During acquisition, the duration of the active step may be extended. You can also terminate the active step and begin the next step in the assay.



NOTE: If the step you want to extend or terminate includes biosensors used in Parallel Reference, Double Reference, or Average Reference subtraction methods, the data will not be analyzed.

To extend the duration of the active step:

1. In the chart window, click the **Extend Current Step** button.
2. In the **Extend Current Step** dialog box (see Figure 8-64), enter the number of seconds to extend the step and click **OK**.

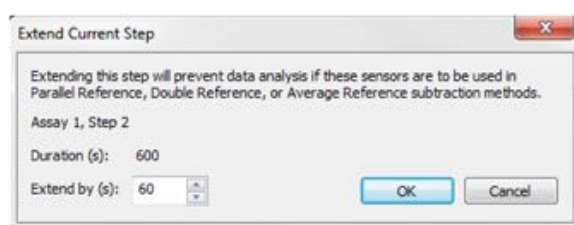


Figure 8-64: Extend Current Step Dialog Box

Terminating a Step to Begin the Next Step

To terminate a step and begin the next step in the assay:

1. In the chart window, click the **Go to Next Step** button.
2. In the **Data Acquisition** dialog box, click **OK**.

Magnifying the Runtime Binding Chart

To magnify the chart, press and hold the mouse button while you draw a box around the chart area to magnify.

To undo the magnification, right-click the chart and select **Undo Zoom**.

Scaling a Runtime Binding Chart

To scale the **Runtime Binding Chart**:

1. Right-click the chart and select **Properties**.
2. In the **Runtime Graph Properties** dialog box, select **Fullscale** or **Autoscale**.

Adding a Runtime Binding Chart Title

To add a **Runtime Binding Chart** title:

1. Right-click the chart and select **Properties**.
2. In the **Runtime Graph Properties** dialog box, enter a graph title or subtitle.

Selecting a Runtime Binding Chart Legend

To select a **Runtime Binding Chart** legend:

1. Right-click the chart and select **Properties**.
2. In the **Runtime Graph Properties** dialog box (see Figure 8-65), select one of the following legends:
 - Sensor Location
 - Sample ID
 - Sensor Information
 - Concentration/Dilution

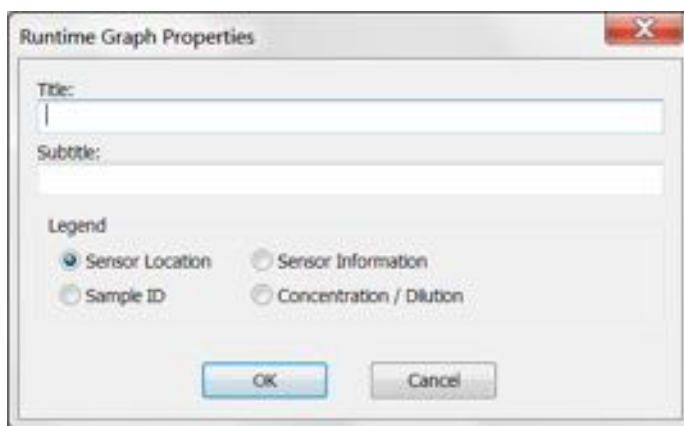


Figure 8-65: Selecting a Runtime Binding Chart Legend



NOTE: Text for **Sample ID**, **Sensor Information**, or **Concentration/Dilution** is taken from the **Plate Definition** and **Sensor Assignment** tabs, and must be entered before the experiment is started.

3. Click **OK**.

Viewing Multiple Runtime Binding Charts

To view multiple Runtime Binding Charts, click **Window > New Window**.

Exporting or Printing the Runtime Binding Chart

To export the **Runtime Binding Chart** as a graphic or data file:

1. Right-click the chart and select **Export Data**.
2. In the **Exporting** dialog box (see Figure 8-66), select the export options and click **Export**.

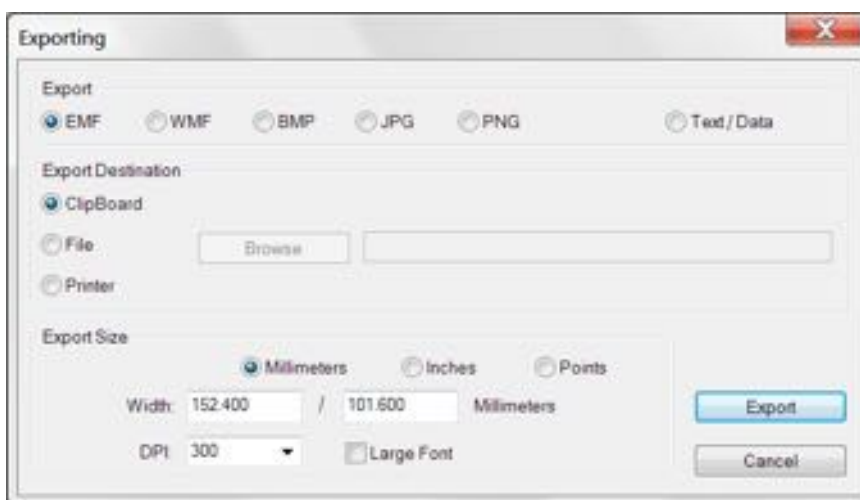


Figure 8-66: Exporting Dialog Box

Table 8-9: Runtime Binding Chart Export Options

Task	Export	Option	Export Destination	Result
	Text/ Data	EMF, WMF, BMP, JPG, or PNG		

Table 8-9: Runtime Binding Chart Export Options (Continued)

Task	Export	Option	Export Destination	Result
Save the binding data	✓		Click File > Browse to select a folder and enter a file name.	Creates a tab-delimited text file of the numerical raw data from each biosensor. Open the file with a text editor such as Notepad.
Export the Runtime Binding Chart to a graphic file		✓	Click File > Browse to select a folder and enter a file name.	Creates a graphic image.
Copy the Runtime Binding Chart		✓	Clipboard	Copies the chart to the system clipboard
Print the Runtime Binding Chart		✓	Printer	Opens the Print dialog box.

MANAGING EXPERIMENT METHOD FILES

After you run an experiment, the Octet System Data Acquisition software automatically saves the method file (.fmf), which includes the sample plate definition, biosensor assignment, and the run parameters. An experiment method file provides a convenient initial template for subsequent experiments. A read-only copy of the method used for an experiment is automatically saved in the experiment folder. Open a method (.fmf) and edit it if necessary.



NOTE: When using the 21 CFR Part 11 version of the Octet System Data Acquisition software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.

Table 8-10: Managing Experiment Method Files

Menu Bar Command/ Toolbar Button	Description
File > Open Method File	Enables you to select and open a method file (.fmf)
File > Save Method File or	Saves one method file or all method files. Saves a method file before the experiment is run.
File > Save Method File As	Saves a method file to a new name so that the original file is not overwritten.

EPITOPE BINNING

The goal of a typical epitope binning or cross-blocking experiment is to identify antibodies which bind to different or identical epitopes on the antigen. Antibodies are tested two at a time for competitive binding to one antigen. By competing antibodies against one another in a pairwise and combinatorial format, antibodies with distinct blocking behaviors can be discriminated and assigned to “bins”. The end result is matrix of pairwise binders and blockers.



An epitope binning or cross-blocking experiment must be run as a kinetic experiment with repeating steps in the Octet Data Acquisition software.



NOTE: Pall ForteBio highly recommends using the Loading, Association or Dissociation assay steps instead of Custom for epitope binning and cross-blocking experiments.

After starting the Octet system and the Octet Data Acquisition software, follow the steps in Table 8-11 to set up and run an epitope binning experiment.

Table 8-11: Octet Data Acquisition Steps for Epitope Binning Assays

Octet Software	Functions
Data Acquisition 	<ol style="list-style-type: none"> 1. Select Epitope Binning under New Kinetics Experiment in the Experiment Wizard. Open a method template from the Experiment Menu or open an existing method file (*.fmf). <hr/> <div>  <p>NOTE: In the Experiment Menu, the Templates command allows users to pick from a set of predefined method templates for Kinetic, Quantitation, or Epitope Binning experiments. Users may also modify existing method templates to suit their experimental conditions and save as a new method file and new method file name.</p> </div> <hr/> <ol style="list-style-type: none"> 2. Define a sample plate or open a sample plate definition. 3. Specify assay steps. 4. Assign biosensors to samples. 5. Run the experiment.

Starting an Experiment

You can start a kinetics experiment using one of the following options:

- Launch the **Experiment Wizard** by clicking **Experiment > New Experiment Wizard**, and selecting **New Kinetics Experiment** and **Epitope Binning**.
- Open a method file (.fmf) by clicking **File > Open Method File**. Method files may be saved and recalled using the File menu and are automatically saved when an experiment is run.
- On the menu bar, click **Experiment > Templates > Epitope Binning**.
- 6. Optional: You can also click **Recent Methods** to display a list of recently used methods. You can open any method file from the list and use it with or without modifications to run a new experiment.

Enter the required information on Tabs 1-5 of the Basic Kinetics Experiment.

Tab 1 (Plate Definition)



NOTE: For the Octet K2 system, wells in sample plate are restricted to rows AB, CD, EF and GH. Sample wells cannot be designated in row pairs BC, DE and FG.

1. Designate layouts for the plate by selecting wells in the plate map and designating sample types. There are several ways to select sample wells in the plate map:
 - Click a column header or select adjacent column headers by click-hold-drag.
 - To select non-adjacent columns, hold the **Ctrl key** and click the column header.
 - Click a row header or select adjacent row headers by click-hold-drag.
 - Click a well or draw a box around a group of wells.
2. Designate well types by right-clicking on selected wells and assigning a sample type:

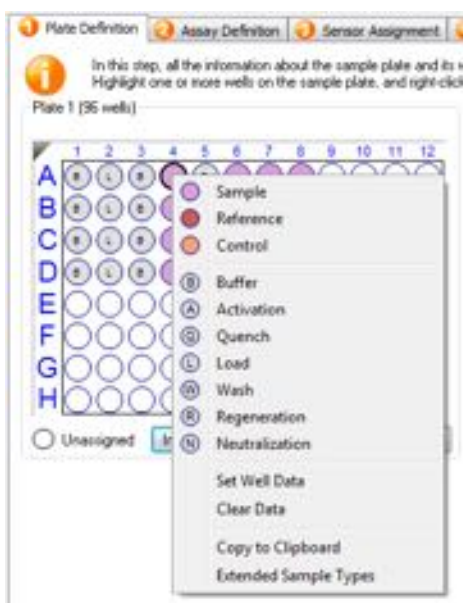


Figure 8-67: Designating Well Types

3. Enter sample information by selecting the table for the plate. There are several ways to enter sample information:
 - Select an individual well in the plate table.
 - Click-drag-hold several wells in the plate table, right-click and choose **Set Well Data**.



NOTE: Assigning sequential alpha-numerical names for Sample ID provides easier sorting of columns and headers for the epitope binning matrix.



NOTE: More information on sample information and annotation can be found in “Entering Sample Information” on page 382.

Tab 2 (Assay Definition)

After completing the plate layout, an Epitope Binning Assay can be defined by building a kinetic assay.

1. Click on Tab 2 (Assay Definition).
2. Add assay step types in the Step Data List:
 - a. Click the **Add** button. The Add Step Definition box will display:



Figure 8-68: Add Step Definition Box

- b. Choose a step type.
- c. Optional: edit step name.
- d. Set the step time and shake speed.
- e. The regeneration step type requires assigning separate parameters. To do this, click the **Regeneration Params** button:

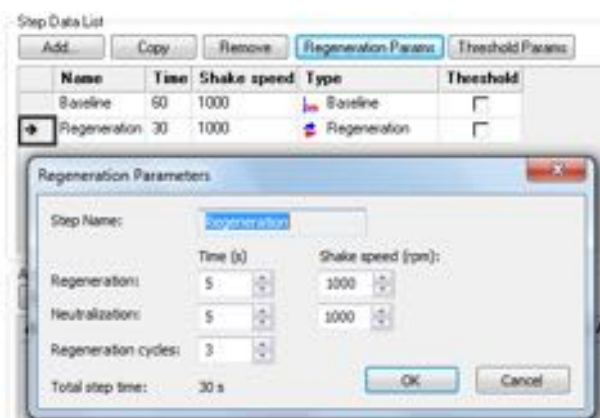


Figure 8-69: Regeneration Parameters Box

- f. Optional: assign a threshold. See “Creating Step Types” on page 398 for more information.
3. Build the assay(s) by assigning steps defined in Step Data List to columns in the plate map(s).



NOTE: Pall ForteBio highly recommends using the Association or Dissociation assay steps instead of Custom for epitope binning and cross-blocking experiments.

- a. Select a step type in the Step Data List.
- b. In the plate map, double-click the columns that you want associated with that step type.
- c. The selected wells will be marked with hatching, and the new step appears in the Assay Steps List:

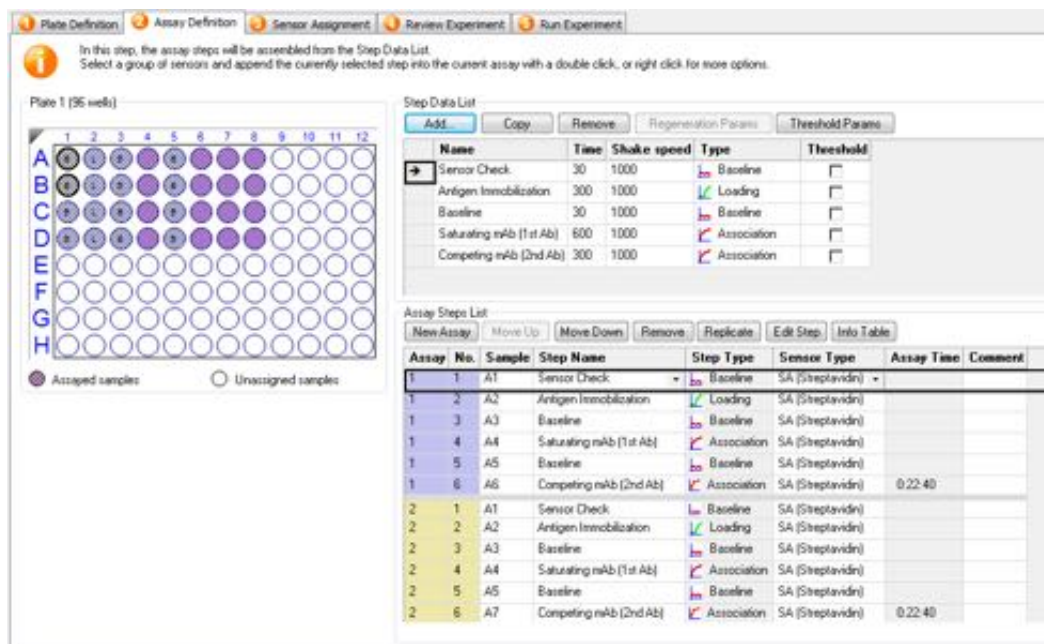


Figure 8-70: Assay Steps List

- Select the correct biosensor from the Sensor Type drop-down list.
- Repeat the previous steps to define other steps in the assay.
- New assays may be added by clicking the **New Assay** button in the Assay Steps List:

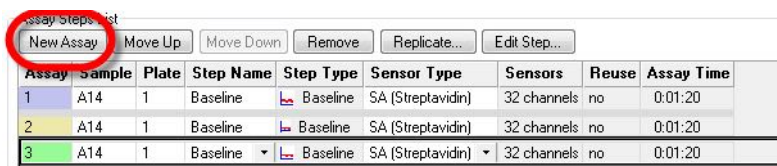


Figure 8-71: New Assay Button



NOTE: More information on assay step editing in Tab 2 (Assay Definition) can be found in "Creating Step Types" on page 398.

Tab 3 (Sensor Assignment):

After completing the assay definition, click on Tab 3 (Sensor Assignment) to verify sensor type(s) for the epitope binning experiment.



NOTE: The Sensor Type for the assay must be selected or changed from the Assay Steps List in the Assay Definition Tab. Changing the Sensor Type from the Sensor Assignment Tab will not update the assay.



NOTE: Full details on biosensor assignment in Tab 3 (Sensor Assignment) can be found in “Assigning Biosensors to Samples” on page 411.

Replacing Biosensors in the Biosensor Tray. After an assay is completed, biosensors can either be returned to the biosensor tray or ejected through the chute. To return them to the tray, click the Replace sensors in tray after use check box:



Figure 8-72: Replace Sensors in Tray After Use Check Box

Tab 4 (Review Experiment)



NOTE: For optimal results, ensure total assay time is less than 3 hours.

Before running the experiment, click on Tab 4 (Review Experiment) to review the sample plate layout, assays and assay steps as well as the biosensors assigned to each assay in the experiment.

Move the slider left or right in the window or click the arrows to highlight the biosensors and samples associated with an assay step:

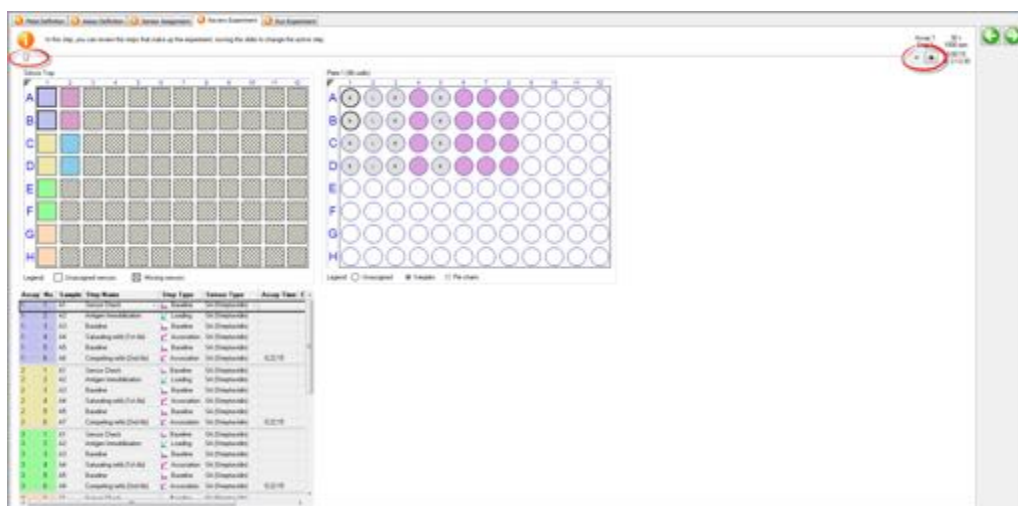


Figure 8-73: Navigating the Review Experiment Tab

Alternatively, select an assay step to view the biosensors and samples associated with it.

Saving Experiments

After an experiment is run, the software automatically saves the experiment information that you specified (sample plate definition, biosensor assignment, assay settings, etc.) to an experiment method file (.fmf).

If you set up an experiment but do not start the run, you can manually save the experiment method. To do this:

1. Select **File > Save Method File**.
2. In the Save dialog box, enter a name and location for the file, and click **Save**.

Loading the Biosensor Tray and Sample Plates

To load the biosensor tray and plate positions 1 and 2:

1. Click **Instrument > Present Stage** to open the door and present the stage. Alternatively, click the **Present Stage** button:

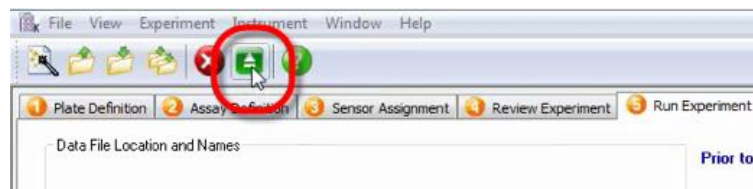


Figure 8-74: Present Stage Button

2. Place the biosensor tray, biosensor wetting plate, Plate 1, and Plate 2 on the appropriate stage so that well A1 is located at the upper right corner.
3. Close the stage and door by clicking the **Present Stage** button again.

Tab 5 (Run Experiment)

1. Click on Tab 5 (Run Experiment) to confirm the default settings or set new settings.
2. To start the experiment, click the **GO** button:

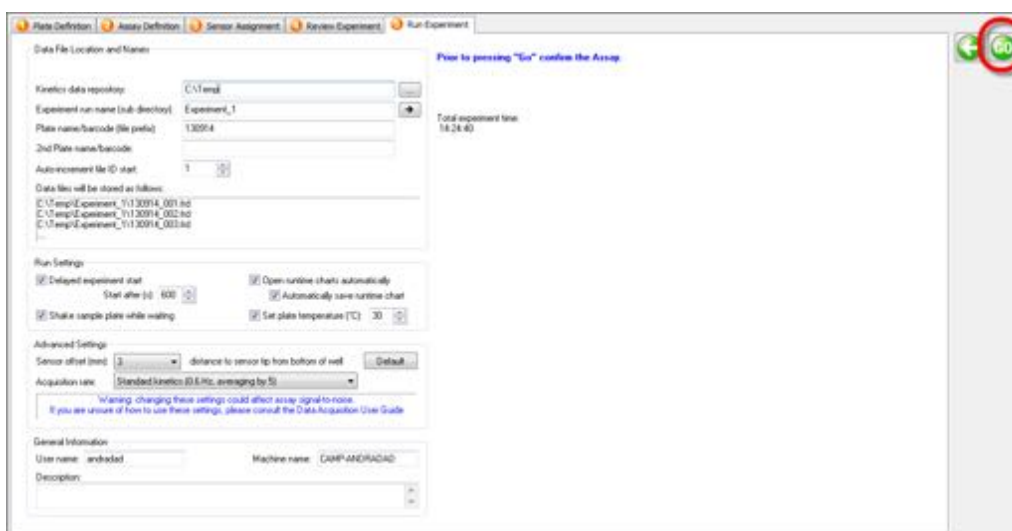


Figure 8-75: GO Button

CHAPTER 9:




Kinetics Experiments: Octet RED96, RED96e, QK^e and QK

Introduction	446
Starting a Basic Kinetics Experiment	447
Defining the Sample Plate	448
Managing Sample Plate Definitions	463
Defining a Kinetic Assay	467
Assigning Biosensors to Samples	481
Reviewing Experiments	490
Saving Experiments	491
Running a Kinetics Experiment	492
Managing the Runtime Binding Chart	500
Managing Experiment Method Files	507
Epitope Binning	508

INTRODUCTION

A basic kinetics experiment enables you to determine the association and dissociation rate of a molecular interaction. After starting the Octet system hardware and the Octet System Data Acquisition software, follow the steps (in Table 9-1) to set up and analyze a quantitation experiment.

Table 9-1: Setting Up and Analyzing a Kinetic Experiment

Software	Step	See
Data Acquisition 	1. Select a kinetics experiment in the Experiment Wizard or open a method file (.fmf).	"Starting a Basic Kinetics Experiment" on page 447
	2. Define a sample plate or import a sample plate definition.	"Defining the Sample Plate" on page 448
	3. Specify assay steps.	"Defining a Kinetic Assay" on page 467
	4. Assign biosensors to samples.	"Assigning Biosensors to Samples" on page 481
	5. Run the experiment.	"Running a Kinetics Experiment" on page 492
Data Analysis  or Data Analysis HT 	6. View and process the raw data.	<i>Octet System Data Analysis Software or Octet Data Analysis HT Software User Guide</i>
	7. Analyze the data.	



NOTE: Before starting an experiment, check the sample plate temperature displayed in the status bar. Confirm that the temperature is appropriate for your experiment and if not set a new temperature. If the Octet System Data Acquisition software is closed, the plate temperature will reset to the default startup value specified in the **Options** window when the software is relaunched.

STARTING A BASIC KINETICS EXPERIMENT



IMPORTANT: Using 96-well half-area plates on the Octet RED96 and RED96e system will result in non-optimal system performance. Pall ForteBio cannot guarantee results within the optimal performance specifications of the system when these plates are used.



You can start a kinetics experiment using one of the following options:

- Launch the **Experiment Wizard**.
- Open a method file (.fmf) by clicking **File > Open Method File**. Method files may be saved and recalled using the **File** menu and are automatically saved when an experiment is run. For more details on method files see “Managing Experiment Method Files” on page 507.
- On the menu bar, click **Experiment > Templates > Kinetics**.



NOTE: When using the 21 CFR Part 11 version of the Octet System Data Acquisition software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.

Starting an Experiment Using the Experiment Wizard

1. If the **Experiment Wizard** is not displayed when the software is launched, click the **Experiment Wizard** toolbar button , or click **Experiment > New Experiment Wizard (Ctrl+N)** from the **Main Menu**.
2. In the **Experiment Wizard**, click **New Kinetics Experiment** (see Figure 9-1, left).
3. Optional: You can also click **Recent Methods** to display a list of recently used methods. You can open any method file from the list and use it with or without modifications to run a new experiment.
4. Click the arrow button () . The **Basic Kinetics Experiment** window displays (Figure 9-1, right).

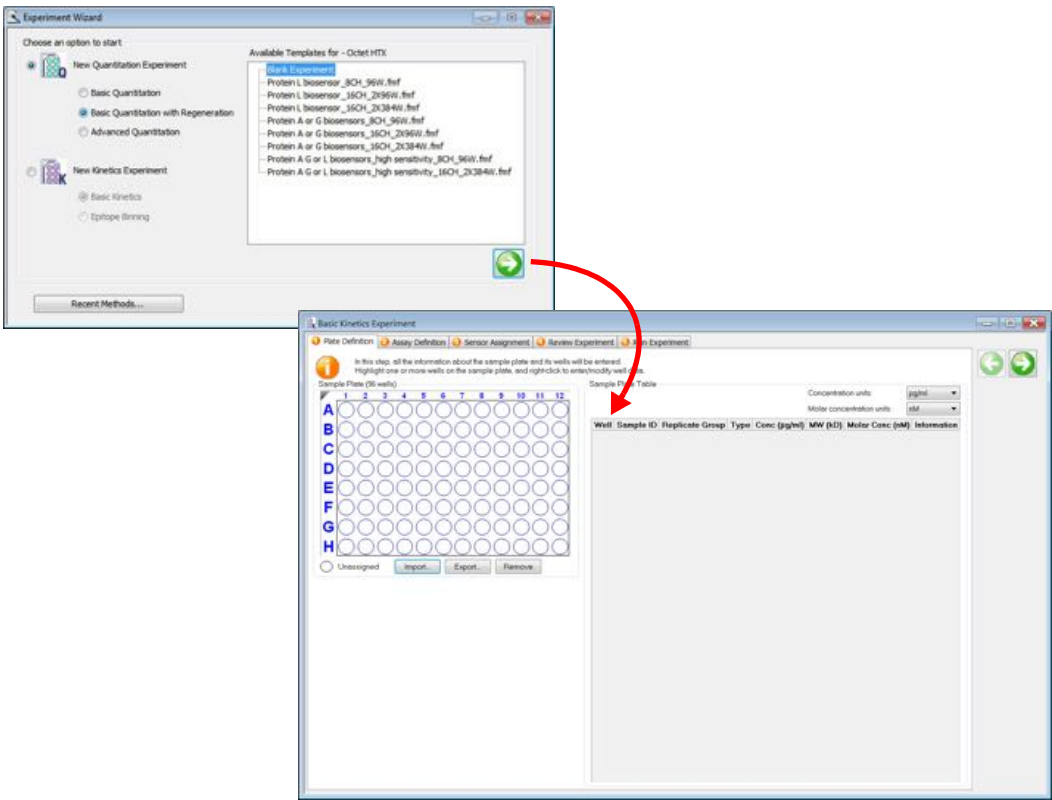


Figure 9-1: Starting a Kinetics Experiment with the Experiment Wizard

DEFINING THE SAMPLE PLATE

The steps to define a sample plate include:

Step	See Page
5. Designate the samples.	448
6. Save the sample plate definition (optional).	463











Designating Samples



NOTE: It is important to define all of the wells that will be used in the assay. Only wells that are selected and defined using one of the sample types in Table 9-2 will be included in the assay.

Table 9-2 displays the well types that can be assigned to a plate map.

Table 9-2: Types of Sample Wells

Icon	Description
 Sample	Any type of sample. For example, an analyte.
 Reference	Reference sample. For example, a buffer-only control biosensor that is used to correct for system drift.
 Controls	A control sample, either positive or negative, of known analyte composition. <ul style="list-style-type: none"> Positive Control: A control sample that contains analyte of known concentration Negative Control: A control sample known not to contain analyte
 Buffer	Any type of buffer. For example, the buffer in a baseline, association, or dissociation step.
 Activation	Activation reagent. Makes the biosensor competent for binding.
 Quench	Quenching reagent. Blocks unreacted immobilization sites on the biosensor surface.
 Load	Ligand to be immobilized (loaded) on the biosensor surface.
 Wash	Wash buffer.
 Regeneration	Regeneration reagents dissociate the analyte from the ligand.
 Neutralization	Neutralization buffer that is used to neutralize the biosensor after the regeneration step.

Selecting Wells in the Sample Plate Map

There are several ways to select wells in the **Sample Plate Map**:

- Click a column header or select adjacent column headers by click-hold-drag. To select non-adjacent columns, hold the **Ctrl** key and click the column header (Figure 9-2 left).
- Click a row header or select adjacent row headers by click-hold-drag (Figure 9-2, center).
- Click a well or draw a box around a group of wells (Figure 9-2, right).

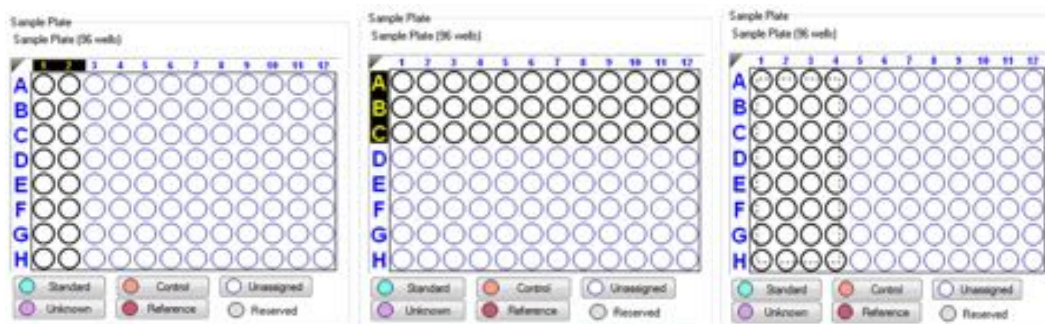


Figure 9-2: Selecting Wells in the Sample Plate Map



NOTE: Shift-clicking in the **Sample Plate Map** mimics the head of the instrument during the selection.

Designating Well Types

In the **Sample Plate Map**, select the wells, right-click and select a sample type (see Figure 9-25).

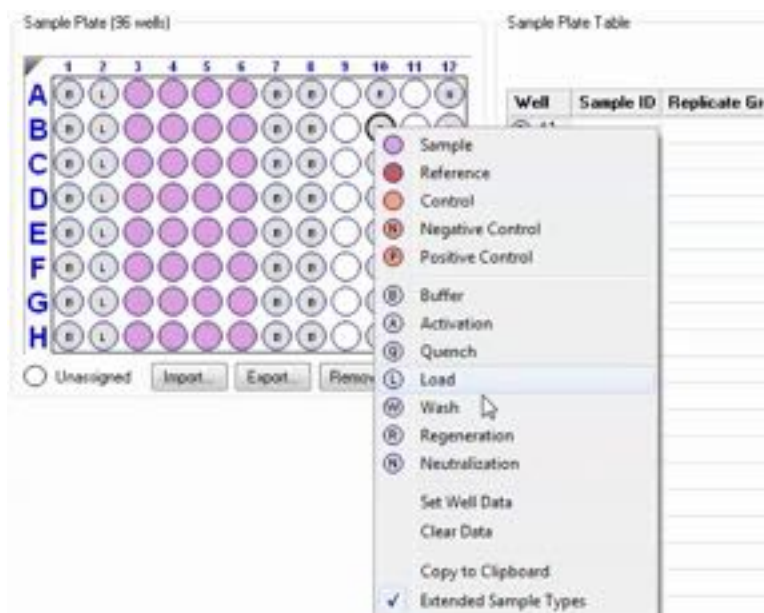


Figure 9-3: Designating a Well Type in the Plate Definition Window

To remove a well designation, in the **Sample Plate Map**, select the well(s) and click **Remove**. Or, right-click the well(s) and select **Clear Data** (see Figure 9-4).

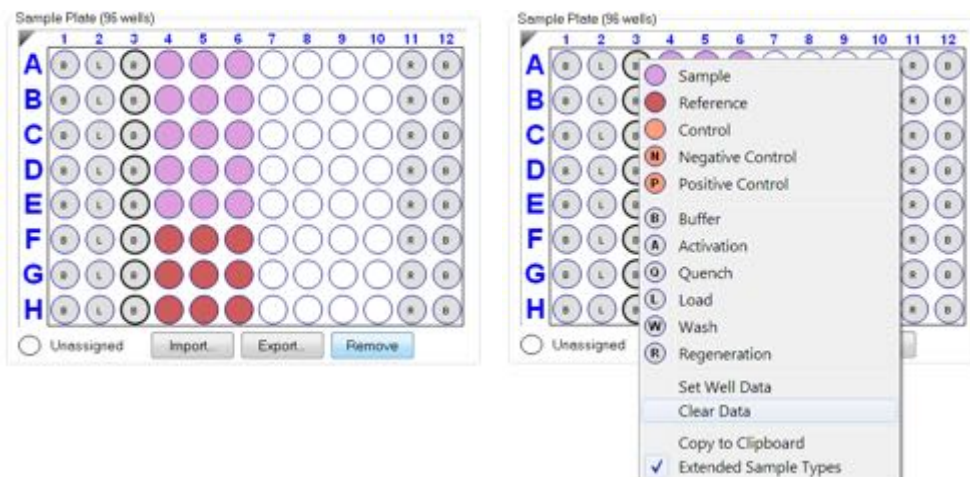


Figure 9-4: Clearing Sample Data from a Sample Plate

Entering Sample Information



NOTE: You must specify sample (analyte) concentration and molecular weight, otherwise the Octet System Data Acquisition software cannot compute a K_D value. If the sample concentration is not specified, only k_d and k_{obs} are calculated. You can also annotate any well with **Sample ID** or **Well Information**, and assign **Replicate Groups**.

Assigning Molecular Weight and Molar Concentration

1. In the **Sample Plate Map**, select the sample wells, right-click and select **Set Well Data**.
2. In the **Set Well Data** dialog box, enter the analyte molecular and molar concentration (Figure 9-5).

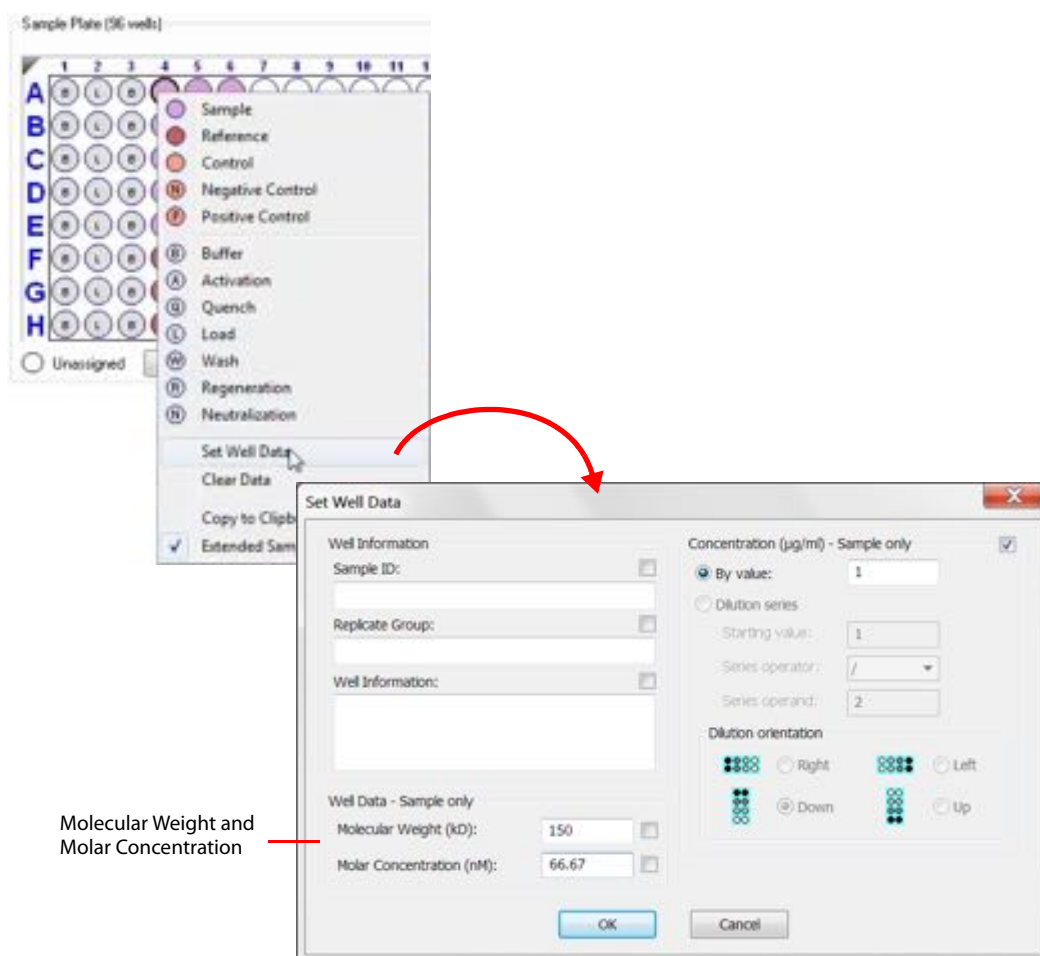


Figure 9-5: Entering Molecular Weight and Molar Concentration from the Sample Plate Map

The information displays in the **Sample Plate Table** (see Figure 9-6).

3. In the **Sample Plate Table**, select the sample concentration units and the molar concentration units.

Sample Plate Table

Concentration units: µg/ml

Molar concentration units: nM

Concentration units

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information
F3			Buffer				
G3			Buffer				
H3			Buffer				
A4			Sample	150	66.67		
B4			Sample	150	33.33		
C4			Sample	150	16.67		
D4			Sample	150	8.333		
E4			Sample	150	4.167		
F4			Reference				
G4			Reference				
H4			Reference				
A5			Sample	150	66.67		
B5			Sample	150	33.33		
C5			Sample	150	16.67		
D5			Sample	150	8.333		
E5			Sample	150	4.167		
F5			Reference				
G5			Reference				
H5			Reference				
A6			Sample	150	66.67		
B6			Sample	150	33.33		
C6			Sample	150	16.67		
D6			Sample	150	8.333		
E6			Sample	150	4.167		
F6			Reference				

Figure 9-6: Entering Molecular Weight and Molar Concentration from the Plate Table

Assigning User Specified Sample Concentrations

To assign sample concentrations using a dilution series:

1. In the **Sample Plate Map**, select the desired wells, right-click and select **Set Well Data**. The **Set Well Data** dialog box displays (see Figure 9-7).
2. Select the **By value** option and enter the starting concentration value.

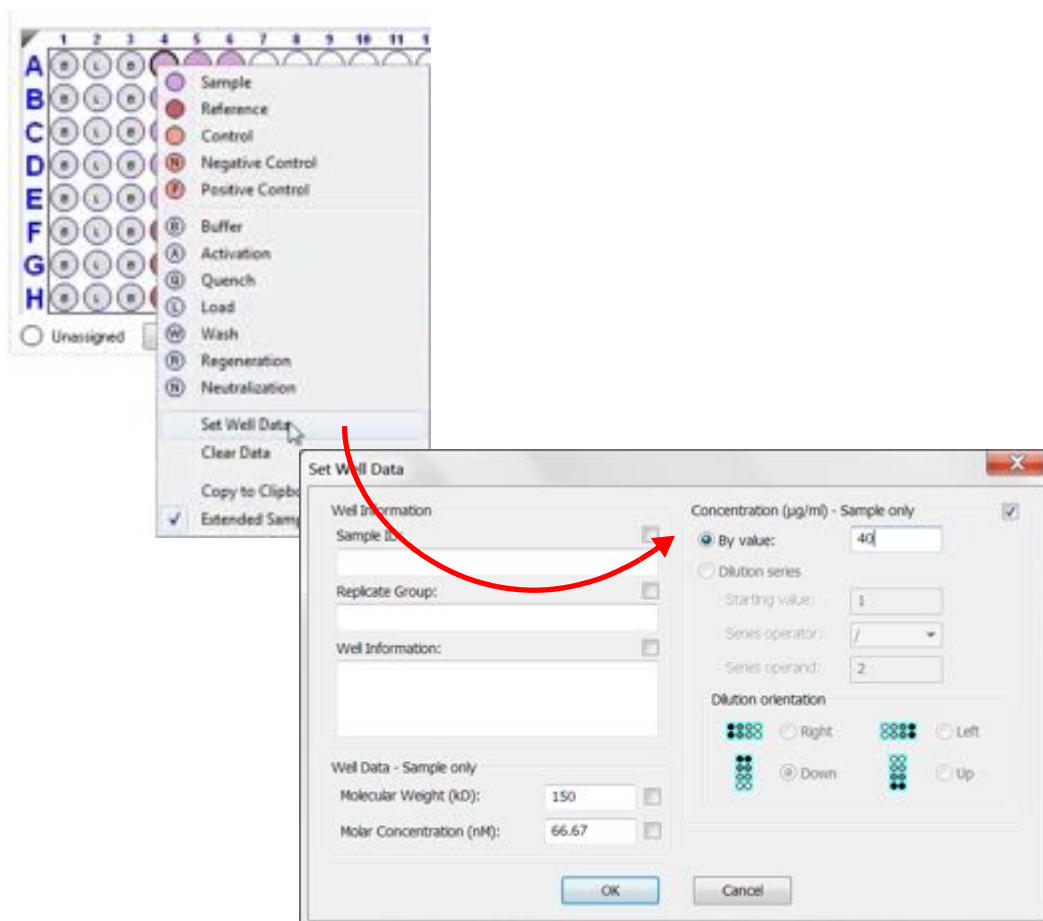


Figure 9-7: Sample Plate Map—Assigning Sample Concentrations by Value

3. Click **OK**. The **Sample Plate Table** will display the entered concentration.

Assigning Concentrations Using a Dilution Series

To assign sample concentrations using a dilution series:

1. In the **Sample Plate Map**, select the wells, right-click, and select **Set Well Data**.
The **Set Well Data** dialog box displays (see Figure 9-8)
2. Select the **Dilution Series** option and enter the starting concentration value.

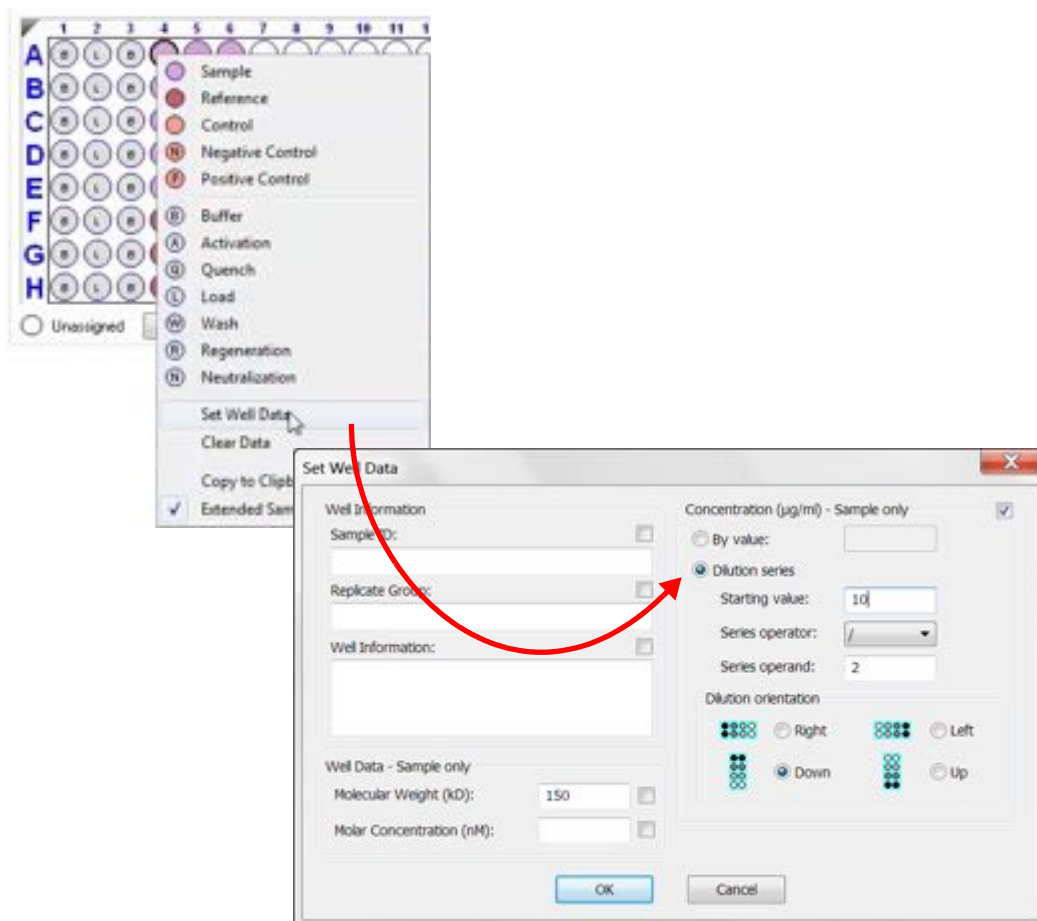


Figure 9-8: Sample Plate Map—Assigning Sample Concentrations Using Dilution Series

3. Select a series operator, enter an operand, and select the appropriate dilution orientation (see Figure 9-9).

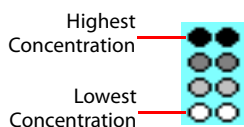


Figure 9-9: Concentration Representation in Dilution Series

4. Click **OK**.

The **Sample Plate Table** displays the standard concentrations.

Annotating Samples

You can enter annotations (notes) for multiple samples in the **Sample Plate Map** or enter information for an individual sample in the **Sample Plate Table**. For greater clarity, annotation text may be displayed as the legend of the **Runtime Binding Chart** during data acquisition, but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it will not be available for display as a legend.

Annotating Wells in the Sample Plate Map

To annotate one or more wells:

1. In the **Sample Plate Map**, select the samples to annotate, right-click and select **Set Well Data**.
2. In the **Set Well Data** dialog box (see Figure 9-10), enter the **Sample ID** and/or **Well Information** and click **OK**.

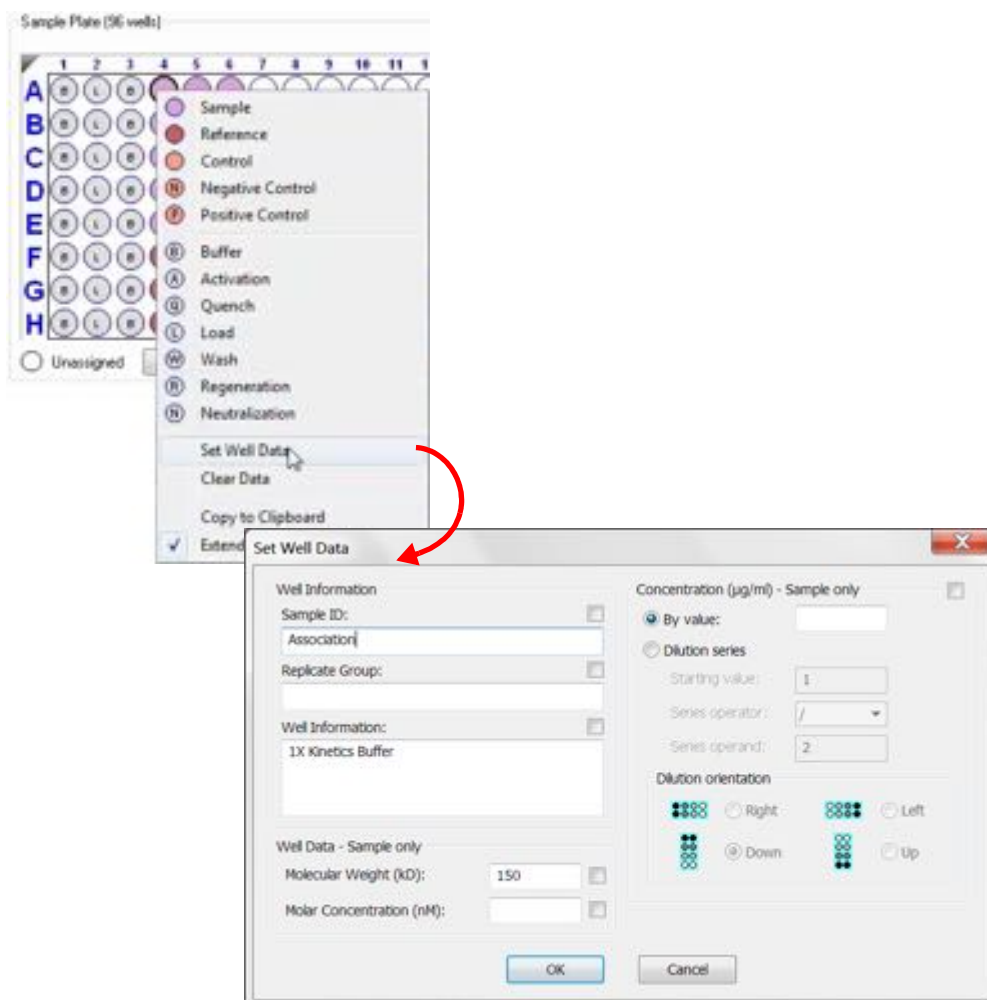


Figure 9-10: Add Sample Annotations from the Sample Plate Map

Annotating Wells in the Sample Plate Table

To annotate an individual well in the **Sample Plate Table**:

1. Double-click the table cell for **Sample ID** or **Well Information**.
2. Enter the desired information in the respective field (see Figure 9-11).



NOTE: A series of Sample IDs may also be assembled in Excel and pasted into the **Sample Plate Table**.

Sample Plate Table

Concentration units:

Molar concentration units:

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information
G3	Dissociation		Buffer				1X Kinetics Buffer
H3	Dissociation		Buffer				1X Kinetics Buffer
A4	Association		Sample	10	150	66.67	1X Kinetics Buffer
B4	Association		Sample	5	150	33.33	1X Kinetics Buffer
C4	Association		Sample	2.5	150	16.67	1X Kinetics Buffer
D4	Association		Sample	1.25	150	8.333	1X Kinetics Buffer
E4	Association		Sample	0.625	150	4.167	1X Kinetics Buffer
F4	Association		Reference				1X Kinetics Buffer
G4	Association		Reference				1X Kinetics Buffer
H4	Association		Reference				1X Kinetics Buffer

Figure 9-11: Add Sample Annotations in the Sample Plate Table



NOTE: Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the **Sample Plate Table**. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the **Sample Plate Map** menu used to designate sample types.

Replicate Groups

Replicate Groups enable data to be organized into custom groups during data analysis (see Figure 9-12).

Index	Include	Color	Sensor Location	Sensor Type	Sensor Info	Replicate Group	Baseline Loc.
20	x		C2	SA (Streptavidin)	3		C3
21	x		C2	SA (Streptavidin)	3		C3
22	x		D2	SA (Streptavidin)	4		D3
23	x		D2	SA (Streptavidin)	4		D3
24	x		E2	SA (Streptavidin)	5		E3
25	x		E2	SA (Streptavidin)	5		E3
26	x		F2	SA (Streptavidin)	6		F3
27	x		F2	SA (Streptavidin)	6		F3
28	x		G2	SA (Streptavidin)	6		G3
29	x		G2	SA (Streptavidin)	6		G3
30	x		H2	SA (Streptavidin)	6		H3
31	x		H2	SA (Streptavidin)	6		H3
32	x		A3	SA (Streptavidin)	1		A3
33	x		A3	SA (Streptavidin)	1		A3
34	x		B3	SA (Streptavidin)	2		B3
35	x		B3	SA (Streptavidin)	2		B3
36	x		C3	SA (Streptavidin)	3		C3
37	x		C3	SA (Streptavidin)	3		C3
38	x		D3	SA (Streptavidin)	4		D3
39	x		D3	SA (Streptavidin)	4		D3

Figure 9-12: Replicate Group Color-Coding



NOTE: Replicate Group information can also be entered in the Octet System Data Analysis software.

Assigning Replicate Groups in the Sample Plate Map

To assign **Replicate Groups** in the **Sample Plate Map**:

1. Select the samples you wish to group, right-click and select **Set Well Data**.
2. In the **Set Well Data** dialog box (see Figure 9-13), enter a name in the **Replicate Group** box and click **OK**.

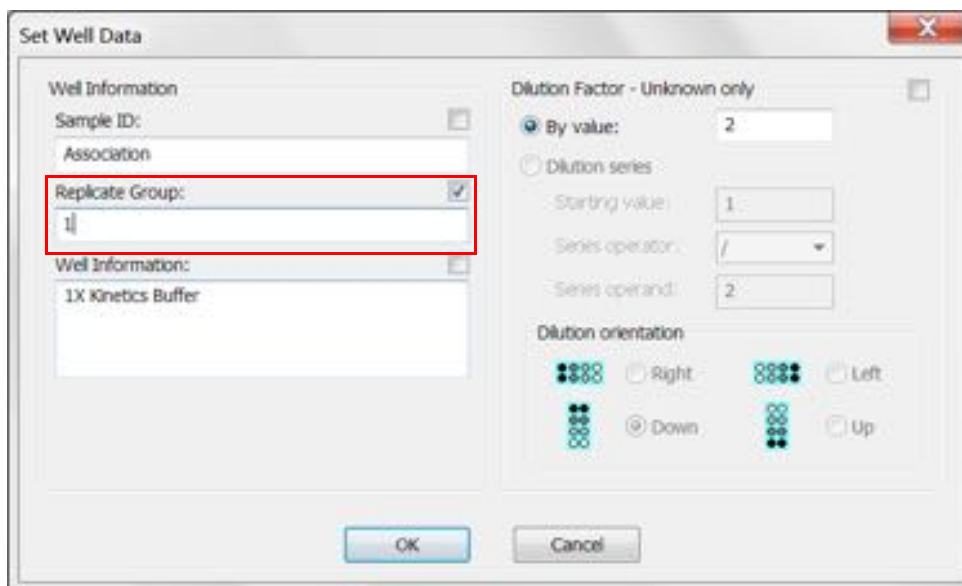


Figure 9-13: Add Replicate Group from the Sample Plate Map

3. Repeat the previous steps to assign new samples to the existing **Replicate Group**, or to designate another set of samples to a new **Replicate Group**. Multiple groups can be used in an experiment.



IMPORTANT: The Octet System Data Analysis software will only recognize and group samples that use the same **Replicate Group** names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.

Wells in the **Sample Plate Map** will show color-coded outlines as a visual indication of which wells are in the same group (see Figure 9-14).

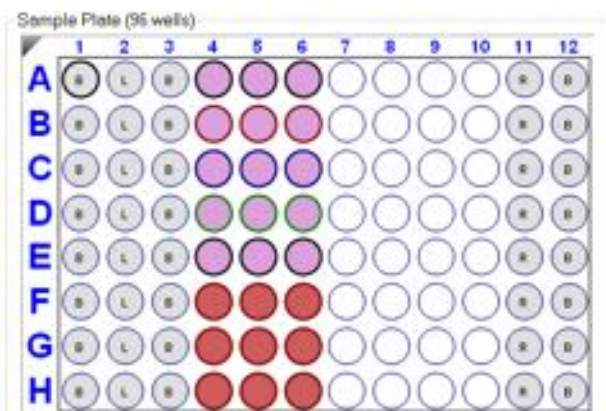


Figure 9-14: Replicate Groups Displayed in Sample Plate Map

The **Sample Plate Table** will update with the **Replicate Group** names entered (see Figure 9-15)

Sample Plate Table

Concentration units:

Molar concentration units:

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information
A4	Association	1	Sample	10	150	66.67	1X Kinetics Buffer
B4	Association	2	Sample	5	150	33.33	1X Kinetics Buffer
C4	Association	3	Sample	2.5	150	16.67	1X Kinetics Buffer
D4	Association	4	Sample	1.25	150	8.333	1X Kinetics Buffer
E4	Association	5	Sample	0.625	150	4.167	1X Kinetics Buffer
F4	Association	6	Reference				1X Kinetics Buffer
G4	Association	6	Reference				1X Kinetics Buffer
H4	Association	6	Reference				1X Kinetics Buffer
A5	Association	1	Sample	10	150	66.67	1X Kinetics Buffer
B5	Association	2	Sample	5	150	33.33	1X Kinetics Buffer
C5	Association	3	Sample	2.5	150	16.67	1X Kinetics Buffer
D5	Association	4	Sample	1.25	150	8.333	1X Kinetics Buffer
E5	Association	5	Sample	0.625	150	4.167	1X Kinetics Buffer
F5	Association	6	Reference				1X Kinetics Buffer
G5	Association	6	Reference				1X Kinetics Buffer
H5	Association	6	Reference				1X Kinetics Buffer

Figure 9-15: Replicate Groups in Sample Plate Table

Assigning Replicate Groups in the Sample Plate Table

To assign **Replicate Groups** in the **Sample Plate Table**:

1. Double-click the desired cell in the **Replicate Group** table column.
2. Enter a group name (see Figure 9-16).

Sample Plate Table

Concentration units:

Molar concentration units:

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information
A4	Association	1	Sample	10	150	66.67	1X Kinetics Buffer
B4	Association	2	Sample	5	150	33.33	1X Kinetics Buffer
C4	Association	3	Sample	2.5	150	16.67	1X Kinetics Buffer
D4	Association	4	Sample	1.25	150	8.333	1X Kinetics Buffer
E4	Association	5	Sample	0.625	150	4.167	1X Kinetics Buffer
F4	Association	6	Reference				1X Kinetics Buffer
G4	Association	6	Reference				1X Kinetics Buffer
H4	Association	6	Reference				1X Kinetics Buffer

Figure 9-16: Add Replicate Group from the Sample Plate Table

Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the **Sample Plate Table**. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the **Sample Plate Map** menu used to designate sample types.

- Repeat the previous steps to assign new samples to the existing **Replicate Group**, or to designate another set of samples to a new **Replicate Group**. Multiple groups can be used in an experiment.



IMPORTANT: The Octet System Data Analysis software will only recognize and group samples that use the same **Replicate Group** names, spacing and capitalization must be identical. For example, samples assigned to **Group 2** and **group2** are treated as two groups.

Editing the Sample Table

Changing Sample Well Designations

To change a well designation, right-click the well in the **Sample Plate Table** and make a new selection (see Figure 9-17).

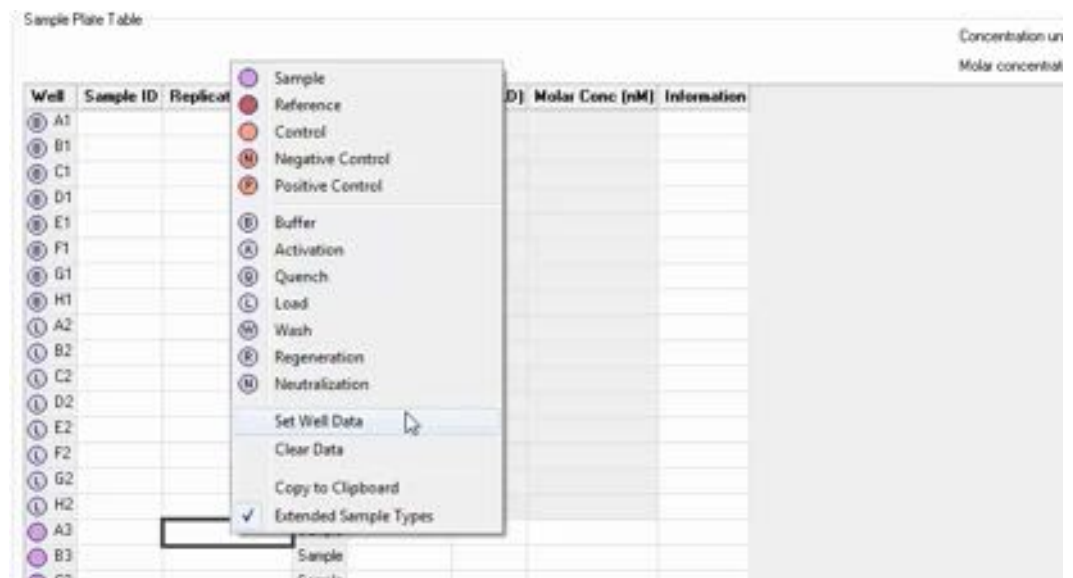


Figure 9-17: Sample Plate Table—Well Designation

Editing Sample Information

To edit sample data in the **Sample Plate Table**, double-click a value and enter a new value (see Figure 9-18).

Sample Plate Table

Concentration units:

Molar concentration units:

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information
H3	Dissociation		Buffer				1X Kinetics Buffer
A4	Association	1	Sample	10	150	66.67	1X Kinetics Buffer
B4	Association	2	Sample	5	150		
C4	Association	3	Sample	2.5	150		
D4	Association	4	Sample	1.25	150		
E4	Association	5	Sample	0.625	150		
F4	Association	6	Reference				
G4	Association	6	Reference				
H4	Association	6	Reference				
A5	Association	1	Sample	10	150		
B5	Association	2	Sample	5	150		
C5	Association	3	Sample	2.5	150		
D5	Association	4	Sample	1.25	150		
E5	Association	5	Sample	0.625	150		
F5	Association	6	Reference				
G5	Association	6	Reference				
H5	Association	6	Reference				

Right-click menu options: Undo, Cut, Copy, Paste, Delete, Select All, Right to left Reading order, Show Unicode control characters, Insert Unicode control character, Open IME, Reconversion.

Figure 9-18: Sample Plate Table—Editing Sample Data

Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the **Sample Plate Table**. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the right-click menu used to designate sample types.

MANAGING SAMPLE PLATE DEFINITIONS



NOTE: After you define a sample plate, you can export and save the plate definition for future use.

Exporting a Plate Definition

To export a plate definition:

1. In the **Sample Plate Map**, click **Export** (see Figure 9-19).

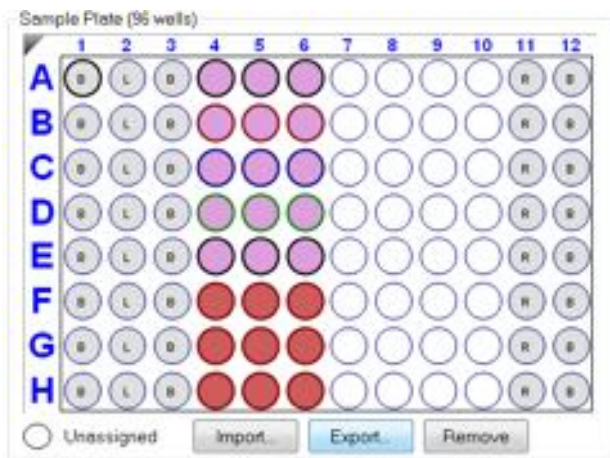


Figure 9-19: Sample Plate Map—Export Button

2. In the **Export Plate Definition** window (see Figure 9-20), select a folder, enter a name for the plate (.csv), and click **Save**.

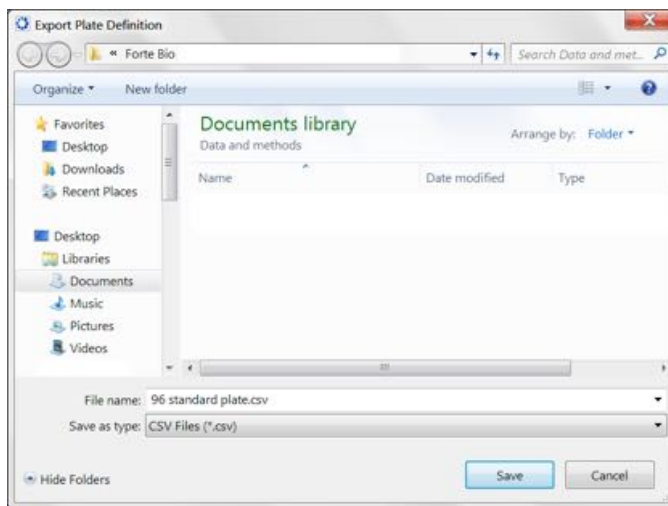


Figure 9-20: Export Plate Definition Window

Importing a Plate Definition

To import a plate definition:

1. In the Sample Plate Definition window (see Figure 9-19: on page 464), click **Import**.

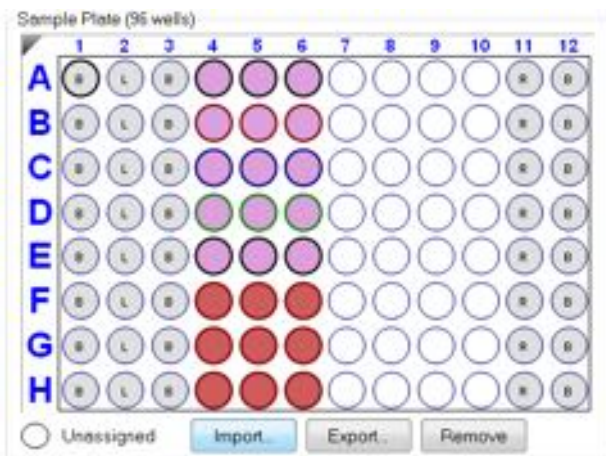


Figure 9-21: Sample Plate Map—**Import** Button

2. In the **Import Plate Definition** window (see Figure 9-22), select the plate definition (.csv), and click **Open**.

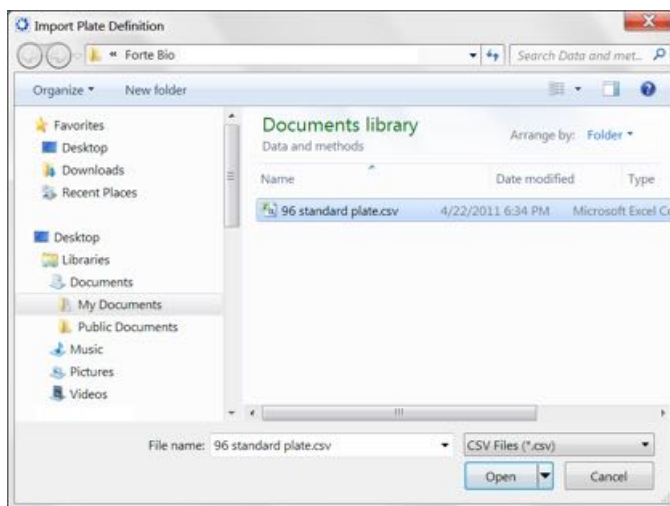


Figure 9-22: Import Plate Definition Window



NOTE: You can also create a .csv file for import. Figure 9-23 shows the appropriate column information layout.

	A	B	C	D	E	F	G	H
1	PlateWells	96						
2	Well	ID	Replicate Group	Group	Concentration (µg/ml)	Molecular Weight (kD)	Molar Concentration (M)	Information
3	A1	Kinetics Buffer		Buffer				1X Kinetics Buffer
4	B1	Kinetics Buffer		Buffer				1X Kinetics Buffer
5	C1	Kinetics Buffer		Buffer				1X Kinetics Buffer
6	D1	Kinetics Buffer		Buffer				1X Kinetics Buffer
7	E1	Kinetics Buffer		Buffer				1X Kinetics Buffer
8	F1	Kinetics Buffer		Buffer				1X Kinetics Buffer
9	G1	Kinetics Buffer		Buffer				1X Kinetics Buffer
10	H1	Kinetics Buffer		Buffer				1X Kinetics Buffer
11	A2	Loading		Load				12.5 ug/ml ProA
12	B2	Loading		Load				12.5 ug/ml ProA

Figure 9-23: Example Plate Definition File (.csv)

Printing a Sample Plate Definition

To print a plate definition:

- 1. In the **Sample Plate Map** (see Figure 9-24), click **Print**.

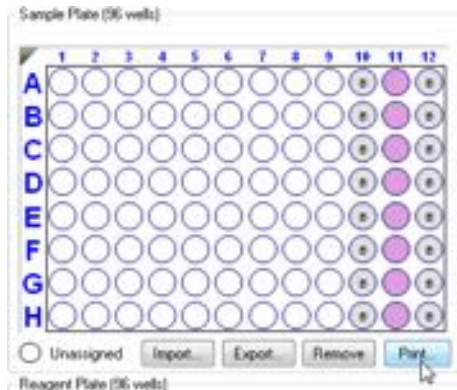


Figure 9-24: Sample Plate Print Button

The associated **Sample Plate Table** information will print.

DEFINING A KINETIC ASSAY

After the sample plate is defined, the assay must be defined. The steps to define a kinetic assay include:

Step	See Page
1. Define the step types.	467
2. Build the assay by assigning a step type to a column(s) in the sample plate.	471
3. Save the sample plate definition (optional).	463

Defining Step Types

Table 9-3 lists the example step types to define a kinetic assay. Use these examples as a starting point to create your own step types.

Table 9-3: Sample Step Types for Kinetic Assays




Step Type	Step Description
Association	Calculates the k_{obs} . Select this step type when binding the second protein of interest (analyte) to the biosensor. This step should be performed at 1,000 rpm.
Dissociation	Calculates the k_d . Select this step type when monitoring the dissociation of the protein complex. This step should be performed at 1,000 rpm.
Baseline	Can be used to align the data. Select this step type when establishing the biosensor baseline in the presence of buffer. This step can be performed with no flow (0 rpm). However, if the baseline step directly precedes an association step, perform the baseline step at 1,000 rpm.
<div>  IMPORTANT: An assay must include a baseline step followed by a set of association/dissociation steps to be analyzed. The Octet System Data Analysis software recognizes the baseline/association/dissociation step series during processing. Data cannot be processed if this sequence is not included in the assay setup. </div>	
Loading	Not used in data analysis. Select this step type when binding the first protein of interest (ligand) to the biosensor.
<div>  NOTE: This step may be performed offline (outside the Octet instrument). </div>	

Table 9-3: Sample Step Types for Kinetic Assays (Continued)

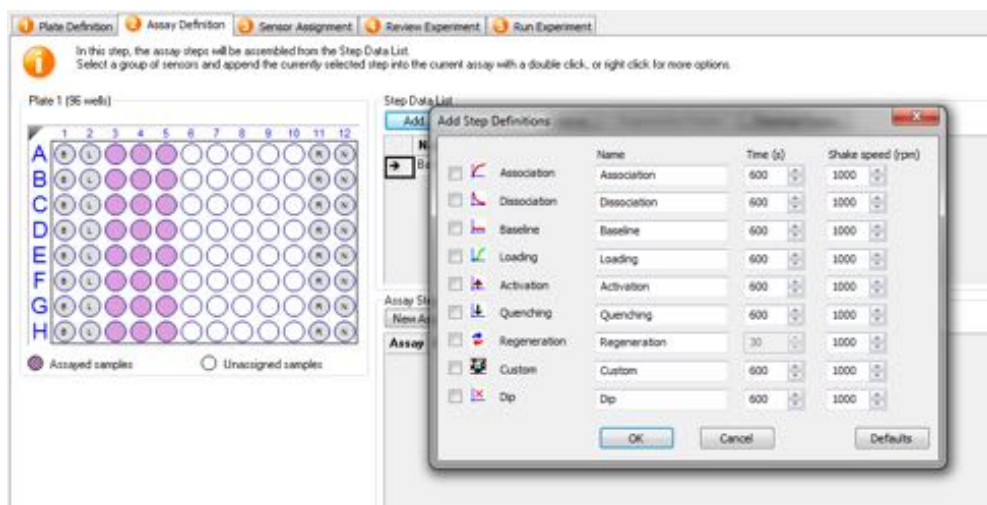
Step Type	Step Description
Activation	Used when employing a reagent to chemically prepare the biosensor for loading.
Quenching	Used to render unreacted immobilization sites on the biosensor inactive.
Regeneration	Used when employing a reagent to chemically regenerate biosensors and remove bound analyte.
Custom	Can be used for an activity not included in any of the above step types.

Creating Step Types

Click the **Assay Definition** tab, or click the  arrow to access the Assay Definition window (see Figure 9-25). The **Step Data List** shows the types of assay steps that are available to build an assay. By default, the list includes a baseline step.

To create different types of assay steps:

1. Click **Add**.
2. In **Assay Step Definition** dialog box (Figure 9-25), specify the step information:
 - a. Choose a step type.
 - b. Optional: Edit the step name.
 - c. Set the step time and shake speed (**Time** range: 2 to 48,000 seconds, **Shake speed** range: 100 to 1,500 rpm or 0).

**Figure 9-25:** Creating an Assay Step Type

3. Apply a threshold to the step:
 - a. In the **Step Data List**, click the **Threshold** check box.

The **Threshold Parameters** dialog box displays (see Figure 9-26).

- b. Set the threshold parameters (refer to Table 9-4 for the parameter definitions).

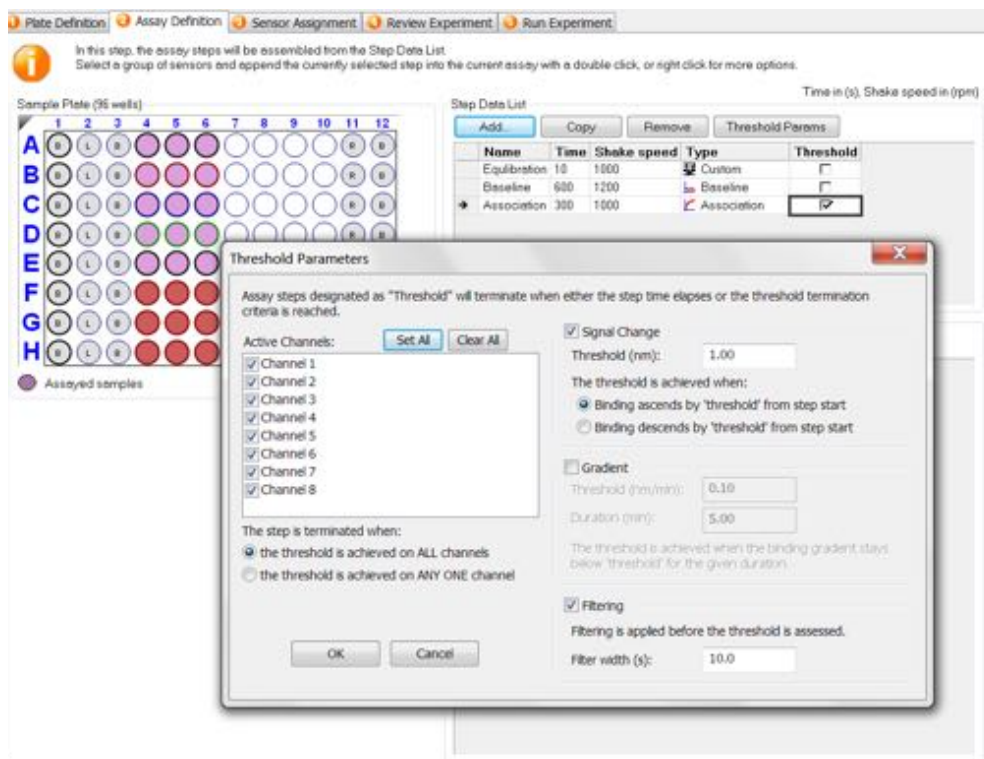


Figure 9-26: Setting Assay Step Threshold Parameters



NOTE: If thresholds are applied, the step is terminated when either the step time elapses or the threshold termination criteria is reached.

Table 9-4: Threshold Parameters

Item	Description
Active Channels	Specifies the instrument channels that monitor the threshold criteria for the assay step. Select an option for terminating the step: <ul style="list-style-type: none"> • The threshold is achieved on ALL channels • The threshold is achieved on ANY ONE channel
Signal Change	The threshold is a user-specified amount of ascending or descending signal change (nm).
Gradient	The threshold is a binding gradient (nm/min) for a user-specified time (min).

Table 9-4: Threshold Parameters (Continued)

Item	Description
Filtering	The amount of data (seconds) to average when computing the signal change or gradient threshold.

- Click **OK** to save the newly-defined step. The new step type appears in the **Step Data List**.
- Repeat the previous steps for each step type to create until all the desired steps are added (see Figure 9-27).

Step Data List









	Name	Time	Shake speed	Type	Threshold
	Baseline	10	1000	 Baseline	<input type="checkbox"/>
	Loading	20	1000	 Loading	<input type="checkbox"/>
	Wash	15	1000	 Custom	<input type="checkbox"/>
	Association	30	1000	 Association	<input type="checkbox"/>
	Long Dissociation	2000	1000	 Dissociation	<input type="checkbox"/>
	Regeneration	24	1000	 Regeneration	<input type="checkbox"/>
	Activation	25	1000	 Activation	<input type="checkbox"/>

Figure 9-27: Step Data List—Displaying Step Types

- To delete a step type from the list, click the corresponding row in the **Step Data List** and click **Remove**, or press the **Delete** key.

Copying and Editing Step Types

To define a step type by copying an existing one, click the step type (row) in the **Step Data List** and click **Copy**. The copied step type appears at the end of the **Step Data List**.

To define a step type by editing an existing one:

- Double-click the cell in the step's **Name**, **Time** or **Shake speed** column and then enter a new value. Or, right-click the cell to display a shortcut menu of editing commands (see Figure 9-28, left).



NOTE: Keyboard commands can also be used (**Ctrl+x=cut**, **Ctrl+c=copy**, **Ctrl+v=paste**, **Ctrl+z=undo**).

- Click the cell in the step's **Type** column, then select another name from the drop-down list (see Figure 9-28, right).

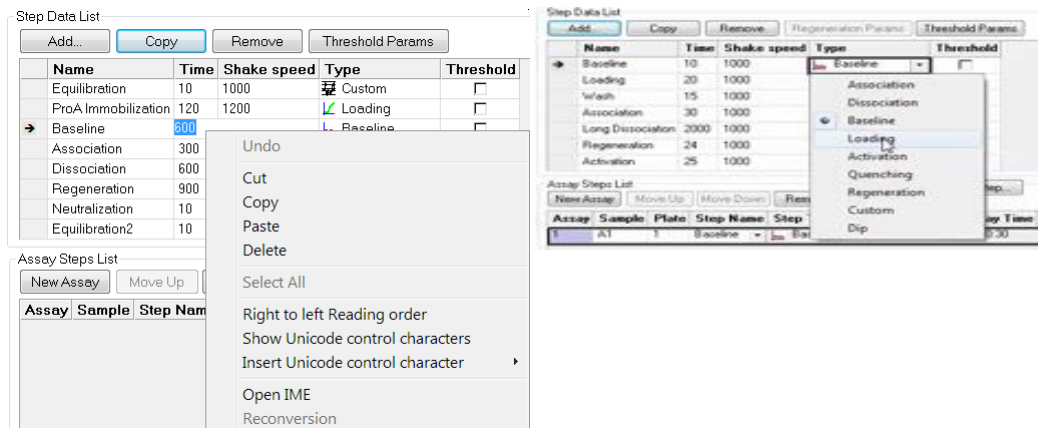


Figure 9-28: Editing a Step Value (left) or Step Type (right)

Building an Assay

After creating the different step types that the assay will use, step types are assigned to columns in the Sample Plate or Reagent Plate maps.

To build an assay:

1. Select a step type in the **Step Data List**.
2. In the **Sample Plate Map**, double-click the column that is associated with the selected step type. For information about sample plate wells, mouse over a well to view a tool tip (see Figure 9-29).

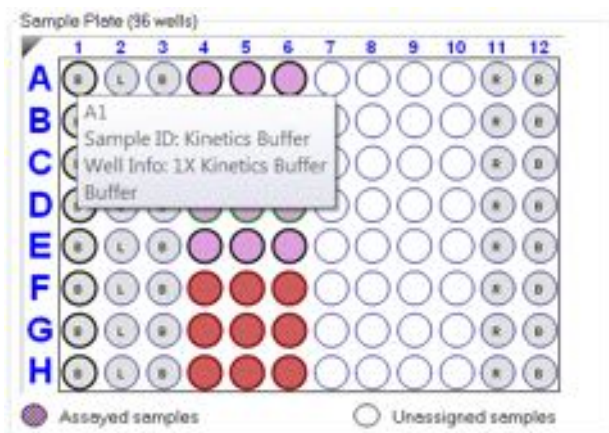



Figure 9-29: Tool Tip of Well Information

The selected wells are marked with hatching (for example, ) and the step appears in the **Assay Steps List** (see Figure 9-30) with an associated **Assay Time**.

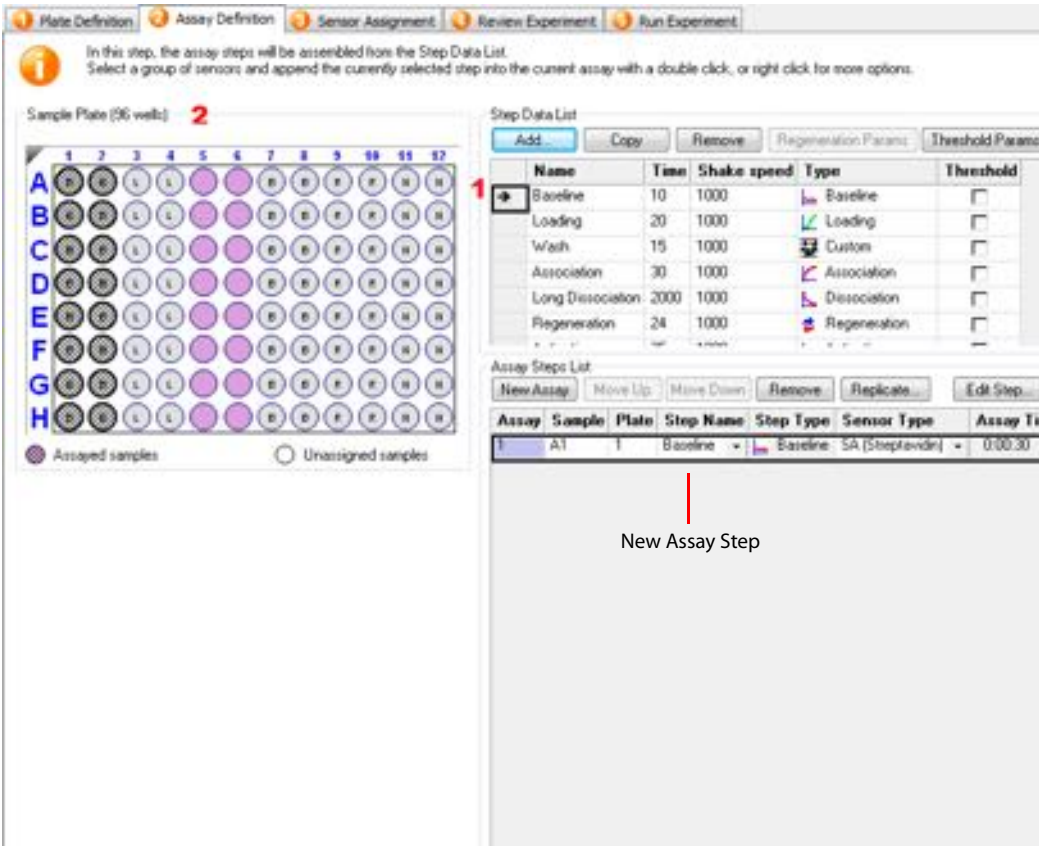


Figure 9-30: Assigning a Step Type to a Column in the Sample Plate

3. Repeat the previous steps to define each step in the assay. As each step is added, the total **Experiment** and **Assay Time** update (see Figure 9-31).

Assay Steps List

New Assay Move Up Move Down Remove Replicate... Edit Step...

Assay	Sample	Step Name	Step Type	Sensor Type	Assay Time
1	1	Baseline	Baseline	SA (Streptavidin)	
1	2	Loading	Loading	SA (Streptavidin)	
1	7	Wash	Custom	SA (Streptavidin)	
1	3	Association	Association	SA (Streptavidin)	
1	8	Long Dissociation	Dissociation	SA (Streptavidin)	
1	10	Regeneration	Regeneration	SA (Streptavidin)	0:35:23
2	1	Baseline	Baseline	SA (Streptavidin)	
2	2	Loading	Loading	SA (Streptavidin)	
2	7	Wash	Custom	SA (Streptavidin)	
2	4	Association	Association	SA (Streptavidin)	
2	8	Long Dissociation	Dissociation	SA (Streptavidin)	0:35:15
3	1	Baseline	Baseline	SA (Streptavidin)	
3	2	Loading	Loading	SA (Streptavidin)	
3	7	Wash	Custom	SA (Streptavidin)	
3	5	Association	Association	SA (Streptavidin)	
3	8	Long Dissociation	Dissociation	SA (Streptavidin)	
3	10	Regeneration	Regeneration	SA (Streptavidin)	0:35:23

Total Assay Time

Figure 9-31: Experiment and Assay Time Updates as Steps Are Added to the Assay



IMPORTANT: If you intend to analyze the data from a sample using the **Inter-step correction** feature in the Octet System Data Acquisition software, the assay must use the same well to perform baseline and dissociation for the sample.

Adding a Regeneration Step

1. In the **Sample Plate Map**, assign wells as **Regeneration** or **Neutralization** (Figure 9-32).



Figure 9-32: Regeneration Step

2. Click **Add** (Figure 9-33) to display the Add Step Definition dialog box (Figure 9-34).

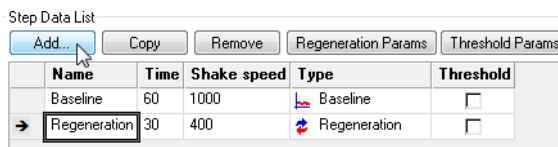


Figure 9-33: Add Button



Figure 9-34: Add Step Definition Dialog Box

3. Select **Regeneration** and click **OK**.
4. Click **Regeneration Params** (Figure 9-35).

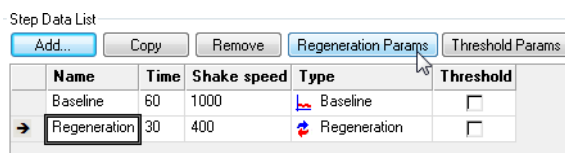


Figure 9-35: Regeneration Params Button

The **Regeneration Parameters** dialog box (Figure 9-36) displays, where you can edit Regeneration parameters as necessary.

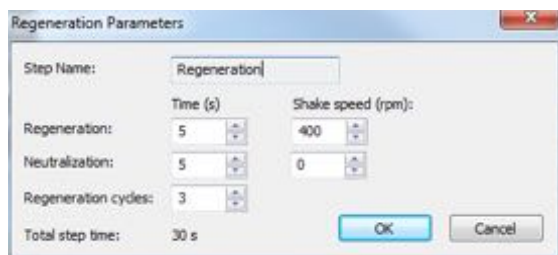


Figure 9-36: Regeneration Parameters Dialog Box

Replicating Steps within an Assay

To copy steps and add them to an assay:

1. In the **Assay Steps List**, select the step(s) to copy and click **Replicate** (for example, in Figure 9-37, step rows 1–4 are selected).

- To select adjacent steps, press and hold the **Shift** key while you click the first and last step in the selection.
 - To select non-adjacent steps, press and hold the **Ctrl** key while you click the desired steps.
2. In the **Replicate Steps** dialog box (see Figure 9-37), click the **Append to current assay** option.
 3. Click the **Offset steps** check box and set the options, as appropriate. (For more details on offset options, see Table 9-5.)

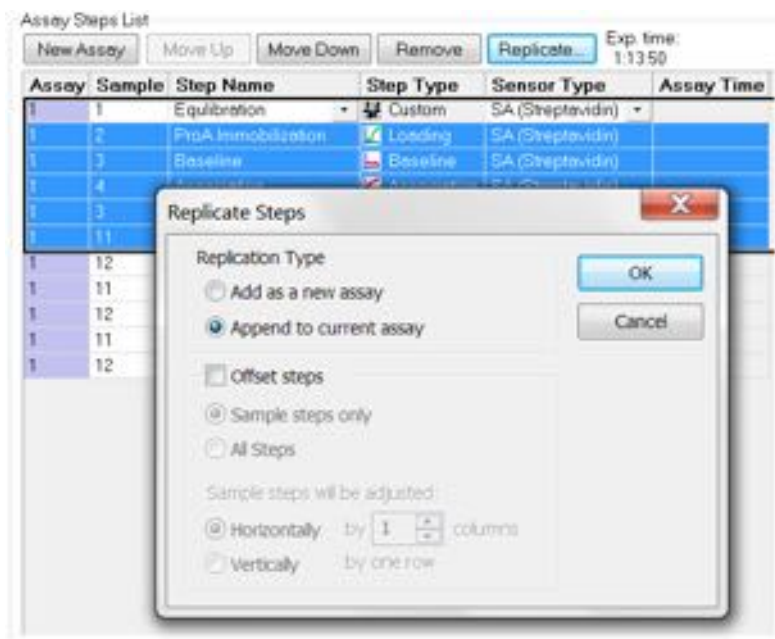


Figure 9-37: Replicating Assay Steps by Appending

4. Click **OK**. The step(s) appear at the end of the assay in the **Assay Steps List**.

Table 9-5: Replicate Steps Options .

Item	Description
Add as a new assay	Adds the replicate step(s) as a new assay to the Assay Steps List .
Append to current assay	Adds the replicate step(s) to the end of the current assay.
Offset steps	Assigns the replicate steps to different columns in the sample plate.
Sample steps only	Applies the offset to the sample plate only.

Table 9-5: Replicate Steps Options (Continued).

Item	Description
All steps	Applies the offset to the sample plate and reagent plate.
	<div> <div>→</div> <div> NOTE: Reagent plates are only available when using an Octet384 or Octet QK384 instrument. </div> </div>
Sample steps will be adjusted horizontally by X columns	Specifies the column in which to add the new step(s). For example, if a step in column 11 is copied and the replicate step should begin in column 12, enter 1 . Enter 0 to apply the step(s) to the same columns.
Sample steps will be adjusted vertically by one row	Applies only to the Octet384 or Octet QK384 instruments.

Starting a New Assay

A new assay will utilize a new set of biosensors. To start a new assay using the next available sensor column:

1. Select a column in the **Sample Plate Map**.
2. Right-click to view the shortcut menu and select **Start New Assay** (see Figure 9-38).
3. Add steps to the assay as described earlier.

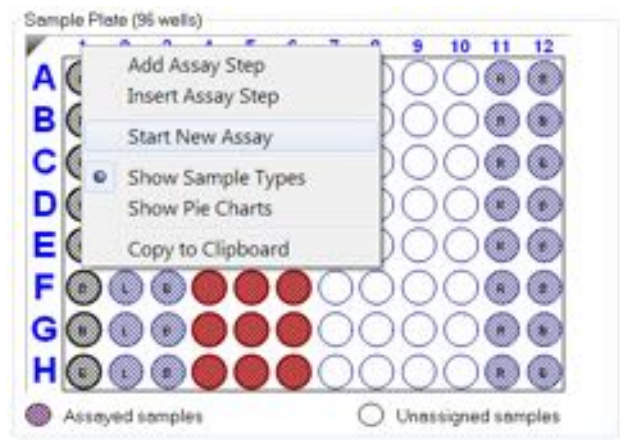


Figure 9-38: Start New Assay

Inserting or Adding an Assay Step

To insert an assay step:

1. Select a step in the **Step Data List**.
2. In the **Assay Steps List**, select the row above where you want to insert the step.
3. In the **Sample Plate Map**, right-click the column to which the step will be applied and select **Insert Assay Step**.

The step is inserted into the **Assay Steps List**.

To add an assay step:

1. Select a step type in the **Step Data List**.
2. In the **Sample Plate Map**, right-click the column to which the step will be applied, and select **Add Assay Step**.

The step is added to the end of the **Assay Steps List**.

Selecting a Biosensor for the Assay

To select the biosensor type associated with the assay, click the **Sensor Type** arrow (▼) for any step in the assay and select a sensor type from the drop-down list (Figure 9-39). The biosensor type will automatically update for every assay step.

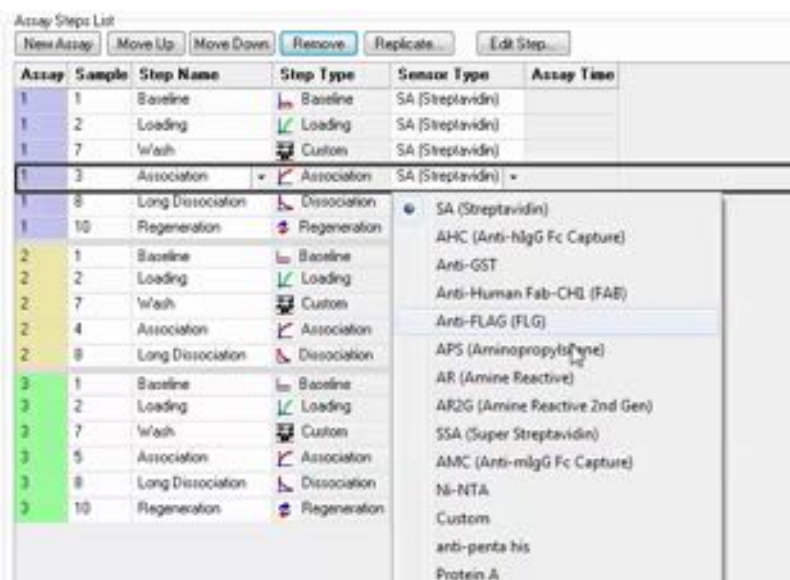


Figure 9-39: Selecting an Assay Sensor Type



NOTE: The **Sensor Type** for the assay must be selected or changed from the **Assay Steps List**. Changing the **Sensor Type** from the **Sensor Assignment Tab** will not update the assay.

Editing an Assay

To edit the step type or the biosensor type:

1. In the **Assay Steps List**:

- To change the step type, click the **Step Name** arrow (▼) and select a step name from the drop-down list (Figure 9-40, top).
- To change the biosensor type, click the **Sensor Type** arrow (▼) for any step in the assay and select a sensor type from the drop-down list (Figure 9-40, bottom). The biosensor type will automatically update for every assay step.



NOTE: The **Step Name** drop-down list includes only the step types defined in the **Step Data List**.

Assay Steps List

New Assay Move Up Move Down Remove Replicate Edit Step...

Assay	Sample	Step Name	Step Type	Sensor Type	Assay Time
1	1	Baseline	Baseline	SA (Streptavidin)	
1	2	Loading	Loading	SA (Streptavidin)	
1	7	Wash	Custom	SA (Streptavidin)	
1	3	Association	Association	SA (Streptavidin)	
1	8	Baseline	Baseline	SA (Streptavidin)	
1	10	Loading	Loading	SA (Streptavidin)	0.35.23
2	1	Wash	Custom	SA (Streptavidin)	
2	2	Associating	Association	SA (Streptavidin)	
2	7	Long Dissociation	Dissociation	SA (Streptavidin)	
2	4	Regeneration	Regeneration	SA (Streptavidin)	0.35.15
2	8	Regeneration	Regeneration	SA (Streptavidin)	
3	1	Baseline	Baseline	SA (Streptavidin)	
3	2	Loading	Loading	SA (Streptavidin)	
3	7	Wash	Custom	SA (Streptavidin)	
3	5	Association	Association	SA (Streptavidin)	
3	8	Long Dissociation	Dissociation	SA (Streptavidin)	
3	10	Regeneration	Regeneration	SA (Streptavidin)	0.35.23

Assay Steps List

New Assay Move Up Move Down Remove Replicate Edit Step...

Assay	Sample	Step Name	Step Type	Sensor Type	Assay Time
1	1	Baseline	Baseline	SA (Streptavidin)	
1	2	Loading	Loading	SA (Streptavidin)	
1	7	Wash	Custom	SA (Streptavidin)	
1	3	Association	Association	SA (Streptavidin)	
1	8	Long Dissociation	Dissociation	SA (Streptavidin)	
1	10	Regeneration	Regeneration	SA (Streptavidin)	
2	1	Baseline	Baseline	AHC (Anti-hlgG Fc Capture)	
2	2	Loading	Loading	Anti-GST	
2	7	Wash	Custom	Anti-Human Fab-CH3 (FAB)	
2	4	Association	Association	Anti-FLAG (FLAG)	
2	8	Long Dissociation	Dissociation	APS (Aminopropylsulfone)	
3	1	Baseline	Baseline	AR (Amine Reactive)	
3	2	Loading	Loading	AR2G (Amine Reactive 2nd Gen)	
3	7	Wash	Custom	SSA (Super Streptavidin)	
3	5	Association	Association	AMC (Anti-mlgG Fc Capture)	
3	8	Long Dissociation	Dissociation	Ni-NTA	
3	10	Regeneration	Regeneration	Custom	
				anti-penta his	
				Protein A	

Figure 9-40: Editing an Assay Step Name (top) or Sensor Type (bottom) in the Assay Steps List

To reorder or remove an assay step:

1. Select a step (row) in the **Assay Steps List**.
2. Click the **Move Up**, **Move Down**, or **Remove** button located above the list.



IMPORTANT: An assay must have a baseline step followed by a set of association/dissociation steps to be analyzed. Octet System Data Analysis software recognizes the baseline/association/dissociation set of steps.

Adding an Assay Through Replication

A sample plate can include multiple assays that are the same (replicates) or different. Each assay utilizes a new set of biosensors. Replicates within a single assay will therefore use the same biosensor and replicates in different assays will use different biosensors.

To add a replicate assay to a plate:

1. In the **Assay Steps List**, select the steps to copy and click **Replicate**.
 - To select adjacent steps, press and hold the **Shift** key while you click the first and last step in the selection.
 - To select non-adjacent steps, press and hold the **Ctrl** key while you click the steps.
2. In the **Replicate Steps** dialog box, click the **Add as a new assay** option (Figure 9-41).

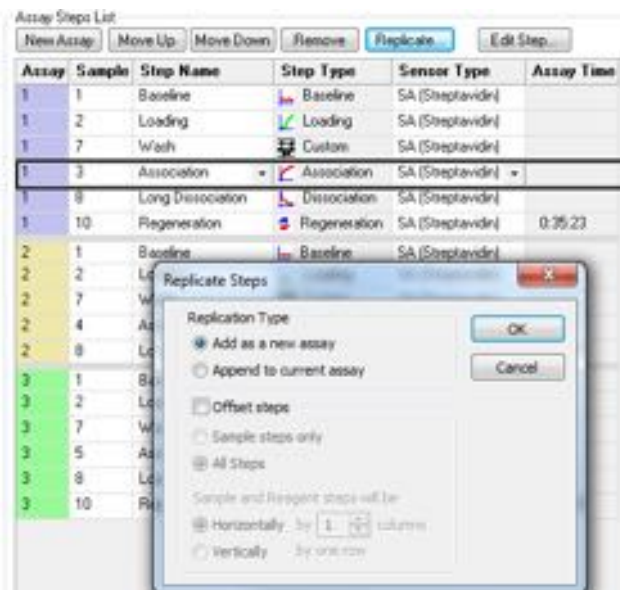



Figure 9-41: Adding a Replicate Assay to a Plate

3. Click the **Offset steps** check box and set the options as appropriate (see Table 9-5 on page 475 for more information). If the replicate assay uses the same sample columns as the original assay, do not choose the **Offset steps** option. If the replicate assay uses a different sample column, select **Offset steps** and the appropriate options.
 - **Sample steps only** offsets the sample wells by the value specified under **Sample steps will be adjusted**. The offset will not be applied to reagent wells such as buffer, loading, regeneration, neutralization and detection.
 - **All Steps** offsets all wells in the assay, including sample and reagent wells, by the value specified under **Sample steps will be adjusted**.
4. Click **OK**. The new assay appears in the **Assay Steps List**.

5. Continue to add assay steps as needed.

ASSIGNING BIOSENSORS TO SAMPLES

After you define the sample plate and assay(s), click the **Sensor Assignment** tab, or click the arrow  to access the Sensor Assignment window. The color-coded **Sensor Tray** and **Sample Plate Map** show the locations of the biosensors associated with the samples (Figure 9-42).



NOTE: If an experiment includes more than one type of biosensor, the software automatically creates a separate sensor tray for each type of biosensor. If the different types of biosensors are in the same tray, change the biosensor type as appropriate.

The biosensor types shown in the **Sensor Type** table column are those designated during the kinetics assay definition. In the example shown in Figure 9-42, the experiment includes three assays in the same wells. The use of those wells by three different biosensors is indicated by the pie chart colors.



NOTE: The **Sensor Type** for the assay must be first be defined in the **Assay Steps List** on the **Assay Definition Tab**. Changing the **Sensor Type** from the **Sensor Assignment Tab** will not update the assay.

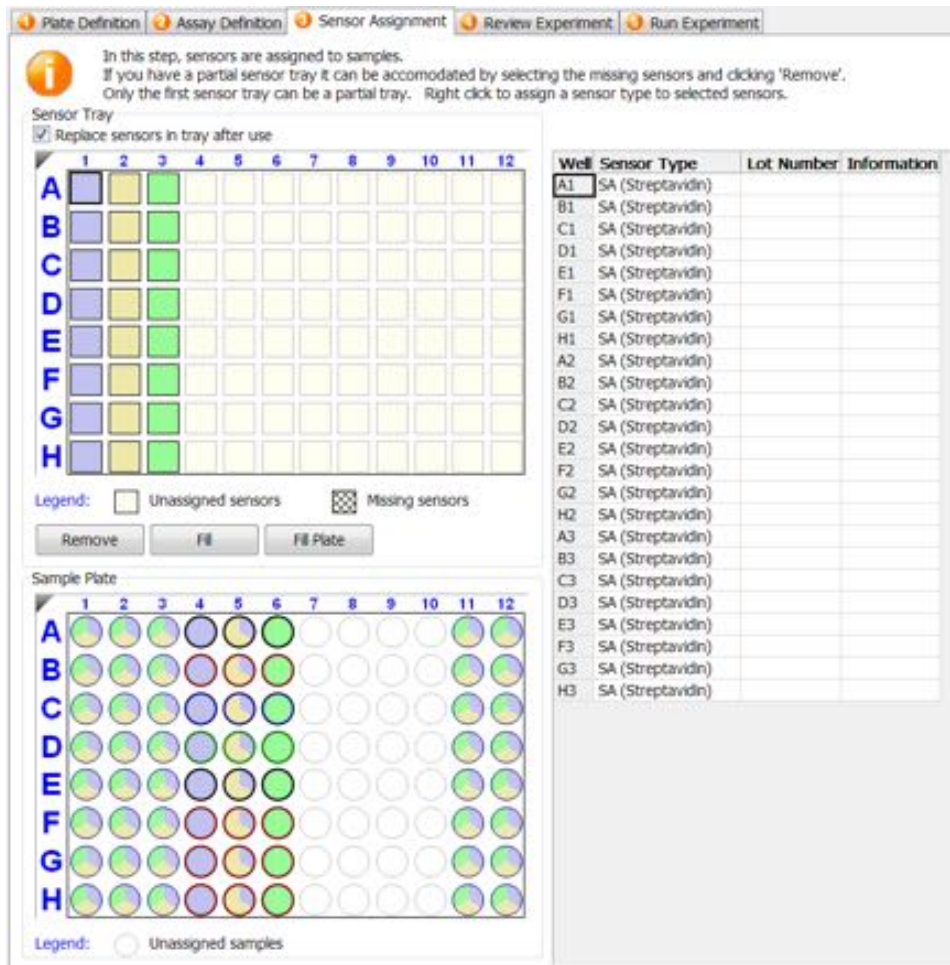


Figure 9-42: Sensor Assignment Window

Hover the cursor over a well in the **Sensor Tray Map** or **Sample Plate Map** to display a tool tip with sample or biosensor information (see Figure 9-43).

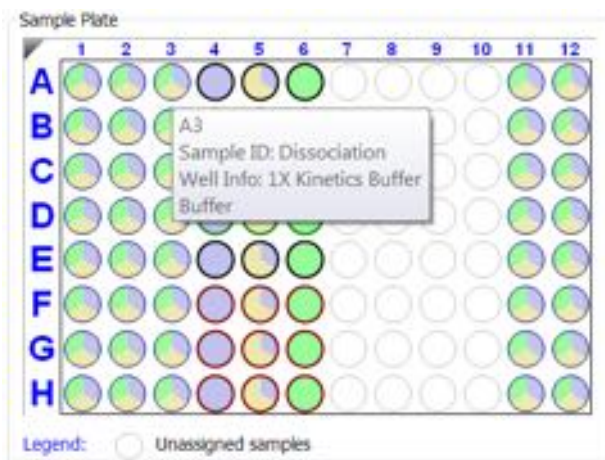


Figure 9-43: Tool Tip of Well Information

Replacing the Biosensors in the Biosensor Tray

After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace sensors in tray after use** check box (see Figure 9-44).

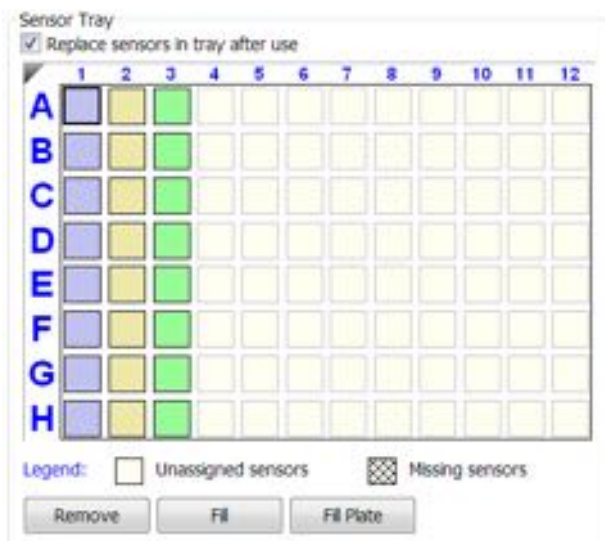


Figure 9-44: Replace Sensors in Tray After Use Check Box



NOTE: Biosensors can be regenerated up to a max of 11 times per experiment.

Entering Biosensor Information

To enter information about a biosensor:

1. Optional: Double-click in any cell in the **Lot Number** column to enter the biosensor lot number. All wells in the **Lot Number** column for that biosensor type will automatically populate with the lot number entered (see Figure 9-45).
2. Optional: Double-click a cell in the **Information** table column. Enter or edit the biosensor information as appropriate (see Figure 9-45).



NOTE: Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

Well	Sensor Type	Lot Number	Information	
A1	SA (Streptavidin)	10102020	Default	
B1	SA (Streptavidin)	10102020		
C1	SA (Streptavidin)	10102020		
D1	SA (Streptavidin)	10102020		
E1	SA (Streptavidin)	10102020		
F1	SA (Streptavidin)	10102020		
G1	SA (Streptavidin)	10102020		
H1	SA (Streptavidin)	10102020		
A2	SA (Streptavidin)	10102020		
B2	SA (Streptavidin)	10102020		
C2	SA (Streptavidin)	10102020		
D2	SA (Streptavidin)	10102020		
E2	SA (Streptavidin)	10102020		
F2	SA (Streptavidin)	10102020		
G2	SA (Streptavidin)	10102020		
H2	SA (Streptavidin)	10102020		

- Undo
- Cut
- Copy
- Paste
- Delete
- Select All
- Right to left Reading order
- Show Unicode control characters
- Insert Unicode control character
- Open IME
- Reconversion

Figure 9-45: Entering or Editing Biosensor Information

Changing the Biosensor Location

If you prefer to not use the default biosensor columns, you can select other column(s) to use. There are two ways to do this:

- **Method 1**—In the **Sensor Tray Map**, **Remove** the columns you do not want to use. The software automatically selects the next available column(s).
- **Method 2**—Remove all columns from the **Sensor Tray Map**, then select the columns you want to use.

Method 1

1. In the **Sensor Tray Map** (see Figure 9-46), select the columns to not use and click **Remove**. Or, right-click the selection and select **Remove** (Figure 9-46 left). The software automatically selects the next available biosensor columns in the tray (Figure 9-46 right).

- Click **Fill Plate** to return the **Sensor Tray Map** to the default layout.

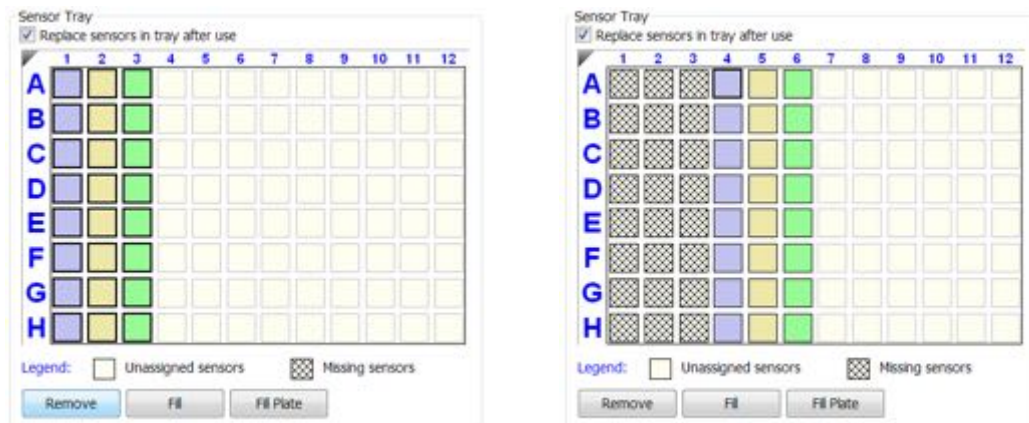


Figure 9-46: Changing Biosensor Location (Method 1)

Method 2

- In the **Sensor Tray Map**, select all of the columns and click **Remove** (Figure 9-47 top left). Or, right-click the selection and select **Remove**. All columns will be shown as **Missing** (Figure 9-47 top right).
- Select the column(s) to use and click **Fill**. Or, right-click the selection and select **Fill** (Figure 9-47 bottom left). The software fills the selected columns in the tray (Figure 9-47 bottom right).

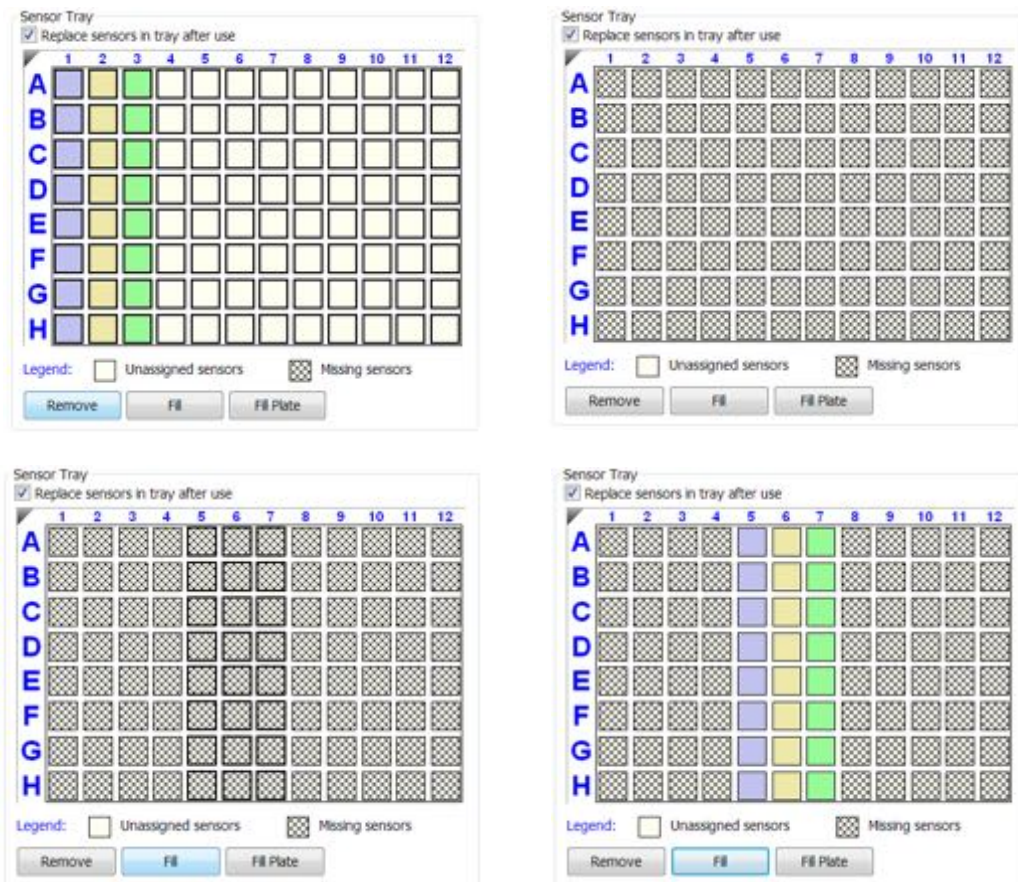


Figure 9-47: Changing Biosensor Location (Method 2)

Click **Fill Plate** to return the **Sensor Tray Map** to the default layout.

Using Heterogeneous Trays

If heterogeneous biosensor trays will be used, the column location of each biosensor type in the tray can be identified in the **Sensor Assignment Tab**. Assignment of biosensors that will not be used in the assay enables the software to auto-assign the biosensors that will be used in the assay by biosensor type.

There are two ways to change the biosensor type:

- Select a column in the **Sensor Tray Map**, right-click and select a biosensor type from the drop-down list (Figure 9-48 left). The associated wells in the **Sensor Type** column will automatically populate with the biosensor type selected.
- Select a cell in the **Sensor Type** table column, click the down arrow and select a biosensor type from the drop-down list (Figure 9-48 right). All other wells in the same column of the **Sensor Tray Map** as the selected cell will automatically populate with the biosensor type selected.

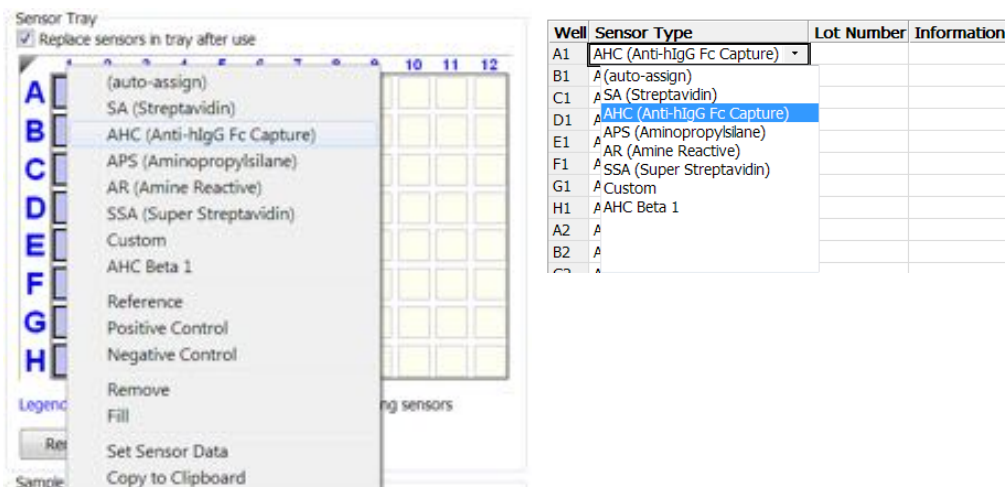


Figure 9-48: Sensor Assignment Window—Changing the Biosensor Type

The biosensor types shown in the **Sensor Assignment** window were specified previously in the **Assay Definition** window, and default locations are assigned automatically. To assign biosensor types for heterogeneous trays:

1. Select the column location of the biosensor type (see Figure 9-49).

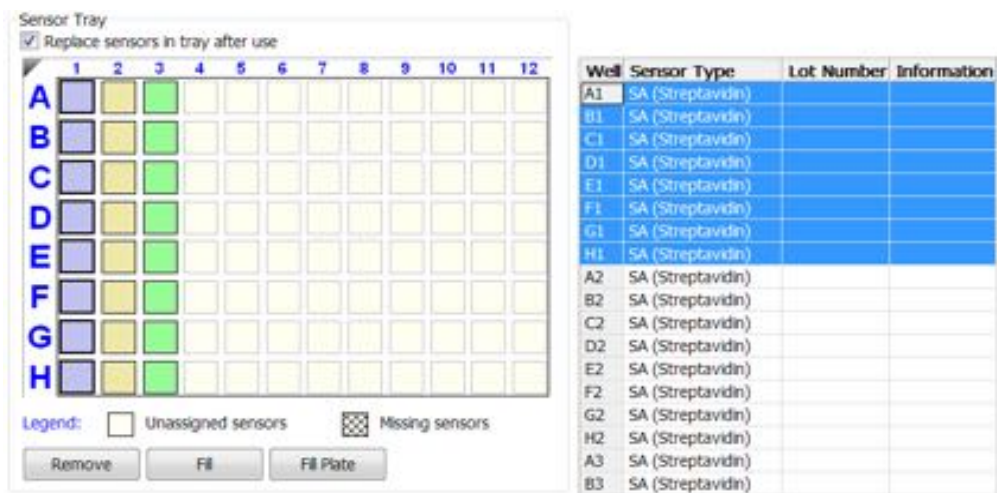


Figure 9-49: Selecting a Sensor Tray Column

2. Right-click in the **Sensor Tray Map** or click in a cell in the **Sensor Type** table column and select a biosensor type from the drop-down list. The biosensor type associated with the assay will shift location accordingly (see Figure 9-50). In the example shown, Streptavidin is the **Sensor Type** used for the current assay. Column 1 was reassigned as AHC according to the heterogeneous tray being used.

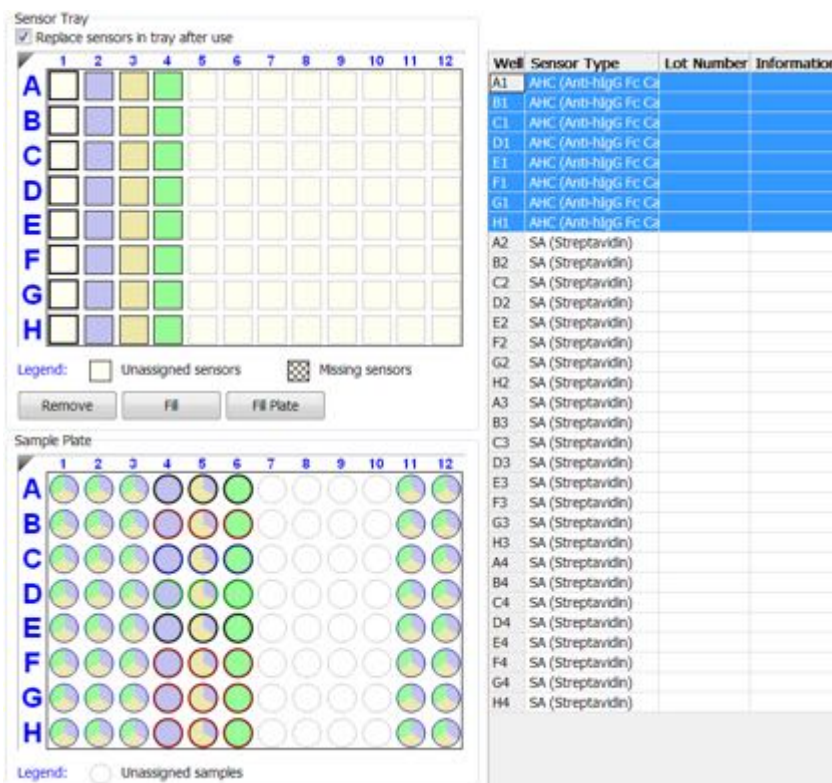


Figure 9-50: Assay Sensor Type Reassignment

- Repeat the previous steps to assign locations for the remaining biosensor types in the tray.



IMPORTANT: Ensure that the biosensor types selected in the **Assay Definition** window have assigned column(s) in the **Sensor Assignment** window or the experiment cannot be run.

Using Partial Biosensor Trays

If you remove biosensors from the **Sensor Tray Map** and there are not enough remaining biosensors for the experiment, the software automatically adds a second tray of biosensors and assigns the biosensors that are required for the assay(s).

The experiment in the example shown in (Figure 9-51) includes three assays, and Tray 1 does not include enough biosensors for the experiment. To view the additional biosensor tray that is required for the assay, select Tray 2 from the **Sensor Tray** drop-down list (Figure 9-51 top). The **Sensor Tray Map** will then display the additional biosensors required for the assay (Figure 9-51 bottom). If necessary, change the location of these biosensors.

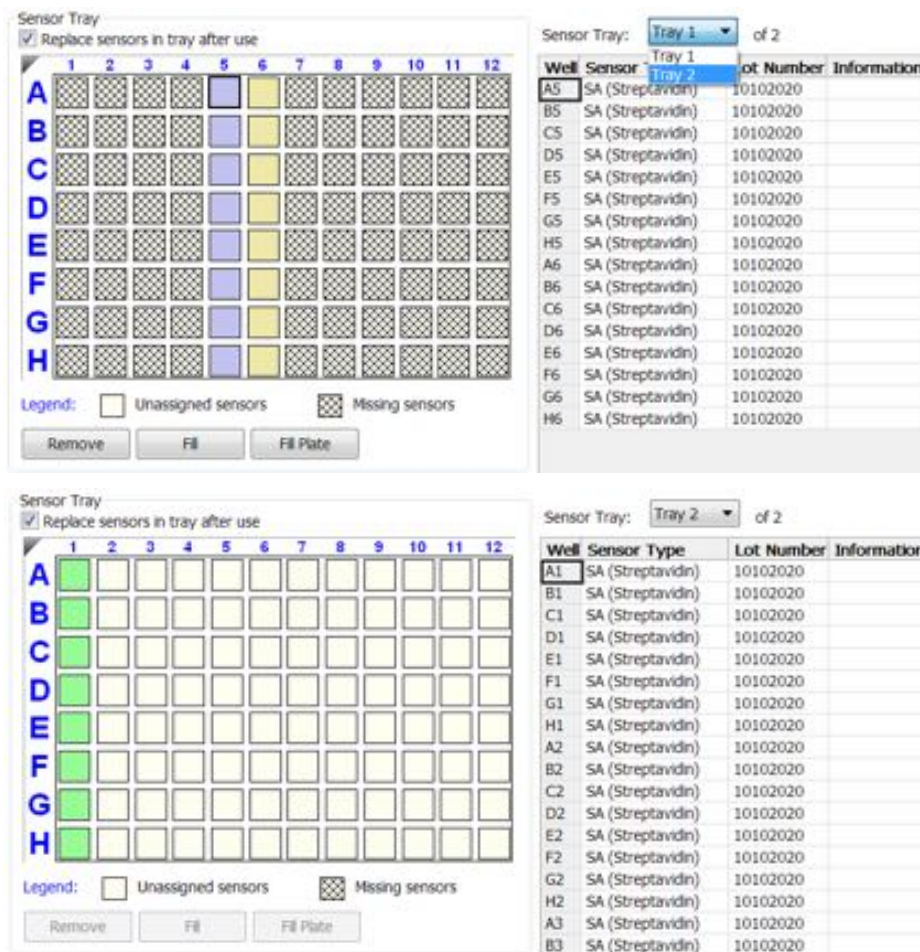


Figure 9-51: Example Experiment Using Two Biosensor Trays



NOTE: Up to two trays may be used per assay, but only the first biosensor tray can be a partial tray. During the experiment run, the software prompts you to insert the appropriate tray in the Octet instrument.

Reference Biosensors

To designate reference biosensors, select the desired biosensors in the **Sensor Tray Map**, right-click and select **Reference**. The reference biosensors are marked with an R.



NOTE: Reference biosensors may also be designated in the **Runtime Binding Chart** during acquisition.

Changing the Biosensor Type

The biosensor type used in the assay must be selected in the **Assay Definition** window. To change the biosensor type:

1. Click the **Assay Definition Tab**.
2. In the **Assay Steps List**, click the cell in the **Sensor Type** column to change.
3. Select from the drop-down list (see Figure 9-52).



IMPORTANT: Ensure that the same biosensor types are selected in both the Assay Definition and the Sensor Assignment windows or the experiment cannot be run.

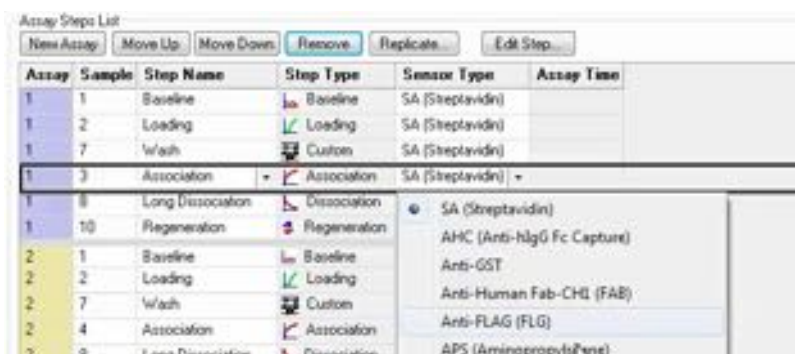


Figure 9-52: Assay Definition Window—Changing the Biosensor Type

REVIEWING EXPERIMENTS

Before running an experiment, you can review the sample plate layout, assays and assay steps as well as the biosensors assigned to each assay in the experiment.

In the **Review Experiment** window (Figure 9-53), move the slider left or right to highlight the biosensors and samples associated with an assay step, or click the arrows. Alternatively, select an assay step to view the biosensors and samples associated with it.

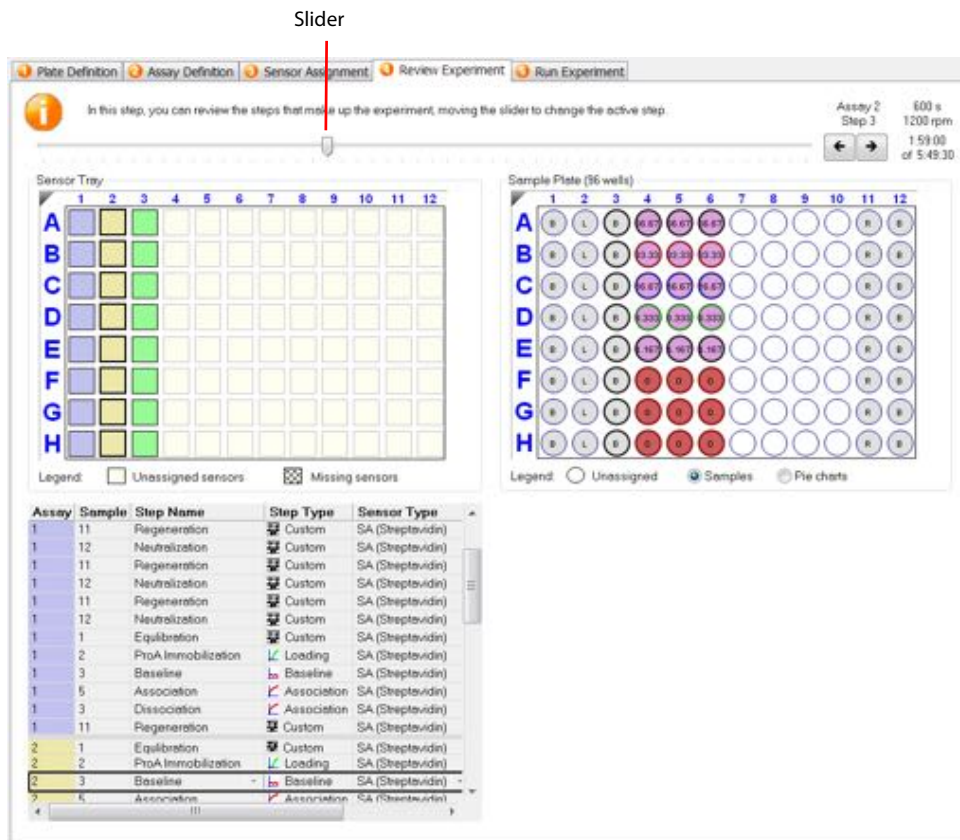


Figure 9-53: Review Experiment Window

SAVING EXPERIMENTS

After an experiment is run, the software automatically saves a read-only copy of the experiment information that you specified (sample plate definition, biosensor assignment, assay settings) to an experiment method file (.fmf). If you set up an experiment, but do not start the run, you can manually save the experiment method.

To manually save an experiment:

1. Click **Save Method File** (📁), or on the main menu, click **File > Save Method File**.

If there is more than one open experiment and you want to save all of them, click **Save All Methods Files** (📁).

2. In the **Save** dialog box, enter a name and location for the file, and click **Save**.



NOTE: If you edit a saved experiment and want to save it without overwriting the original file, click **File > Save Method File As** and enter a new name for the experiment.

Saving an Experiment to the Template Folder

If you save an experiment to the factory-installed Template folder, the experiment will be available for selection. To view templates, select **Experiment > Templates > Kinetics > Experiment Name** (Figure 9-54).

Follow the steps above to save an experiment to the Template folder located at C:\Program Files\ForteBio\DataAcquisition\TemplateFiles.



IMPORTANT: Do not change the location of the Template folder. If the Template folder is moved from the factory-set location, the software may not function properly.

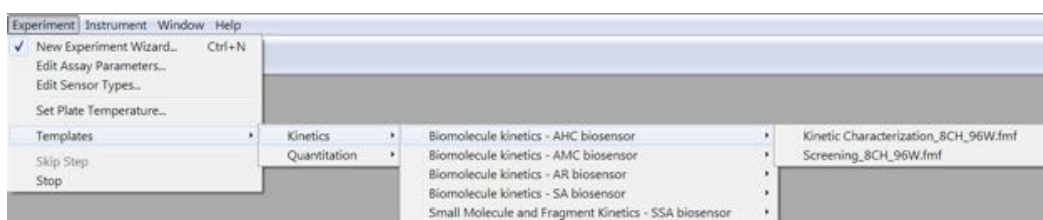


Figure 9-54: Saved Experiments in the Template Folder

RUNNING A KINETICS EXPERIMENT



IMPORTANT: Before starting an experiment, ensure that the biosensors are properly rehydrated. For details on how to prepare biosensors, see the appropriate biosensor product insert.

Loading the Biosensor Tray and Sample Plate

To load the biosensor tray and sample plate:

1. Open the Octet instrument door (lift the handle up).
2. Place the biosensor tray on the biosensor stage (left side) so that well A1 is located at the upper right corner (see Figure 9-55).
3. Place the sample plate on the sample stage (right side) so that well A1 is located at the upper right corner (see Figure 9-55).

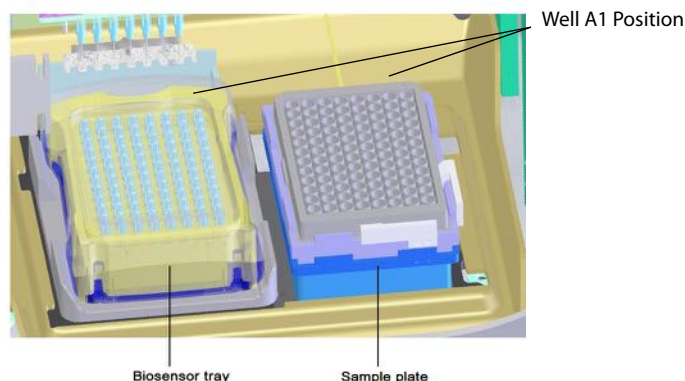


Figure 9-55: Biosensor Stage (left) and Sample Stage (right)



IMPORTANT: Make sure that the bottom of the sample plate and biosensor tray are flat on the stages.

4. **Octet RED96e only, optional.** Cover the microplate with the evaporation cover as recommended below to prevent evaporation from samples during analysis and lengthen the experiment time (only applies to RED96e instrument). For more information, see “Microplate Evaporation Cover” on page 51.
5. Close the Octet instrument door.
6. Allow the plate to equilibrate.

The time required for temperature equilibration depends on the temperature that your application requires and the initial temperature of the sample plate. For specific biosensor rehydration times, see the appropriate biosensor product insert. We recommend delaying the experiment time by 20 minutes to ensure the samples have equilibrated to the desired temperature, especially if you’re cooling the samples to 15 °C or heating to 30 °C from an earlier experiment at 15 °C.

Starting the Experiment

To start the experiment:

1. Click the **Run Experiment** tab, or click the arrow (➡) to access the Run Experiment window (see Figure 9-56).

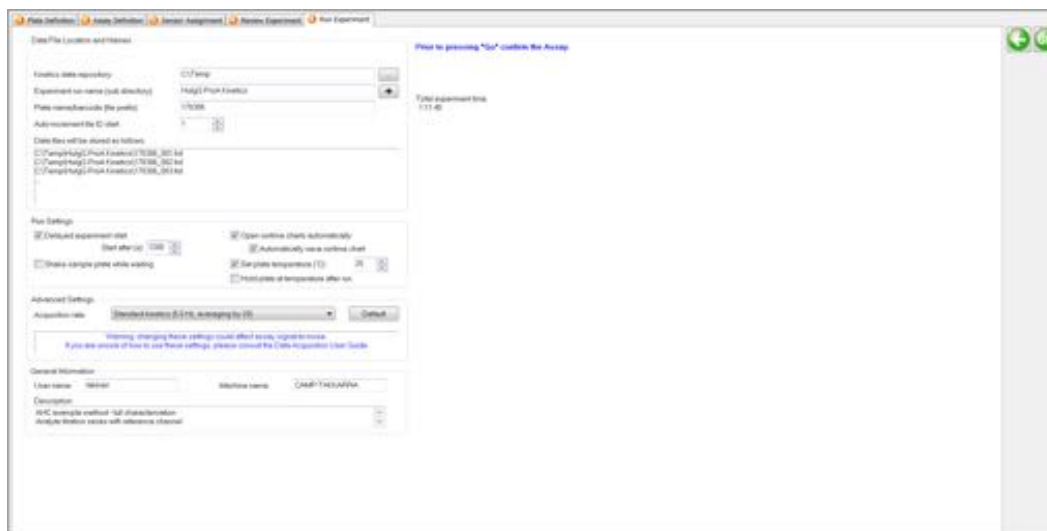


Figure 9-56: Run Experiment Window—Octet RED96

2. Confirm the default settings or enter new settings. See “Run Experiment Window Settings” on page 497 for more information on experimental settings.



NOTE: If you delay the experiment start, you have the option to shake the plate until the experiment starts. We recommend delaying the experiment time by 20 minutes to ensure the samples have equilibrated to the desired temperature, especially if you’re cooling the samples to 15 °C or heating to 30 °C from an earlier experiment at 15 °C.

3. **Optional if you are using a microplate evaporation cover.** Hold plate at temperature after run is pertinent when you are running very long experiments with the evaporation cover. If you are running a 10-12 hour assay and want to ensure that the plate temperature remains at the set plate temperature, then check **Hold plate at temperature after run**. If it is acceptable for the plate to go back to room temperature post-run, then leave that option unchecked.
4. To start the experiment, click **GO**.

If you specified a delayed experiment start, a message box displays the remaining time until the experiment starts.

If you select the **Open runtime charts automatically** option, the **Runtime Binding Chart** window displays the binding data in real-time, as well as the experiment progress (Figure 9-57).



NOTE: For more details about the *Runtime Binding Chart*, see “Managing the Runtime Binding Chart” on page 500.

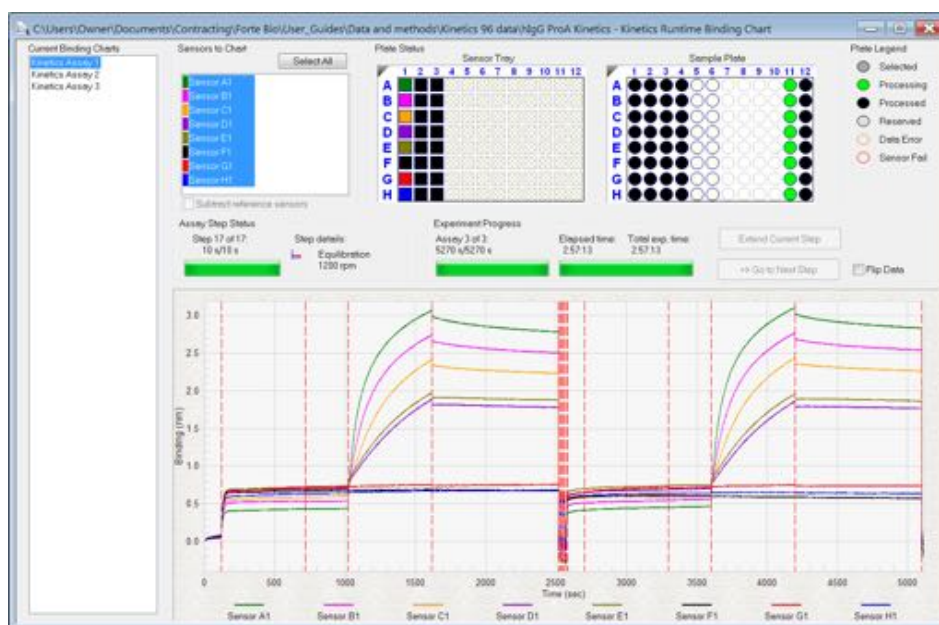


Figure 9-57: Runtime Binding Chart

5. Optional: Click **View > Instrument Status** to view the log file (see Figure 9-58).

The experiment temperature is recorded at the beginning of every experiment as well as each time the manifold picks up a new set of biosensors. Instrument events such as biosensor pick up, manifold movement, integration time, biosensor ejection and sample plate temperature are recorded in the log file.

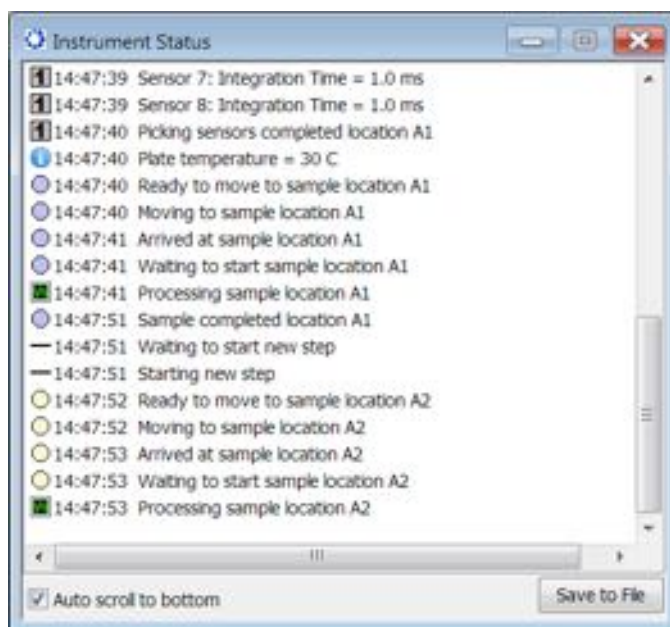


Figure 9-58: Instrument Status Log



WARNING: Do not open the Octet instrument door when an experiment is in progress. If the door is opened, the data from the active biosensors is lost. The data already acquired is saved, however the assay is aborted and cannot be restarted without ejecting the biosensors and starting from the beginning.



WARNING: N'ouvrez pas la porte de l'instrument Octet lorsqu'une analyse est en cours. En cas d'ouverture de la porte, les données issues de l'étape d'acquisition active seront perdues et cela entraînera l'échec de la procédure.




WARNING: Öffnen Sie die Instrumentenklappe des Octet-Systems nicht während eines laufenden Experiments. Wird die Klappe geöffnet, gehen die Daten des aktiven Erfassungsschritts verloren und das Experiment wird abgebrochen.

Run Experiment Window Settings


The following **Data File Location and Name** settings are available on the **Run Experiment** Tab:

Table 9-6: Data File Location and Name

Item	Description
Assay type	The name of the selected assay.
Kinetics data repository	The location where the subdirectory will be created. The subdirectory contains the data (.frd) files. Click Browse to select another data location.
<div> NOTE: It is recommended that you save the data to the local machine first, then transfer to a network drive.</div>	
Experiment Run Name (sub-directory)	Specifies a subdirectory name for the data files (.frd). The software generates one data file for each biosensor that includes the data from all steps the biosensor performs.
Plate name/ barcode (file prefix)	A user-defined field where you can enter text or a barcode (barcode reader required).
2nd Plate name/barcode	A user-defined field where you can enter text or a barcode (barcode reader required) for a second plate. This field is also used to generate the path of the saved directory.
Auto Increment File ID Start	Each file is saved with a number after the plate name. For example, if the Auto Increment File ID Start number is 1, the first file name is xxx_001.frd.

The following **Run Settings** are available on the **Run Experiment** Tab:

Table 9-7: Run Settings

<i>Item</i>	<i>Description</i>
Delayed experiment start	Specifies a time delay for the start of the experiment. Enter the number of seconds to wait before the experiment starts after you click  .
Start after	Enter the number of seconds to delay the start of the experiment.
Shake sample plate while waiting	If the experiment has a delayed start time, this setting shakes the plate until the experiment starts.
Open runtime charts automatically	Displays the Runtime Binding Chart for the current biosensor during data acquisition.
Automatically save runtime chart	Saves an image (.jpg) of the Runtime Binding Chart . The binding data (.frd) is saved as a text file, regardless of whether a chart image is created.
Set plate temperature (°C)	Specifies a plate temperature and enters the temperature in the dialog box. If not selected, the plate temperature is set to the default temperature specified in File > Options . The factory set default temperature is 30 °C.



NOTE: If the actual plate temperature is not equal to the set plate temperature, a warning displays and the Octet System Data Acquisition software provides the option to wait until the set temperature is reached before proceeding with the run, continue without waiting until the set temperature is reached, or cancel the run.


Advanced settings are available for the Octet QK^e, Octet RED, Octet RED96 and Octet RED96e systems. The signal to noise ratio of the assay can be optimized by selecting different acquisition rates. The acquisition rate refers to the number of binding signal data points reported by the Octet system per minute and is reported in Hertz (per second). A higher acquisition rate generates more data points per second and monitors faster binding events better than a slower acquisition rate. A lower acquisition rate allows the software enough time to perform more averages of the collected data. Typically, more averaging leads to

reduced noise and thus, better signal-to-noise ratios. The choice of a setting should be determined based upon consideration of the binding rate and the amount of signal generated in your assay, and some experimentation with the settings.

Table 9-8: Advanced Settings Octet QK^e, Octet RED, Octet RED96 and Octet RED96e

Item	Description
Acquisition rate Octet QK ^e	<ul style="list-style-type: none"> High sensitivity kinetics (0.3 Hz, averaging by 40) - The average of 40 data frames is reported as one data point. One data point is reported every 3.3 seconds. Standard kinetics (0.6 Hz, averaging by 5) - The average of five data frames is reported as one data point. One data point is reported every 1.6 seconds.
Acquisition rate Octet RED96 and Octet RED96e	<ul style="list-style-type: none"> High sensitivity kinetics (2 Hz, averaging by 50): - The average of 50 data frames is reported as one data point. Two data points are reported per second. Standard kinetics (5 Hz, averaging by 20 - The average of 20 data frames is reported as one data point.
Sensor offset (mm) - Octet QK ^e only	Recommended sensor offset: Large molecule kinetics—4 mm
Default	Sets acquisition rate and sensor offset to the defaults.

Stopping an Experiment

To stop an experiment in progress, click  or click **Experiment > Stop**.

The experiment is aborted. The data for the active biosensor is lost, the biosensor is ejected into the waste tray, and the event is recorded in the experimental log.



NOTE: After the experiment is run, the software automatically saves the experiment method (.fmf).

MANAGING THE RUNTIME BINDING CHART

If the **Open runtime charts automatically** check box is selected in the Run Experiment window (Figure 9-59), the Runtime Binding Charts are automatically displayed when data acquisition starts. The **Runtime Binding Chart** window displays the assay step status, experiment progress, and the elapsed experiment time.

The **Runtime Binding Chart** is updated at the start of each experimental step. The active biosensor column is color-coded (A=green, B=magenta, C=orange, D=purple, E=olive, F=black, G=red, H=blue) within the **Sensor Tray Map**. Used sensor columns that are inactive are colored black. Active sample columns are colored green. Each assay in the experiment is represented by **Assay X** in the **Current Binding Charts** box.

To selectively display data for particular assay:

1. Click the corresponding **Assay** number.
2. Select a subset of sensors for a displayed column under **Sensors to Chart** box (see Figure 9-59).



IMPORTANT: Do not close the **Runtime Binding Chart** window until the experiment is complete and all data is acquired. If the window is closed, the charts are not saved. To remove the chart from view, minimize the window. The Octet System Data Acquisition software saves the **Runtime Binding Chart** as displayed at the end of the experiment. For example, modifying a chart by hiding the data for a particular biosensor will cause this data not to be included in the bitmap image generated at the end of the run.

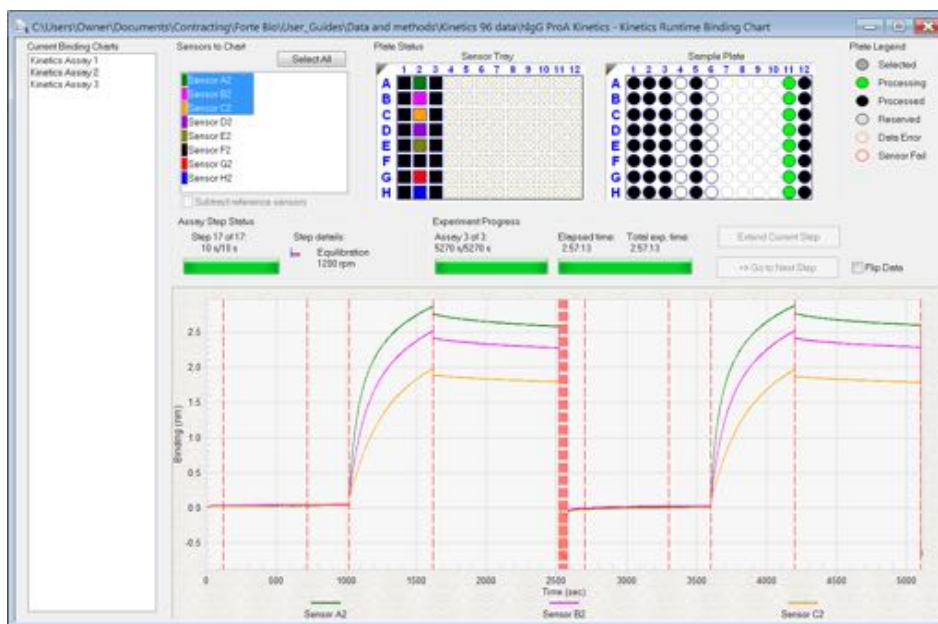


Figure 9-59: Runtime Binding Chart Window

Opening the Runtime Binding Chart

After an experiment is run, you can open and review the **Runtime Binding Chart** at any time:

1. Click **File > Open Experiment**.
2. In the dialog box that appears, select an experiment folder and click **Select**.

Viewing Reference-Subtracted Data

If the experiment includes reference biosensors, you can display reference-subtracted data in the chart by clicking the **Subtract Reference Biosensor** check box in the chart window. To view raw data, remove the check mark next to this option.

Reference biosensors can be designated:

- During experiment setup in the **Sensor Assignment** tab
- During acquisition in the Runtime Binding Chart **Sensors to Chart** box
- During analysis in the **Data Selection** tab

Designating a Reference Biosensor During Acquisition

To designate a reference biosensor during acquisition:

1. In the **Sensors to Chart** list or the **Sensor Tray**, right-click a biosensor and select **Reference** (see Figure 9-60).

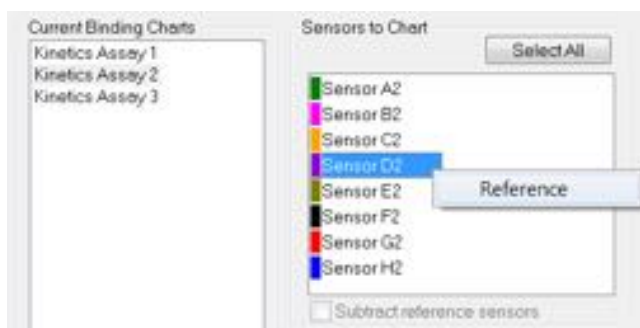


Figure 9-60: Designating a Reference Biosensor in the Runtime Binding Chart

The selected biosensor will be shown with an **R** in the **Sensors to Chart** list and **Sensor Tray** (see Figure 9-61).

2. Click the **Subtract reference sensors** check box (see Figure 9-61).

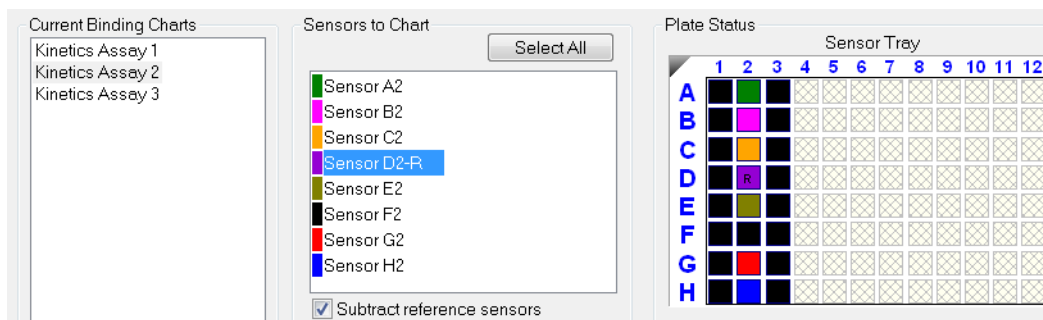


Figure 9-61: Subtract Reference Sensors check box in the Runtime Binding Chart



NOTE: Subtracting reference data in the **Runtime Binding Chart** only makes a visual change to the data on the screen. The actual raw data is unaffected and the reference subtraction must be repeated during data analysis if needed.

Viewing Inverted Data

The data displayed in the **Runtime Binding Chart** can be inverted during real-time data acquisition or data analysis after the experiment has completed. To invert data, select the **Flip Data** check box (see Figure 9-62). Uncheck the box to return to the default data display.

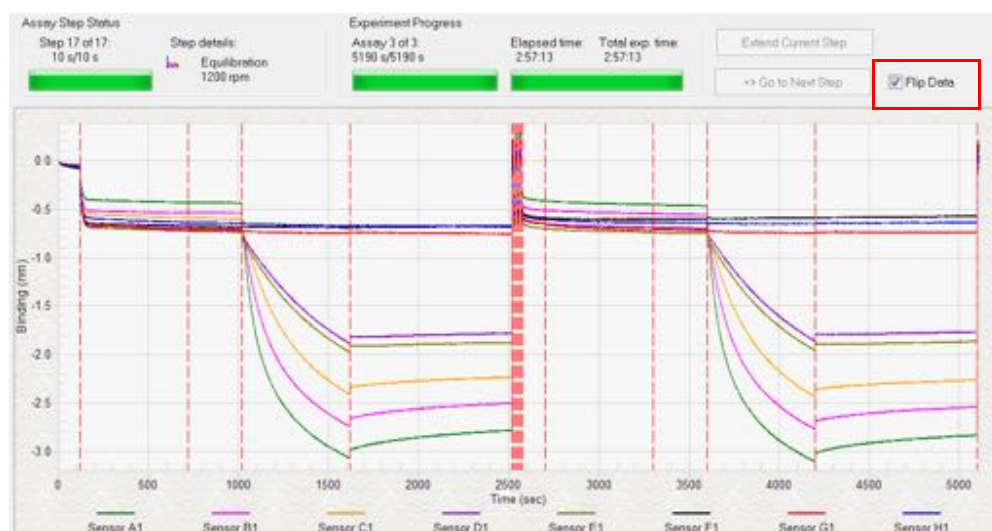


Figure 9-62: Data Inverted Using Flip Data Function

Aligning Data by a Selected Step

To align the binding data to the beginning of a user-selected step, in the **Runtime Binding Chart** (see Figure 9-63), right-click a step and select **Align to Step <number>**.

To remove the step alignment, right-click the step and select **Unaligned**.

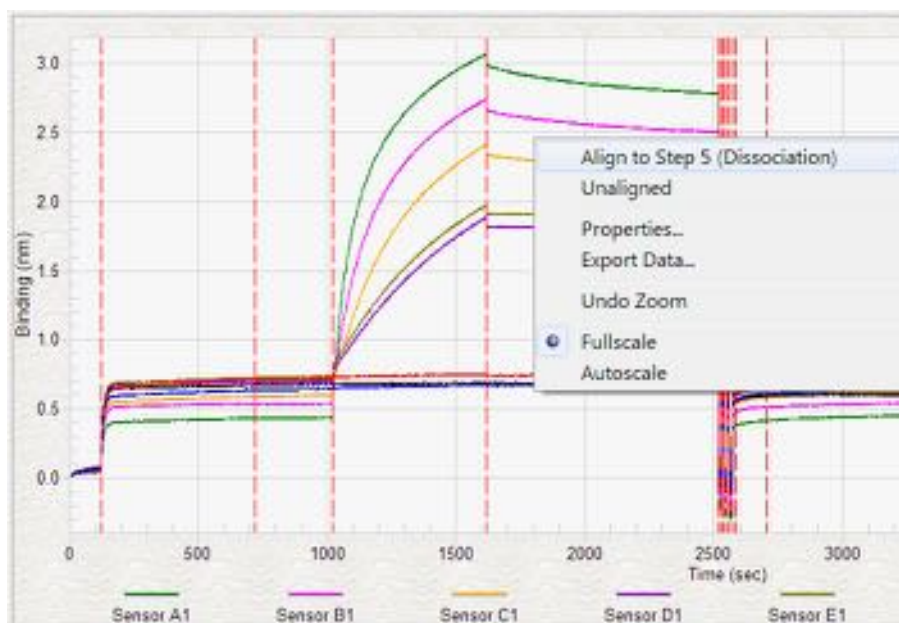


Figure 9-63: Runtime Binding Chart—Aligning the Data to a User-Selected Step

Aligning Data to a Specific Time

1. To align the binding data to a specific time, in the **Runtime Binding Chart** (see Figure 9-64), right-click and select **Align at time**.

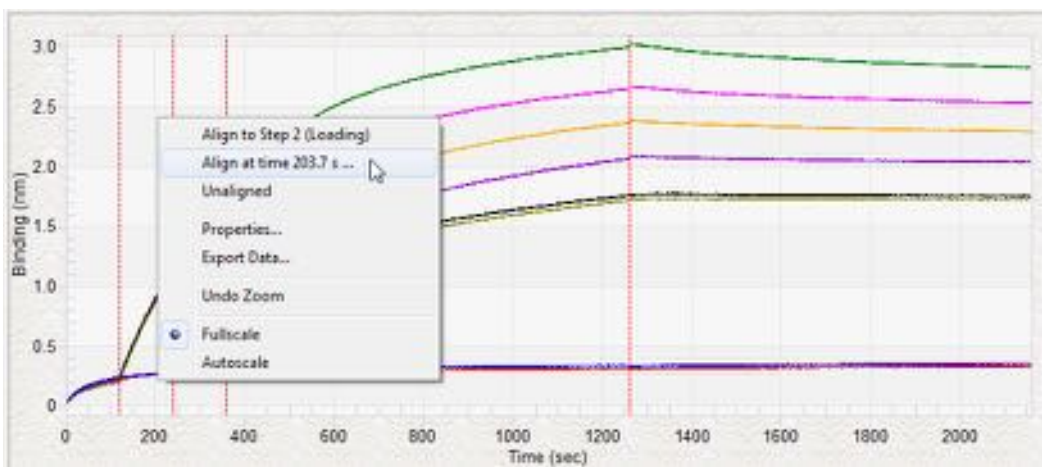


Figure 9-64: Runtime Binding Chart—Aligning the Data to a User-Specified Time

The Align at Time dialog box displays (Figure 9-65).

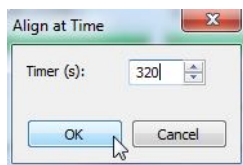


Figure 9-65: Align at Time Dialog Box

2. Enter the time point you want to align to and click **OK**. The binding chart will then align to the time point specified.

To remove the time alignment, right-click and select **Unaligned**.

Extending or Skipping an Assay Step

During acquisition, the duration of the active step may be extended. You can also terminate the active step and begin the next step in the assay.



NOTE: If the step you want to extend or terminate includes biosensors used in Parallel Reference, Double Reference, or Average Reference subtraction methods, the data will not be analyzed.

To extend the duration of the active step:

1. In the chart window, click the **Extend Current Step** button.
2. In the **Extend Current Step** dialog box (see Figure 9-66), enter the number of seconds to extend the step and click **OK**.

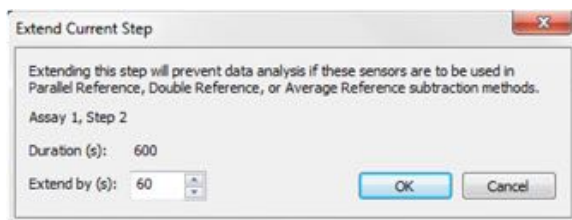


Figure 9-66: Extend Current Step Dialog Box

Terminating a Step to Begin the Next Step

To terminate a step and begin the next step in the assay:

1. In the chart window, click the **Go to Next Step** button.
2. In the **Data Acquisition** dialog box, click **OK**.

Magnifying the Runtime Binding Chart

To magnify the chart, press and hold the mouse button while you draw a box around the chart area to magnify.

To undo the magnification, right-click the chart and select **Undo Zoom**.

Scaling a Runtime Binding Chart

To scale the **Runtime Binding Chart**:

1. Right-click the chart and select **Properties**.
2. In the **Runtime Graph Properties** dialog box, select **Fullscale** or **Autoscale**.

Adding a Runtime Binding Chart Title

To add a **Runtime Binding Chart** title:

1. Right-click the chart and select **Properties**.
2. In the **Runtime Graph Properties** dialog box, enter a graph title or subtitle.

Selecting a Runtime Binding Chart Legend

To select a **Runtime Binding Chart** legend:

1. Right-click the chart and select **Properties**.
2. In the **Runtime Graph Properties** dialog box (see Figure 9-67), select one of the following legends:
 - Sensor Location
 - Sample ID
 - Sensor Information
 - Concentration/Dilution

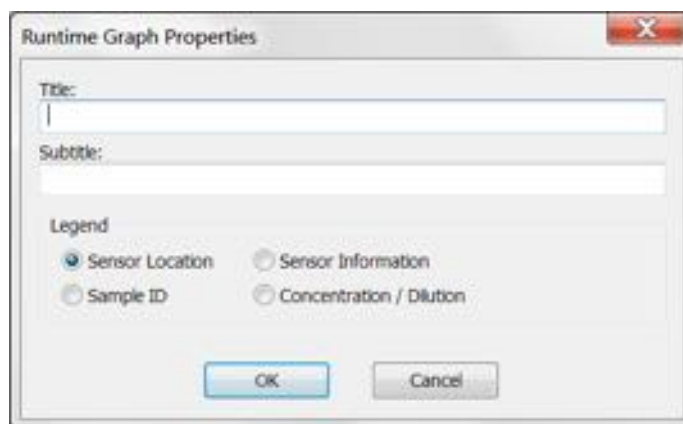


Figure 9-67: Selecting a Runtime Binding Chart Legend



NOTE: Text for **Sample ID**, **Sensor Information**, or **Concentration/Dilution** is taken from the **Plate Definition** and **Sensor Assignment** tabs, and must be entered before the experiment is started.

3. Click **OK**.

Viewing Multiple Runtime Binding Charts

To view multiple Runtime Binding Charts, click **Window > New Window**.

Exporting or Printing the Runtime Binding Chart

To export the **Runtime Binding Chart** as a graphic or data file:

1. Right-click the chart and select **Export Data**.
2. In the **Exporting** dialog box (see Figure 9-68), select the export options and click **Export**.

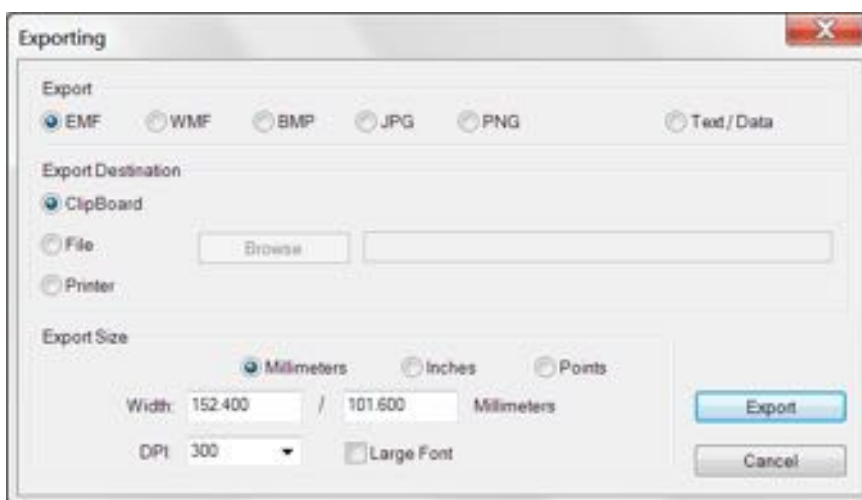


Figure 9-68: Exporting Dialog Box

Table 9-9: Runtime Binding Chart Export Options

Task	Export	Option	Export Destination	Result
	Text/ Data	EMF, WMF, BMP, JPG, or PNG		

Table 9-9: Runtime Binding Chart Export Options (Continued)

Task	Export	Option	Export Destination	Result
Save the binding data	✓		Click File > Browse to select a folder and enter a file name.	Creates a tab-delimited text file of the numerical raw data from each biosensor. Open the file with a text editor such as Notepad.
Export the Runtime Binding Chart to a graphic file		✓	Click File > Browse to select a folder and enter a file name.	Creates a graphic image.
Copy the Runtime Binding Chart		✓	Clipboard	Copies the chart to the system clipboard
Print the Runtime Binding Chart		✓	Printer	Opens the Print dialog box.

MANAGING EXPERIMENT METHOD FILES

After you run an experiment, the Octet System Data Acquisition software automatically saves the method file (.fmf), which includes the sample plate definition, biosensor assignment, and the run parameters. An experiment method file provides a convenient initial template for subsequent experiments. A read-only copy of the method used for an experiment is automatically saved in the experiment folder. Open a method (.fmf) and edit it if necessary.



NOTE: When using the 21 CFR Part 11 version of the Octet System Data Acquisition software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.

Table 9-10: Managing Experiment Method Files

Menu Bar Command/ Toolbar Button	Description
File > Open Method File	Enables you to select and open a method file (.fmf)
File > Save Method File or	Saves one method file or all method files. Saves a method file before the experiment is run.
File > Save Method File As	Saves a method file to a new name so that the original file is not overwritten.

EPITOPE BINNING

The goal of a typical epitope binning or cross-blocking experiment is to identify antibodies which bind to different or identical epitopes on the antigen. Antibodies are tested two at a time for competitive binding to one antigen. By competing antibodies against one another in a pairwise and combinatorial format, antibodies with distinct blocking behaviors can be discriminated and assigned to “bins”. The end result is matrix of pairwise binders and blockers.



An epitope binning or cross-blocking experiment must be run as a kinetic experiment with repeating steps in the Octet Data Acquisition software.



NOTE: Pall ForteBio highly recommends using the Loading, Association or Dissociation assay steps instead of Custom for epitope binning and cross-blocking experiments.

After starting the Octet system and the Octet Data Acquisition software, follow the steps in Table 9-11 to set up and run an epitope binning experiment.

Table 9-11: Octet Data Acquisition Steps for Epitope Binning Assays

Octet Software	Functions
Data Acquisition 	<ol style="list-style-type: none"> 1. Select Epitope Binning under New Kinetics Experiment in the Experiment Wizard. Open a method template from the Experiment Menu or open an existing method file (*.fmf). <hr/> <div>  <p>NOTE: In the Experiment Menu, the Templates command allows users to pick from a set of predefined method templates for Kinetic, Quantitation, or Epitope Binning experiments. Users may also modify existing method templates to suit their experimental conditions and save as a new method file and new method file name.</p> </div> <hr/> <ol style="list-style-type: none"> 2. Define a sample plate or open a sample plate definition. 3. Specify assay steps. 4. Assign biosensors to samples. 5. Run the experiment.

Starting an Experiment

You can start a kinetics experiment using one of the following options:

- Launch the **Experiment Wizard** by clicking **Experiment > New Experiment Wizard**, and selecting **New Kinetics Experiment** and **Epitope Binning**.
- Open a method file (.fmf) by clicking **File > Open Method File**. Method files may be saved and recalled using the File menu and are automatically saved when an experiment is run.
- On the menu bar, click **Experiment > Templates > Epitope Binning**.
- 6. Optional: You can also click **Recent Methods** to display a list of recently used methods. You can open any method file from the list and use it with or without modifications to run a new experiment.

Enter the required information on Tabs 1-5 of the Basic Kinetics Experiment.

Tab 1 (Plate Definition)



NOTE: The Sample plate and the Reagent plate are now referred to as "Plate 1" and "Plate 2" in the software.

1. Designate layouts for the plate by selecting wells in the plate map and designating sample types. There are several ways to select sample wells in the plate map:
 - Click a column header or select adjacent column headers by click-hold-drag.
 - To select non-adjacent columns, hold the **Ctrl key** and click the column header.
 - Click a row header or select adjacent row headers by click-hold-drag.
 - Click a well or draw a box around a group of wells.
2. Designate well types by right-clicking on selected wells and assigning a sample type:



Figure 9-69: Designating Well Types

3. Enter sample information by selecting the table for the plate. There are several ways to enter sample information:
 - Select an individual well in the plate table.
 - Click-drag-hold several wells in the plate table, right-click and choose **Set Well Data**.



NOTE: Assigning sequential alpha-numerical names for Sample ID provides easier sorting of columns and headers for the epitope binning matrix.



NOTE: More information on sample information and annotation can be found in “Entering Sample Information” on page 451.

Tab 2 (Assay Definition)

After completing the plate layout, an Epitope Binning Assay can be defined by building a kinetic assay.

1. Click on Tab 2 (Assay Definition).
2. Add assay step types in the Step Data List:
 - a. Click the **Add** button. The Add Step Definition box will display:

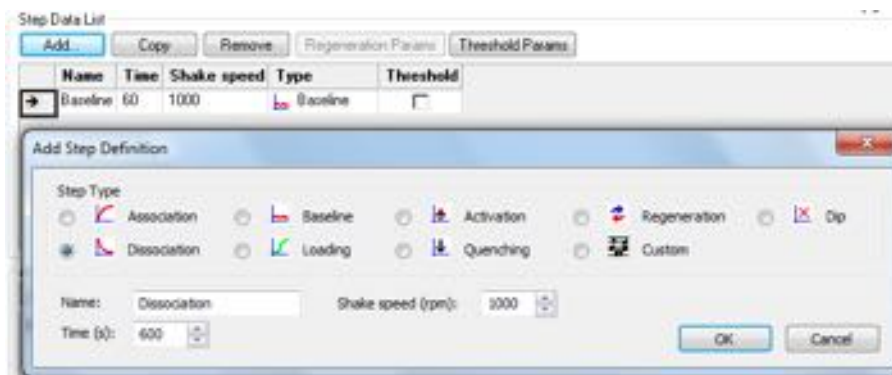


Figure 9-70: Add Step Definition Box

- b. Choose a step type.
- c. Optional: edit step name.
- d. Set the step time and shake speed.
- e. The regeneration step type requires assigning separate parameters. To do this, click the **Regeneration Params** button:

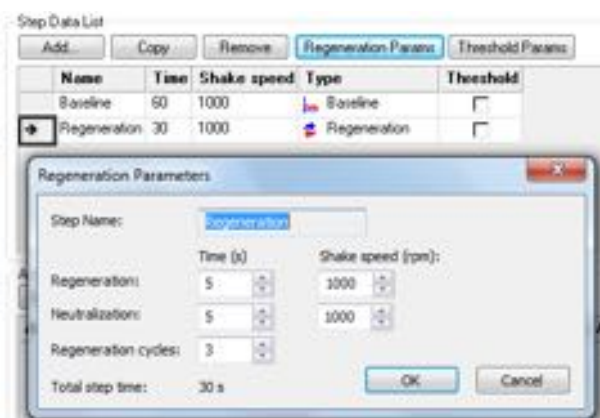


Figure 9-71: Regeneration Parameters Box

- f. Optional: assign a threshold. See “Creating Step Types” on page 468 for more information.
3. Build the assay(s) by assigning steps defined in Step Data List to columns in the plate map(s).



NOTE: Pall ForteBio highly recommends using the Associate or Dissociate assay steps instead of Custom for epitope binning and cross-blocking experiments.

- a. Select a step type in the Step Data List.
- b. In the plate map, double-click the columns that you want associated with that step type.
- c. The selected wells will be marked with hatching, and the new step appears in the Assay Steps List:

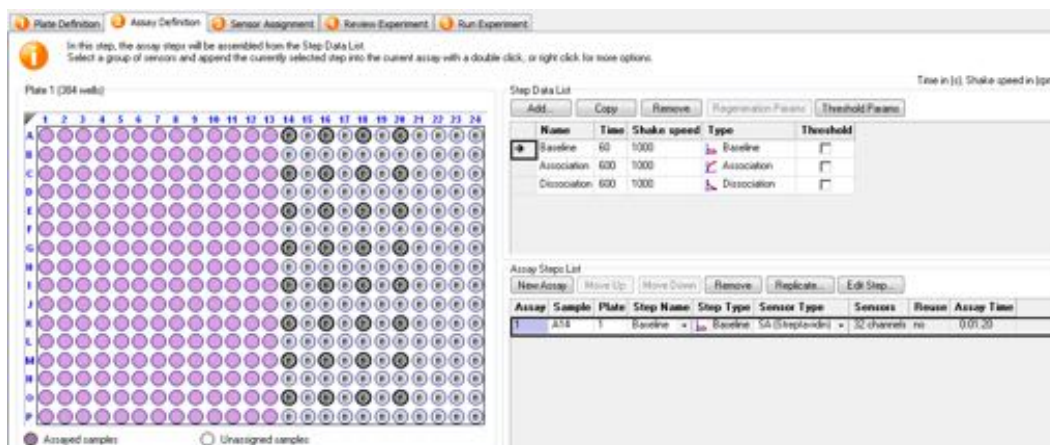


Figure 9-72: Assay Steps List

- d. Select the correct biosensor from the Sensor Type drop-down list. The Sensors column shows the Read Head selection made in Tab 1 (Assay Definition).
- e. Repeat the previous steps to define other steps in the assay.
- f. New assays may be added by clicking the **New Assay** button in the Assay Steps List:

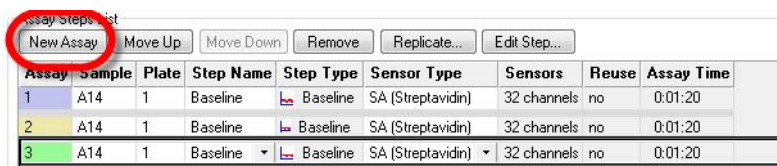


Figure 9-73: New Assay Button



NOTE: More information on assay step editing in Tab 2 (Assay Definition) can be found in “Creating Step Types” on page 468.

Tab 3 (Sensor Assignment):

After completing the assay definition, click on Tab 3 (Sensor Assignment) to verify sensor type(s) for the epitope binning experiment.



NOTE: The Sensor Type for the assay must be selected or changed from the Assay Steps List in the Assay Definition Tab. Changing the Sensor Type from the Sensor Assignment Tab will not update the assay.



NOTE: Full details on biosensor assignment in Tab 3 (Sensor Assignment) can be found in “Assigning Biosensors to Samples” on page 481.

Replacing Biosensors in the Biosensor Tray. After an assay is completed, biosensors can either be returned to the biosensor tray or ejected through the chute. To return them to the tray, click the Replace sensors in tray after use check box:



Figure 9-74: Replace Sensors in Tray After Use Check Box

Tab 4 (Review Experiment)

Before running the experiment, click on Tab 4 (Review Experiment) to review the sample plate layout, assays and assay steps as well as the biosensors assigned to each assay in the experiment.

Move the slider left or right in the window or click the arrows to highlight the biosensors and samples associated with an assay step:



Figure 9-75: Navigating the Review Experiment Tab

Alternatively, select an assay step to view the biosensors and samples associated with it.

Saving Experiments

After an experiment is run, the software automatically saves the experiment information that you specified (sample plate definition, biosensor assignment, assay settings, etc.) to an experiment method file (.fmf).

If you set up an experiment but do not start the run, you can manually save the experiment method. To do this:

1. Select **File > Save Method File**.
2. In the Save dialog box, enter a name and location for the file, and click **Save**.

Loading the Biosensor Tray and Sample Plates

To load the biosensor tray and plate positions 1 and 2:

1. Click **Instrument > Present Stage** to open the door and present the stage. Alternatively, click the **Present Stage** button:

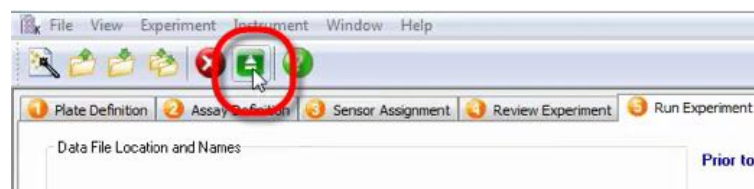


Figure 9-76: Present Stage Button

2. Place the biosensor tray, biosensor wetting plate, Plate 1, and Plate 2 on the appropriate stage so that well A1 is located at the upper right corner.
3. Close the stage and door by clicking the **Present Stage** button again.

Tab 5 (Run Experiment)

1. Click on Tab 5 (Run Experiment) to confirm the default settings or set new settings.
2. To start the experiment, click the **GO** button:

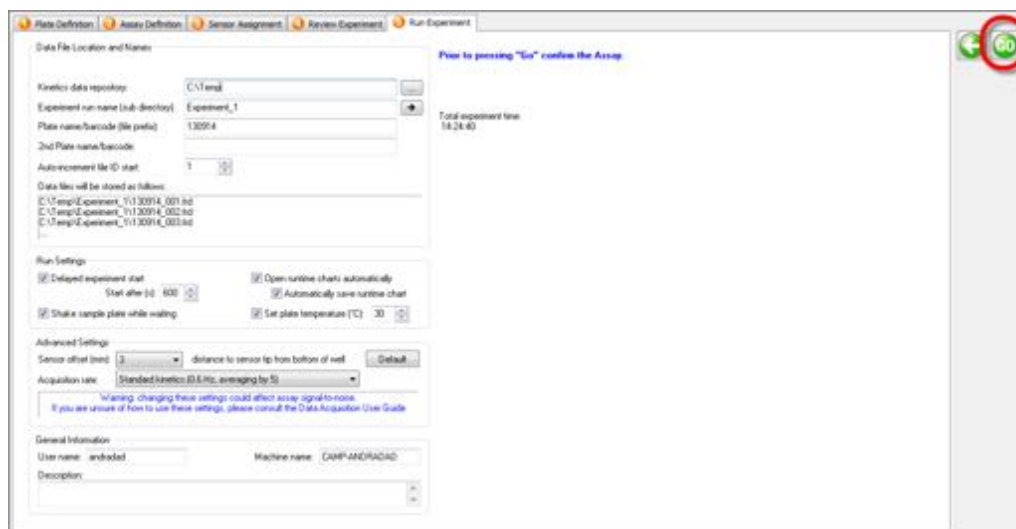


Figure 9-77: GO Button

CHAPTER 10:

Kinetics Experiments: Octet RED384, QK384 and HTX

Introduction	518
Starting a Basic Kinetics Experiment: Octet RED384 and QK384	519
Starting a Basic Kinetics Experiment: Octet HTX	571
Reviewing Experiments	589
Saving Experiments	590
Running a Kinetics Experiment	591
Managing the Runtime Binding Chart	599
Managing Experiment Method Files	607
Epitope Binning	608




INTRODUCTION

A basic kinetics experiment enables you to determine the association and dissociation rate of a molecular interaction. After starting the Octet system hardware and the Octet System Data Acquisition software, follow the steps (in Table 10-1) to set up and analyze a quantitation experiment.



NOTE: Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet Data Acquisition software versions 8.0 and higher.

Table 10-1: Setting Up and Analyzing a Kinetic Experiment

Software	Step	See
Data Acquisition 	1. Select a kinetics experiment in the Experiment Wizard or open a method file (.fmf).	"Starting a Basic Kinetics Experiment: Octet RED384 and QK384" on page 519
	2. Define a sample plate or import a sample plate definition.	"Defining the Sample Plate" on page 520
	3. Define a or import a reagent plate (optional).	"Printing a Sample Plate Definition" on page 543
	4. Specify assay steps.	"Defining a Kinetic Assay" on page 547
	5. Assign biosensors to samples.	"Assigning Biosensors to Samples" on page 561
	6. Run the experiment.	"Running a Kinetics Experiment" on page 591
Data Analysis  or Data Analysis HT 	7. View and process the raw data.	<i>Octet System Data Analysis Software or Octet Data Analysis HT Software User Guide</i>
	8. Analyze the data.	



NOTE: Before starting an experiment, check the sample plate temperature displayed in the status bar. Confirm that the temperature is appropriate for your experiment and if not set a new temperature. If the Octet System Data Acquisition software is closed, the plate temperature will reset to the default startup value specified in the **Options** window when the software is relaunched.

STARTING A BASIC KINETICS EXPERIMENT: OCTET RED384 AND QK384

You can start a kinetics experiment using one of the following options:



- Launch the **Experiment Wizard**.
- Open a method file (.fmf) by clicking **File > Open Method File**. Method files may be saved and recalled using the **File** menu and are automatically saved when an experiment is run. For more details on method files see “Managing Experiment Method Files” on page 607.
- On the menu bar, click **Experiment > Templates > Kinetics**.



NOTE: When using the 21 CFR Part 11 version of the Octet System Data Acquisition software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.

Starting an Experiment Using the Experiment Wizard

To start an experiment from the **Experiment Wizard**:

1. If the **Experiment Wizard** is not displayed when the software is launched, click the **Experiment Wizard** toolbar button , or click **Experiment > New Experiment Wizard (Ctrl+N)** from the **Main Menu**.
2. In the **Experiment Wizard**, click **New Kinetics Experiment** (see Figure 10-1, left).
3. Optional: You can also click **Recent Methods** to display a list of recently used methods. You can open any method file from the list and use it with or without modifications to run a new experiment.
4. Click the arrow button(). The **Basic Kinetics Experiment** window displays (Figure 10-1, right).

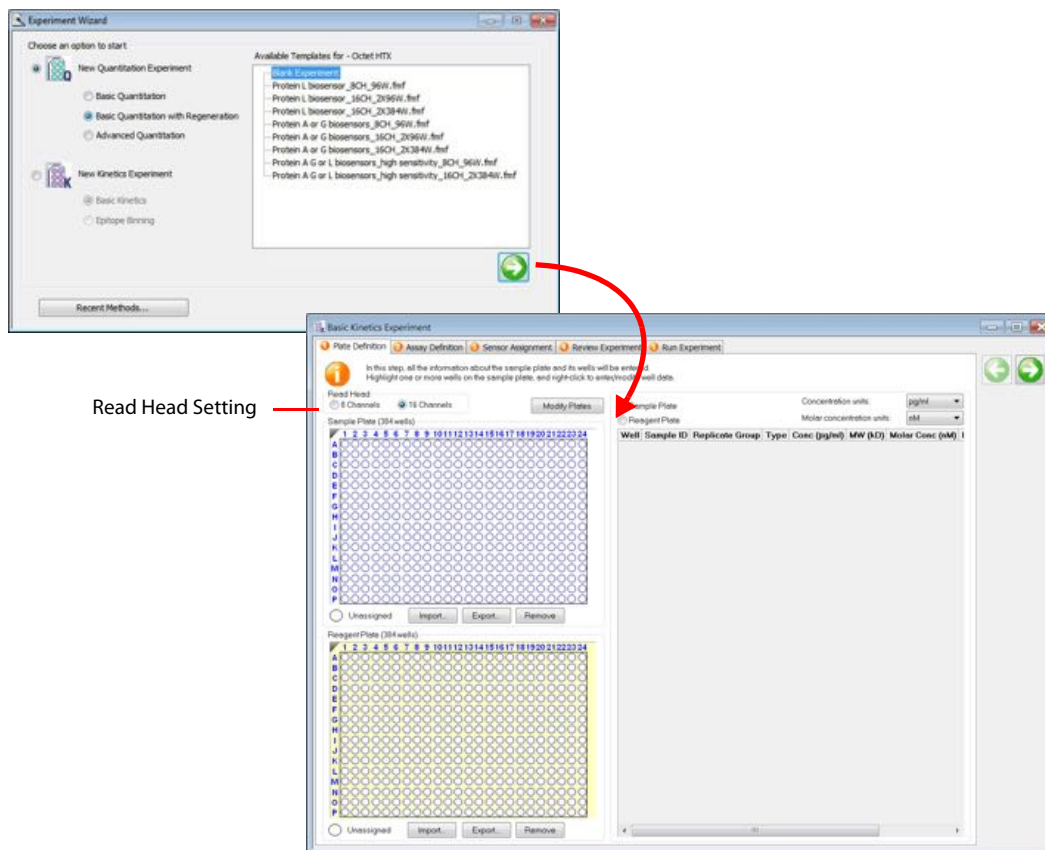


Figure 10-1: Starting a Kinetics Experiment with the Experiment Wizard

Defining the Sample Plate



NOTE: Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet Data Acquisition software versions 8.0 and higher (Figure 10-2).

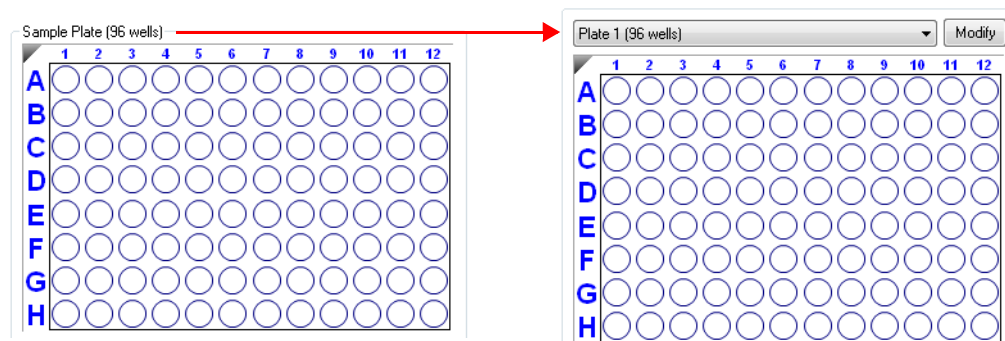


Figure 10-2: Sample Plate Renamed Plate 1 in Software Versions 8.0 and Higher

The steps to define a sample plate include:

Step	See Page
1. Select the instrument read head configuration (8 or 16 channels).	521
2. Select the sample plate format (96 or 384 wells).	523
3. Designate the samples.	523
4. Save the sample plate definition (optional).	540

Read Head Configuration and Plate Layout

The Octet read head contains the collection optics. If the read head is set to 8 channels, one column of 8 biosensors interrogate 8 plate wells. If the read head is set to 16 channels, two columns of biosensors interrogate 16 wells (see Figure 10-3). The read head configuration and the plate format (96 or 384 wells) determine the plate layout (see example Figure 10-3).

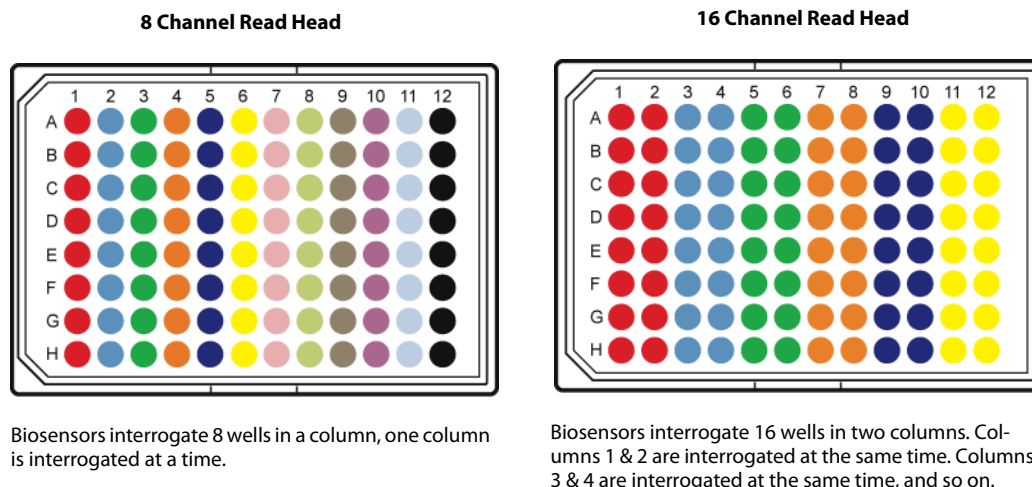


Figure 10-3: Color-Coded Wells Display How Biosensors Interrogate a 96-well Plate, 8 Channel or 16-Channel Read Head

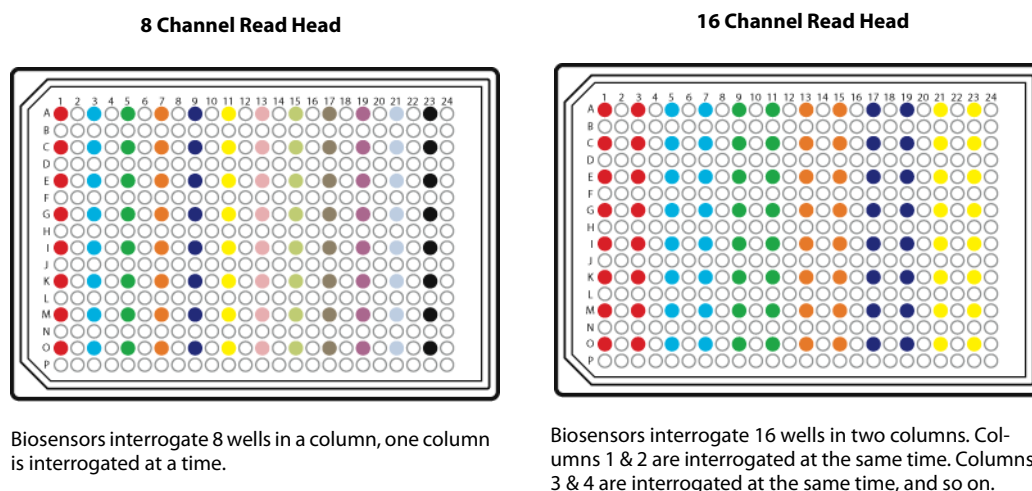


Figure 10-4: Color-Coded Wells Display How Biosensors Interrogate a 384-well Plate, 8 Channel or 16-Channel Read Head



NOTE: Keep the read head configuration in mind when laying out the sample plate. While reading a 384-well sample plate, both the 8 channel and 16 channel read heads can freely step through the plate by either moving left or right to step across columns or step one row up or down.

Changing the Sample Plate Format



NOTE: Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet Data Acquisition software versions 8.0 and higher.

To change the sample plate format:

1. Click **Modify** (above the plate map).
2. In the **Modify Plates** dialog box, select **96 Well** or **384 Well** format.

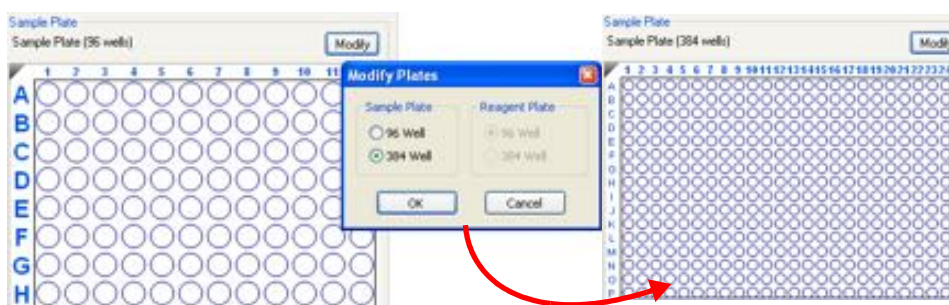


Figure 10-5: Changing the Sample Plate Format

Designating Samples



NOTE: It is important to define all of the wells that will be used in the assay. Only wells that are selected and defined using one of the sample types in Table 10-2 will be included in the assay.



NOTE: Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet Data Acquisition software versions 8.0 and higher.

Table 10-2 displays the well types that can be assigned to a plate map.

Table 10-2: Types of Sample Wells











Icon	Description
 Sample	Any type of sample. For example, an analyte.
 Reference	Reference sample. For example, a buffer-only control biosensor that is used to correct for system drift.

Table 10-2: Types of Sample Wells

Icon	Description
 Controls	<p>A control sample, either positive or negative, of known analyte composition. Data from the well is not used to generate a standard curve during analysis.</p> <ul style="list-style-type: none"> • Positive Control: A control sample that contains analyte of known concentration • Negative Control: A control sample known not to contain analyte
 Buffer	Any type of buffer. For example, the buffer in a baseline, association, or dissociation step.
 Activation	Activation reagent. Makes the biosensor competent for binding.
 Quench	Quenching reagent. Blocks unreacted immobilization sites on the biosensor surface.
 Load	Ligand to be immobilized (loaded) on the biosensor surface.
 Wash	Wash buffer.
 Regeneration	Regeneration reagents dissociate the analyte from the ligand.
 Neutralization	Neutralization buffer that is used to neutralize the biosensor after the regeneration step.

Selecting Wells in the Sample Plate Map



NOTE: Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet Data Acquisition software versions 8.0 and higher.

There are several ways to select wells in the **Sample Plate Map**:

- Click a column header or select adjacent column headers by click-hold-drag (Figure 10-6 left). To select non-adjacent columns, hold the **Ctrl** key and click the column header.
- Click a row header or select adjacent row headers by click-hold-drag (Figure 10-6, center).
- Click a well or draw a box around a group of wells (Figure 10-6, right).

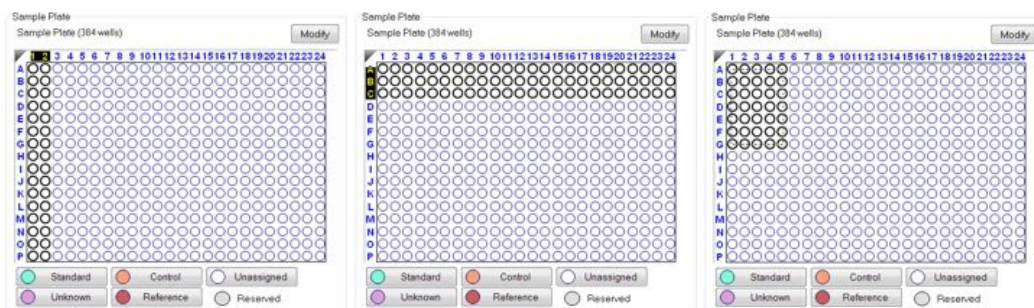


Figure 10-6: Selecting Wells in the Sample Plate Map



NOTE: Shift-clicking in the **Sample Plate Map** mimics the head of the instrument during the selection.

Designating Well Types



NOTE: Sample plate and Reagent plate designations have been renamed **Plate 1** and **Plate 2** in Octet Data Acquisition software versions 8.0 and higher.

In the **Sample Plate Map**, select the wells, right-click and select a sample type. (Figure 10-7).

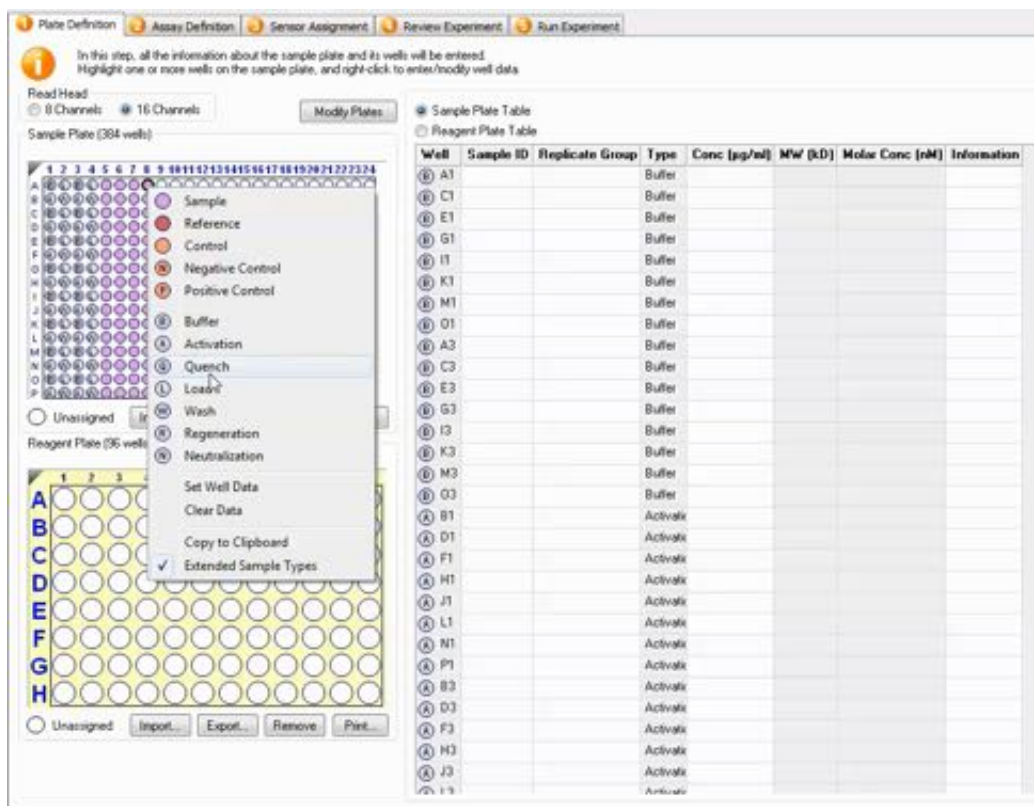


Figure 10-7: Designating a Well Type in the Plate Definition Window

To remove a well designation, in the **Sample Plate Map**, select the well(s) and click **Remove**. Or, right-click the well(s) and select **Clear Data** (see Figure 10-8).

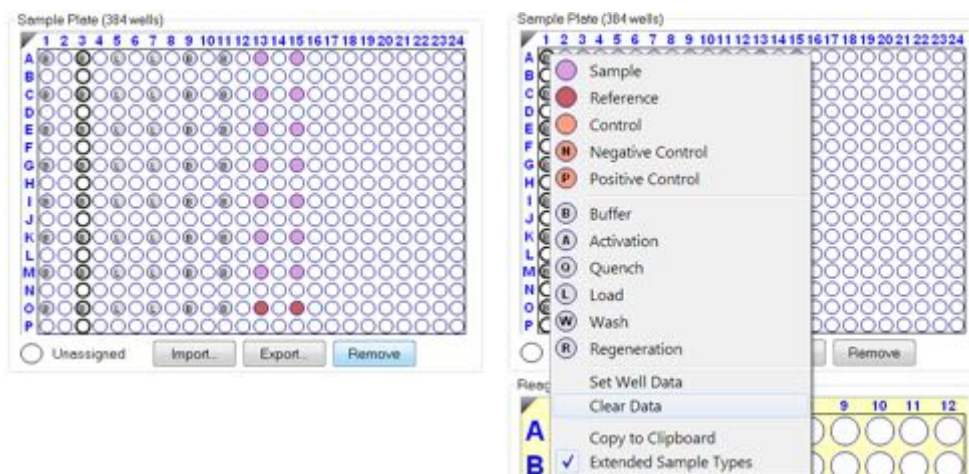


Figure 10-8: Clearing Sample Data from a Sample Plate

Entering Sample Information



NOTE: You must specify sample (analyte) concentration and molecular weight; otherwise, the Octet System Data Acquisition software cannot compute a K_D value. If the sample concentration is not specified, only k_d and k_{obs} are calculated. You can also annotate any well with **Sample ID** or **Well Information**, and assign **Replicate Groups**.

Assigning Molecular Weight and Molar Concentration

1. In the **Sample Plate Map**, select the sample wells, right-click and select **Set Well Data**.
2. In the **Set Well Data** dialog box, enter the analyte molecular and molar concentration (Figure 10-9).

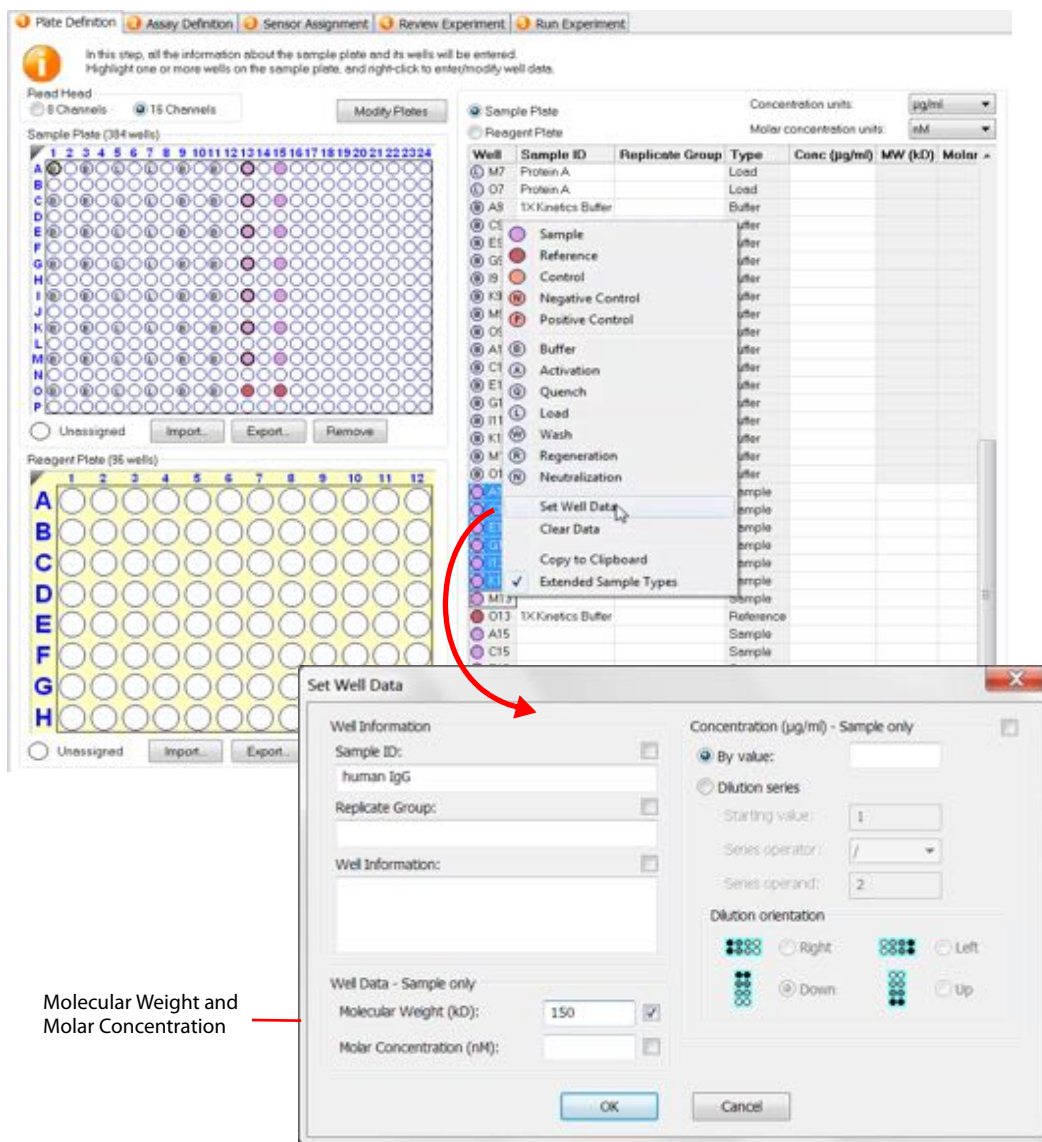


Figure 10-9: Entering Molecular Weight and Molar Concentration from the Sample Plate Map

The information displays in the **Sample Plate Table** (see Figure 10-10).

3. In the **Sample Plate Table**, select the sample concentration units and the molar concentration units.

☒ Sample Plate
☐ Reagent Plate

Concentration units: µg/ml
 Molar concentration units: nM

Concentration units

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	MW (kD)	Molar Conc (nM)	Information
L7	Protein A		Load	12.5			
M7	Protein A		Load	12.5			
O7	Protein A		Load	12.5			
A8	TX Kinetics Buffer		Buffer				
C8	TX Kinetics Buffer		Buffer				
E8	TX Kinetics Buffer		Buffer				
G8	TX Kinetics Buffer		Buffer				
I8	TX Kinetics Buffer		Buffer				
K8	TX Kinetics Buffer		Buffer				
M8	TX Kinetics Buffer		Buffer				
O8	TX Kinetics Buffer		Buffer				
A11	TX Kinetics Buffer		Buffer				
C11	TX Kinetics Buffer		Buffer				
E11	TX Kinetics Buffer		Buffer				
G11	TX Kinetics Buffer		Buffer				
I11	TX Kinetics Buffer		Buffer				
K11	TX Kinetics Buffer		Buffer				
M11	TX Kinetics Buffer		Buffer				
O11	TX Kinetics Buffer		Buffer				
A13	human IgG		Sample	40	150	266.7	
C13	human IgG		Sample	20	150	133.3	
E13	human IgG		Sample	10	150	66.67	
G13	human IgG		Sample	5	150	33.33	
I13	human IgG		Sample	2.5	150	16.67	
K13	human IgG		Sample	1.25	150	8.333	
M13	human IgG		Sample	0.625	150	4.167	
O13	TX Kinetics Buffer		Reference				

Figure 10-10: Entering Molecular Weight and Molar Concentration from the Plate Table

Assigning User Specified Sample Concentrations

To assign sample concentrations using a dilution series:

1. In the **Sample Plate Map**, select the desired wells, right-click and select **Set Well Data**. The **Set Well Data** dialog box displays (see Figure 10-11).
2. Select the **By value** option and enter the starting concentration value.

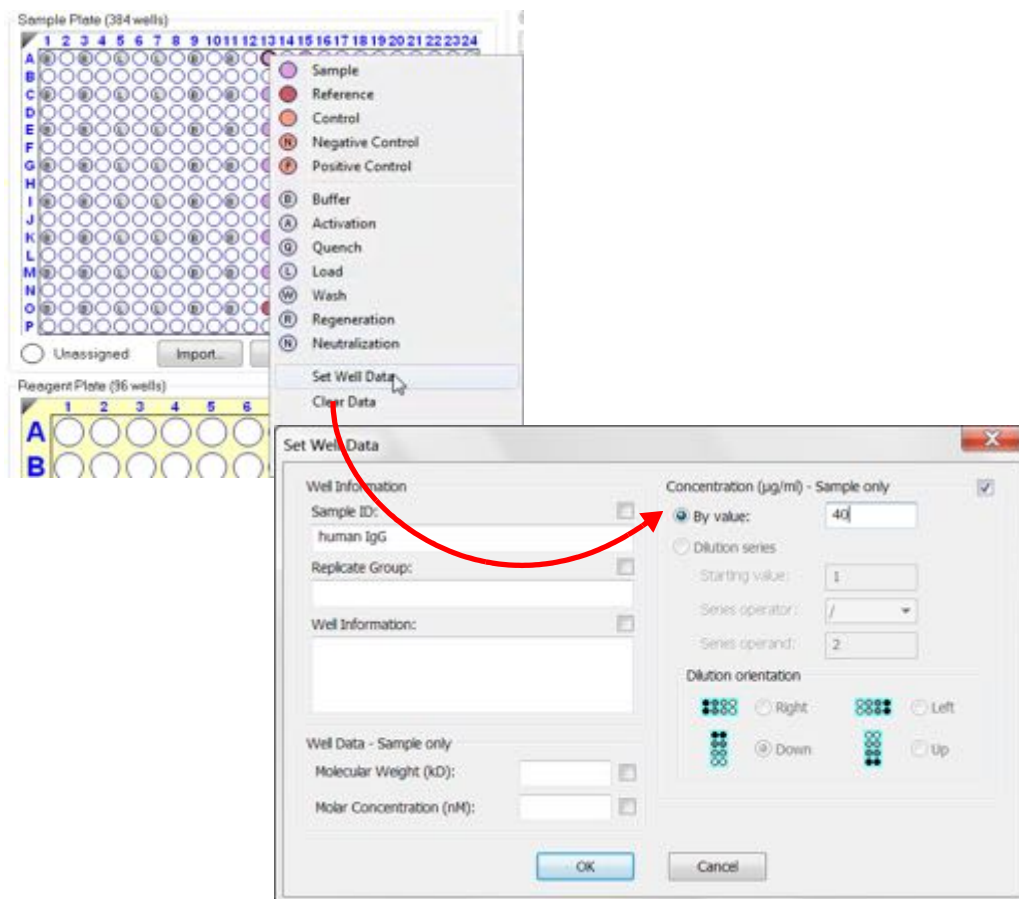


Figure 10-11: Sample Plate Map—Assigning Sample Concentrations by Value

3. Click **OK**. The **Sample Plate Table** will display the entered concentration.

Assigning Concentrations Using a Dilution Series

To assign sample concentrations using a dilution series:

1. In the **Sample Plate Map**, select the wells, right-click, and select **Set Well Data**.
The **Set Well Data** dialog box displays (see Figure 10-12)
2. Select the **Dilution Series** option and enter the starting concentration value.

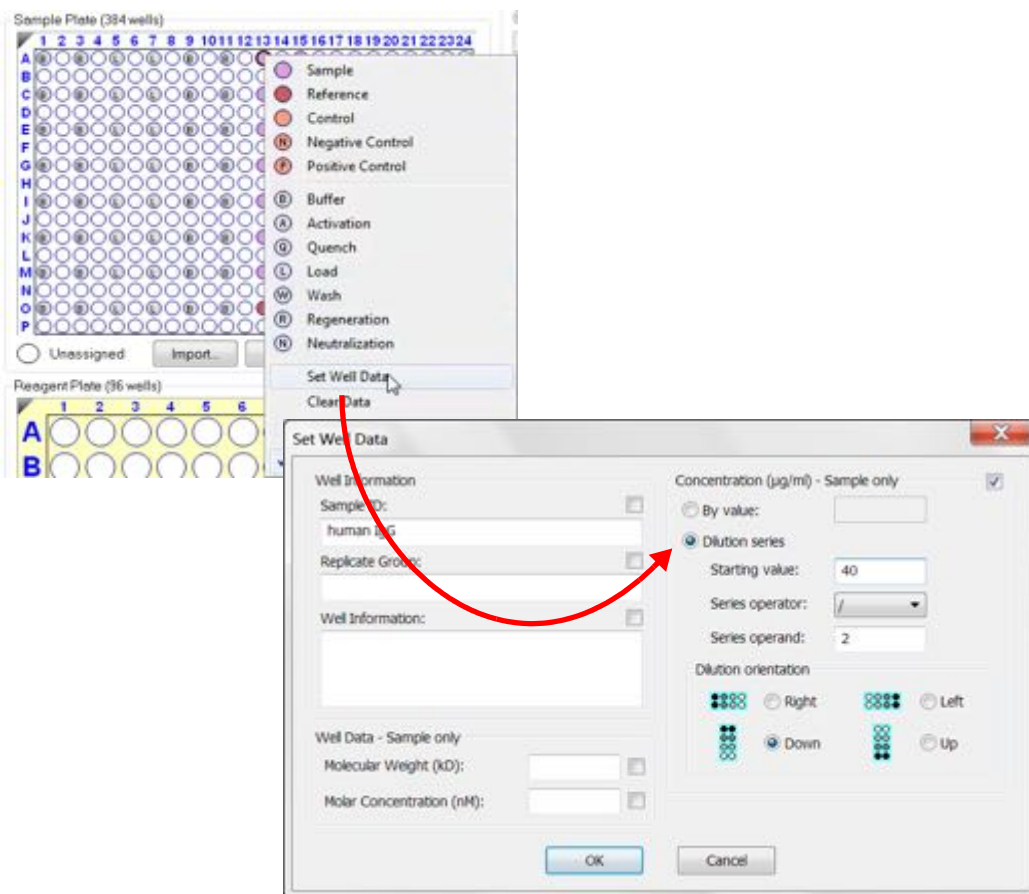


Figure 10-12: Sample Plate Map—Assigning Sample Concentrations Using Dilution Series

3. Select a series operator, enter an operand, and select the appropriate dilution orientation (see Figure 10-13).

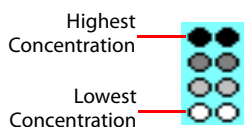


Figure 10-13: Concentration Representation in Dilution Series:

4. Click **OK**.

The **Sample Plate Table** displays the standard concentrations.

Annotating Samples

You can enter annotations (notes) for multiple samples in the **Sample Plate Map** or enter information for an individual sample in the **Sample Plate Table**. For greater clarity, annotation text may be displayed as the legend of the **Runtime Binding Chart** during data acquisition, but annotations must be entered before the experiment is started. If the annotation is entered after the experiment is started, it will not be available for display as a legend.

Annotating Wells in the Sample Plate Map

To annotate one or more wells:

1. In the **Sample Plate Map**, select the samples to annotate, right-click and select **Set Well Data**.
2. In the **Set Well Data** dialog box (see Figure 10-14), enter the **Sample ID** and/or **Well Information** and click **OK**.

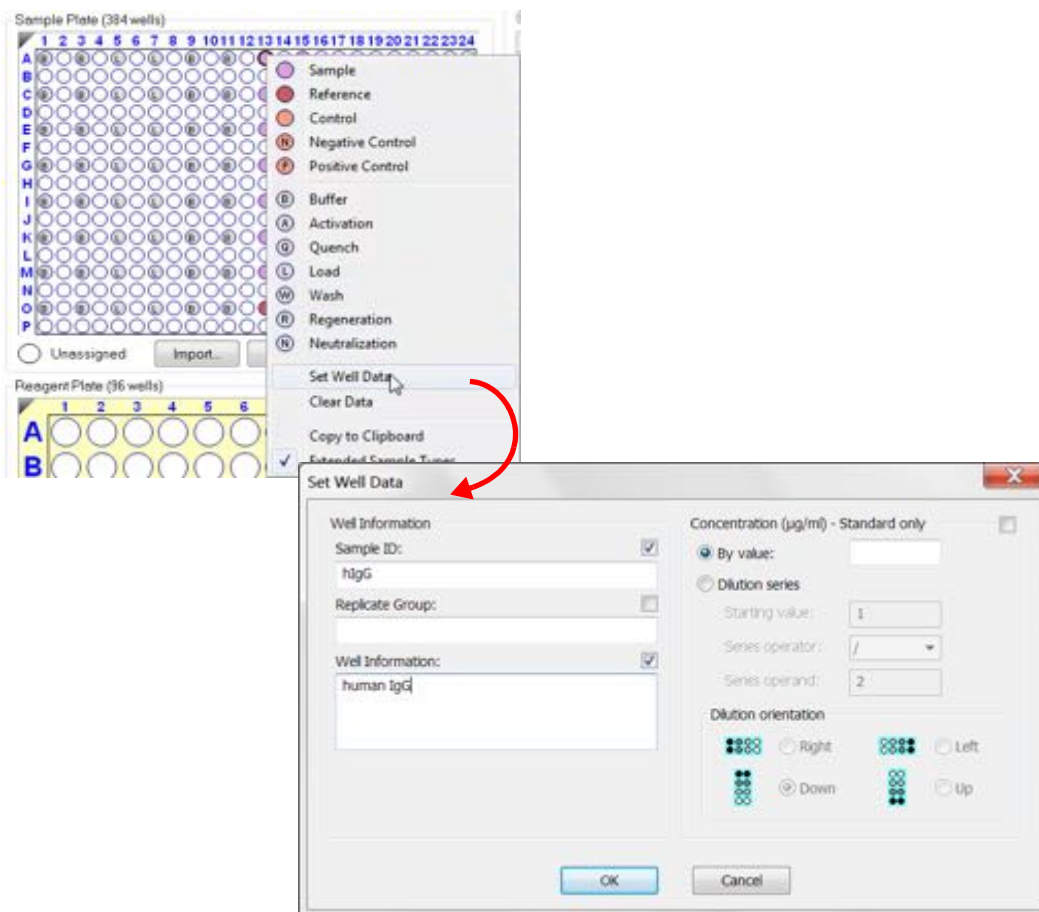


Figure 10-14: Add Sample Annotations from the Sample Plate Map

Annotating Wells in the Sample Plate Table

To annotate an individual well in the **Sample Plate Table**:

1. Double-click the table cell for **Sample ID** or **Well Information**.
2. Enter the desired information in the respective field (see Figure 10-15).



NOTE: A series of Sample IDs may also be assembled in Excel and pasted into the **Sample Plate Table**.

Sample Plate Table

Concentration units: µg/ml Export... Import...

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	Dilution Factor	Information
A1	hlgG		Standard	200	n/a	human IgG
C1			Standard	100	n/a	
E1			Standard	50	n/a	
G1			Standard	25	n/a	
I1			Standard	10	n/a	
K1			Standard	5	n/a	

Figure 10-15: Add Sample Annotations in the Sample Plate Table

Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the **Sample Plate Table**. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the **Sample Plate Map** menu used to designate sample types.

Replicate Groups

Replicate Groups enable data to be organized into custom groups during data analysis (see Figure 10-16).

Index	Include	Color	Sensor Location	Sensor Type	Sensor Info	Replicate Group	Baseline Loc.
20	x		C2	SA (Streptavidin)	3		C3
21	x		C2	SA (Streptavidin)	3		C3
22	x		D2	SA (Streptavidin)	4		D3
23	x		D2	SA (Streptavidin)	4		D3
24	x		E2	SA (Streptavidin)	5		E3
25	x		E2	SA (Streptavidin)	5		E3
26	x		F2	SA (Streptavidin)	6		F3
27	x		F2	SA (Streptavidin)	6		F3
28	x		G2	SA (Streptavidin)	6		G3
29	x		G2	SA (Streptavidin)	6		G3
30	x		H2	SA (Streptavidin)	6		H3
31	x		H2	SA (Streptavidin)	6		H3
32	x		A3	SA (Streptavidin)	1		A3
33	x		A3	SA (Streptavidin)	1		A3
34	x		B3	SA (Streptavidin)	2		B3
35	x		B3	SA (Streptavidin)	2		B3
36	x		C3	SA (Streptavidin)	3		C3
37	x		C3	SA (Streptavidin)	3		C3
38	x		D3	SA (Streptavidin)	4		D3
39	x		D3	SA (Streptavidin)	4		D3

Figure 10-16: Replicate Group Color-Coding



NOTE: Replicate Group information can also be entered in the Octet System Data Analysis software.

Assigning Replicate Groups in the Sample Plate Map

To assign **Replicate Groups** in the **Sample Plate Map**:

1. Select the samples you wish to group, right-click and select **Set Well Data**.
2. In the **Set Well Data** dialog box (see Figure 10-17), enter a name in the **Replicate Group** box and click **OK**.

The screenshot shows the 'Set Well Data' dialog box. The 'Replicate Group' field is highlighted with a red box and contains the text 'Group 1'. Other fields include 'Sample ID' (human IgG), 'Well Information', 'Concentration (µg/ml) - Sample only' (By value: 40), 'Dilution series' (Starting value: 1, Series operator: /, Series operand: 2), and 'Dilution orientation' (Right, Left, Down, Up).

Figure 10-17: Add Replicate Group from the Sample Plate Map

- Repeat the previous steps to assign new samples to the existing **Replicate Group**, or to designate another set of samples to a new **Replicate Group**. Multiple groups can be used in an experiment.



IMPORTANT: The Octet System Data Analysis software will only recognize and group samples that use the same Replicate Group names, spacing and capitalization must be identical. For example, samples assigned to Group 2 and group2 are treated as two groups.

Wells in the **Sample Plate Map** will show color-coded outlines as a visual indication of which wells are in the same group (see Figure 10-18).

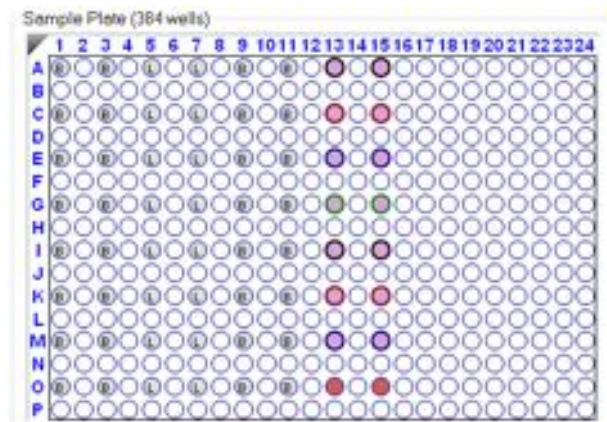


Figure 10-18: Replicate Groups Displayed in Sample Plate Map

The **Sample Plate Table** will update with the **Replicate Group** names entered (see Figure 10-19)

☒ Sample Plate
 ☐ Reagent Plate

Concentration units:
 Molar concentration units:

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	MW (kD)	Molar
M7	Protein A		Load	12.5		
O7	Protein A		Load	12.5		
A8	1X Kinetics Buffer		Buffer			
C8	1X Kinetics Buffer		Buffer			
E8	1X Kinetics Buffer		Buffer			
G8	1X Kinetics Buffer		Buffer			
I8	1X Kinetics Buffer		Buffer			
K8	1X Kinetics Buffer		Buffer			
M8	1X Kinetics Buffer		Buffer			
O8	1X Kinetics Buffer		Buffer			
A11	1X Kinetics Buffer		Buffer			
C11	1X Kinetics Buffer		Buffer			
E11	1X Kinetics Buffer		Buffer			
G11	1X Kinetics Buffer		Buffer			
I11	1X Kinetics Buffer		Buffer			
K11	1X Kinetics Buffer		Buffer			
M11	1X Kinetics Buffer		Buffer			
O11	1X Kinetics Buffer		Buffer			
A13	human IgG	Group 1	Sample	40		
C13	human IgG	Group 2	Sample	20		
E13	human IgG	Group 3	Sample	10		
G13	human IgG	Group 4	Sample	5		
I13	human IgG	Group 5	Sample	2.5		
K13	human IgG	Group 6	Sample	1.25		
M13	human IgG	Group 7	Sample	0.625		
O13	1X Kinetics Buffer		Reference			
A15	human IgG	Group 1	Sample	40		
C15	human IgG	Group 2	Sample	20		
E15	human IgG	Group 3	Sample	10		
G15	human IgG	Group 4	Sample	5		
I15	human IgG	Group 5	Sample	2.5		
K15	human IgG	Group 6	Sample	1.25		
M15	human IgG	Group 7	Sample	0.625		

Figure 10-19: Replicate Groups in Sample Plate Table

Assigning Replicate Groups in the Sample Plate Table

To assign **Replicate Groups** in the **Sample Plate Table**:

1. Double-click the desired cell in the **Replicate Group** table column.
2. Enter a group name (see Figure 10-20).

☒ Sample Plate
 Concentration units:
☐ Reagent Plate
 Molar concentration units:

Well	Sample ID	Replicate Group	Type	Conc (µg/ml)	MW (kD)	Molar
M7	Protein A		Load	12.5		
O7	Protein A		Load	12.5		
A9	1X Kinetics Buffer		Buffer			
C9	1X Kinetics Buffer		Buffer			
E9	1X Kinetics Buffer		Buffer			
G9	1X Kinetics Buffer		Buffer			
I9	1X Kinetics Buffer		Buffer			
K9	1X Kinetics Buffer		Buffer			
M9	1X Kinetics Buffer		Buffer			
O9	1X Kinetics Buffer		Buffer			
A11	1X Kinetics Buffer		Buffer			
C11	1X Kinetics Buffer		Buffer			
E11	1X Kinetics Buffer		Buffer			
G11	1X Kinetics Buffer		Buffer			
I11	1X Kinetics Buffer		Buffer			
K11	1X Kinetics Buffer		Buffer			
M11	1X Kinetics Buffer		Buffer			
O11	1X Kinetics Buffer		Buffer			
A13	human IgG	Group 1	Sample	40		
C13	human IgG	Group 2	Sample	20		
E13	human IgG	Group 3	Sample	10		

Figure 10-20: Add Replicate Group from the Sample Plate Table

Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the **Sample Plate Table**. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the **Sample Plate Map** menu used to designate sample types.

- Repeat the previous steps to assign new samples to the existing **Replicate Group**, or to designate another set of samples to a new **Replicate Group**. Multiple groups can be used in an experiment.



IMPORTANT: The Octet System Data Analysis software will only recognize and group samples that use the same **Replicate Group** names, spacing and capitalization must be identical. For example, samples assigned to **Group 2** and **group2** are treated as two groups.

Editing the Sample Table

Changing Sample Well Designations

To change a well designation, right-click the well in the **Sample Plate Table** and make a new selection (see Figure 10-21).

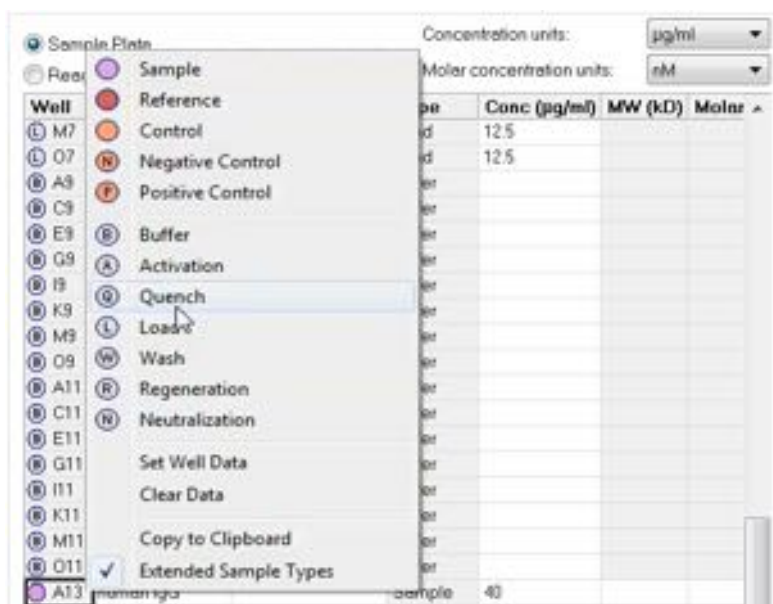


Figure 10-21: Sample Plate Table—Well Designation

Editing Sample Information

To edit sample data in the **Sample Plate Table**, double-click a value and enter a new value (see Figure 10-22).

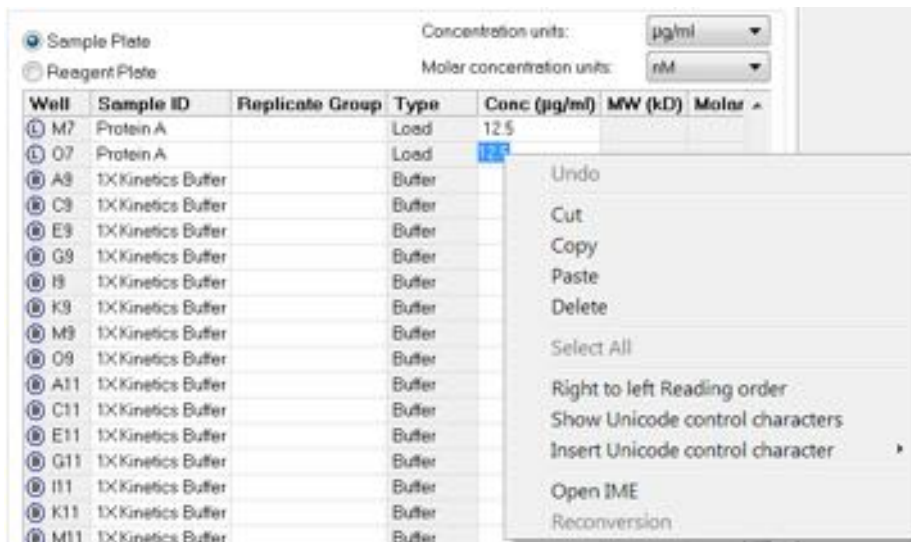


Figure 10-22: Sample Plate Table—Editing Sample Data

Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the **Sample Plate Table**. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.



NOTE: The right-click menu is context-dependent. Right-clicking on a cell where the value is not highlighted and in edit mode opens the right-click menu used to designate sample types.

Managing Sample Plate Definitions



NOTE: After you define a sample plate, you can export and save the plate definition for future use.



NOTE: Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet Data Acquisition software versions 8.0 and higher.

Exporting a Plate Definition

To export a plate definition:

1. In the **Sample Plate Map**, click **Export** (see Figure 10-23).

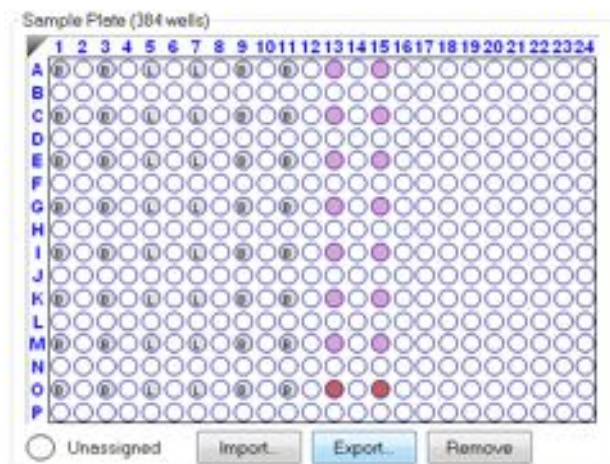


Figure 10-23: Sample Plate Map — Export Button

2. In the **Export Plate Definition** window (see Figure 10-24), select a folder, enter a name for the plate (.csv), and click **Save**.

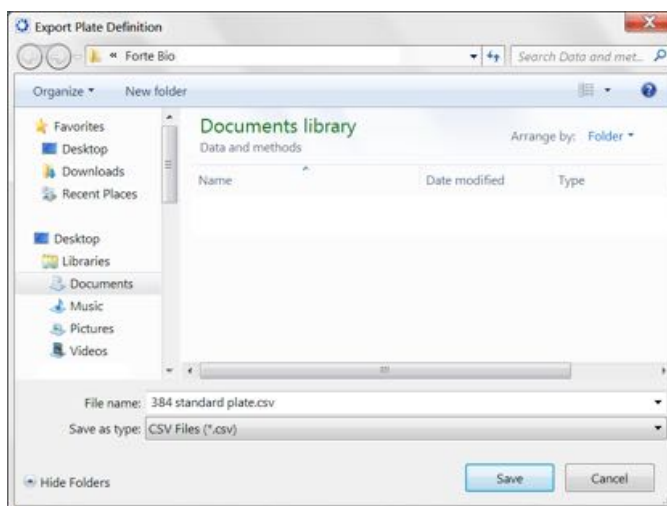


Figure 10-24: Export Plate Definition Window

Importing a Plate Definition

To import a plate definition:

1. In the Plate Definition window (see Figure 10-23: on page 541), click **Import**.

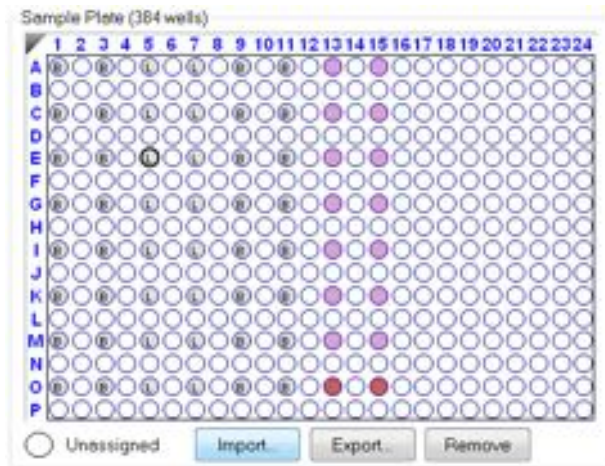


Figure 10-25: Sample Plate Map—Import Button

2. In the **Import Plate Definition** window (see Figure 10-26), select the plate definition (.csv), and click **Open**.

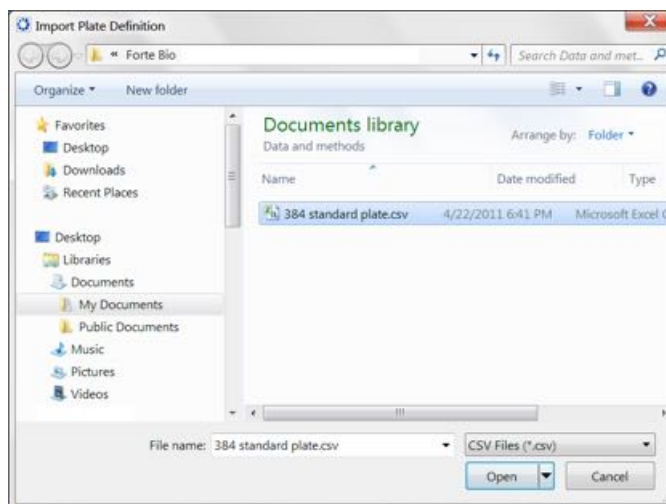


Figure 10-26: Import Plate Definition Window



NOTE: You can also create a .csv file for import. Figure 10-27 shows the appropriate column information layout.

	A	B	C	D	E	F	G	H
1	PlateWells	384						
2	Well	ID	Replicate Group	Group	Concentration (µg/ml)	Molecular Weight (kD)	Molar Concentration (M)	Information
3	A1	1X Kinetics Buffer		Buffer				
4	C1	1X Kinetics Buffer		Buffer				
5	E1	1X Kinetics Buffer		Buffer				
6	G1	1X Kinetics Buffer		Buffer				
7	I1	1X Kinetics Buffer		Buffer				
8	K1	1X Kinetics Buffer		Buffer				
9	M1	1X Kinetics Buffer		Buffer				
10	O1	1X Kinetics Buffer		Buffer				
11	A3	1X Kinetics Buffer		Buffer				
12	C3	1X Kinetics Buffer		Buffer				
13	E3	1X Kinetics Buffer		Buffer				
14	G3	1X Kinetics Buffer		Buffer				
15	I3	1X Kinetics Buffer		Buffer				
16	K3	1X Kinetics Buffer		Buffer				
17	M3	1X Kinetics Buffer		Buffer				
18	O3	1X Kinetics Buffer		Buffer				
19	A5	Protein A		Load	12.5			
20	C5	Protein A		Load	12.5			

Figure 10-27: Example Plate Definition File (.csv)

Printing a Sample Plate Definition

To print a plate definition:

1. In the **Sample Plate/Plate 1 Map** (see Figure 10-28), click **Print**.

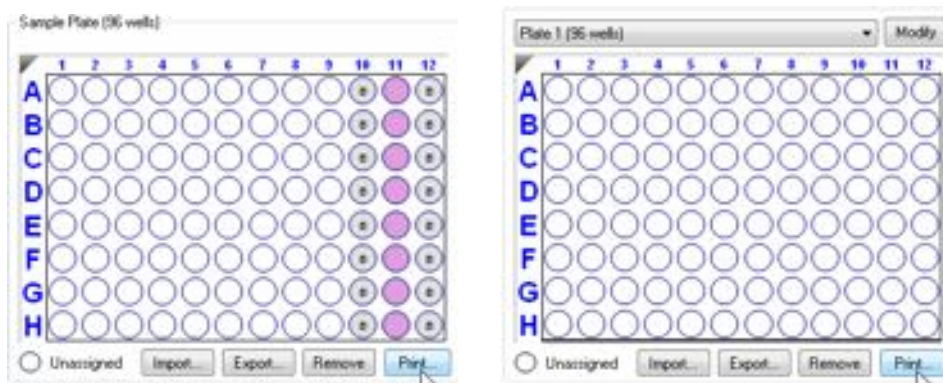


Figure 10-28: Sample Plate/Plate 1 Print Button

The associated **Sample Plate Table** information will print.

Working with a Reagent Plate



NOTE: Sample plate and Reagent plate designations have been renamed Plate 1 and Plate 2 in Octet Data Acquisition software versions 8.0 and higher (Figure 10-29).

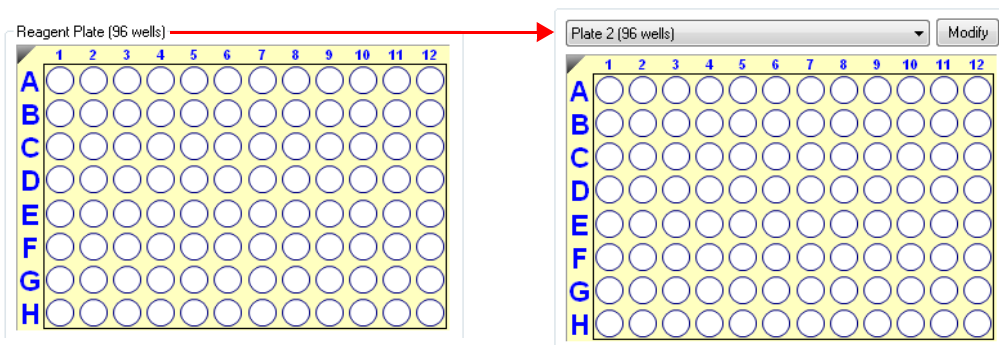


Figure 10-29: Reagent Plate Renamed Plate 2 in Software Versions 8.0 and Higher

You can include an optional reagent plate in a Basic Kinetics experiment. Using a reagent plate enables higher sample throughput since no reagents are included in the sample plate. An experiment can include any combination of sample and reagent plate formats (96- or 384-well). The reagent plate can be used for reagents but not samples, references or controls.



NOTE: Reagent plates can only contain reagents. Samples, references and controls must be assigned to the sample plate.



NOTE: The reagent plate format (96- or 384-well) and the read head configuration (8 or 16 channels) determine the reagent plate layout. For more details, see "Read Head Configuration and Plate Layout" on page 521.

To modify a reagent plate:

2. Click **Modify Plates** above the **Sample Plate Map**. The **Modify Plates** dialog box displays (see Figure 10-30).

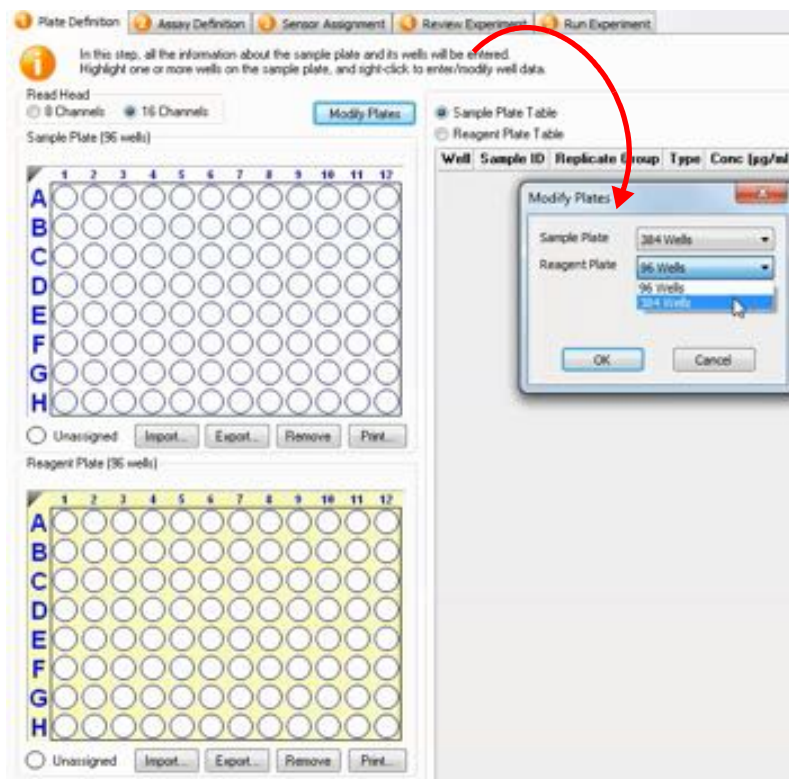


Figure 10-30: Modifying the Reagent Plate

3. Select a reagent plate format (**96 Well** or **384 Well**) and click **OK**.
4. Select the **Reagent Plate** radio button above the plate table. This will display the **Reagent Plate Table**.
5. In the **Reagent Plate Map**, right-click a column to use and select **Buffer**, **Activation**, **Quench**, **Load**, **Wash**, or **Regeneration** from the shortcut menu (see Figure 10-31). The well designations appear in the **Reagent Plate Table**. Repeat this step to define other wells in the reagent plate.

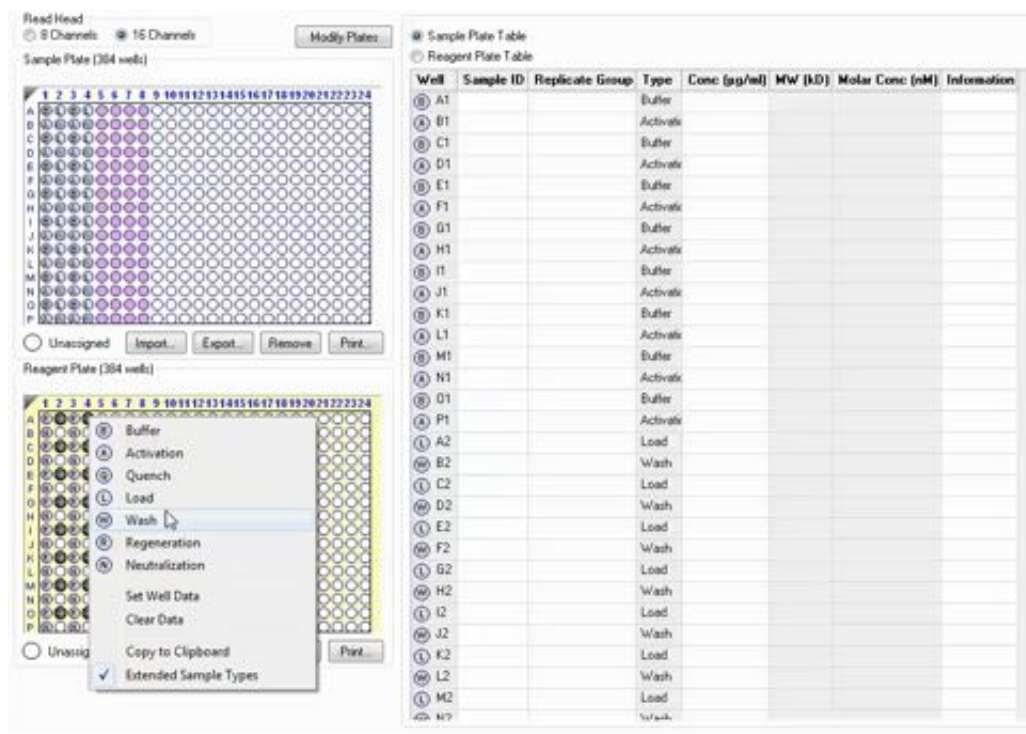


Figure 10-31: Defining Wells in the Reagent Plate

6. Optional: Enter well data or reagent information in the **Reagent Plate Table**.

To remove well designations, select the column(s) and click **Remove**, or right-click and choose **Clear Data**.

Saving a Reagent Plate Definition

Exporting and saving reagent plate definition is done in the same manner as you would for sample plates. For details “Managing Sample Plate Definitions” on page 540.

Printing a Reagent Plate Definition

To print a plate definition:

1. In the **Reagent Plate/Plate 2 Map** (see Figure 10-32), click **Print**.

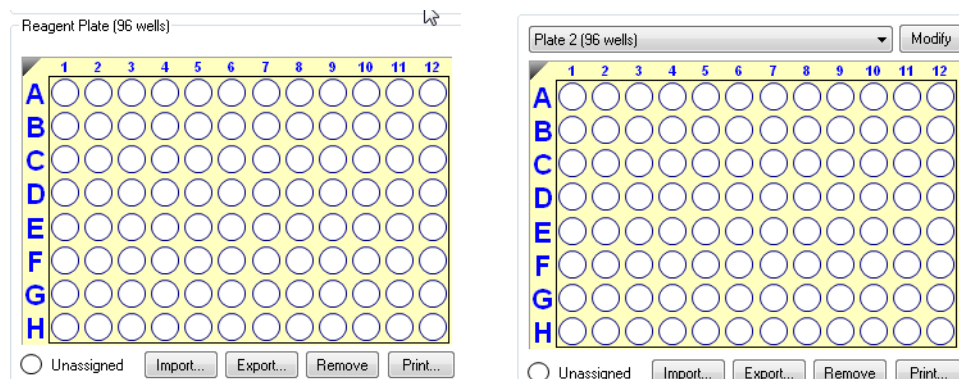


Figure 10-32: Reagent Plate/Plate 2 Print Button

The associated **Reagent Plate Table** information will print.

Defining a Kinetic Assay

After the sample plate is defined, the assay must be defined. The steps to define a kinetic assay include:

Step	See Page
1. Define the step types.	547
2. Build the assay by assigning a step type to a column(s) in the sample plate.	551
3. Save the sample plate definition (optional).	540



Defining Step Types

Table 10-3 lists the example step types to define a kinetic assay. Use these examples as a starting point to create your own step types.


Table 10-3: Sample Step Types for Kinetic Assays .

Step Type	Step Description
Association	Calculates the k_{obs} . Select this step type when binding the second protein of interest (analyte) to the biosensor. This step should be performed at 1,000 rpm.
Dissociation	Calculates the k_d . Select this step type when monitoring the dissociation of the protein complex. This step should be performed at 1,000 rpm.

Table 10-3: Sample Step Types for Kinetic Assays (Continued).

Step Type	Step Description
Baseline	Can be used to align the data. Select this step type when establishing the biosensor baseline in the presence of buffer. This step can be performed with no flow (0 rpm). However, if the baseline step directly precedes an association step, perform the baseline step at 1,000 rpm.
	 IMPORTANT: An assay must include a baseline step followed by a set of association/dissociation steps to be analyzed. The Octet System Data Analysis software recognizes the baseline/association/dissociation step series during processing. Data cannot be processed if this sequence is not included in the assay setup.
Loading	Not used in data analysis. Select this step type when binding the first protein of interest (ligand) to the biosensor.
	 NOTE: This step may be performed offline (outside the Octet instrument).
Activation	Used when employing a reagent to chemically prepare the biosensor for loading.
Quenching	Used to render unreacted immobilization sites on the biosensor inactive.
Regeneration	Used when employing a reagent to chemically regenerate biosensors and remove bound analyte.
Custom	Can be used for an activity not included in any of the above step types.

Creating Step Types

Click the **Assay Definition** tab, or click the  arrow to access the Assay Definition window (see Figure 10-33). The **Step Data List** shows the types of assay steps that are available to build an assay. By default, the list includes a baseline step.

To create different types of assay steps:

1. Click **Add**.
2. In **Assay Step Definition** dialog box (Figure 10-33), specify the step information:
 - a. Choose a step type.
 - b. Optional: Edit the step name.
 - c. Set the step time and shake speed (**Time** range: 2 to 48,000 seconds, **Shake speed** range: 100 to 1,500 rpm or 0).

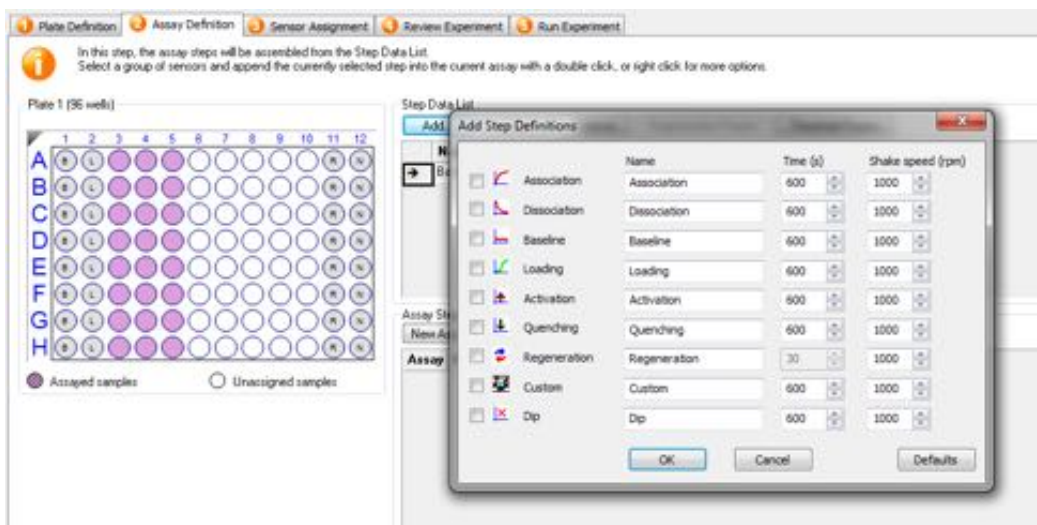


Figure 10-33: Creating an Assay Step Type

3. Apply a threshold to the step:

- a. In the **Step Data List**, click the **Threshold** check box.

The **Threshold Parameters** dialog box displays (see Figure 10-34).

- b. Set the threshold parameters (refer to Table 10-4 for the parameter definitions).

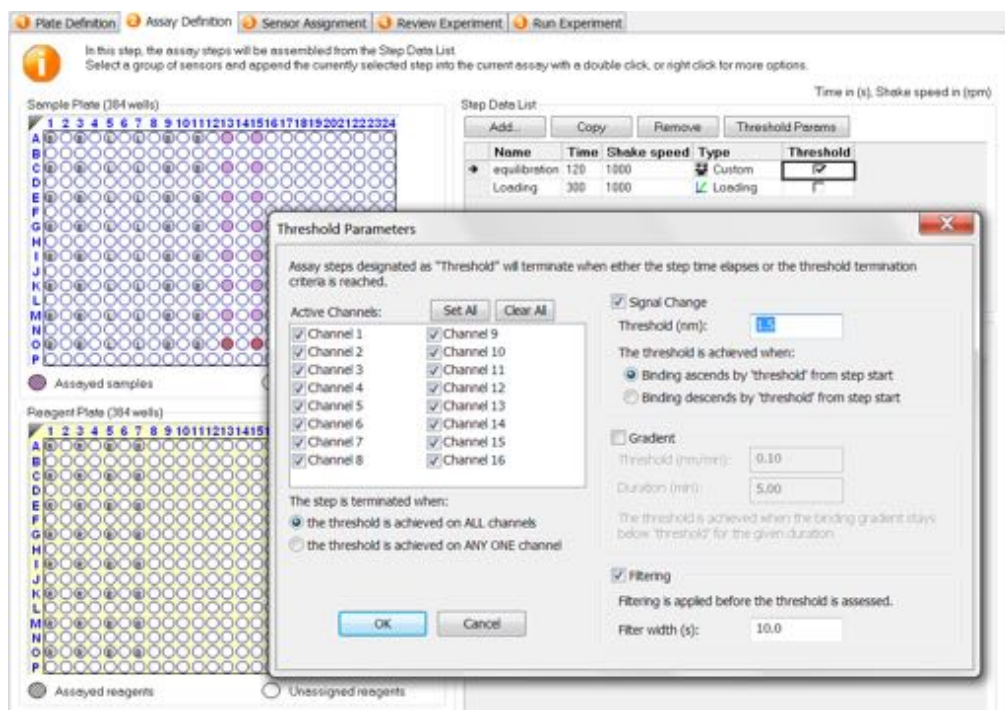


Figure 10-34: Setting Assay Step Threshold Parameters



NOTE: If thresholds are applied, the step is terminated when either the step time elapses or the threshold termination criteria is reached.

Table 10-4: Threshold Parameters

Item	Description
Active Channels	Specifies the instrument channels that monitor the threshold criteria for the assay step. Select an option for terminating the step: <ul style="list-style-type: none"> • The threshold is achieved on ALL channels • The threshold is achieved on ANY ONE channel
Signal Change	The threshold is a user-specified amount of ascending or descending signal change (nm).
Gradient	The threshold is a binding gradient (nm/min) for a user-specified time (min).
Filtering	The amount of data (seconds) to average when computing the signal change or gradient threshold.

- Click **OK** to save the newly-defined step. The new step type appears in the **Step Data List**.
- Repeat the previous steps for each step type to create until all the desired steps are added (see Figure 10-35).

Step Data List

Add... Copy Remove Threshold Params

Name	Time	Shake speed	Type	Threshold
equilibration	120	1000	Custom	<input type="checkbox"/>
Loading	300	1000	Loading	<input checked="" type="checkbox"/>
Baseline	300	1000	Baseline	<input type="checkbox"/>
Association	300	1000	Association	<input type="checkbox"/>
Dissociation	600	1000	Dissociation	<input type="checkbox"/>
Regeneration	20	1000	Custom	<input type="checkbox"/>
Neutralization	20	1000	Custom	<input type="checkbox"/>

Figure 10-35: Step Data List—Displaying Step Types

- To delete a step type from the list, click the corresponding row in the **Step Data List** and click **Remove**, or press the **Delete** key.

Copying and Editing Step Types

To define a step type by copying an existing one, click the step type (row) in the **Step Data List** and click **Copy**. The copied step type appears at the end of the **Step Data List**.

To define a step type by editing an existing one:

1. Double-click the cell in the step's **Name**, **Time**, or **Shake speed** column and then enter a new value. Or, right-click the cell to display a shortcut menu of editing commands (see Figure 10-36, left).



NOTE: Keyboard commands can also be used (**Ctrl+x=cut**, **Ctrl+c=copy**, **Ctrl+v=paste**, **Ctrl+z=undo**).

2. Click the cell in the step's **Type** column, then select another name from the drop-down list (see Figure 10-36, right).

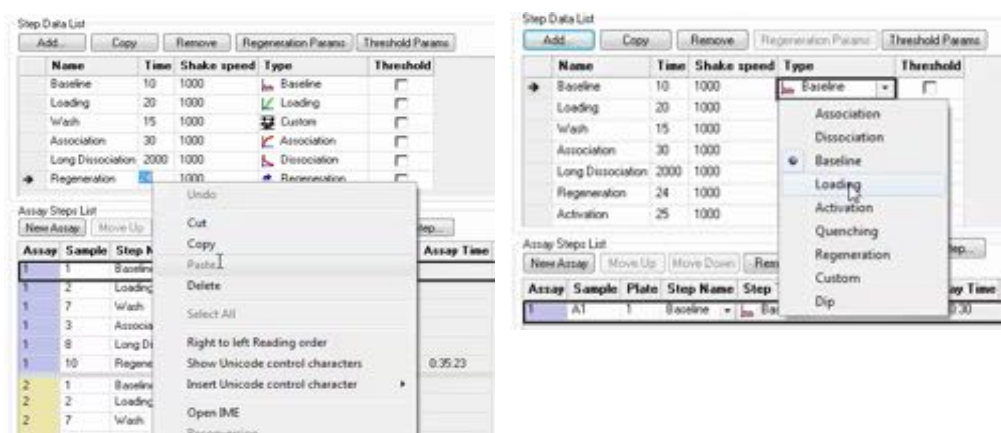


Figure 10-36: Editing a Step Value (left) or Step Type (right)

Building an Assay


After creating the different step types that the assay will use, step types are assigned to columns in the Sample Plate or Reagent Plate maps.

To build an assay:

1. Select a step type in the **Step Data List**.
2. In the **Sample Plate** or **Reagent Plate Map**, double-click the column that is associated with the selected step type. For information about sample or reagent plate wells, mouse over a well to view a tool tip (see Figure 10-37).



Figure 10-37: Tool Tip of Well Information

The selected wells are marked with hatching (for example, ) and the step appears in the **Assay Steps List** (see Figure 10-38) with an associated **Assay Time**.



NOTE: In the **Assay Steps List**, Plate 1 is the Sample Plate and Plate 2 is the Reagent Plate.

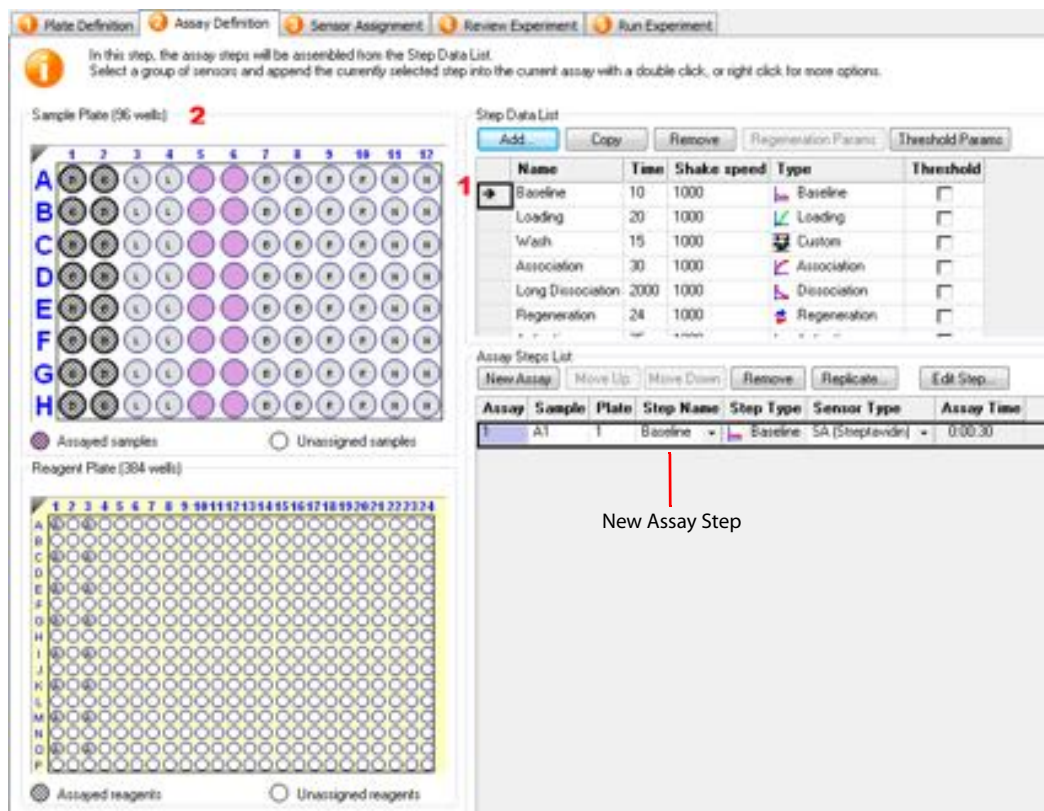


Figure 10-38: Assigning a Step Type to a Column in the Sample Plate

- Repeat the previous steps to define each step in the assay. As each step is added, the total **Experiment** and **Assay Time** update (see Figure 10-39).

Assay Steps List

New Assay Move Up Move Down Remove Replicate... Edit Step...

Assay	Sample	Step Name	Step Type	Sensor Type	Assay Time
1	1	Baseline	Baseline	SA (Streptavidin)	
1	2	Loading	Loading	SA (Streptavidin)	
1	7	Wash	Custom	SA (Streptavidin)	
1	3	Association	Association	SA (Streptavidin)	
1	8	Long Dissociation	Dissociation	SA (Streptavidin)	
1	10	Regeneration	Regeneration	SA (Streptavidin)	0:35:23
2	1	Baseline	Baseline	SA (Streptavidin)	
2	2	Loading	Loading	SA (Streptavidin)	
2	7	Wash	Custom	SA (Streptavidin)	
2	4	Association	Association	SA (Streptavidin)	
2	8	Long Dissociation	Dissociation	SA (Streptavidin)	0:35:15
3	1	Baseline	Baseline	SA (Streptavidin)	
3	2	Loading	Loading	SA (Streptavidin)	
3	7	Wash	Custom	SA (Streptavidin)	
3	5	Association	Association	SA (Streptavidin)	
3	8	Long Dissociation	Dissociation	SA (Streptavidin)	
3	10	Regeneration	Regeneration	SA (Streptavidin)	0:35:23

Total Assay Time

Figure 10-39: Experiment and Assay Time Updates as Steps Are Added to the Assay



IMPORTANT: If you intend to analyze the data from a sample using the **Inter-step correction** feature in the Octet System Data Acquisition software, the assay must use the same well to perform baseline and dissociation for the sample.

Adding a Regeneration Step

1. In the **Sample Plate Map**, assign wells as **Regeneration** or **Neutralization** (Figure 10-40).



Figure 10-40: Regeneration Step

2. Click **Add** (Figure 10-41) to display the Add Step Definition dialog box (Figure 10-42).

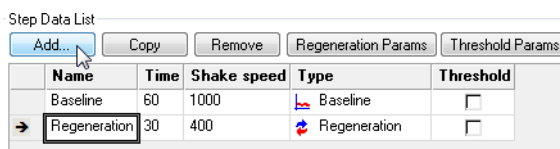


Figure 10-41: Add Button



Figure 10-42: Add Step Definition Dialog Box

3. Select **Regeneration** and click **OK**.
4. Click **Regeneration Params** (Figure 10-43).

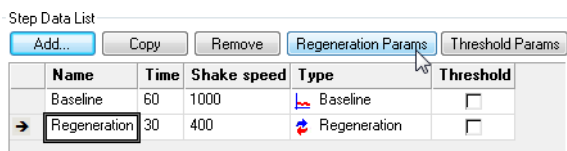


Figure 10-43: Regeneration Params Button

The **Regeneration Parameters** dialog box (Figure 10-44) displays, where you can edit Regeneration parameters as necessary.

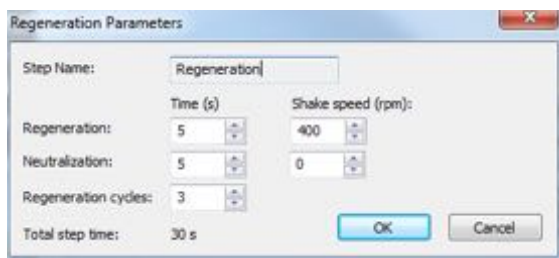


Figure 10-44: Regeneration Parameters Dialog Box

Replicating Steps Within an Assay

To copy steps and add them to an assay:

1. In the **Assay Steps List**, select the step(s) to copy and click **Replicate** (for example, in Figure 10-45, step rows 1–4 are selected).
 - To select adjacent steps, press and hold the **Shift** key while you click the first and last step in the selection.
 - To select non-adjacent steps, press and hold the **Ctrl** key while you click the desired steps.
2. In the **Replicate Steps** dialog box (see Figure 10-45), click the **Append to current assay** option.
3. Click the **Offset steps** check box and set the options, as appropriate. (For more details on offset options, see Table 10-5.)

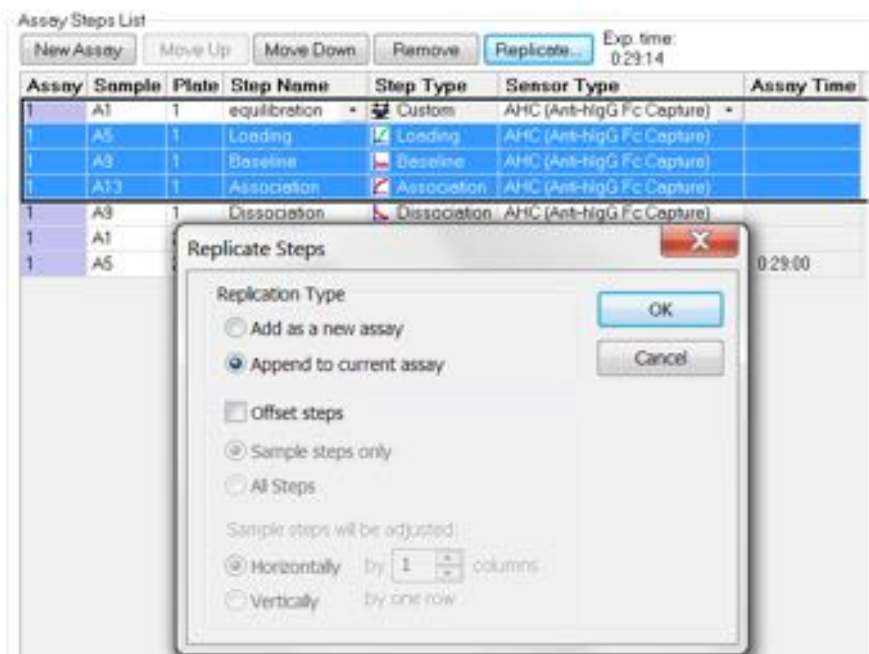


Figure 10-45: Replicating Assay Steps by Appending

- Click **OK**. The step(s) appear at the end of the assay in the **Assay Steps List**.

Table 10-5: Replicate Steps Options .

Item	Description
Add as a new assay	Adds the replicate step(s) as a new assay to the Assay Steps List .
Append to current assay	Adds the replicate step(s) to the end of the current assay.
Offset steps	Assigns the replicate steps to different columns in the sample plate.
Sample steps only	Applies the offset to the sample plate only.
All steps	Applies the offset to the sample plate and reagent plate.
Sample steps will be adjusted horizontally by X columns	Specifies the column in which to add the new step(s). For example, if a step in column 11 is copied and the replicate step should begin in column 12, enter 1. Enter 0 to apply the step(s) to the same columns.
Sample steps will be adjusted vertically by one row	Choose this option to put the replicate step in the same column, but the next row.

Starting a New Assay

A new assay will utilize a new set of biosensors. To start a new assay using the next available sensor column:

1. Select a column in the **Sample Plate Map**.
2. Right-click to view the shortcut menu and select **Start New Assay** (see Figure 10-46).
3. Add steps to the assay as described earlier.



Figure 10-46: Start New Assay

Inserting or Adding an Assay Step

To insert an assay step:

1. Select a step in the **Step Data List**.
2. In the **Assay Steps List**, select the row above where you want to insert the step.
3. In the **Sample Plate Map**, right-click the column to which the step will be applied and select **Insert Assay Step**.

The step is inserted into the **Assay Steps List**.

To add an assay step:

1. Select a step type in the **Step Data List**.
2. In the **Sample Plate Map**, right-click the column to which the step will be applied and select **Add Assay Step**.

The step is added to the end of the **Assay Steps List**.

Selecting a Biosensor for the Assay

To select the biosensor type associated with the assay, click the **Sensor Type** arrow (▼) for any step in the assay and select a sensor type from the drop-down list (Figure 10-47). The biosensor type will automatically update for every assay step.

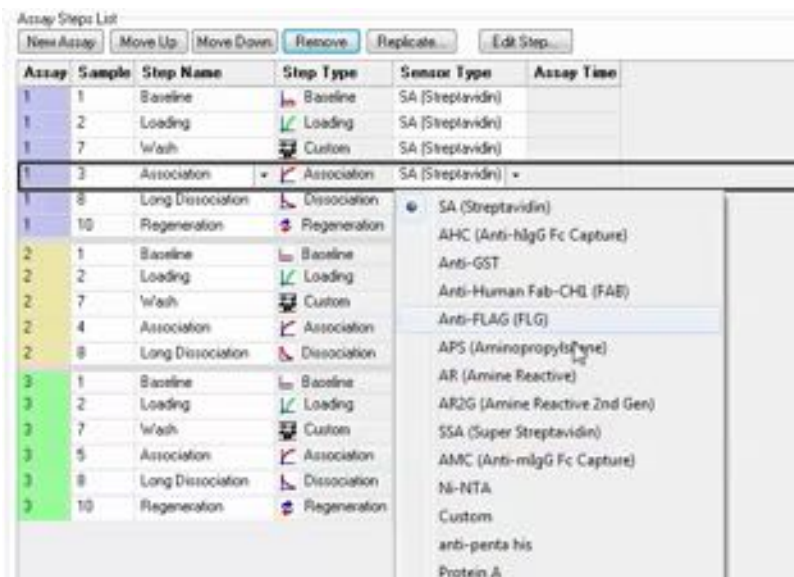


Figure 10-47: Selecting an Assay Sensor Type



NOTE: The **Sensor Type** for the assay must be selected or changed from the **Assay Steps List**. Changing the **Sensor Type** from the **Sensor Assignment Tab** will not update the assay.

Editing an Assay

To edit the step type or the biosensor type:

In the **Assay Steps List**:

- To change the step type, click the **Step Name** arrow (▼) and select a step name from the drop-down list (Figure 10-48, top).
- To change the biosensor type, click the **Sensor Type** arrow (▼) for any step in the assay and select a sensor type from the drop-down list (Figure 10-48, bottom). The biosensor type will automatically update for every assay step.



NOTE: The **Step Name** drop-down list includes only the step types defined in the **Step Data List**.

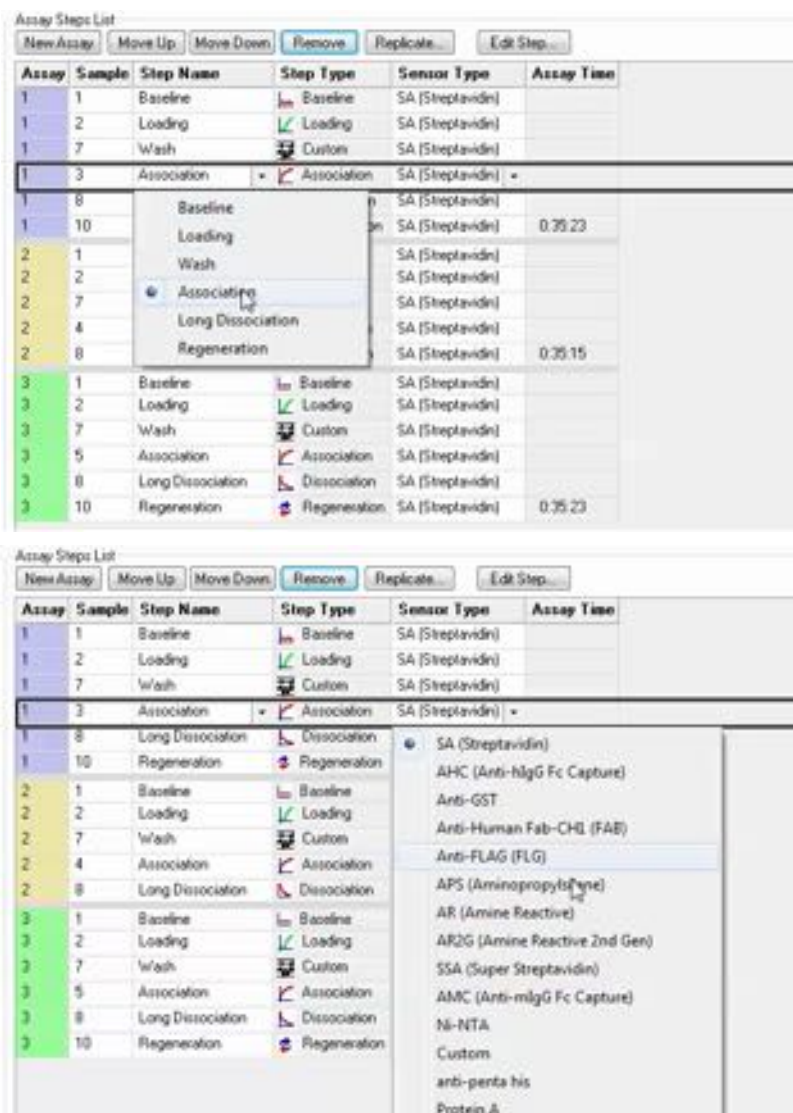


Figure 10-48: Editing an Assay Step Name (top) or Sensor Type (bottom) in the Assay Steps List

To reorder or remove an assay step:

1. Select a step (row) in the **Assay Steps List**.
2. Click the **Move Up**, **Move Down**, or **Remove** button located above the list.



IMPORTANT: An assay must have a baseline step followed by a set of association/dissociation steps to be analyzed. Octet System Data Analysis software recognizes the baseline/association/dissociation set of steps.

Adding an Assay Through Replication

A sample plate can include multiple assays that are the same (replicates) or different. Each assay utilizes a new set of biosensors. Replicates within a single assay will therefore use the same biosensor and replicates in different assays will use different biosensors.

To add a replicate assay to a plate:

1. In the **Assay Steps List**, select the steps to copy and click **Replicate**.
 - To select adjacent steps, press and hold the **Shift** key while you click the first and last step in the selection.
 - To select non-adjacent steps, press and hold the **Ctrl** key while you click the steps.
2. In the **Replicate Steps** dialog box, click the **Add as a new assay** option (Figure 10-49).

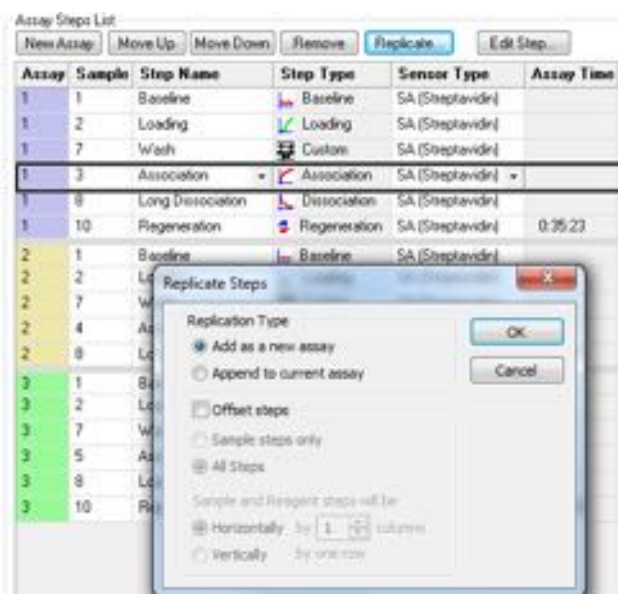



Figure 10-49: Adding a Replicate Assay to a Plate

3. Click the **Offset steps** check box and set the options as appropriate (see Table 10-5 on page 556 for more information). If the replicate assay uses the same sample columns as the original assay, do not choose the **Offset steps** option. If the replicate assay uses a different sample column, select **Offset steps** and the appropriate options.
 - **Sample steps only** offsets the sample wells by the value specified under **Sample steps will be adjusted**. The offset will not be applied to reagent wells such as buffer, loading, regeneration, neutralization and detection.
 - **All Steps** offsets all wells in the assay, including sample and reagent wells, by the value specified under **Sample steps will be adjusted**.
4. Click **OK**. The new assay appears in the **Assay Steps List**.
5. Continue to add assay steps as needed.

Assigning Biosensors to Samples

After you define the sample plate and assay(s), click the **Sensor Assignment** tab or click the arrow  to access the Sensor Assignment window. The color-coded **Sensor Tray** and **Sample Plate Map** show the locations of the biosensors associated with the samples (Figure 10-50).



NOTE: When using a 96-well plate with the 8 channel read head, do not put biosensors in columns 2, 4, 6, 8, 10, and 12 if the biosensors will be returned to the biosensor tray and not discarded. If the biosensors will be ejected, biosensors can be placed in all columns.



NOTE: If an experiment includes more than one type of biosensor, the software automatically creates a separate sensor tray for each type of biosensor. If the different types of biosensors are in the same tray, change the biosensor type as appropriate.

The biosensor types shown in the **Sensor Type** table column are those designated during the kinetics assay definition. In the example shown in Figure 10-50, the experiment includes two assays in the same wells. The use of those wells by two different biosensors is indicated by the pie chart colors.



NOTE: The **Sensor Type** for the assay must be selected or changed from the **Assay Steps List** in the **Assay Definition Tab**. Changing the **Sensor Type** from the **Sensor Assignment Tab** will not update the assay.

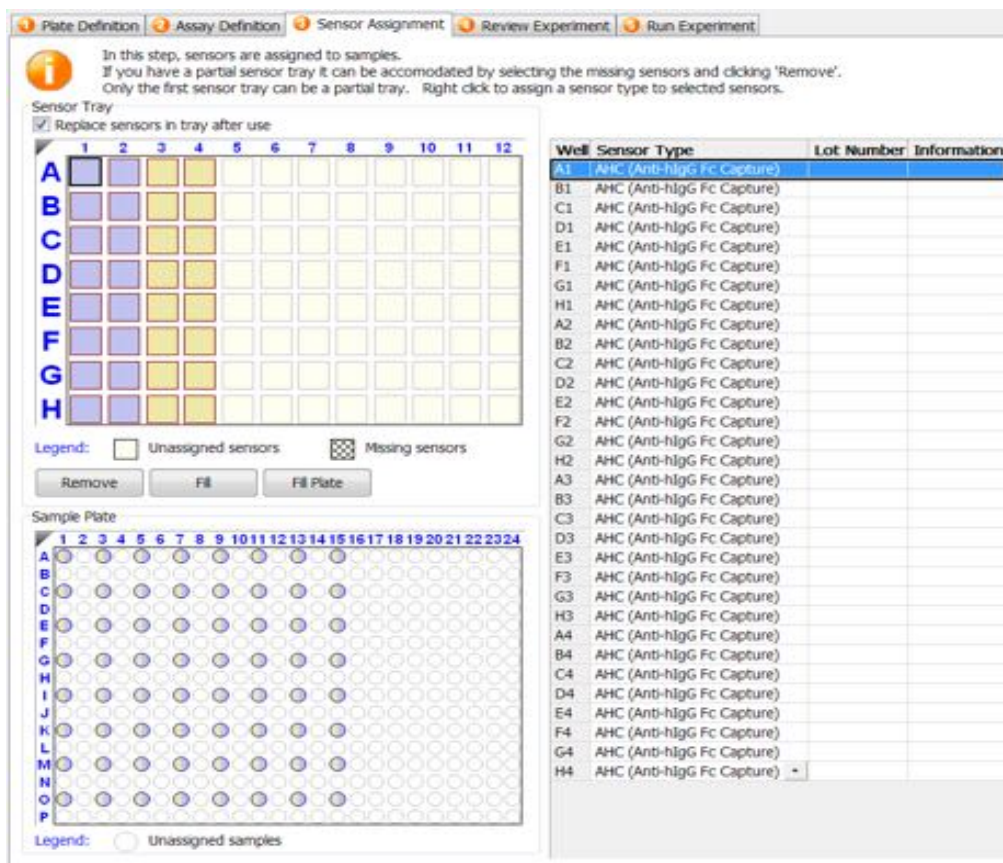


Figure 10-50: Sensor Assignment Window

Hover the cursor over a well in the **Sensor Tray Map** or **Sample Plate Map** to display a tool tip with sample or biosensor information (see Figure 10-51).

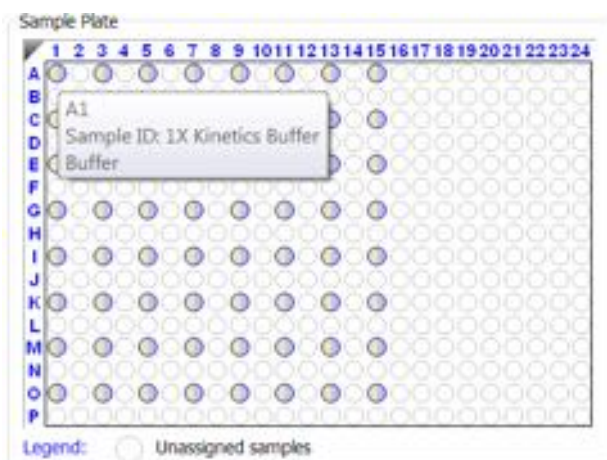


Figure 10-51: Tool Tip of Well Information

Replacing the Biosensors in the Biosensor Tray

After an assay is completed, the biosensors can be returned to the biosensor tray or ejected through the biosensor chute to an appropriate waste container. To return the biosensors to the tray, click the **Replace sensors in tray after use** check box (see Figure 10-52).

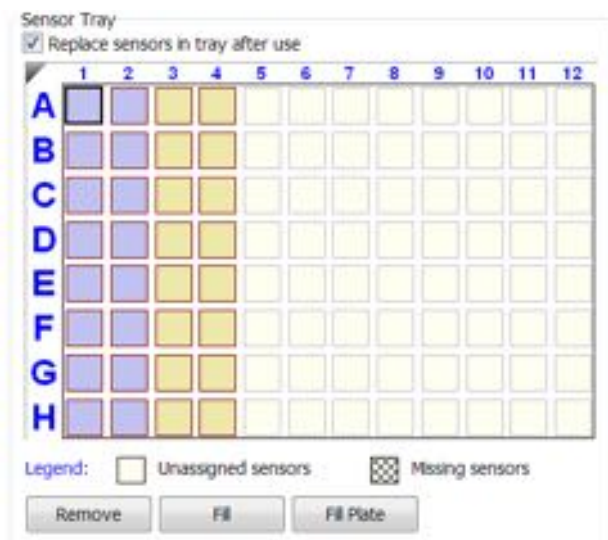


Figure 10-52: Replace Sensors in Tray After Use Check Box



NOTE: Biosensors can be regenerated up to a max of 11 times per experiment.

Entering Biosensor Information

To enter information about a biosensor:

1. Optional: Double-click in any cell in the **Lot Number** column to enter the biosensor lot number. All wells in the **Lot Number** column for that biosensor type will automatically populate with the lot number entered (see Figure 10-53).
2. Optional: Double-click a cell in the **Information** table column. Enter or edit the biosensor information as appropriate (see Figure 10-53).



NOTE: Edit commands (**Cut**, **Copy**, **Paste**, **Delete**) and shortcut keys (**Cut** - **Ctrl+x**, **Copy** - **Ctrl+c**, **Paste** - **Ctrl+v**, **Undo** - **Ctrl+z**) are available in the table. To view edit commands, double-click the cell. This highlights the value and allows it to be edited. Next, right-click to view the edit menu.

Well	Sensor Type	Lot Number	Information
A1	AHC (Anti-hIgG Fc Capture)	10102020	Default Biosensor
B1	AHC (Anti-hIgG Fc Capture)	10102020	
C1	AHC (Anti-hIgG Fc Capture)	10102020	
D1	AHC (Anti-hIgG Fc Capture)	10102020	
E1	AHC (Anti-hIgG Fc Capture)	10102020	
F1	AHC (Anti-hIgG Fc Capture)	10102020	
G1	AHC (Anti-hIgG Fc Capture)	10102020	
H1	AHC (Anti-hIgG Fc Capture)	10102020	
A2	AHC (Anti-hIgG Fc Capture)	10102020	
B2	AHC (Anti-hIgG Fc Capture)	10102020	
C2	AHC (Anti-hIgG Fc Capture)	10102020	
D2	AHC (Anti-hIgG Fc Capture)	10102020	
E2	AHC (Anti-hIgG Fc Capture)	10102020	
F2	AHC (Anti-hIgG Fc Capture)	10102020	
G2	AHC (Anti-hIgG Fc Capture)	10102020	
H2	AHC (Anti-hIgG Fc Capture)	10102020	

- Undo
- Cut
- Copy
- Paste
- Delete
- Select All
- Right to left Reading order
- Show Unicode control characters
- Insert Unicode control character
- Open IME
- Reconversion

Figure 10-53: Entering or Editing Biosensor Information

Changing the Biosensor Location

If you prefer to not use the default biosensor columns, you can select other column(s) to use. There are two ways to do this:

- **Method 1**—In the **Sensor Tray Map**, **Remove** the columns you do not want to use. The software automatically selects the next available column(s).
- **Method 2**—**Remove** all columns from the **Sensor Tray Map**, then select the columns you want to use.

Method 1

In the **Sensor Tray Map**, select the columns to not use and click **Remove**. Or, right-click the selection and select **Remove** (Figure 10-54 left). The software automatically selects the next available biosensor columns in the tray (Figure 10-54 right).

Click **Fill Plate** to return the **Sensor Tray Map** to the default layout.

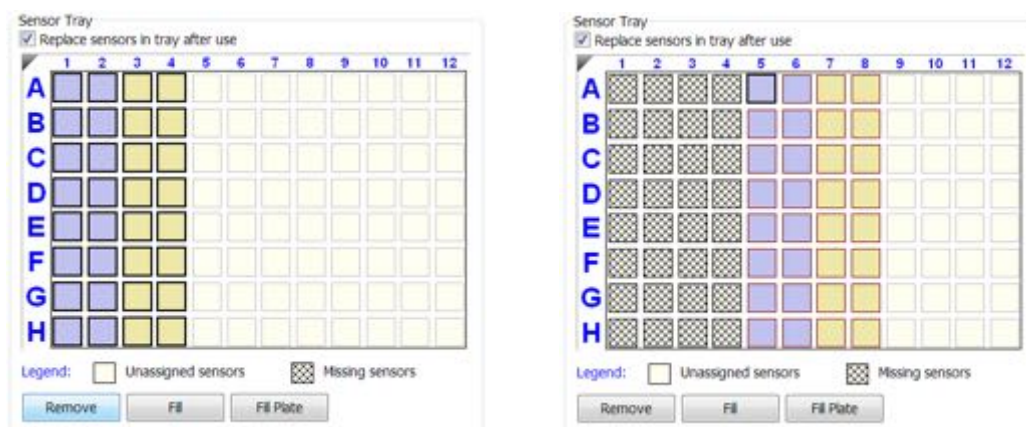


Figure 10-54: Changing Biosensor Location (Method 1)

Method 2

1. In the **Sensor Tray Map**, select all of the columns and click **Remove** (Figure 10-55 top left). Or, right-click the selection and select **Remove**. All columns will be shown as **Missing** (Figure 10-55 top right).
2. Select the column(s) to use and click **Fill**. Or, right-click the selection and select **Fill** (Figure 10-55 bottom left). The software fills the selected columns in the tray (Figure 10-55 bottom right).



Figure 10-55: Changing Biosensor Location (Method 2)

Click **Fill Plate** to return the **Sensor Tray Map** to the default layout.

Using Heterogeneous Trays

If heterogeneous biosensor trays will be used, the column location of each biosensor type in the tray can be identified in the **Sensor Assignment Tab**. Assignment of biosensors that will not be used in the assay enables the software to auto-assign the biosensors that will be used in the assay by biosensor type.

There are two ways to change the biosensor type:

- Select a column in the **Sensor Tray Map**, right-click and select a biosensor type from the drop-down list (Figure 10-56 left). The associated wells in the **Sensor Type** column will automatically populate with the biosensor type selected.
- Select a cell in the **Sensor Type** table column, click the down arrow and select a biosensor type from the drop-down list (Figure 10-56 right). All other wells in the same column of the **Sensor Tray Map** as the selected cell will automatically populate with the biosensor type selected.

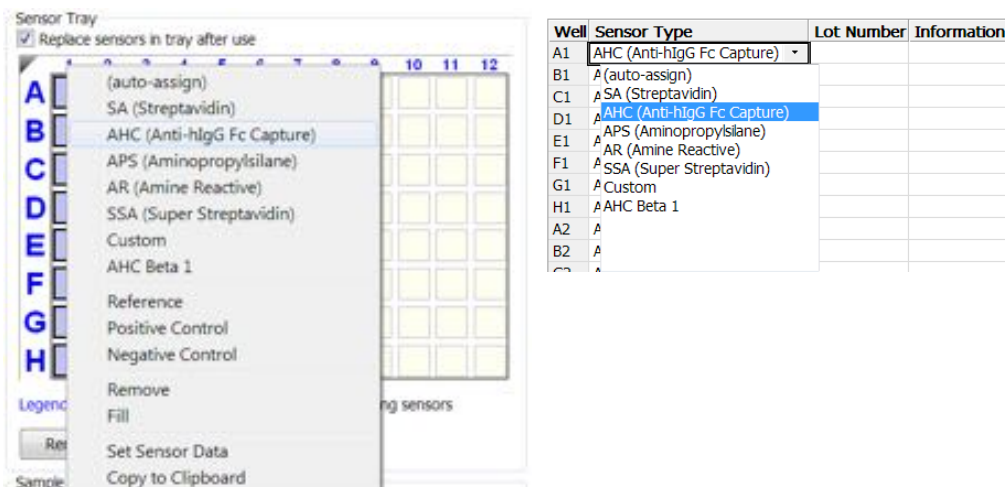


Figure 10-56: Sensor Assignment Window—Changing the Biosensor Type

The biosensor types shown in the **Sensor Assignment** window were specified previously in the **Assay Definition** window, and default locations are assigned automatically. To assign biosensor types for heterogeneous trays:

1. Select the column location of the biosensor type (see Figure 10-57).



Figure 10-57: Selecting a Sensor Tray Column

- 2. Right-click in the **Sensor Tray Map** or click in a cell in the **Sensor Type** table column and select a biosensor type from the drop-down list. The biosensor type associated with the assay will shift location accordingly (see Figure 10-58). In the example shown, AHC is the **Sensor Type** used for the current assay. Columns 1 and 2 were reassigned as Streptavidin according to the heterogeneous tray being used.

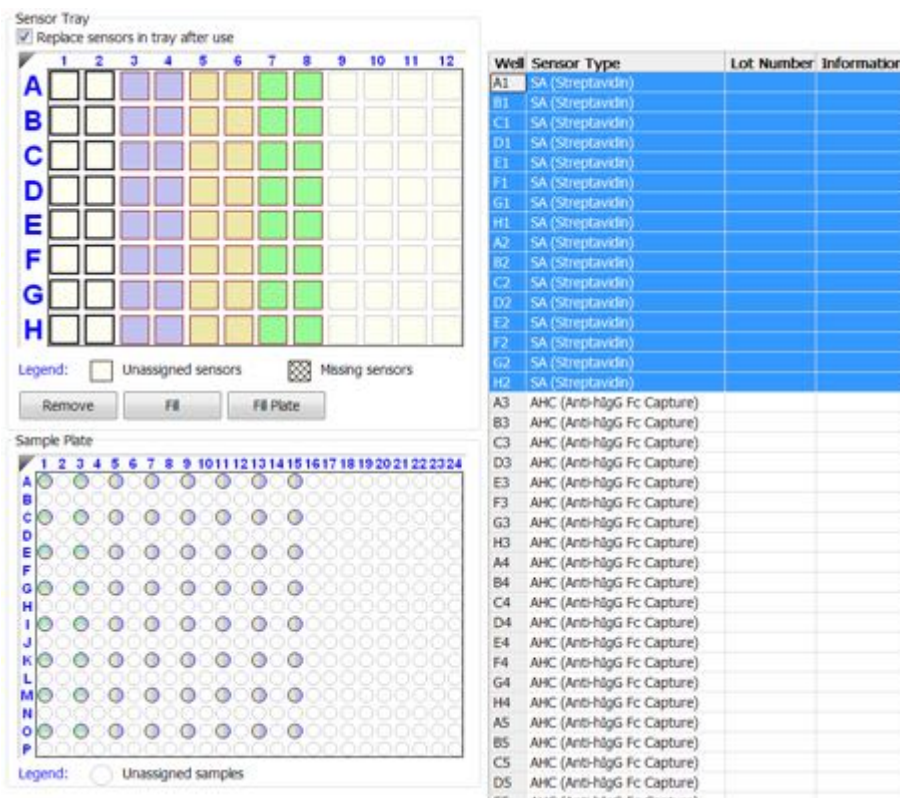


Figure 10-58: Assay Sensor Type Reassignment

- Repeat the previous steps to assign locations for the remaining biosensor types in the tray.



IMPORTANT: Ensure that the biosensor types selected in the **Assay Definition** window have assigned column(s) in the **Sensor Assignment** window or the experiment cannot be run.

Using Partial Biosensor Trays

If you remove biosensors from the **Sensor Tray Map** and there are not enough remaining biosensors for the experiment, the software automatically adds a second tray of biosensors and assigns the biosensors that are required for the assay(s).

The experiment in the example shown in (Figure 10-59) includes two assays, and Tray 1 does not include enough biosensors for the experiment. To view the additional biosensor tray that is required for the assay, select Tray 2 from the **Sensor Tray** drop-down list (Figure 10-59 top). The **Sensor Tray Map** will then display the additional biosensors required for the assay (Figure 10-59 bottom). If necessary, change the location of these biosensors.

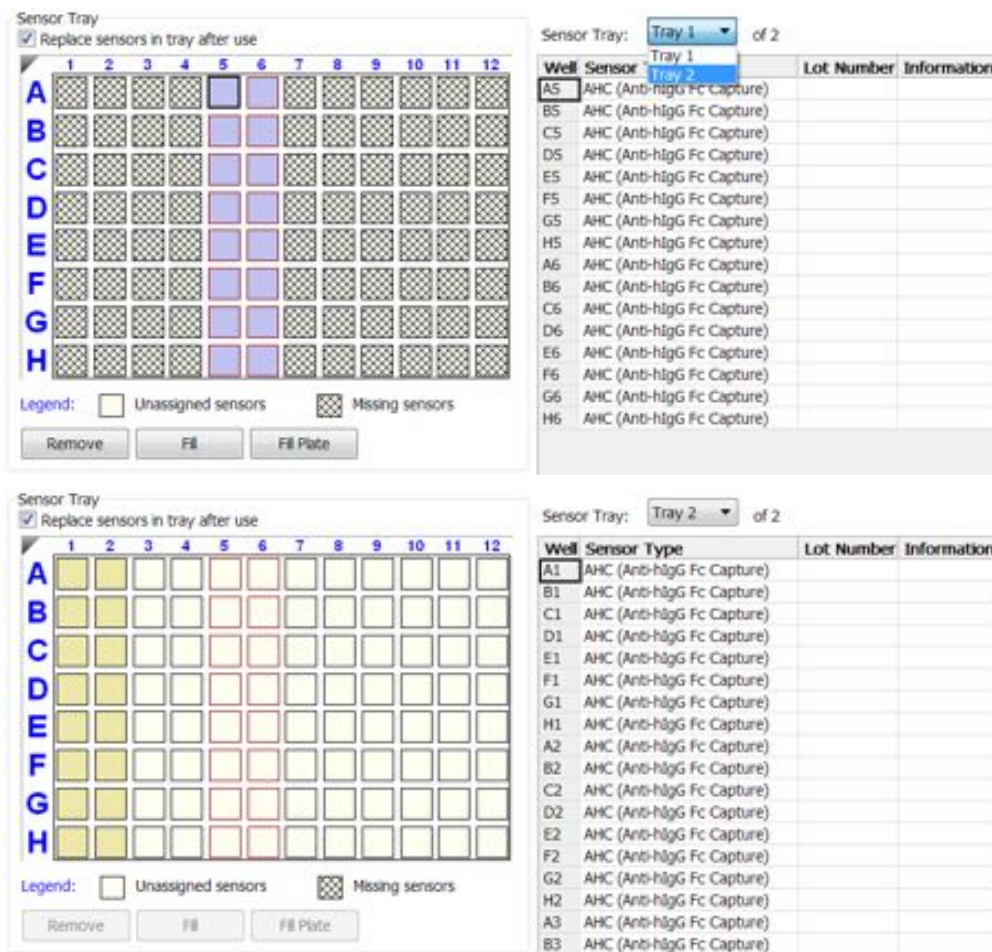


Figure 10-59: Example Experiment Using Two Biosensor Trays



NOTE: Up to two trays may be used per assay, but only the first biosensor tray can be a partial tray. During the experiment run, the software prompts you to insert the appropriate tray in the Octet instrument.

Reference Biosensors

To designate reference biosensors, select the desired biosensors in the **Sensor Tray Map**, right-click and select **Reference**. The reference biosensors are marked with an R.



NOTE: Reference biosensors may also be designated in the **Runtime Binding Chart** during acquisition.

Changing the Biosensor Type

The biosensor type used in the assay must be selected in the **Assay Definition** window. To change the biosensor type:

1. Click the **Assay Definition Tab**.
2. In the **Assay Steps List**, click the cell in the **Sensor Type** column to change.
3. Select from the drop-down list (see Figure 10-60).



IMPORTANT: Ensure that the same biosensor types are selected in both the Assay Definition and the Sensor Assignment windows or the experiment cannot be run.

Assay Steps List

Exp. time: 0:29:14



Assay	Sample	Plate	Step Name	Step Type	Sensor Type	Assay Time
1	A1	1	equilibration	Custom	AHC (Anti-hlgG Fc Capture)	
1	A5	1	Loading	Loading	SA (Streptavidin)	
1	A9	1	Baseline	Baseline	AHC (Anti-hlgG Fc Capture)	
1	A13	1	Association	Association	APS (Aminopropylsilane)	
1	A9	1	Dissociation	Dissociation	AR (Amine Reactive)	
1	A1	2	Regeneration	Custom	SSA (Super Streptavidin)	
1	A5	2	Neutralization	Custom	Custom	
					AHC Beta 1	0:29:00

Figure 10-60: Assay Definition Window—Changing the Biosensor Type

STARTING A BASIC KINETICS EXPERIMENT: OCTET HTX

The user-selectable Read Head can be used for kinetic experiments and provides the flexibility to choose multiple configurations in a single experiment, or given Method file (*.fmf). After starting the Octet HTX system and the Octet Data Acquisition software, follow the steps below to set up and run a kinetic experiment with multiple Read Head configurations.

Table 10-6: Octet Data Acquisition Steps for Kinetic Assays

Octet Software	Functions
Data Acquisition 	<ol style="list-style-type: none"> 1. Select a kinetics experiment in the Experiment Wizard. Open a method template from the Experiment Menu or open an existing method file (*.fmf). <hr/> <div>  <p>NOTE: In the Experiment Menu, the Templates command allows users to pick from a set of predefined method templates for Kinetic, Quantitation, or Epitope Binning experiments. Users may also modify existing method templates to suit their experimental conditions and save as a new method file and new method file name.</p> </div> <hr/> <ol style="list-style-type: none"> 2. Define a sample plate or open a sample plate definition. 3. Specify assay steps. 4. Assign biosensors to samples. 5. Run the experiment.

Starting an Experiment

You can start a kinetics experiment using one of the following options:

- Launch the **Experiment Wizard** by clicking **Experiment > New Experiment Wizard**, and selecting **New Kinetics Experiment**.
- Open an existing method file (.fmf) by clicking **File > Open Method File**. Method files may be saved and recalled using the File menu and are automatically saved when an experiment is run.
- On the menu bar, click **Experiment > Templates > Kinetics**.

Enter the required information on Tabs 1-5 of the Basic Kinetics Experiment.

Read Head Configuration and Plate Layout

The Octet HTX has a user-selectable Read Head for monitoring 8, 16, 32, 48, or 96 wells in parallel so you can tailor your assay design to maximize either throughput or detection sensitivity.

The 96 biosensor mode uses multiplexer switching to read 96 wells simultaneously either in a 96- or 384-well plate, with similar sensitivity as the Octet QK384 system. Large sample sets are analyzed in the shortest amount of time using this Read Head setting, which is also ideal for rapid, whole plate analysis and biosensor loading in multi-step assays.

Figure 10-61 shows the biosensor layout in a 96- and 384-well plate with the 96-channels Read Head setting. Biosensors interrogate 96 wells in 12 columns at the same time.

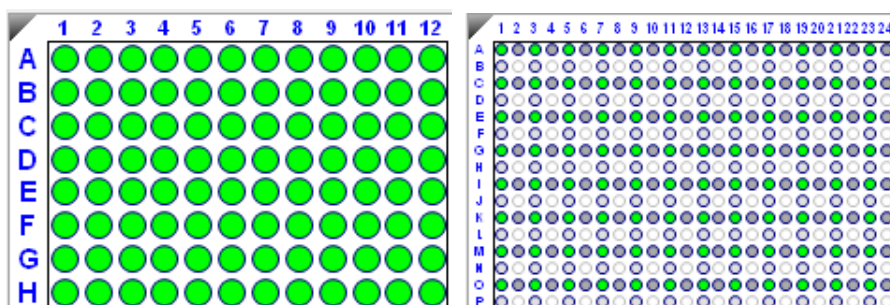


Figure 10-61: Biosensor Layout in 96- and 384-well Plates Using 96-channels Read Head Setting.



NOTE: A column of 16 wells is read in two sets of interrogations. Biosensors interrogate 8 wells in a column at a time: rows A, C, E, G, I, K, M and O are read first followed by rows B, D, F, H, J, L, N and P.

The 32 and 48 biosensor modes also use multiplexer switching to read 32 and 48 wells in parallel, with sensitivity equivalent to the Octet QK384 system. Cross-blocking experiments as large as 32 x 32 or larger may be accomplished with the 32 or 48 biosensor modes combined with 384-well tilted-bottom plates in a shorter amount of time compared to other Octet systems.

In Figure 10-62, biosensors interrogate 32 wells in 4 columns at a time or 48 wells in 6 columns at a time. Columns 1, 3, 5 and 7 are interrogated at the same time, and so on for the 32-channels setting. Columns 1, 3, 5, 7, 9 and 11 are interrogated at the same time, and so on for the 48-channel setting:



Figure 10-62: Biosensor Layout in 384-well Plates Using 32 (left) and 48 (right) Channels Read Head Setting.

The 8 and 16 biosensor modes provide high sensitivity for measuring small molecule binding interactions and protein quantitation down to 50 ng/mL, similar to the Octet RED96 and RED384 systems. These two modes are best for assays requiring a wide dynamic range or fine signal resolution, and may be combined with the other Read Head options in a single experiment.

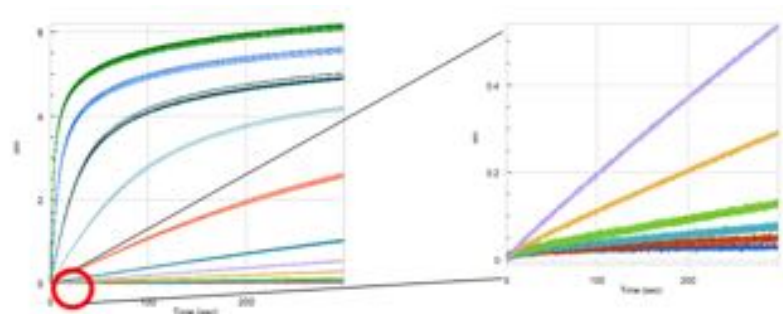


Figure 10-63: Zoomed View of Closely Overlaid Traces Shows Fine Signal Resolution for Human IgG Quantitation Assay with Protein A Biosensors

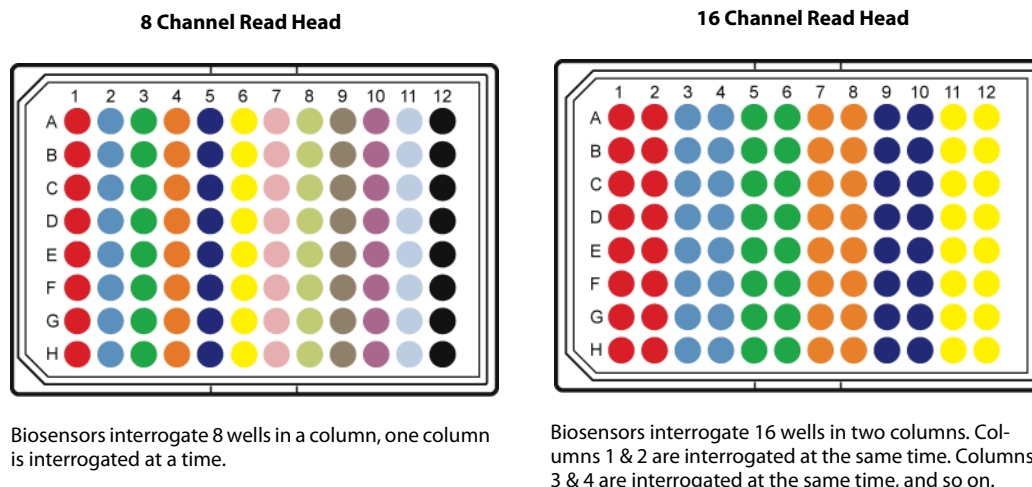


Figure 10-64: Color-Coded Wells Display How Biosensors Interrogate a 96-well Plate, 8 Channel or 16-Channel Read Head

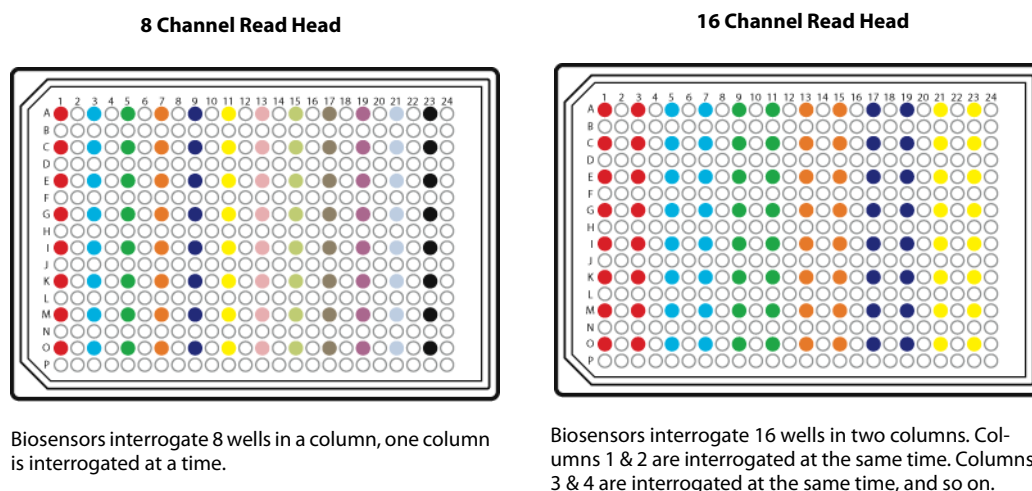


Figure 10-65: Color-Coded Wells Display How Biosensors Interrogate a 384-well Plate, 8 Channel or 16-Channel Read Head

Tab 1 (Plate Definition)

1. Choose the number of simultaneous wells to be read from the **Read Head** drop down list:

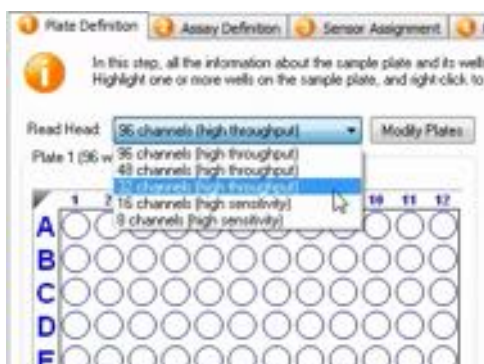


Figure 10-66: Select Wells to be Read



NOTE: A column of 16 wells is read in two sets of interrogations. Biosensors interrogate 8 wells in a column at a time: rows A, C, E, G, I, K, M and O are read first followed by rows B, D, F, H, J, L, N and P.

- Choose a plate format for Plate 1 and Plate 2 by clicking **Modify Plates**. Select either the 96- or 384 well format for each plate:



Figure 10-67: Select Plate 1 and Plate 2 Formats

- Designate plate layouts for Plate 1 and Plate 2 by selecting wells in the plate maps and designating sample types.



NOTE: It is important to define all of the wells that will be used in the assay. Only wells that are selected and defined using one of the sample types in Table 10-2 will be included in the assay.

Table 10-7: Types of Sample Wells

Icon	Description
Sample	Any type of sample. For example, an analyte.
Reference	Reference sample. For example, a buffer-only control biosensor that is used to correct for system drift.
Controls	<p>A control sample, either positive or negative, of known analyte composition. Data from the well is not used to generate a standard curve during analysis.</p> <ul style="list-style-type: none"> Positive Control: A control sample that contains analyte of known concentration Negative Control: A control sample known not to contain analyte
Buffer	Any type of buffer. For example, the buffer in a baseline, association, or dissociation step.
Activation	Activation reagent. Makes the biosensor competent for binding.
Quench	Quenching reagent. Blocks unreacted immobilization sites on the biosensor surface.
Load	Ligand to be immobilized (loaded) on the biosensor surface.
Wash	Wash buffer.
Regeneration	Regeneration reagents dissociate the analyte from the ligand.
Neutralization	Neutralization buffer that is used to neutralize the biosensor after the regeneration step.

There are several ways to select sample wells in either plate map:

- Click a column header or select adjacent column headers by click-hold-drag (Figure 10-68, top left).
- To select non-adjacent columns, hold the **Ctrl key** and click the column header (Figure 10-68, top right).
- Click a row header or select adjacent row headers by click-hold-drag (Figure 10-68, bottom left).
- Click a well or draw a box around a group of wells (Figure 10-68, bottom right).

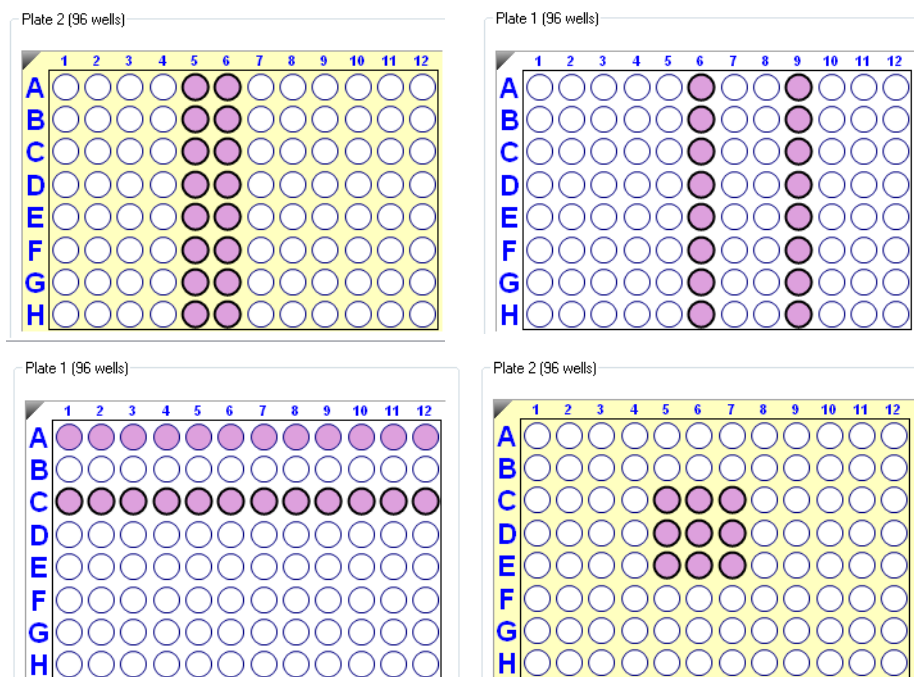


Figure 10-68: Selecting Sample Wells in a Plate Map

- Designate well types by right-clicking on selected wells and assigning a sample type:



Figure 10-69: Designating Well Types

5. To remove a well designation in either plate map, select the well(s) and click **Remove**. Or, right-click the well(s) and select **Clear Data**:

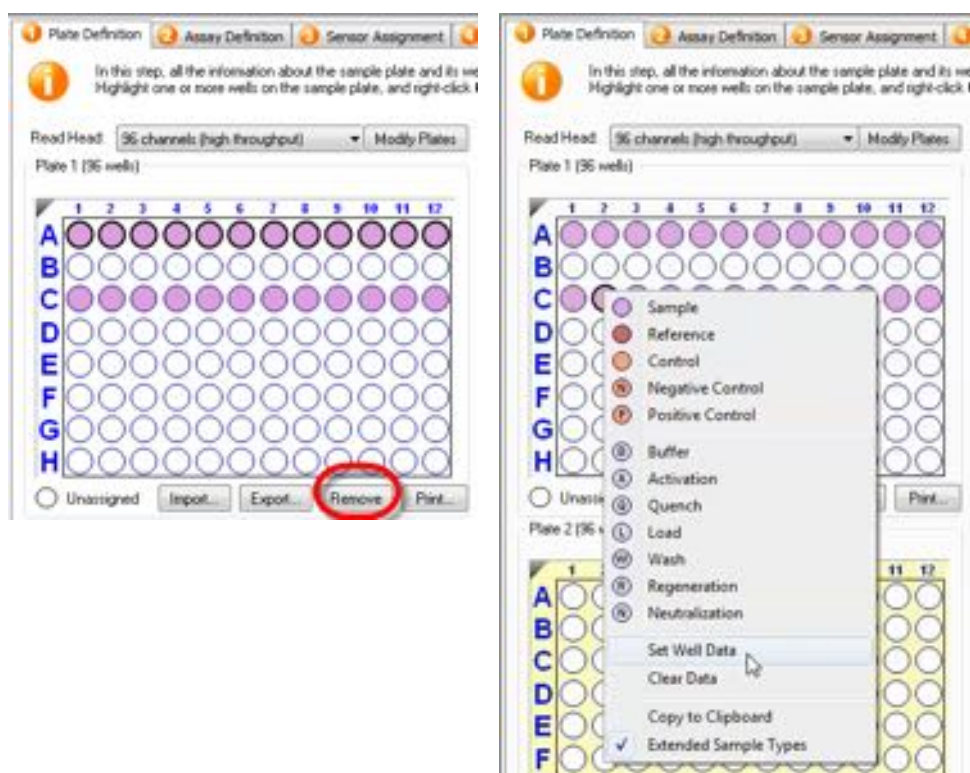


Figure 10-70: Clearing Sample Well Designations from the Plate Map



NOTE: Shift-clicking in the plate map simultaneously selects a set of wells equal to the number of channels chosen in the read head option.



NOTE: All sample types can be placed in either plate position 1 or 2, or both.

6. Enter sample information.



NOTE: You must specify sample (analyte) concentration and molecular weight to allow Octet Data Analysis software to compute a K_D value. If the sample concentration is not specified, only k_d and k_{obs} are calculated. You can also annotate any well with Sample ID or Well Information, and assign Replicate Groups.

Select the table for either Plate 1 or Plate 2. There are several ways to enter sample information:

- Select an individual well in the plate table and enter information per well.
- Click-drag-hold several wells in the plate table, right-click and choose **Set Well Data**:

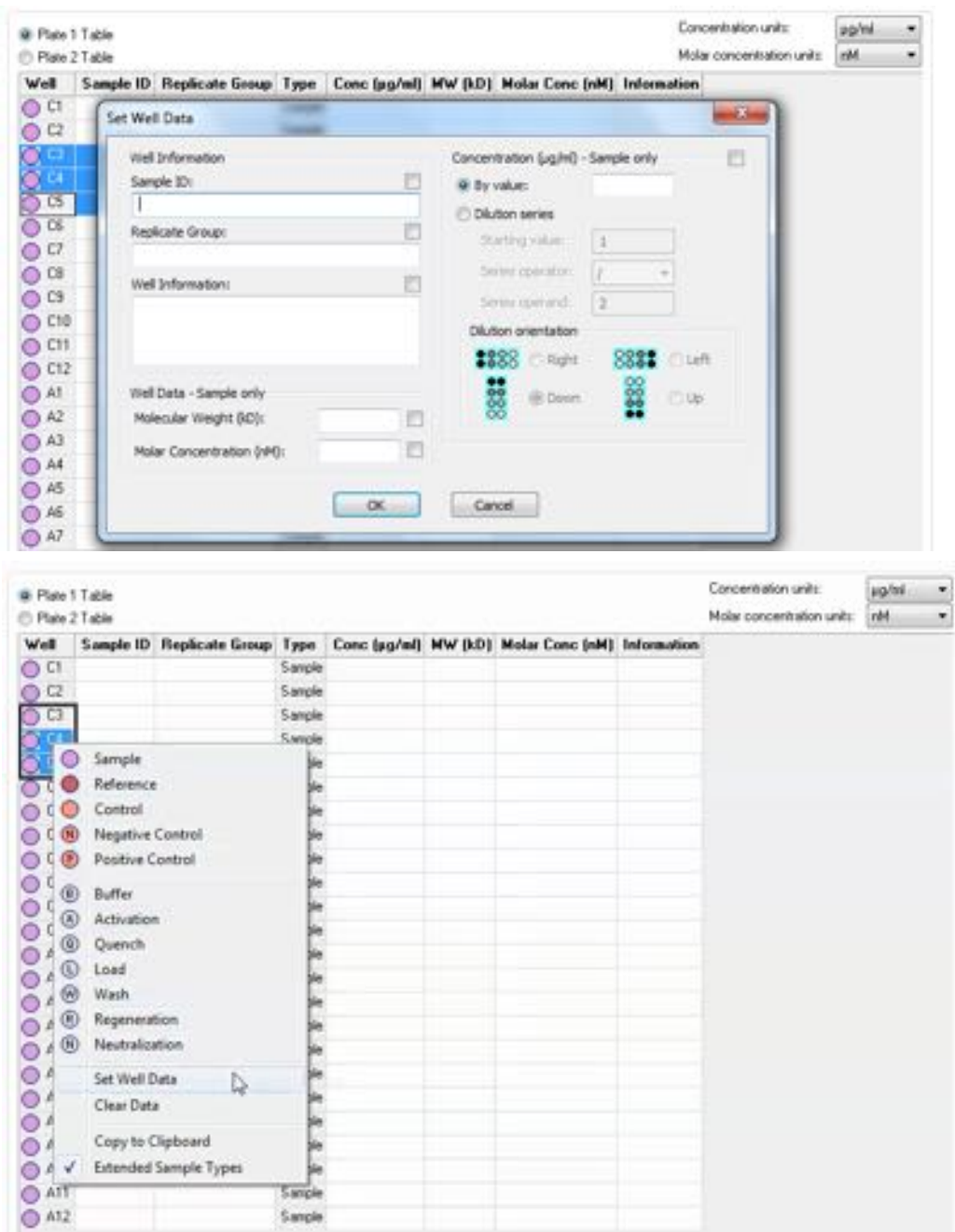


Figure 10-71: Entering Molecular Weight and Molar Concentration



NOTE: More information on sample information and annotation can be found in “Entering Sample Information” on page 527.

Tab 2 (Assay Definition)

After completing the plate layout(s), a Kinetic Assay can be defined:

1. Click on Tab 2 (Assay Definition).
2. Add assay step types in the Step Data List:
 - a. Click **Add**. The Add Step Definition box will display:



Figure 10-72: Add Step Definition Box

- b. Choose a step type.
- c. Optional: Edit step name.
- d. Set the step time and shake speed.
- e. The regeneration step type requires assigning separate parameters. To do this, click the **Regeneration Params** button:

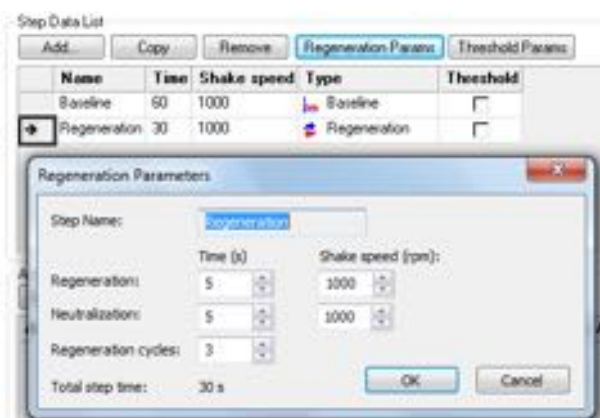


Figure 10-73: Regeneration Parameters Box

- f. Optional: Assign a threshold. See “Creating Step Types” on page 548 for more information.
3. Build the assay(s) by assigning steps defined in Step Data List to columns in the plate map(s).



NOTE: Each assay color group must use the same Read Head setting for each of their steps, as listed in the Sensors column.



NOTE: Individual assays are differentiated by color in the Assay column.



NOTE: Individual assays may have different Read Head settings.

- a. Select a step type in the Step Data List.
- b. In the Plate 1 or Plate 2 map, double-click the columns that you want associated with that step type.
- c. The selected wells will be marked with hatching, and the new step appears in the Assay Steps List:

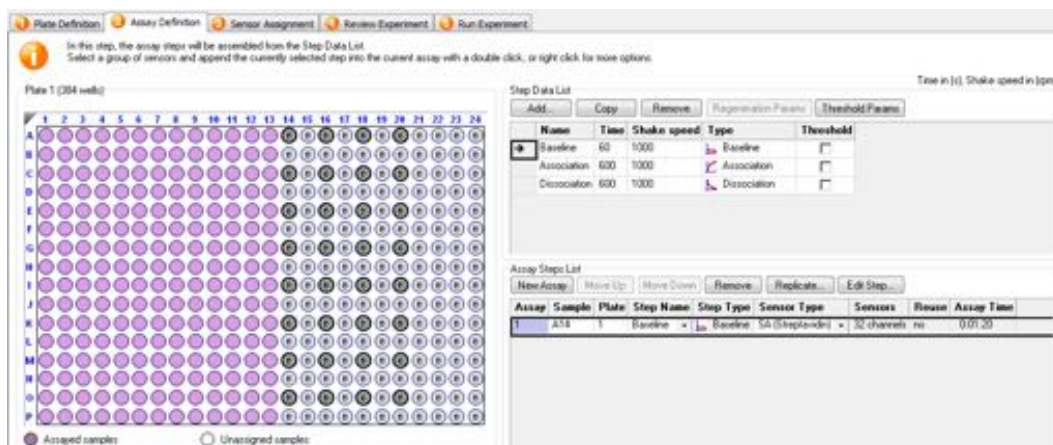


Figure 10-74: Assay Steps List

- d. Select the correct biosensor from the Sensor Type drop-down list. The Sensors column shows the Read Head selection made in Tab 1 (Assay Definition). The number of biosensors listed must remain the same for that assay color group.
- e. Repeat the previous steps to define other steps in the assay.
- f. New assays may be added by clicking the **New Assay** button in the Assay Steps List:

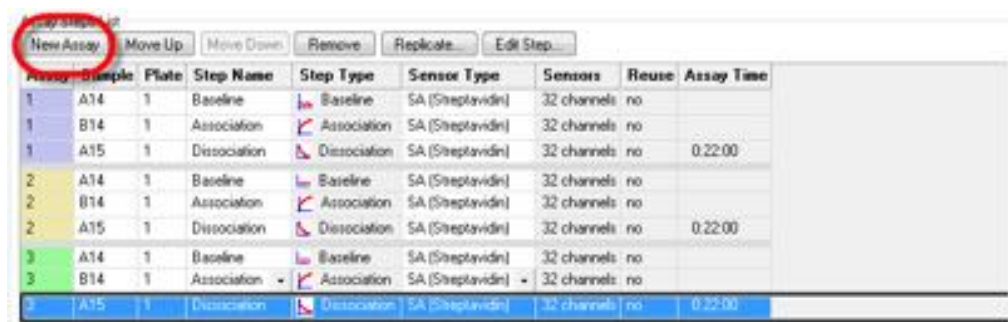


Figure 10-75: New Assay Button

4. Change the Read Head setting for an individual assay by clicking the **Edit Step** button to bring up the edit step dialogue:

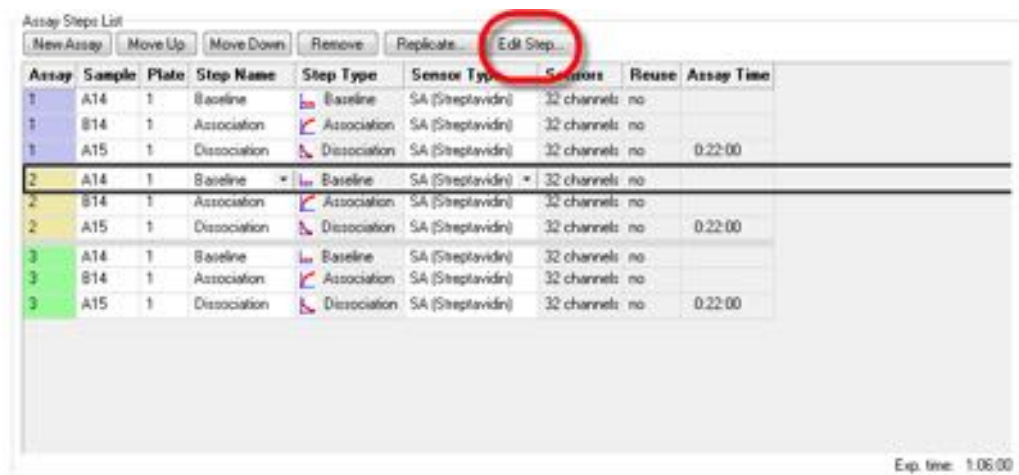


Figure 10-76: Edit Step Button

- Choose a new setting from the Read Head drop-down list, then click **OK**:

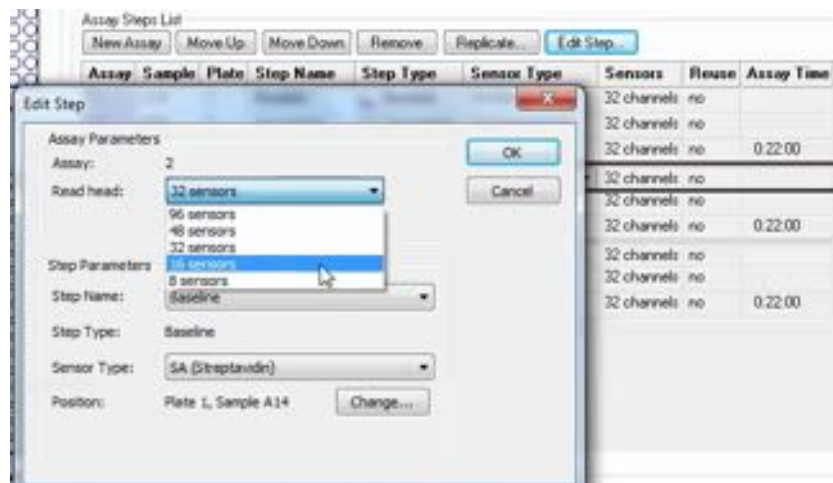


Figure 10-77: Setting Read Head Sensors

- Repeat for new Read Head settings for other assays in the experiment:



Figure 10-78: Setting Read Head Sensors for Experiment Assays

7. Edit or change the columns associated with a step type by selecting the individual step and clicking the **Edit Step** button:

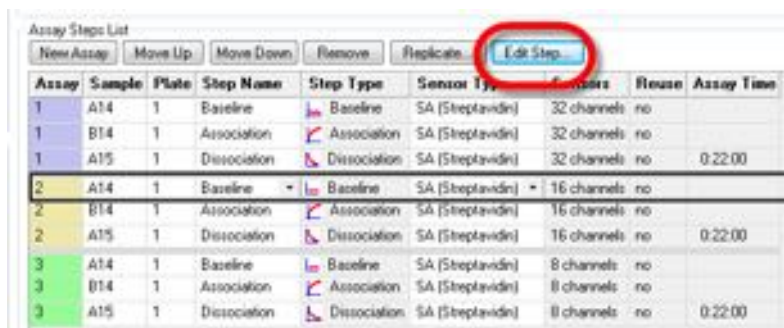


Figure 10-79: Edit Step Button

8. Click the **Change** button, then click **OK**:



Figure 10-80: Edit Step Change Button

9. This will bring up the Set Position plate map. Click on the new column(s) associated with that step:

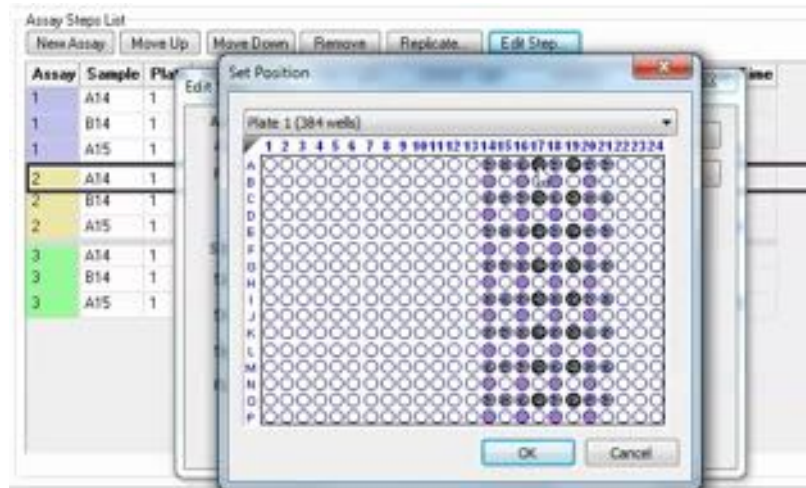


Figure 10-81: Set Position Plate Map

10. You can use new biosensors or reuse the same biosensors for the next color assay group. The default **Reuse** selection is no, which will use new biosensors:

Assay Steps List

New Assay Move Up Move Down Remove Replicate Edit Step

Assay	Sample	Plate	Step Name	Step Type	Sensor Type	Sensors	Reuse	Assay Time
1	A14	1	Baseline	Baseline	SA (Streptavidin)	32 channels	no	
1	B14	1	Association	Association	SA (Streptavidin)	32 channels	no	
1	A15	1	Dissociation	Dissociation	SA (Streptavidin)	32 channels	no	0:22:00
2	A17	1	Baseline	Baseline	SA (Streptavidin)	16 channels	no	
2	B14	1	Association	Association	SA (Streptavidin)	16 channels	no	
2	A15	1	Dissociation	Dissociation	SA (Streptavidin)	16 channels	no	0:22:00
3	A14	1	Baseline	Baseline	SA (Streptavidin)	8 channels	no	
3	B14	1	Association	Association	SA (Streptavidin)	8 channels	no	
3	A15	1	Dissociation	Dissociation	SA (Streptavidin)	8 channels	no	0:22:00

Figure 10-82: Default Biosensor Reuse Selection



NOTE: The Reuse option is only available for the Octet HTX system at this time.



NOTE: Pall ForteBio recommends adding Regeneration steps at the end of the current assay before reusing the same biosensors on the next color assay group.

To reuse the biosensors from the current assay color group for the next color assay group, select a step in the current assay and click the **Edit Step** button. Select the **Reuse sensors** box and click **OK**. The **Reuse** selection will now be set to yes.

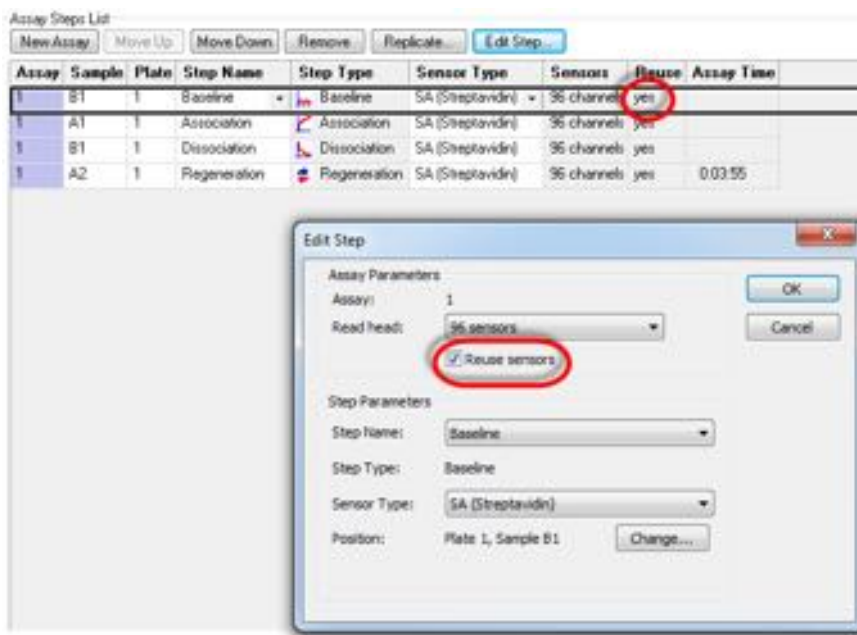


Figure 10-83: Changing the Biosensor Reuse Selection

Tab 3 (Sensor Assignment)

After completing the assay definition, click on Tab 3 (Sensor Assignment) to assign sensor type(s) for the kinetic experiment.



NOTE: The Sensor Type for the assay must be selected or changed from the Assay Steps List in the Assay Definition Tab. Changing the Sensor Type from the Sensor Assignment Tab will not update the assay.



NOTE: Full details on biosensor assignment in Tab 3 (Sensor Assignment) can be found in "Assigning Biosensors to Samples" on page 561.

Replacing Biosensors in the Biosensor Tray

After an assay is completed, biosensors can either be returned to the biosensor tray or ejected through the chute. To return them to the tray, click the **Replace sensors in tray after use** check box:

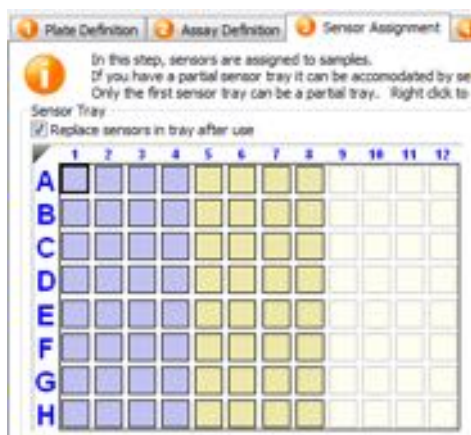




Figure 10-84: Replace Sensors in Tray After Use Check Box

REVIEWING EXPERIMENTS

Before running an experiment, you can review the sample plate layout, assays and assay steps as well as the biosensors assigned to each assay in the experiment.

In the **Review Experiment** window (Figure 10-85), move the slider left or right to highlight the biosensors and samples associated with an assay step, or click the   arrows. Alternatively, select an assay step to view the biosensors and samples associated with it.

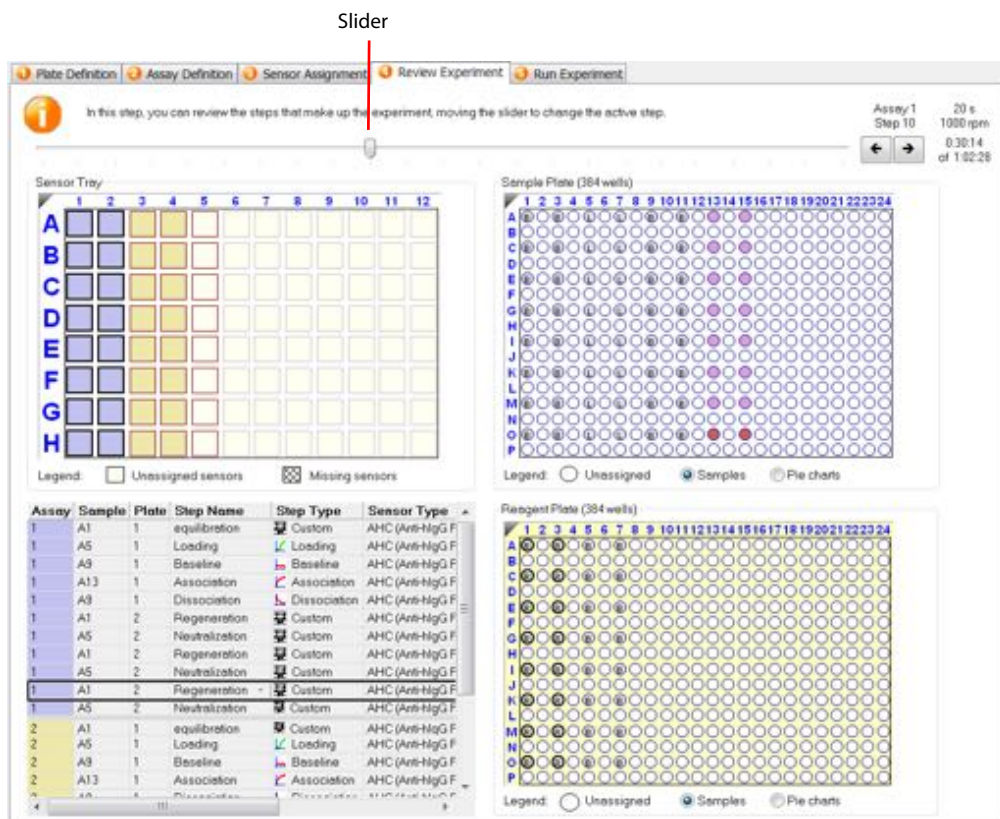


Figure 10-85: Review Experiment Window

SAVING EXPERIMENTS

After an experiment is run, the software automatically saves a read-only copy of the experiment information that you specified (sample plate definition, biosensor assignment, assay settings) to an experiment method file (.fmf). If you set up an experiment, but do not start the run, you can manually save the experiment method.

To manually save an experiment:

1. Click **Save Method File** (📁), or on the main menu, click **File > Save Method File**.

If there is more than one open experiment and you want to save all of them, click **Save All Methods Files** (📁).

2. In the **Save** dialog box, enter a name and location for the file, and click **Save**.



NOTE: If you edit a saved experiment and want to save it without overwriting the original file, click **File > Save Method File As** and enter a new name for the experiment.

Saving an Experiment to the Template Folder

If you save an experiment to the factory-installed Template folder, the experiment will be available for selection. To view templates, select **Experiment > Templates > Kinetics > Experiment Name** (see Figure 10-86).

Follow the steps above to save an experiment to the Template folder located at C:\Program Files\ForteBio\DataAcquisition\TemplateFiles.



IMPORTANT: Do not change the location of the Template folder. If the Template folder is moved from the factory-set location, the software may not function properly.

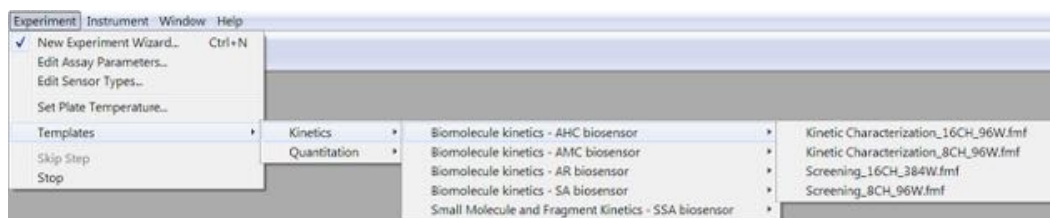


Figure 10-86: Saved Experiments in the Template Folder

RUNNING A KINETICS EXPERIMENT



IMPORTANT: Before starting an experiment, ensure that the biosensors are properly rehydrated. For details on how to prepare biosensors, see the appropriate biosensor product insert.

Loading the Biosensor Tray, Sample, and Reagent Plates

To load the biosensor tray, sample plate, and reagent plate:

1. Open the Octet instrument door (lift the handle up) and present the instrument stage (click the **Present Stage** button).
2. Place the biosensor tray, sample plate, and reagent plate on the appropriate stage so that well A1 is located at the upper right corner (see Figure 10-87):
 - a. Place the rehydration plate and biosensor tray on the biosensor stage (left platform).
 - b. Place the sample plate on the sample stage (middle platform).
 - c. Place the reagent plate on the reagent stage (right platform).

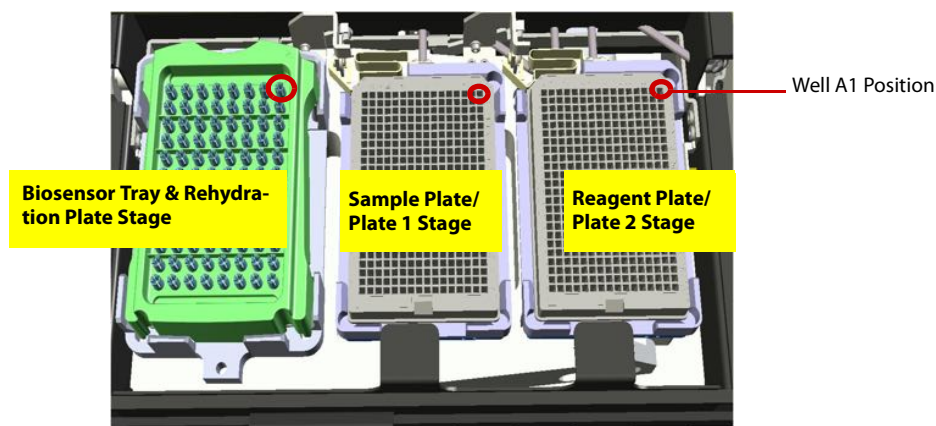



Figure 10-87: Octet Instrument Stage Platform




IMPORTANT: Ensure that the bottom of the sample plate, reagent plate and biosensor tray are flat on the stages.

3. Click  to close the Octet instrument door.
4. Allow the plate to equilibrate.

The time required for temperature equilibration depends on the temperature that your application requires and the initial temperature of the sample plate. For specific biosensor rehydration times, see the appropriate biosensor product insert.

Starting the Experiment

To start the experiment:

1. Click the **Run Experiment** tab, or click the arrow () to access the Run Experiment window (see Figure 10-88).

The screenshot displays the 'Run Experiment' tab of the Octet RED384 software interface. The window is divided into several sections:

- Data File Location and Names:**
 - Kinetics data repository: C:\Users\Owner\Documents\ForteBio
 - Experiment run name (sub directory): Experiment_1
 - Plate name/barcode (file prefix): FB100
 - 2nd Plate name/barcode: (empty)
 - Auto-increment file ID start: 1
 - Data files will be stored as follows:
 - Documents\ForteBio\Experiment_1\FB100_001.nd
 - Documents\ForteBio\Experiment_1\FB100_002.nd
 - Documents\ForteBio\Experiment_1\FB100_003.nd
- Run Settings:**
 - ☒ Delayed experiment start (Start after (s): 300)
 - ☒ Open runtime charts automatically
 - ☒ Shake sample plate while waiting
 - ☒ Automatically save runtime chart
 - ☒ Set plate temperature (°C): 30
- Advanced Settings:**
 - Acquisition rate: Standard kinetics (5.8 Hz, averaging by 20)
 - Sensor offset (mm): 4 (distance to sensor tip from bottom of well)
 - Warning: changing these settings could affect assay signal-to-noise. If you are unsure of how to use these settings, please consult the Data Acquisition User Guide.
- General Information:**
 - User name: Owner
 - Machine name: J-RICHARDS
 - Description: (empty)

On the right side, there is a status area with a 'Prior to pressing *Go* confirm the Assay.' message and a 'Total experiment time: 1:02:28'. At the bottom right, there are 'GO' and 'GO' buttons.

Figure 10-88: Run Experiment Tab—Octet RED384

- Confirm the default settings or enter new settings. See “Run Experiment Window Settings” on page 595 for more information on experimental settings.



NOTE: If you delay the experiment start, you have the option to shake the plate until the experiment starts.

- To start the experiment, click **GO**.

If you specified a delayed experiment start, a message box displays the remaining time until the experiment starts.

If you select the **Open runtime charts automatically** option, the **Runtime Binding Chart** window displays the binding data in real-time, as well as the experiment progress (Figure 10-89).



NOTE: For more details about the **Runtime Binding Chart**, see “Managing the Runtime Binding Chart” on page 599.

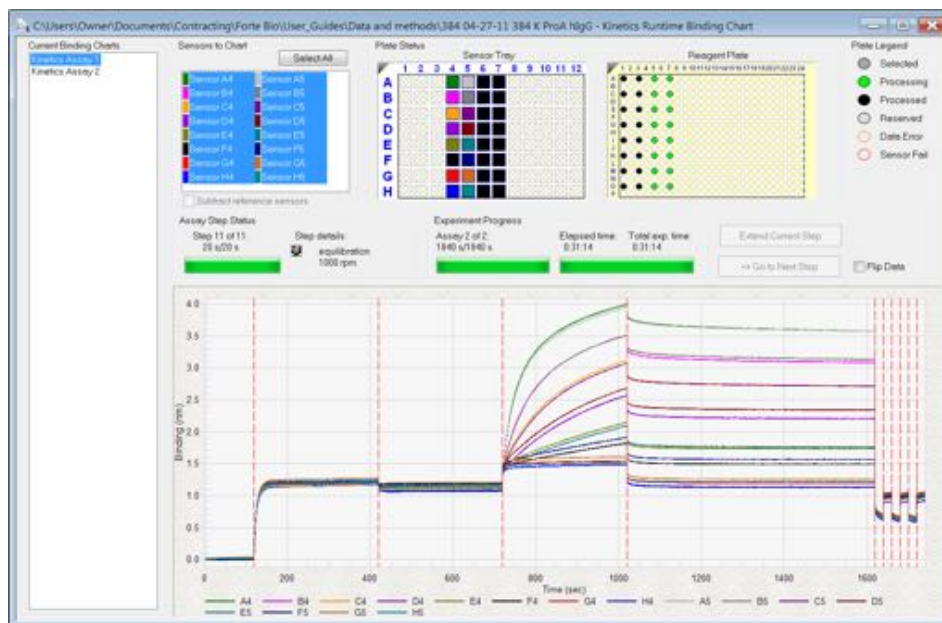


Figure 10-89: Runtime Binding Chart

4. Optional: Click **View > Instrument Status** to view the log file (see Figure 10-90).

The experiment temperature is recorded at the beginning of every experiment as well as each time the manifold picks up a new set of biosensors. Instrument events such as biosensor pick up, manifold movement, integration time, biosensor ejection and sample plate temperature are recorded in the log file.

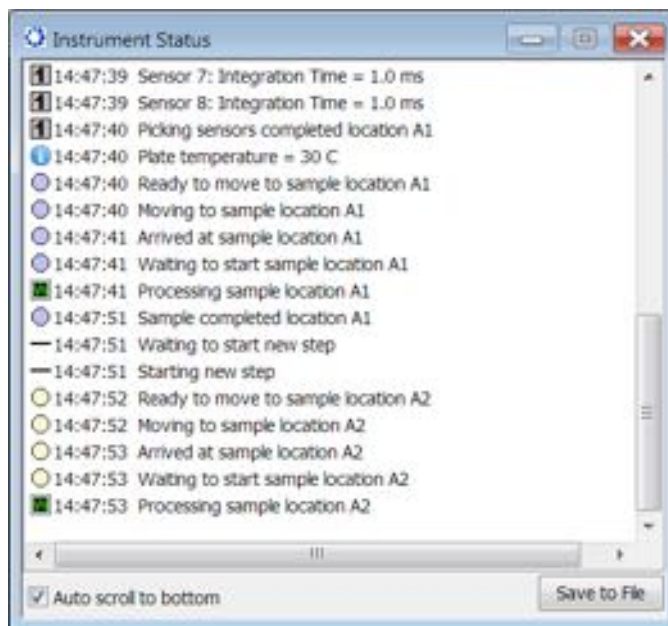


Figure 10-90: Instrument Status Log



WARNING: Do not open the Octet instrument door when an experiment is in progress. If the door is opened, the data from the active biosensors is lost. The data already acquired is saved, however the assay is aborted and cannot be restarted without ejecting the biosensors and starting from the beginning.



WARNING: N'ouvrez pas la porte de l'instrument Octet lorsqu'une analyse est en cours. En cas d'ouverture de la porte, les données issues de l'étape d'acquisition active seront perdues et cela entraînera l'échec de la procédure.



WARNING: Öffnen Sie die Instrumentenklappe des Octet-Systems nicht während eines laufenden Experiments. Wird die Klappe geöffnet, gehen die Daten des aktiven Erfassungsschritts verloren und das Experiment wird abgebrochen.

Run Experiment Window Settings

The following **Data File Location and Name** settings are available on the **Run Experiment** Tab:

Table 10-8: Data File Location and Name



Item	Description
Assay type	The name of the selected assay.
Kinetics data repository	The location where the subdirectory will be created. The subdirectory contains the data (.frd) files. Click Browse to select another data location.
<div>  NOTE: It is recommended that you save the data to the local machine first, then transfer to a network drive. </div>	
Experiment Run Name (sub-directory)	Specifies a subdirectory name for the data files (.frd). The software generates one data file for each biosensor that includes the data from all steps the biosensor performs.
Plate name/ barcode (file prefix)	A user-defined field where you can enter text or a barcode (barcode reader required).

Table 10-8: Data File Location and Name (Continued)

<i>Item</i>	<i>Description</i>
2nd Plate name/barcode	A user-defined field where you can enter text or a barcode (barcode reader required) for a second plate. This field is also used to generate the path of the saved directory.
Auto Increment File ID Start	Each file is saved with a number after the plate name. For example, if the Auto Increment File ID Start number is 1, the first file name is xxx_001.frd.

The following **Run Settings** are available on the **Run Experiment** Tab:

Table 10-9: Run Settings

<i>Item</i>	<i>Description</i>
Delayed experiment start	Specifies a time delay for the start of the experiment. Enter the number of seconds to wait before the experiment starts after you click  .
Start after	Enter the number of seconds to delay the start of the experiment.
Shake sample plate while waiting	If the experiment has a delayed start time, this setting shakes the plate until the experiment starts.
Open runtime charts automatically	Displays the Runtime Binding Chart for the current biosensor during data acquisition.
Automatically save runtime chart	Saves an image (.jpg) of the Runtime Binding Chart . The binding data (.frd) is saved as a text file, regardless of whether a chart image is created.
Set plate temperature (°C)	Specifies a plate temperature and enters the temperature in the dialog box. If not selected, the plate temperature is set to the default temperature specified in File > Options . The factory set default temperature is 30 °C.



NOTE: If the actual plate temperature is not equal to the set plate temperature, a warning displays and the Octet System Data Acquisition software provides the option to wait until the set temperature is reached before proceeding with the run, continue without waiting until the set temperature is reached, or cancel the run.

Advanced settings are available for Octet RED384 and Octet QK384 systems. The signal to noise ratio of the assay can be optimized by selecting different acquisition rates. The acquisition rate refers to the number of binding signal data points reported by the Octet system per second and is reported in Hertz (per second). A higher acquisition rate generates more data points per second and monitors faster binding events better than a slower acquisition rate. A lower acquisition rate allows the software enough time to perform more averages of the collected data. Typically, more averaging leads to reduced noise and thus, better signal-to-noise ratios. Therefore, the frequency setting should be determined based on consideration of the binding rate, the amount of signal generated in your assay and some experimentation with the settings.

The following **Advanced Settings** are available for the Octet384 system:

Table 10-10: Advanced Settings Octet RED384

Item	Description
Acquisition rate	<ul style="list-style-type: none"> High sensitivity kinetics (2.0 Hz, averaging by 50)—The average of 50 data frames is reported as one data point. Two data points are reported per second. Standard kinetics (5.0 Hz, averaging by 20)—The average of 50 data frames is reported as one data point. Five data points are reported per second. Fast kinetics (10.0 Hz, averaging by 5)- The average of 5 data frames is reported as one data point. Ten data points are reported per second.
Sensor off set (mm)	Recommended sensor offset: Large molecule kinetics—4 mm
Default	Sets the acquisition speed and sensor offset at the default settings.

The following **Advanced Settings** are available for the OctetQK384 system:

Table 10-11: Advanced Settings Octet QK384

Item	Description
Acquisition rate	<ul style="list-style-type: none"> High sensitivity kinetics (0.3 Hz, averaging by 40) - The average of 40 data frames is reported as one data point. One data point is reported every 3.3 seconds. Standard kinetics (0.6 Hz, averaging by 5) - The average of 5 data frames is reported as one data point. One data point is reported every 1.6 seconds.
Sensor off set (mm)	Recommended sensor offset: Large molecule kinetics—4 mm
Default	Sets the acquisition speed and sensor offset at the default settings.

The following **General Settings** are available on the **Run Experiment** Tab:


Table 10-12: General Settings

Item	Description
Machine name	The computer name that controls the Octet instrument and acquires the data.
User name	The user logon name.

Table 10-12: General Settings (Continued)

Item	Description
Description	A user-specified description of the assay or assay purpose. The description is saved with the method file (.fmf).

Stopping an Experiment

To stop an experiment in progress, click  or click **Experiment > Stop**.

The experiment is aborted. The data for the active biosensor is lost, the biosensor is ejected into the waste tray, and the event is recorded in the experimental log.



NOTE: After the experiment is run, the software automatically saves the experiment method (.fmf).

MANAGING THE RUNTIME BINDING CHART

If the **Open runtime charts automatically** check box is selected in the Run Experiment window, the Runtime Binding Charts are automatically displayed when data acquisition starts (see Figure 10-91). The **Runtime Binding Chart** window displays the assay step status, experiment progress, and the elapsed experiment time.

The **Runtime Binding Chart** is updated at the start of each experimental step. The active biosensor column is color-coded (A=green, B=magenta, C=orange, D=purple, E=olive, F=black, G=red, H=blue) within the **Sensor Tray Map**. Used sensor columns that are inactive are colored black. Active sample columns are colored green. Each assay in the experiment is represented by **Assay X** in the **Current Binding Charts** box.

To selectively display data for particular assay:

1. Click the corresponding **Assay** number.
2. Select a subset of sensors for a displayed column under **Sensors to Chart** box (see Figure 10-91).



IMPORTANT: Do not close the **Runtime Binding Chart** window until the experiment is complete and all data is acquired. If the window is closed, the charts are not saved. To remove the chart from view, minimize the window. The Octet System Data Acquisition software saves the **Runtime Binding Chart** as displayed at the end of the experiment. For example, modifying a chart by hiding the data for a particular biosensor will cause this data not to be included in the bitmap image generated at the end of the run.

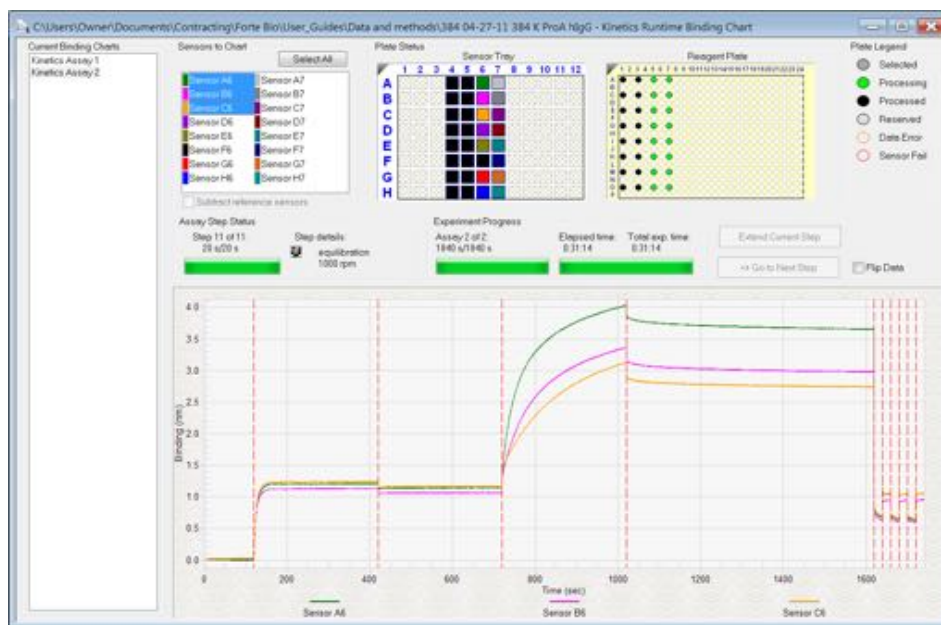


Figure 10-91: Runtime Binding Chart Window

Opening the Runtime Binding Chart

After an experiment is run, you can open and review the **Runtime Binding Chart** at any time:

1. Click **File > Open Experiment**.
2. In the dialog box that appears, select an experiment folder and click **Select**.

Viewing Reference-Subtracted Data

If the experiment includes reference biosensors, you can display reference-subtracted data in the chart by clicking the **Subtract Reference Biosensor** check box in the chart window. To view raw data, remove the check mark next to this option.

Reference biosensors can be designated:

- During experiment setup in the **Sensor Assignment** tab
- During acquisition in the Runtime Binding Chart **Sensors to Chart** box
- During analysis in the **Data Selection** tab

Designating a Reference Biosensor During Acquisition

To designate a reference biosensor during acquisition:

1. In the **Sensors to Chart** list or the **Sensor Tray**, right-click a biosensor and select **Reference** (see Figure 10-92).



Figure 10-92: Designating a Reference Biosensor in the Runtime Binding Chart

The selected biosensor will be shown with an **R** in the **Sensors to Chart** list and **Sensor Tray** (see Figure 10-93).

2. Click the **Subtract reference sensors** check box (see Figure 10-93).

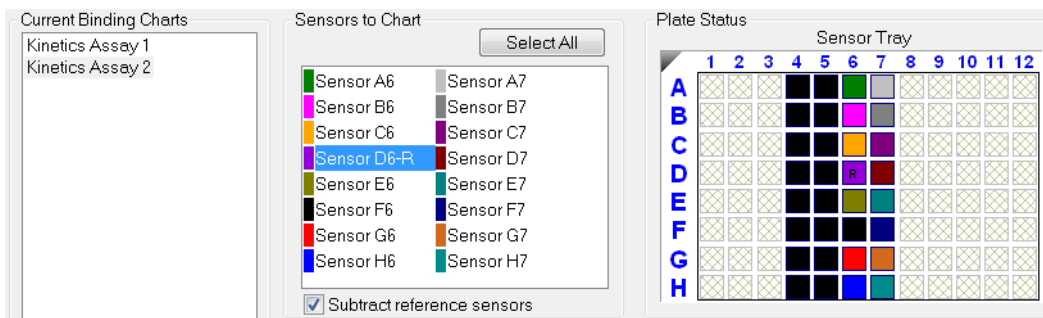


Figure 10-93: Subtract Reference Sensors check box in the Runtime Binding Chart



NOTE: Subtracting reference data in the **Runtime Binding Chart** only makes a visual change to the data on the screen. The actual raw data is unaffected and the reference subtraction must be repeated during data analysis if needed.

Viewing Inverted Data

The data displayed in the **Runtime Binding Chart** can be inverted during real-time data acquisition or data analysis after the experiment has completed. To invert data, select the **Flip Data** check box (see Figure 10-94). Uncheck the box to return to the default data display.

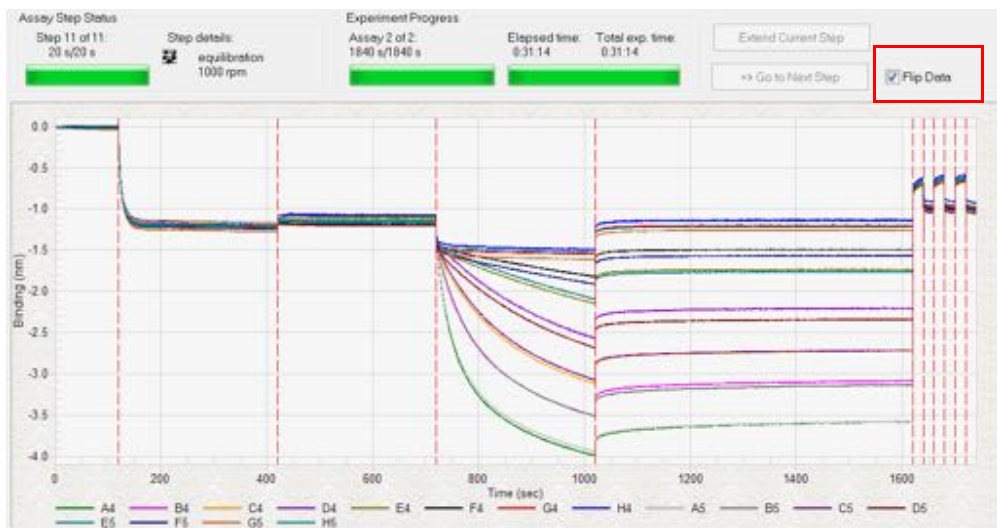


Figure 10-94: Data Inverted Using Flip Data Function

Aligning Data by a Selected Step

To align the binding data to the beginning of a user-selected step, in the **Runtime Binding Chart** (see Figure 10-95), right-click a step and select **Align to Step <number>**.

To remove the step alignment, right-click the step and select **Unaligned**.

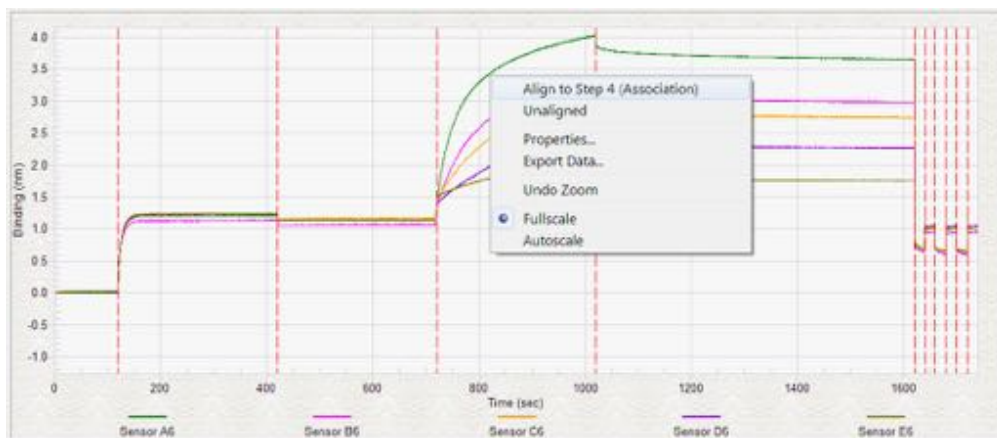


Figure 10-95: Runtime Binding Chart—Aligning the Data to a User-Selected Step

Aligning Data to a Specific Time

1. To align the binding data to a specific time, in the **Runtime Binding Chart** (see Figure 10-96), right-click and select **Align at time**.

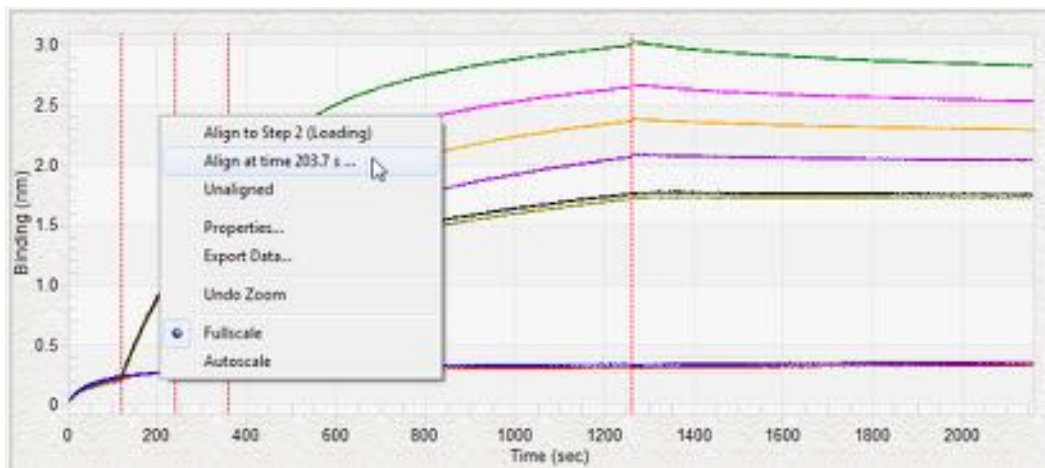


Figure 10-96: Runtime Binding Chart—Aligning the Data to a User-Specified Time

The Align at Time dialog box displays (Figure 10-97).

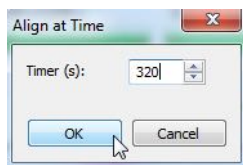


Figure 10-97: Align at Time Dialog Box

2. Enter the time point you want to align to and click **OK**. The binding chart will then align to the time point specified.

To remove the time alignment, right-click and select **Unaligned**.

Extending or Skipping an Assay Step

During acquisition, the duration of the active step may be extended. You can also terminate the active step and begin the next step in the assay.



NOTE: If the step you want to extend or terminate includes biosensors used in Parallel Reference, Double Reference, or Average Reference subtraction methods, the data will not be analyzed.

To extend the duration of the active step:

1. In the chart window, click the **Extend Current Step** button.
2. In the **Extend Current Step** dialog box (see Figure 10-98), enter the number of seconds to extend the step and click **OK**.

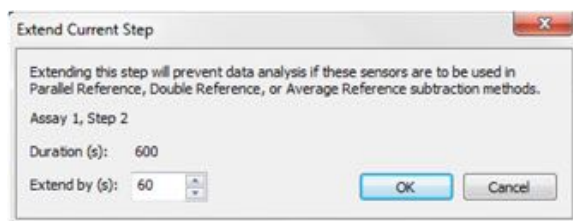


Figure 10-98: *Extend Current Step Dialog Box*

To terminate a step and begin the next step in the assay:

1. In the chart window, click the **Go to Next Step** button.
2. In the **Data Acquisition** dialog box, click **OK**.

Magnifying the Runtime Binding Chart

To magnify the chart, press and hold the mouse button while you draw a box around the chart area to magnify.

To undo the magnification, right-click the chart and select **Undo Zoom**.

Scaling a Runtime Binding Chart

To scale the Runtime Binding Chart:

1. Right-click the Runtime Binding Chart and select **Properties**.
2. In the **Runtime Graph Properties** dialog box, select **Fullscale** or **Autoscale**.

Adding a Runtime Binding Chart Title

To add a **Runtime Binding Chart** title:

1. Right-click the chart and select **Properties**.
2. In the **Runtime Graph Properties** dialog box, enter a graph title or subtitle.

Selecting a Runtime Binding Chart Legend

To select a **Runtime Binding Chart** legend:

1. Right-click the chart and select **Properties**.
2. In the **Runtime Graph Properties** dialog box, select one of the following legends:
 - Sensor Location
 - Sample ID
 - Sensor Information
 - Concentration/Dilution

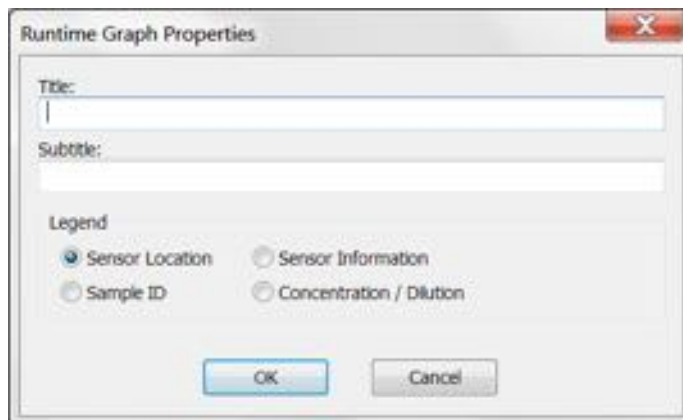


Figure 10-99: Selecting a Runtime Binding Chart Legend



NOTE: Text for **Sample ID**, **Sensor Information**, or **Concentration/Dilution** is taken from the **Plate Definition** and **Sensor Assignment** tabs, and must be entered before the experiment is started.

3. Click **OK**.

Viewing Multiple Runtime Binding Charts

To view multiple Runtime Binding Charts, click **Window > New Window**.

Exporting or Printing the Runtime Binding Chart

To export the **Runtime Binding Chart** as a graphic or data file:

1. Right-click the chart and select **Export Data**.
2. In the **Exporting** dialog box (see Figure 10-100), select the export options and click **Export**.

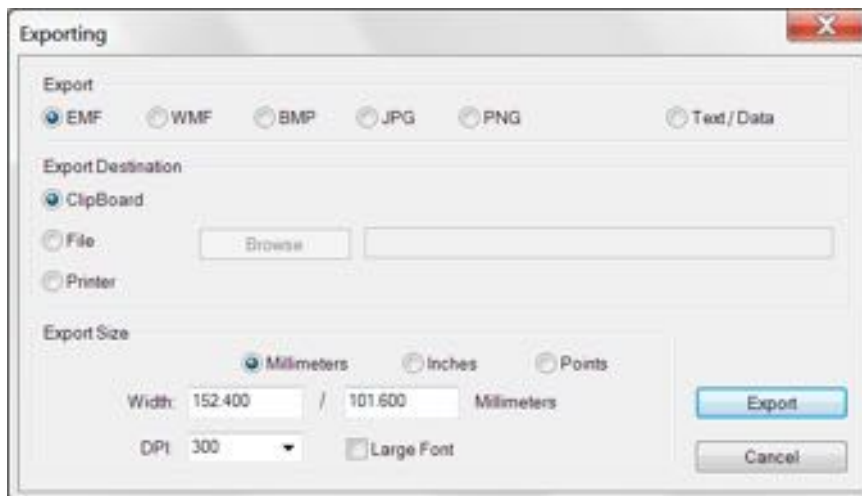


Figure 10-100: Exporting Dialog Box

Table 10-13: Runtime Binding Chart Export Options

Task	Export	Option	Export Destination	Result
	Text/ Data	EMF, WMF, BMP, JPG, or PNG		
Save the binding data	✓		Click File > Browse to select a folder and enter a file name.	Creates a tab-delimited text file of the numerical raw data from each biosensor. Open the file with a text editor such as Notepad.
Export the Runtime Binding Chart to a graphic file		✓	Click File > Browse to select a folder and enter a file name.	Creates a graphic image.

Table 10-13: Runtime Binding Chart Export Options (Continued)

Task	Export	Option	Export Destination	Result
Copy the Runtime Binding Chart		✓	Clipboard	Copies the chart to the system clipboard
Print the Runtime Binding Chart		✓	Printer	Opens the Print dialog box.

MANAGING EXPERIMENT METHOD FILES

After you run an experiment, the Octet System Data Acquisition software automatically saves the method file (.fmf), which includes the sample plate definition, biosensor assignment, and the run parameters. An experiment method file provides a convenient initial template for subsequent experiments. A read-only copy of the method used for an experiment is automatically saved in the experiment folder. Open a method (.fmf) and edit it if necessary.



NOTE: When using the 21 CFR Part 11 version of the Octet System Data Acquisition software, only 21 CFR Part 11 compliant experiments and method files generated using the 21 CFR Part 11 version of the software can be opened. Files generated using the non-compliant version of the software or with a non-compliant system cannot be opened, and a message indicating this will be presented.

Table 10-14: Managing Experiment Method Files

Menu Bar Command/ Toolbar Button	Description
File > Open Method File	Enables you to select and open a method file (.fmf)
File > Save Method File or	Saves one method file or all method files. Saves a method file before the experiment is run.
File > Save Method File As	Saves a method file to a new name so that the original file is not overwritten.

EPITOPE BINNING

The goal of a typical epitope binning or cross-blocking experiment is to identify antibodies which bind to different or identical epitopes on the antigen. Antibodies are tested two at a time for competitive binding to one antigen. By competing antibodies against one another in a pairwise and combinatorial format, antibodies with distinct blocking behaviors can be discriminated and assigned to “bins”. The end result is matrix of pairwise binders and blockers.



An epitope binning or cross-blocking experiment must be run as a kinetic experiment with repeating steps in the Octet Data Acquisition software.



NOTE: Pall ForteBio highly recommends using the Loading, Association or Dissociation assay steps instead of Custom for epitope binning and cross-blocking experiments.

After starting the Octet system and the Octet Data Acquisition software, follow the steps in Table 10-15 to set up and run an epitope binning experiment.

Table 10-15: Octet Data Acquisition Steps for Epitope Binning Assays

Octet Software	Functions
Data Acquisition 	<ol style="list-style-type: none"> 1. Select Epitope Binning under New Kinetics Experiment in the Experiment Wizard. Open a method template from the Experiment Menu or open an existing method file (*.fmf). <hr/> <p>  NOTE: In the Experiment Menu, the Templates command allows users to pick from a set of predefined method templates for Kinetic, Quantitation, or Epitope Binning experiments. Users may also modify existing method templates to suit their experimental conditions and save as a new method file and new method file name. </p> <hr/> <ol style="list-style-type: none"> 2. Define a sample plate or open a sample plate definition. 3. Specify assay steps. 4. Assign biosensors to samples. 5. Run the experiment.

Starting an Experiment: Octet RED384 or QK384

You can start a kinetics experiment using one of the following options:

- Launch the **Experiment Wizard** by clicking **Experiment > New Experiment Wizard**, and selecting **New Kinetics Experiment** and **Epitope Binning**.
 - Open a method file (.fmf) by clicking **File > Open Method File**. Method files may be saved and recalled using the File menu and are automatically saved when an experiment is run.
 - On the menu bar, click **Experiment > Templates > Epitope Binning**.
6. Optional: You can also click **Recent Methods** to display a list of recently used methods. You can open any method file from the list and use it with or without modifications to run a new experiment.

Enter the required information on Tabs 1-5 of the Basic Kinetics Experiment.

Tab 1 (Plate Definition)



NOTE: The Sample plate and the Reagent plate are now referred to as "Plate 1" and "Plate 2" in the software.

1. For Octet QK384 and RED384, choose a plate format for both plate positions by clicking **Modify Plates**. Select either the 96- or 384 well format for each plate:



Figure 10-101: Select Plate Formats

2. Designate layouts for both plates by selecting wells in the plate maps and designating sample types. There are several ways to select sample wells in either plate map:
 - Click a column header or select adjacent column headers by click-hold-drag.
 - To select non-adjacent columns, hold the **Ctrl key** and click the column header.
 - Click a row header or select adjacent row headers by click-hold-drag.
 - Click a well or draw a box around a group of wells.
3. Designate well types by right-clicking on selected wells and assigning a sample type:



Figure 10-102: Designating Well Types

4. Enter sample information by selecting the table for either plate. There are several ways to enter sample information:
 - Select an individual well in the plate table.
 - Click-drag-hold several wells in the plate table, right-click and choose **Set Well Data**.



NOTE: Assigning sequential alpha-numerical names for Sample ID provides easier sorting of columns and headers for the epitope binning matrix.



NOTE: More information on sample information and annotation can be found in "Entering Sample Information" on page 527.

Tab 2 (Assay Definition)

After completing the plate layout(s), an Epitope Binning Assay can be defined by building a kinetic assay.

1. Click on Tab 2 (Assay Definition).
2. Add assay step types in the Step Data List:

- a. Click the **Add** button. The Add Step Definition box will display:



Figure 10-103: Add Step Definition Box

- b. Choose a step type.
 c. Optional: edit step name.
 d. Set the step time and shake speed.
 e. The regeneration step type requires assigning separate parameters. To do this, click the **Regeneration Params** button:

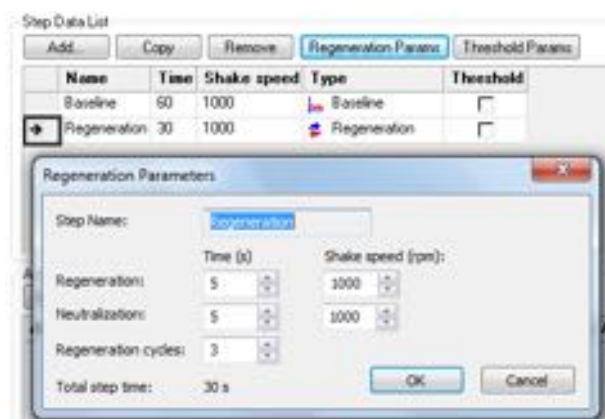


Figure 10-104: Regeneration Parameters Box

- f. Optional: assign a threshold. See “Creating Step Types” on page 548 for more information.
3. Build the assay(s) by assigning steps defined in Step Data List to columns in the plate map(s).



NOTE: We highly recommend using the Associate or Dissociate assay steps instead of Custom for epitope binning and cross-blocking experiments.

- Select a step type in the Step Data List.
- In the plate map, double-click the columns that you want associated with that step type.
- The selected wells will be marked with hatching, and the new step appears in the Assay Steps List:

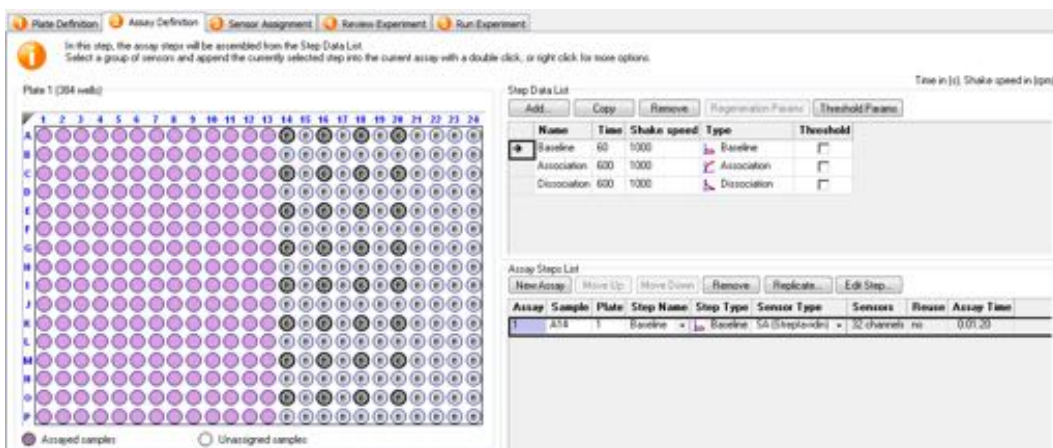


Figure 10-105: Assay Steps List

- Select the correct biosensor from the Sensor Type drop-down list. The Sensors column shows the Read Head selection made in Tab 1 (Assay Definition).
- Repeat the previous steps to define other steps in the assay.
- New assays may be added by clicking the **New Assay** button in the Assay Steps List:

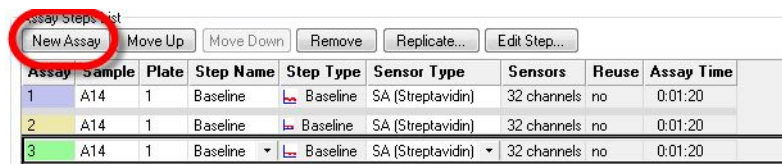


Figure 10-106: New Assay Button



NOTE: More information on assay step editing in Tab 2 (Assay Definition) can be found in "Creating Step Types" on page 548.

Tab 3 (Sensor Assignment):

After completing the assay definition, click on Tab 3 (Sensor Assignment) to verify sensor type(s) for the epitope binning experiment.



NOTE: The Sensor Type for the assay must be selected or changed from the Assay Steps List in the Assay Definition Tab. Changing the Sensor Type from the Sensor Assignment Tab will not update the assay.



NOTE: Full details on biosensor assignment in Tab 3 (Sensor Assignment) can be found in "Assigning Biosensors to Samples" on page 561.

Replacing Biosensors in the Biosensor Tray. After an assay is completed, biosensors can either be returned to the biosensor tray or ejected through the chute. To return them to the tray, click the Replace sensors in tray after use check box:



Figure 10-107: Replace Sensors in Tray After Use Check Box

Tab 4 (Review Experiment)

Before running the experiment, click on Tab 4 (Review Experiment) to review the sample plate layout, assays and assay steps as well as the biosensors assigned to each assay in the experiment.

Move the slider left or right in the window or click the arrows to highlight the biosensors and samples associated with an assay step:



Figure 10-108: Navigating the Review Experiment Tab

Alternatively, select an assay step to view the biosensors and samples associated with it.

Saving Experiments

After an experiment is run, the software automatically saves the experiment information that you specified (sample plate definition, biosensor assignment, assay settings, etc.) to an experiment method file (.fmf).

If you set up an experiment but do not start the run, you can manually save the experiment method. To do this:

1. Select **File > Save Method File**.
2. In the Save dialog box, enter a name and location for the file, and click **Save**.

Loading the Biosensor Tray and Sample Plates

To load the biosensor tray and plate positions 1 and 2:

1. Click **Instrument > Present Stage** to open the door and present the stage. Alternatively, click the **Present Stage** button:

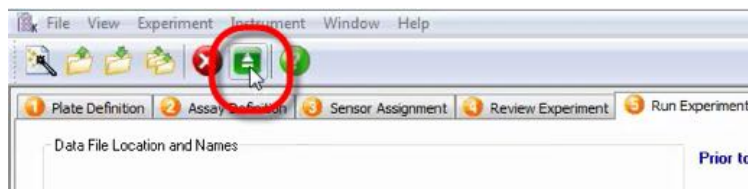


Figure 10-109: Present Stage Button

2. Place the biosensor tray, biosensor wetting plate, Plate 1, and Plate 2 on the appropriate stage so that well A1 is located at the upper right corner.
3. Close the stage and door by clicking the **Present Stage** button again.

Tab 5 (Run Experiment)

1. Click on Tab 5 (Run Experiment) to confirm the default settings or set new settings.
2. To start the experiment, click the **GO** button:

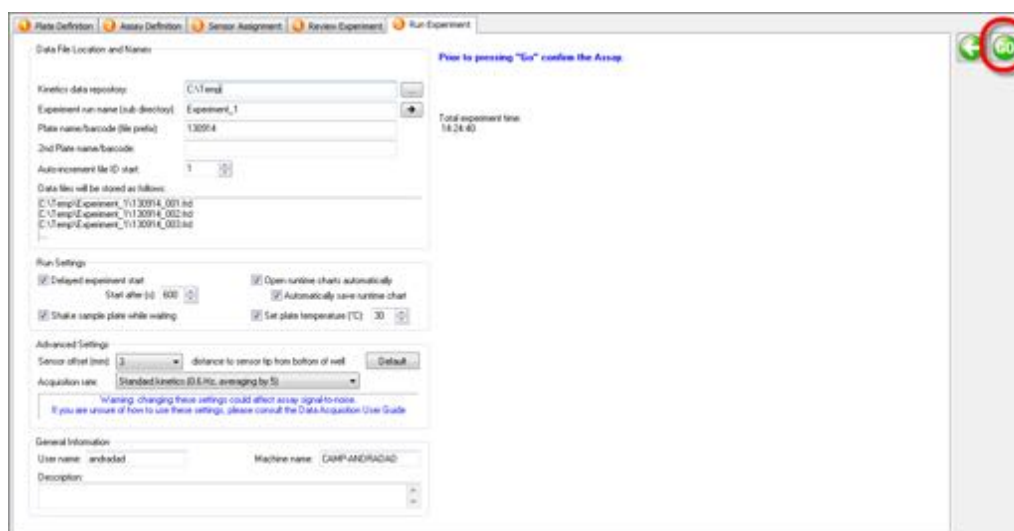


Figure 10-110: GO Button

Starting an Experiment: Octet HTX

You can start a kinetics experiment using one of the following options:

- Launch the **Experiment Wizard** by clicking **Experiment > New Experiment Wizard**, and selecting **New Kinetics Experiment**.
- Open a method file (.fmf) by clicking **File > Open Method File**. Method files may be saved and recalled using the File menu and are automatically saved when an experiment is run.
- On the menu bar, click **Experiment > Templates > Epitope Binning**.

Enter the required information on Tabs 1-5 of the Basic Kinetics Experiment.

Tab 1 (Plate Definition)

1. Choose the number of simultaneous wells to be read from the **Read Head** drop down list:

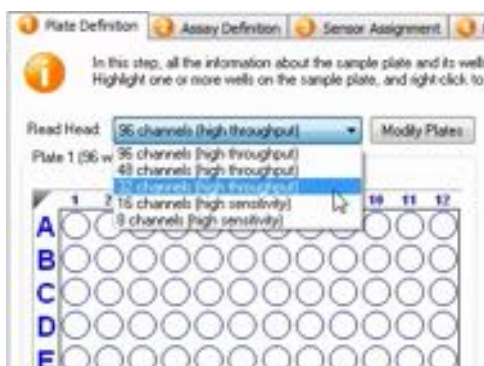


Figure 10-111: Select Wells to be Read

See “Read Head Configuration and Plate Layout” on page 572 for biosensor configurations.

2. Choose a plate format for Plate 1 and Plate 2 by clicking **Modify Plates**. Select either the 96- or 384 well format for each plate:



Figure 10-112: Select Plate 1 and Plate 2 Formats

3. Designate plate layouts for Plate 1 and Plate 2 by selecting wells in the plate maps and designating sample types. There are several ways to select sample wells in either plate map:
 - Click a column header or select adjacent column headers by click-hold-drag.
 - To select non-adjacent columns, hold the **Ctrl key** and click the column header.
 - Click a row header or select adjacent row headers by click-hold-drag.
 - Click a well or draw a box around a group of wells.
4. Designate well types by right-clicking on selected wells and assigning a sample type:



Figure 10-113: Designating Well Types

5. Enter sample information by selecting the table for either Plate 1 or Plate 2. There are several ways to enter sample information:
 - Select an individual well in the plate table.
 - Click-drag-hold several wells in the plate table, right-click and choose **Set Well Data**.



NOTE: Assigning sequential alpha-numerical names for Sample ID provides easier sorting of columns and headers for the epitope binning matrix.



NOTE: More information on sample information and annotation can be found in “Entering Sample Information” on page 467.

Tab 2 (Assay Definition)

After completing the plate layout(s), an Epitope Binning Assay can be defined by building a kinetic assay.

1. Click on Tab 2 (Assay Definition).
2. Add assay step types in the Step Data List:

- a. Click the **Add** button. The Add Step Definition box will display:



Figure 10-114: Add Step Definition Box

- b. Choose a step type.
 c. Optional: edit step name.
 d. Set the step time and shake speed.
 e. The regeneration step type requires assigning separate parameters. To do this, click the **Regeneration Params** button:

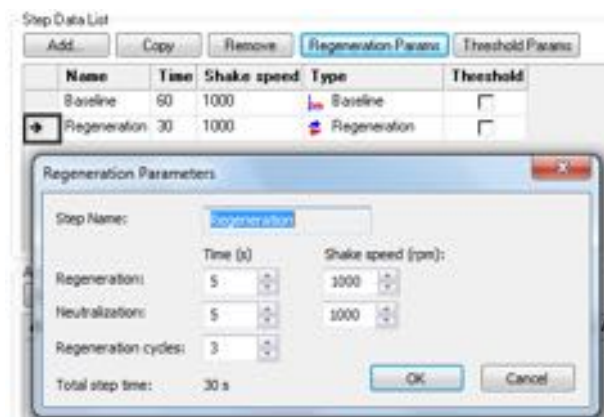


Figure 10-115: Regeneration Parameters Box

- f. Optional: assign a threshold. See “Creating Step Types” on page 488 for more information.
3. Build the assay(s) by assigning steps defined in Step Data List to columns in the plate map(s).



NOTE: We highly recommend using the Associate or Dissociate assay steps instead of Custom for epitope binning and cross-blocking experiments.

- Select a step type in the Step Data List.
- In the Plate 1 or Plate 2 map, double-click the columns that you want associated with that step type.
- The selected wells will be marked with hatching, and the new step appears in the Assay Steps List:

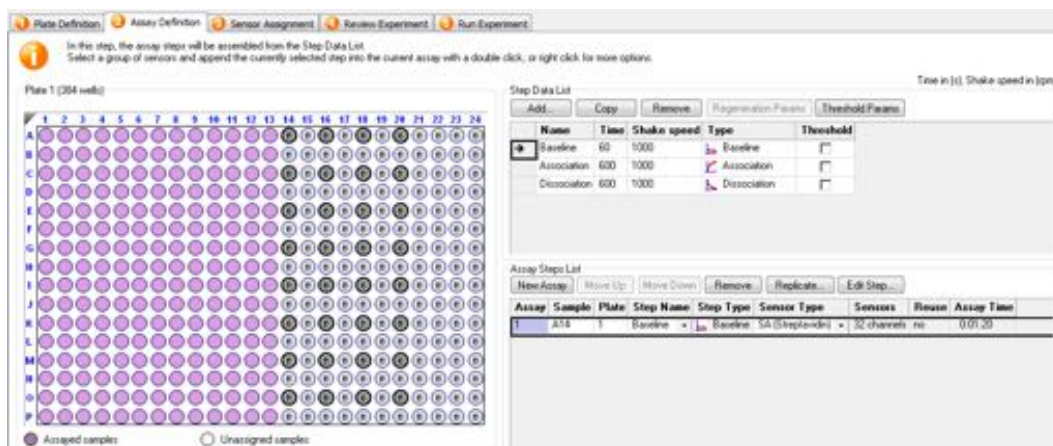


Figure 10-116: Assay Steps List

- Select the correct biosensor from the Sensor Type drop-down list. The Sensors column shows the Read Head selection made in Tab 1 (Assay Definition). The number of biosensors listed must remain the same for that assay color group.
- Repeat the previous steps to define other steps in the assay.
- New assays may be added by clicking the **New Assay** button in the Assay Steps List:

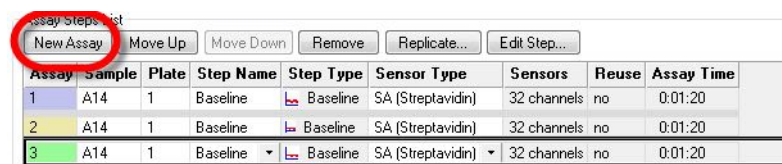


Figure 10-117: New Assay Button

- You can use new biosensors or reuse the same biosensors for the next color assay group. The default **Reuse** selection is no, which will use new biosensors:

Assay Steps List

New Assay Move Up Move Down Remove Replicate Edit Step

Assay	Sample	Plate	Step Name	Step Type	Sensor Type	Sensors	Reuse	Assay Time
1	A14	1	Baseline	Baseline	SA (Streptavidin)	32 channels	no	
1	B14	1	Association	Association	SA (Streptavidin)	32 channels	no	
1	A15	1	Dissociation	Dissociation	SA (Streptavidin)	32 channels	no	0:22:00
2	A17	1	Baseline	Baseline	SA (Streptavidin)	16 channels	no	
2	B14	1	Association	Association	SA (Streptavidin)	16 channels	no	
2	A15	1	Dissociation	Dissociation	SA (Streptavidin)	16 channels	no	0:22:00
3	A14	1	Baseline	Baseline	SA (Streptavidin)	8 channels	no	
3	B14	1	Association	Association	SA (Streptavidin)	8 channels	no	
3	A15	1	Dissociation	Dissociation	SA (Streptavidin)	8 channels	no	0:22:00

Figure 10-118: Default Biosensor Reuse Selection



NOTE: The Reuse option is only available for the Octet HTX system at this time.



NOTE: We recommend adding Regeneration steps at the end of the current assay before reusing the same biosensors on the next color assay group.



NOTE: We recommend not reusing biosensors ('no' in Reuse column) with epitope binning experiments. Regeneration is recommended within an individual color group assay, but start the next assay color group with next set of biosensors.

To reuse the biosensors from the current assay color group for the next color assay group, select a step in the current assay and click the **Edit Step** button. Select the **Reuse sensors** box and click **OK**. The **Reuse** selection will now be set to yes.

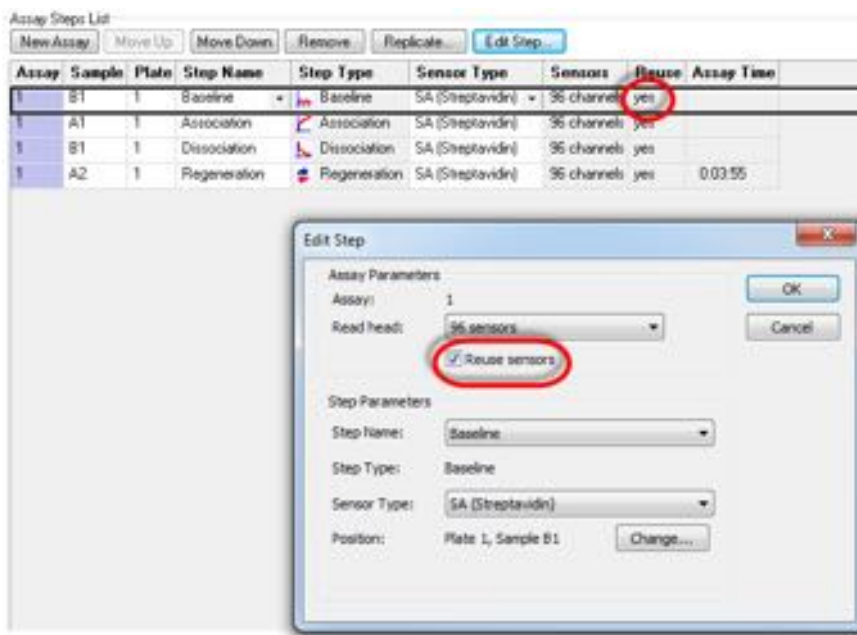


Figure 10-119: Changing the Biosensor Reuse Selection



NOTE: More information on assay step editing in Tab 2 (Assay Definition) can be found in “Creating Step Types” on page 488.

Tab 3 (Sensor Assignment)

After completing the assay definition, click on Tab 3 (Sensor Assignment) to assign sensor type(s) for the epitope binning experiment.



NOTE: The Sensor Type for the assay must be selected or changed from the Assay Steps List in the Assay Definition Tab. Changing the Sensor Type from the Sensor Assignment Tab will not update the assay.



NOTE: Full details on biosensor assignment in Tab 3 (Sensor Assignment) can be found in “Assigning Biosensors to Samples” on page 502.

Replacing Biosensors in the Biosensor Tray. After an assay is completed, biosensors can either be returned to the biosensor tray or ejected through the chute. To return them to the tray, click the Replace sensors in tray after use check box:

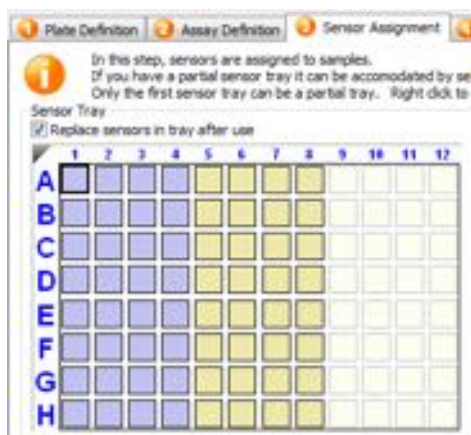


Figure 10-120: Replace Sensors in Tray After Use Check Box

Tab 4 (Review Experiment)

Before running the experiment, click on Tab 4 (Review Experiment) to review the sample plate layout, assays and assay steps as well as the biosensors assigned to each assay in the experiment.

Move the slider left or right in the window or click the arrows to highlight the biosensors and samples associated with an assay step:



Figure 10-121: Navigating the Review Experiment Tab

Alternatively, select an assay step to view the biosensors and samples associated with it.

Saving Experiments

After an experiment is run, the software automatically saves the experiment information that you specified (sample plate definition, biosensor assignment, assay settings, etc.) to an experiment method file (.fmf).

If you set up an experiment but do not start the run, you can manually save the experiment method. To do this:

1. Select **File > Save Method File**.
2. In the Save dialog box, enter a name and location for the file, and click **Save**.

Loading the Biosensor Tray and Sample Plates

To load the biosensor tray and plate positions 1 and 2:

1. Click **Instrument > Present Stage** to open the door and present the stage. Alternatively, click the **Present Stage** button:

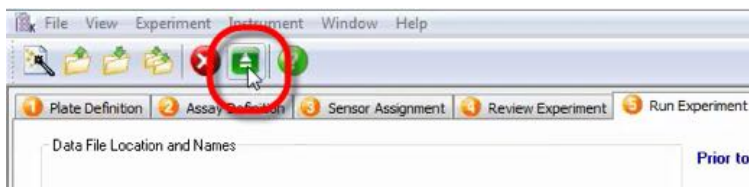


Figure 10-122: Present Stage Button

2. Place the biosensor tray, biosensor wetting plate, Plate 1, and Plate 2 on the appropriate stage so that well A1 is located at the upper right corner.
3. Close the stage and door by clicking the **Present Stage** button again.

Tab 5 (Run Experiment)

1. Click on Tab 5 (Run Experiment) to confirm the default settings or set new settings.
2. To start the experiment, click the **GO** button:

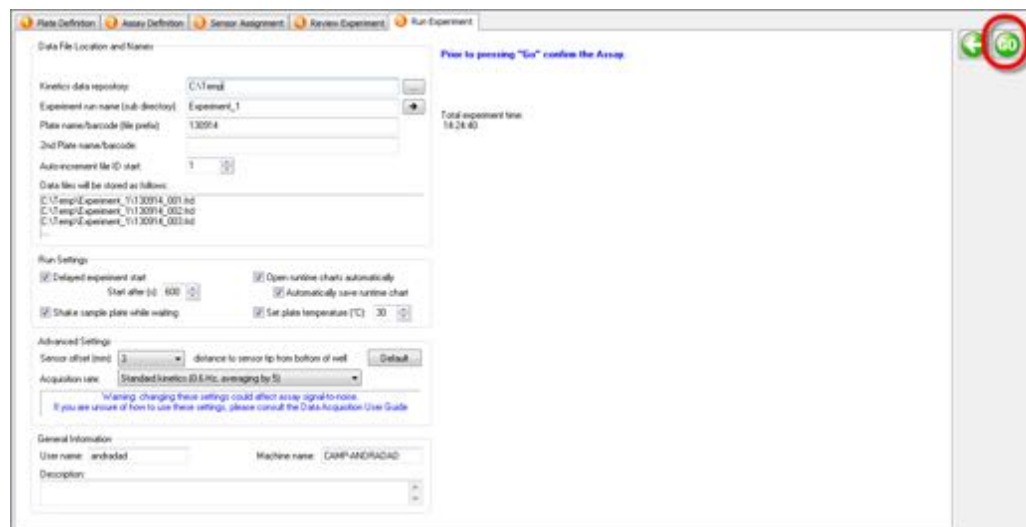


Figure 10-123: GO Button

CHAPTER 11:

Maintenance

Troubleshooting and Service	626
Octet K2, RED96, RED96 ^e and QK ^e Systems	626
Octet RED384 and Octet QK384 Systems	629



NOTE: For Octet HTX system maintenance-related questions, please contact your local Pall ForteBio representative or Technical Support at fortebio_support@pall.com or +1-650-322-1360.

TROUBLESHOOTING AND SERVICE

For troubleshooting and service requests, please contact your local Pall ForteBio representative or Technical Support at fortebio_support@pall.com or +1-650-322-1360.

OCTET K2, RED96, RED96e AND QK^e SYSTEMS

Cleaning the Octet Instrument



WARNING: Sample platform may be hot if the instrument has been in operation. Wait for the platform to cool before attempting to clean.



WARNING: Il se peut que la plateforme d'analyse des échantillons chauffe si l'appareil est en train de fonctionner. Attendez que la plateforme refroidisse avant de tenter de la nettoyer.



WARNING: War das Gerät in Betrieb, ist die Probenplattform möglicherweise heiß. Lassen Sie die Plattform vor der Reinigung abkühlen.



NOTE: If you use the Octet instrument regularly, clean the interior horizontal surfaces daily with a Kimwipe® tissue moistened with a 30–60% isopropyl alcohol solution. Otherwise, clean once a week or as needed.

Routine cleaning of the Octet instrument:

1. Turn off the power to the instrument
2. Open the system door.
3. Wipe the biosensor and sample platform (Figure 11-1).
4. Carefully wipe the eight biosensor pickup tips.
5. Allow the surfaces to dry for at least one minute with the door open.

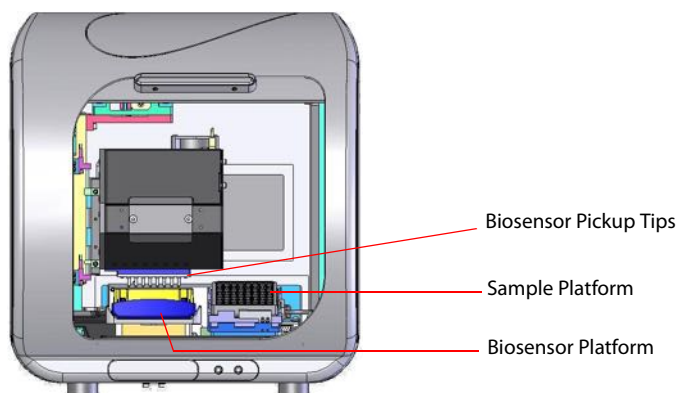


Figure 11-1: Octet Instrument

Cleaning Guidelines



WARNING: System users are responsible for appropriate decontamination in case of spillage of hazardous materials on or inside of the equipment. If there are any doubts about the compatibility of a cleaning agent or decontamination procedure with the materials making up the Octet system, please consult with Pall ForteBio before proceeding.



WARNING: Il incombe aux utilisateurs du système de procéder à une décontamination adéquate en cas de débordement de produits dangereux sur ou à l'intérieur de l'équipement. En cas de doute sur la compatibilité d'un détergent ou d'une procédure de décontamination avec les matériaux composant le système Octet, veuillez vous adresser à Pall ForteBio avant toute intervention.



WARNING: Die angemessene Dekontamination des Systems im Falle der Freisetzung gefährlicher Substanzen auf dem oder innerhalb des Geräts liegt in der Verantwortung des Systembenutzers. Sollten Zweifel über die Kompatibilität eines Reinigungsprodukts oder Dekontaminationsverfahrens im Hinblick auf die Werkstoffe bestehen, aus denen das Octet-System gefertigt ist, wenden Sie sich vor der Reinigung bitte an Pall ForteBio.

- In case of accidental spills inside the instrument enclosure, use a dry paper towel or cloth to absorb the liquid.



WARNING: *If a large volume of liquid has been spilled in or near the instrument, turn off the power prior to cleaning, and wait at least 24 hours before attempting to restart the instrument. Never place anything on top of the instrument.*



WARNING: *En cas de débordement important de liquide à l'intérieur ou aux abords de l'instrument, éteignez l'appareil avant de procéder au nettoyage et attendez au moins 24 heures avant de tenter de le redémarrer. Ne posez rien sur l'instrument.*



WARNING: *Wenn große Mengen Flüssigkeit im Gerät oder in der Nähe des Geräts verschüttet wurden, schalten Sie das Gerät vor der Reinigung zunächst aus, und warten Sie mindestens 24 Stunden, bevor Sie es wieder in Betrieb nehmen. Platzieren Sie niemals Objekte auf der Oberseite des Gerät.*

- Dirt, stains, or residue left after removing the liquid can be removed by gently wiping with a damp paper towel or cloth.
- Mild liquid soap can be added to the damp paper towel or cloth to remove difficult stains or debris from the exterior surface.
- No organic solvents should be used to clean the enclosure surface.
- Biological contaminants can be removed by wiping the surface of the instrument with a general disinfectant such as a 10% bleach solution or a Virkon (1%) solution using a damp paper towel or cloth, and leaving for a minimum contact time of 10 minutes. The selection and effectiveness of the disinfectant will depend on the type of contaminant and should be considered by the system user.

Emptying the Waste Container

To empty the waste container:

1. Press on the container to open it (Figure 11-2).
2. Pull the container out and completely remove it from the instrument.
3. Remove the container insert with the biosensor tips and dispose of both in a biohazard container suitable for sharp objects.



NOTE: *Pall ForteBio recommends that the waste container be emptied after every run of a 96-biosensor tray.*

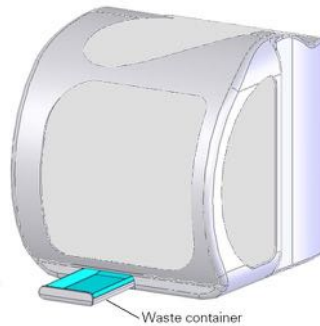


Figure 11-2: Waste Container for the Octet Instrument

Replacing Fuses



WARNING: All fuse replacements need to be performed by Pall ForteBio service personnel. Pall ForteBio is not responsible for personal injury incurred by unqualified personnel during fuse replacement or any other repair.



WARNING: Chaque remplacement de fusible doit être effectué par le personnel de maintenance de Pall ForteBio. Pall ForteBio décline toute responsabilité en cas de blessures dues au recours à du personnel non qualifié pour assurer le remplacement des fusibles ou toute autre réparation.



WARNING: Das Auswechseln von Sicherungen muss stets von Servicepersonal von Pall ForteBio vorgenommen werden. Pall ForteBio übernimmt keine Verantwortung für Personenschäden, die infolge der Auswechslung von Sicherungen oder der Durchführung sonstiger Reparaturen durch ungeschultes Personal entstehen.

OCTET RED384 AND OCTET QK384 SYSTEMS

Cleaning the Octet Instrument



NOTE: If you use the Octet instrument regularly, clean the interior horizontal surfaces daily with a Kimwipe moistened with a 30–60% isopropyl alcohol solution. Otherwise, clean once a week or as needed.

To clean the Octet instrument:

1. Present the sample plate stage (Figure 11-3).
2. Turn off the power to the instrument.
3. Open the system door.
4. Wipe the biosensor and sample platform.
5. Allow the surfaces to dry for at least one minute with the door open.

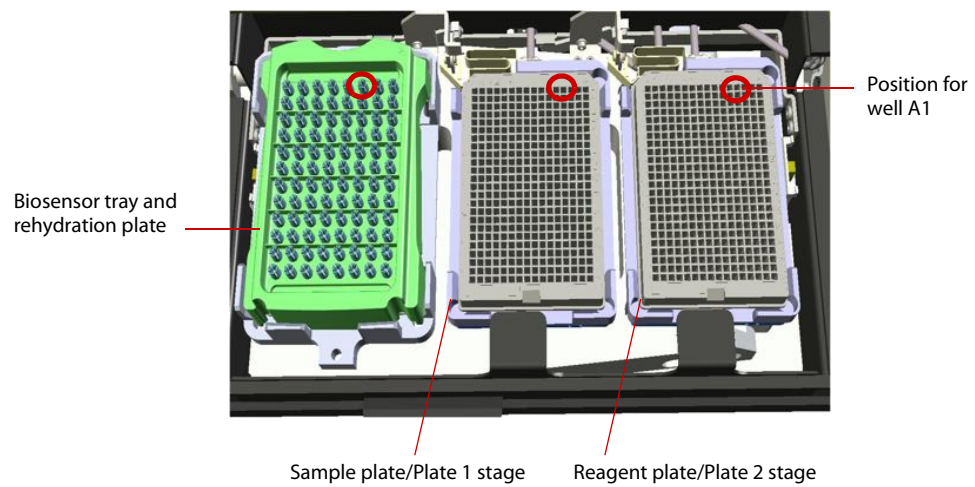


Figure 11-3: Octet RED384 and QK384 Stage Platform

Cleaning the Biosensor Pickup Tips

The biosensor pickup tips hold the biosensors during an assay. Pall ForteBio now offers Biosensor Mount Cleaning Trays (Part No: 18-5133, pack of 12) to perform periodic, regular, automated cleaning of biosensor mounts on Octet HTX, RED384 and QK384 instruments.

With normal instrument use, plastic residue from BLI biosensor hubs can accumulate on the tips of the metal biosensor mounts inside the instrument. This accumulation can potentially lead to incorrect loading of biosensors and inconsistencies in data sensing. It is very important to periodically remove the plastic residue in order to ensure continued optimal performance of the Octet system.

Important notes:

- The Biosensor Mount cleaning procedure is meant to be a preventative measure and will not remove excessive accumulations of plastic residue.
- If Biosensor Mounts are particularly dirty (indicated by visible thick white film around the tip of the mounts), OR if the instrument has been used for an extended period of time without cleaning, we recommend first scheduling a Preventative Maintenance visit with one of our service engineers before initiating regular cleanings with Cleaning Trays.
- Cleaning Trays are to be utilized for automated cleaning only with the designated method in Octet Data Acquisition Software version 9 and up.
- Cleaning Trays are recommended for single use only.

Cleaning Procedure

1. Remove the Cleaning Tray from plastic bag.
2. Spray the top of the Cleaning Tray sponge three times with 70% ethanol in a spray bottle. Do not over moisten!
3. Open Octet Data Acquisition software and wait for the instrument to initialize.
4. From the Instrument menu, select **Clean Biosensor Mounts**. The instrument stage will present automatically.
5. Place the moistened Cleaning Tray in the Tray position on the instrument stage and click **OK**.
6. Follow the simple prompts in the software dialogue to begin the cleaning protocol. The full automated cleaning cycle will take approximately 8 minutes to complete.
7. Once the cleaning cycle is complete, the instrument stage will present again. Remove the used Cleaning Tray from the stage and discard.

Cleaning Frequency

For instruments under moderate use (a few to several assays per week), running the cleaning procedure once per month is recommended. For heavily utilized instruments (daily use for screening applications), cleaning may need to be performed as often as once per week.

Minimally, the cleaning procedure should be performed after every 100 biosensor pickups. Octet Data Acquisition software will display a reminder message to run the cleaning procedure after it has recorded 100 biosensor pickups.

Replacing Fuses



WARNING: All fuse replacements need to be performed by Pall ForteBio service personnel. Pall ForteBio is not responsible for personal injury incurred by unqualified personnel during fuse replacement or any other repair.



WARNING: Chaque remplacement de fusible doit être effectué par le personnel de maintenance de Pall ForteBio. Pall ForteBio décline toute responsabilité en cas de blessures dues au recours à du personnel non qualifié pour assurer le remplacement des fusibles ou toute autre réparation.



WARNING: Das Auswechseln von Sicherungen muss stets von Servicepersonal von Pall ForteBio vorgenommen werden. Pall ForteBio übernimmt keine Verantwortung für Personenschäden, die infolge der Auswechslung von Sicherungen oder der Durchführung sonstiger Reparaturen durch ungeschultes Personal entstehen.

APPENDIX A:

Using Octet384 and HTX Systems with an Automation Interface

Overview	634
Design of the Automation Interface	634
Automation Commands	637

OVERVIEW

The Octet Data Acquisition software provides support for an automation interface using a COM port (RS-232) or a Transmission Control Protocol/Internet Protocol (TCP/IP) socket/port.

An example application for testing the automation interface, called **AutomationClient.exe**, is included in the applications and Dynamic Link Libraries (DLLs) installed with the Octet Data Acquisition software. The file is located in the C:\Program Files\ForteBio\DataAcquisition directory.



NOTES:

The automation interface can be used with Octet384 systems only.

The examples that follow are illustrated using a TCP/IP connection, but the serial port connection behaves identically.

DESIGN OF THE AUTOMATION INTERFACE

The automation interface is designed to be as universal as possible, making no assumptions about the communication medium or the language of the client application connecting to the Octet System Data Acquisition software.

The following guidelines apply:

- All commands and responses are ASCII strings, one per line.
- All lines are terminated with both carriage-return and line-feed characters ("r\n").
- Each command starts with the name of the command and may then be followed by required and optional parameters.
- Each parameter starts with a switch definition (a la dos/unix command line) followed by the parameter itself, which allows parameters to be sent in any order.
- The command or response is terminated with a new line (CR/LF) sequence.
- Parameters containing embedded spaces need to be enclosed in double quotes.

Automation Interface Control Setup

Before the Octet System Data Acquisition software can be controlled using an automation interface, the correct automation options must be set. To do this, go to **File > Options** (Figure A-1) and select the appropriate port in the **Automation** box.



NOTE: *The Octet System Data Acquisition software can be controlled via automation interface through a serial port (RS-232) or a TCP/IP socket.*

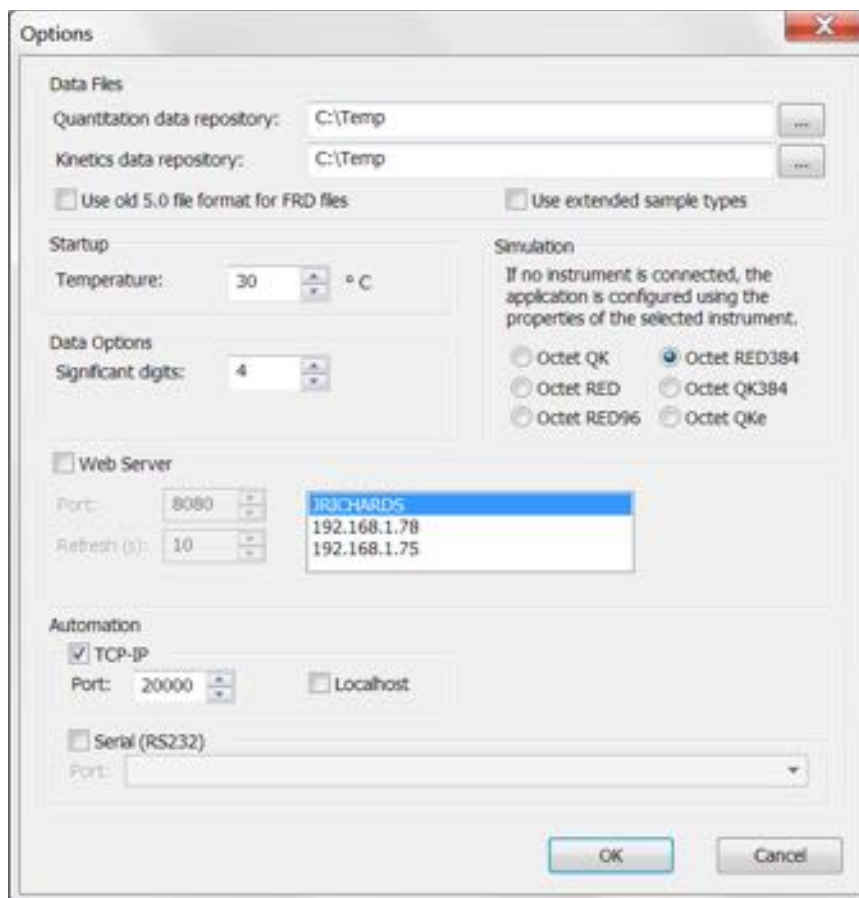


Figure A-1: Options Dialog Box—Automation Interface Selection



NOTE: The **Localhost** option can be useful in developing the automation client on the same computer that runs the Octet System Data Acquisition software.



NOTE: Pall ForteBio recommends that the **Data File** repositories be set using shared folders addressed by “UNC” folder names so that the internal path used by the Data Acquisition application corresponds to the external path used to access/retrieve the data files recorded during the experiment. Alternatively, the path returned by the **GetRunInfo** command to access the data files from another computer on the LAN.

Automation Client Example Application

The **Automation Client** example application can connect to the Octet System Data Acquisition software via serial port (RS-232) port or TCP/IP socket.

To connect the Automation Client example application:

1. In the Octet System Data Acquisition software, go to **File > Options** (see Figure A-1).
2. In the **Automation** box, select the communication port to be used (either TCP/IP or RS232, see Figure A-1).
3. Launch **AutomationClient.exe** located in the C:\Program Files\ForteBio\DataAcquisition directory to display the **Automation Client** dialog box (Figure A-2).

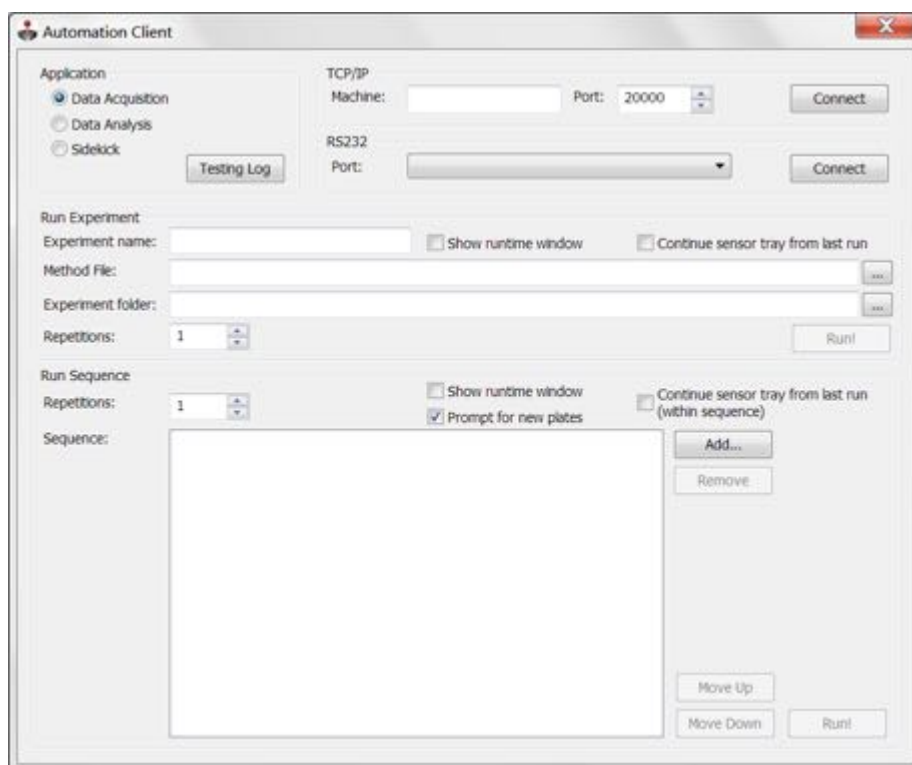


Figure A-2: Automation Client Window

4. Select the TCP/IP or RS-232 port selected previously in the Octet Data Acquisition software **Options** dialog box (Figure A-1). To connect locally using **localhost**, leave the **Machine** field blank.
5. Click **Connect**.

If the port is successfully opened, the automation client dialog will be minimized and remain minimized, indicating that the connection succeeded and the port is open. Otherwise, the automation client dialog will minimize and come back again, indicating that the connection attempt failed.

- After a successful connection is established, send the default **Version** command (in the **Send Commands—Command** field) and then click **Send!**.

A response similar to the following should appear in the **Response** box:

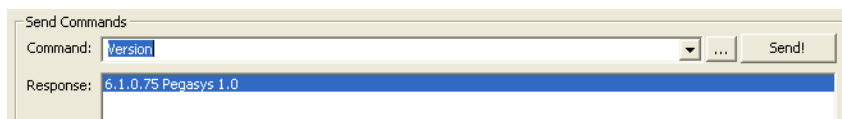


Figure A-3: Send Commands—Command Field

The response indicates that the **Automation Client** has connected to the Octet System Data Acquisition software. This example indicates that version 6.1.0.75 of the Data Acquisition software is controlling an Octet instrument using version 1.0 of the automation interface.

AUTOMATION COMMANDS

Table A-1 summarizes the commands supported by the Octet System Data Acquisition software automation interface.



NOTE: The symbolic names are provided for C++ clients who connect using the interface as defined in the *AutomationAPI.h* header file.

Table A-1: Commands Supported by the Automation Interface

Command	Symbolic Name	Purpose
Version	AUT_CMD_VERSION	Returns the version of the application being automated, the type of instrument it is controlling, and the automation API version.
Reset	AUT_CMD_RESET	Stops any running experiment and resets the instrument.
GetMethodInfo	AUT_CMD_GETMETHODINFO	Returns information about the resources required by given method file.
Run	AUT_CMD_RUN	Runs an experiment using a given method file.
GetRunInfo	AUT_CMD_GETRUNINFO	Returns information about the experiment currently running.

Table A-1: Commands Supported by the Automation Interface (Continued)

<i>Command</i>	<i>Symbolic Name</i>	<i>Purpose</i>
Stop	AUT_CMD_STOP	Stops a running experiment, ejecting the sensors if necessary.
Status	AUT_CMD_STATUS	Returns status during a running experiment: OK = ready Busy = running Waiting = waiting for a condition to be resolved Error = experiment was terminated by an error Busy is followed by descriptive information on the progress of the experiment (% complete)
Present	AUT_CMD_PRESENT	(Octet 384 only) Open the door and move the stage to the presentation position.
Resume	AUT_CMD_RESUME	Indicates that the “Waiting” condition has been resolved (new sensor tray installed). Continues the experiment.
Close	AUT_CMD_CLOSE	Closes the door if it is open. Homes the read head on an Octet 384 instrument.
Cleanup	AUT_CMD_CLEANUP	Closes open MDI windows. Only valid when not busy. Useful when using the Run command without the -s option.

Typical Automation Session

The following example is a typical automation session that illustrates the use of the automation commands to run an experiment.



NOTE: Commands sent from the client application are designated as **SEND:**. Responses received from the Octet System Data Acquisition software are designated as **RECV:**.

Connecting to the Data Acquisition Software

```
SEND: Version\r\n
RECV: 6.1.0.30 Pegasys 1.0
SEND: Status\r\n
RECV: OK
```

Preparing for an Experiment

```
SEND: Cleanup
RECV: OK
SEND: GetMethodInfo -mC:\MethodFiles\Q001.fmf\r\n
RECV: OK -p96,0 -t1 -s"Anti-Human IgG Fc"
```

Starting the Experiment

```
SEND: Version\r\n
RECV: 6.1.0.30 Pegasys 1.0
SEND: Run\r\n
RECV: OK
```

Getting Information about the Experiment

```
SEND: Version\r\n
RECV: 6.1.0.30 Pegasys 1.0
SEND: GetRunInfo\r\n
RECV: OK -n"Experiment 1" -p"\\fbdata\Quantitation\Experiment 1"
```

Monitoring the Experiment

```
bool bBusy = true;
while (bBusy)
{
    Send("Status\r\n");
    response = Recv();

    if (response==OK)
```

```
        bBusy = false;
    else
        Sleep(1000); // sleep for a second
}
```

```
SEND: Status\r\n
RECV: Running (5%)
```

```
SEND: Status\r\n
RECV: Running (25%)
```

```
SEND: Status\r\n
RECV: Running (45%)
```

```
SEND: Status\r\n
RECV: Running (75%)
```

```
SEND: Status\r\n
RECV: Running (95%)
```

```
SEND: Status\r\n
RECV: OK
```

Stopping the Experiment and Presenting the Plate for Unloading

Both the **Stop** and the **Present** commands are asynchronous—they initially return **OK** to indicate that the command was accepted and started OK, but status must be polled until **OK** is returned to indicate completion.

```
SEND: Stop\r\n
RECV: OK
```

```
SEND: Status\r\n
RECV: Busy
```

```
SEND: Status\r\n
RECV: Busy
```

```
SEND: Status\r\n
RECV: OK
```

```
SEND: Present\r\n
RECV: OK
```

```
SEND: Status\r\n
```

```
RECV: Busy
```

```
SEND: Status\r\n
```

```
RECV: Busy
```

```
SEND: Status\r\n
```

```
RECV: OK
```

Advanced Automation Session

If an experiment is sufficiently complex it may require more than one tray of sensors to complete the experiment. This can be detected at the start of the experiment by checking the **-tN** response from the **GetMethodInfo** command. If *N* is greater than **1**, then the experiment requires more than one tray of sensors to complete.

If this is the case, initially the experiment will start as before, but halfway through the experiment the **Status** command will return **LoadSensors** indicating that the first tray of sensors has been exhausted and another tray of sensors needs to be loaded. At this point, you must issue the **Present** command to allow access to the sensor plate (polled for completion) and then once the new sensor tray is in place, the **Resume** command must be sent to resume the experiment.

Connecting to Data Acquisition

```
SEND: Version\r\n
```

```
RECV: 6.1.0.30 Pegasys 1.0
```

```
SEND: Status\r\n
```

```
RECV: OK
```

Preparing for an Experiment

```
SEND: Cleanup
```

```
RECV: OK
```

```
SEND: GetMethodInfo -mC:\MethodFiles\Q002.fmf\r\n
```

```
RECV: OK -p96,0 -t2 -s"Anti-Human IgG Fc"
```

Starting the Experiment

```
SEND: Run -mC:\MethodFiles\Q002.fmf -bP0001 -s\r\n
```

```
RECV: OK
```

Getting Information about the Experiment

```
SEND: GetRunInfo\r\n
```

```
RECV: OK -n"Experiment 2" -p"\\fbdata\Quantitation\Experiment 2"\r\n
```

Monitoring the Experiment

```

bool MonitorExperiment(CCmdTransport *pPort)
{
    // Poll the experiment until it is done.
    for (;;)
    {
        Sleep(200);

        if (!SendRecv(pPort, AUT_CMD_STATUS + AUT_EOL, csResp))
            return false;

        int nStart = 0;
        CString csStatus = csResp.Tokenize(" ", nStart);

        if (csStatus == AUT_OK)
            break; // SUCCESS
        else if (csStatus == AUT_STOPPED)
            break; // SUCCESS
        else if (csStatus == AUT_RUNNING)
            ;
        else if (csStatus == AUT_WAITING)
            ;
        else if (csStatus == AUT_LOADSENSORS)
        {
            if (!LoadSensors(pPort))
                return false;
        }
        else if (csStatus == AUT_BUSY)
            ;
        else if (csStatus == AUT_ERROR)
            return false;
    }
}

bool LoadSensors(CCmdTransport *pPort)
{
    if (!SendRecv(pPort, AUT_CMD_PRESENT + AUT_EOL, csResp))
        return false;

    if (csResp != AUT_OK)
        return false;
}

```



```
if (!WaitNotBusy(pPort))
    return false;

// At this point the robot replaces the sensor tray.
AfxMessageBox("Robot changes sensor tray...");

if (!SendRecv(pPort, AUT_CMD_RESUME + AUT_EOL, csResp))
    return false;

if (csResp != AUT_OK)
    return false;

return WaitNotBusy(pPort);
}

bool WaitNotBusy(CClientResponder *pPort)
{
    CCountdownTimer Timer(c_uBusyTimeoutMS);
    CString csResp;
    while (!Timer.IsDone())
    {
        Sleep(200);

        if (!SendRecv(pPort, AUT_CMD_STATUS + AUT_EOL, csResp))
            return false;

        int nStart = 0;
        CString csStatus = csResp.Tokenize(" ", nStart);

        if (csStatus == AUT_OK)
            return true;
        else if (csStatus == AUT_STOPPED)
            return false;
        else if (csStatus == AUT_RUNNING)
            return true;
        else if (csStatus == AUT_WAITING)
            return true;
        else if (csStatus == AUT_LOADSENSORS)
            return true;
    }
}
```

```

        else if (csStatus == AUT_BUSY)
            ;
        else if (csStatus == AUT_ERROR)
            return false;
    }
    TRACE1("Timeout waiting for not busy after %d ms\n",
Timer.GetElapsed());
    return false;
}

```

Automation API.H

```

//
*****
//
//      Copyright (c) 2011 ForteBio.
//      All rights reserved.
//
//
*****
// HEADER:  AutomationAPI.h
// PURPOSE: Defines the commands supported by the automation API.
// AUTHOR:  BHI   Nov 2008
//
#ifdef INC_ACQUISITION_AUTOMATIONAPI_H
#define INC_ACQUISITION_AUTOMATIONAPI_H

// NOTES:
// Do not position the Octet instrument such that it is difficult to dis-
connect the power.
// The automation interface is string based. Commands and responses are
strings, one per line.
// Each command starts with the name of the command and may then be fol-
lowed by required and optional parameters.
// Each parameter starts with a switch definition (a la dos/unix command
line) followed by the parameter itself. This allows parameters to be sent
in any order.
// The command or response is terminated with a new line (CR/LF) sequence.
// Parameters containing embedded spaces must be enclosed in double
quotes.
// Response items containing embedded spaces will be enclosed in double
quotes.

// REVISIONS:
// 1.0      First release

```

```

// 1.1      Added (-p) plate file parameter to "Run" and "GetMethodInfo"
// commands
//          Added (-u) use-last-sensor-tray option to the "Run" command.
//          Added "SetValue" command to set the temperature target.

// Version of the API described in this header file.
const char AUT_API_VERSION[] = "1.1";

// Status return values
const char AUT_OK[]          = "OK";
const char AUT_STOPPED[]     = "Stopped";
const char AUT_RUNNING[]     = "Running";
const char AUT_WAITING[]     = "Waiting";
const char AUT_LOADSENSORS[] = "LoadSensors";
const char AUT_BUSY[]        = "Busy";           // Resetting, Presenting
const char AUT_ERROR[]       = "ERROR";
const char AUT_EOL[]         = "\r\n";

// Parameter switches for the Run command
const char AUT_SWITCH_METHOD      = 'm';        // Method file to load
(required)
const char AUT_SWITCH_FOLDER      = 'f';        // Root folder for experiment
data (optional)
const char AUT_SWITCH_EXPERIMENT  = 'e';        // Override for the experiment
name in the FMF file (optional)
const char AUT_SWITCH_PLATEFILE   = 'p';        // Plate file to import after
method file is loaded (optional)
const char AUT_SWITCH_BARCODE     = 'b';        // Bar code of Sample plate
(optional)
const char AUT_SWITCH_BARCODE1    = '1';        // Alias for AUT_SWITCH_BAR-
CODE (optional)
const char AUT_SWITCH_BARCODE2    = '2';        // Bar code of Reagent plate
(optional)
const char AUT_SWITCH_LOTNUMBER   = 'l';        // Lot number of sensors
(optional)
const char AUT_SWITCH_SILENT      = 's';        // Don't open the runtime win-
dow (optional)
const char AUT_SWITCH_USELAST     = 'u';        // Reuse the sensor tray as
it was left after last run (optional)
const char AUT_SWITCH_VERBOSE     = 'v';        // Send back verbose status
information

// Parameter switches for the SetValue command
const char AUT_SWITCH_TEMPERATURE = 't';

```

```

// Response parameter switches for the GetMethodInfo command
const char AUT_RESPONSE_PLATEWELLS = 'p';
const char AUT_RESPONSE_SENSORTRAYS = 't';
const char AUT_RESPONSE_SENSORTYPE = 's';
const char AUT_RESPONSE_EXPTYPE = 'e';
const char AUT_RESPONSE_RERACKING = 'r';

// Response parameter switches for the GetRunInfo command
const char AUT_RESPONSE_EXPNAME = 'n';
const char AUT_RESPONSE_EXPPATH = 'p';

const char AUT_CMD_VERSION[] = "Version";
// Returns the version of the app being automated, the hardware platform
it controls, and the API version.
// Args: (none)
// Response: App product version (e.g. "6.0.0.120 Pegasys 1.0\r\n")

const char AUT_CMD_RESET[] = "Reset";
// Stops any running experiment and resets the instrument.
// Args: (none)
// Response:
//   "OK\r\n"
//   "Error: <reason>\r\n"

const char AUT_CMD_GETMETHODINFO[] = "GetMethodInfo";
// Returns info about a method file
// Args:
//   -m <path>      Method file name (required)
// Response:
//   "OK -r<bool> -t<int> -s<name>\r\n"
// e.g. OK -p96,0 -t2 -s"SA (Streptavidin)\r\n"
// Response params:
//   -p<int>,<int>  Sizes of the plates in use          e.g. -
//                  p384,96
//   -t<int>        Number of sensor trays required (0 .. 5)   e.g. -t2
//   -s<name>       Name of first sensor in the tray          e.g. -s"SA
//                  (Streptavidin)"
//   "Error: load method\r\n"
//   "Error: bad method\r\n"

const char AUT_CMD_RUN[] = "Run";

```

```

// Runs an experiment
// Args:
//   -m <path>      Method file name (required)
//   -p <path>      Plate file to update sample plate in method settings
//                  (optional)
//   -b <barcode>   Sample plate bar code (optional)
//   -1 <barcode>   Sample plate bar code (optional)
//   -2 <barcode>   Reagent plate bar code (optional)
//   -l <lotnumber> Sensor tray lot number (optional)
//   -s             Silent - does not open the runtime view (optional)
//   -u             Use the state of the sensor tray as it was left after
//                  last run
// Response:
//   "OK\r\n"
//   "Error: not ready\r\n"
//   "Error: bad method\r\n"
//   "Error: bad barcode\r\n"

const char AUT_CMD_GETRUNINFO[] = "GetRunInfo";
// Returns information about an experiment that is currently running
// Args: (none)
// Response:
//   "OK -n"Experiment 1" -p"\\fbdata\\Quantitation\\Experiment 1"\r\n"
//   "Error: <reason>\r\n"
// Response params:
//   -n<experiment name> Name of the experiment (folder name in repository)
//                       e.g. -n"Experiment 1"
//   -p<experiment path> Full path to experiment folder in repository
//                       e.g. -p"\\fbdata\\Quantitation\\Experiment 1"

const char AUT_CMD_STOP[] = "Stop";
// Stops a running experiment
// Args: (none)
// Response:
//   "OK\r\n"
//   "Error: <reason>\r\n"

const char AUT_CMD_SETVALUE[] = "SetValue";
// Sets a value
// Args:
//   -t <temp>      Sets heater target temperature (DegC)
// Response:

```

```
// "OK\r\n"
// "Error: <reason>\r\n"

const char AUT_CMD_STATUS[] = "Status";
// Returns status: OK=ready, Busy=running, Error=Experiment was terminated
// by an error.
// Busy is followed by descriptive information on the progress of the
// experiment (% complete)
// Args: (none)
// Response:
// "OK\r\n"
// "Waiting\r\n"
// "Busy\r\n"
// "Running (nn%)\r\n"
// "LoadSensors\r\n"
// "Error: <reason>\r\n"

const char AUT_CMD_PRESENT[] = "Present"; // Pegasys only
// Open the door and move the stage to the presentation position.
// Args: (none)
// Response:
// "OK\r\n"
// "Error: <reason>\r\n"
// N.B.: Poll status waiting for "Waiting" condition to reappear

const char AUT_CMD_RESUME[] = "Resume";
// Indicates that the "Waiting" condition has been resolved (new sensor
// tray installed). Continues the experiment.
// Args: (none)
// Response:
// "OK\r\n"
// "Error: <reason>\r\n"
// Status will indicate busy until door is closed, then will return to
// Running state.

const char AUT_CMD_CLOSE[] = "Close";
// Closes the stage if it is open.
// Args: (none)
// Response:
// "OK\r\n"
// "Error: <reason>\r\n"
// Status will indicate busy until door is closed.
```

```

const char AUT_CMD_CLEANUP[] = "Cleanup";
// Closes open MDI windows. Only valid when not busy.
// Args: (none)
// Response:
//   "OK\r\n"
//   "Error: busy\r\n";

```

```

#endif // INC_ACQUISITION_AUTOMATIONAPI_H

```

Analysis Automation API

```

//
*****
//
//   Copyright (c) 2011 ForteBio.
//   All rights reserved.
//
//
*****
// HEADER: AutomationAPI.h
// PURPOSE: Defines the commands supported by the automation API.
// AUTHOR: BHI Nov 2008
//
#ifndef INC_ANALYSIS_AUTOMATIONAPI_H
#define INC_ANALYSIS_AUTOMATIONAPI_H

// NOTES:
// * The automation interface is string based. Commands and responses are
// strings, one per line.
// * Each command starts with the name of the command and may then be
// followed by required and
// optional parameters.
// * Each parameter starts with a switch definition (a la dos/unix command
// line) followed by the
// parameter itself. This allows parameters to be sent in any order.
// * The command or response is terminated with a new line (CR/LF)
// sequence.
// * Parameters containing embedded spaces must be enclosed in double
// quotes.
// * Response items containing embedded spaces will be enclosed in double
// quotes.

```

```

// Version of thew API described in this header file.
const char AUT_API_VERSION[] = "1.0";

// Status return values
const char AUT_OK[]          = "OK";
const char AUT_RUNNING[]     = "Running";
const char AUT_ERROR[]       = "ERROR";
const char AUT_BUSY[]        = "Busy";
const char AUT_STOPPED[]     = "Stopped"; // Stopped by user.
const char AUT_EOL[]         = "\r\n";

// Parameter switches for the LOAD command
const char AUT_SWITCH_DATASET = 'd';

// Parameter switches for the ANALYZE command
const char AUT_SWITCH_PARAMS  = 'p';
const char AUT_SWITCH_XMLINFO = 'x';

// COMMAND API
// =====

const char AUT_CMD_VERSION[] = "Version";
// Returns the version of the app being automated, and the API version.
// Args: (none)
// Response: App product version (e.g. "6.3.1.12 1.0\r\n")

const char AUT_CMD_LOAD[]    = "Load";
// Loads an experiment
// Args:
//   -d <path>      Path to experiment data files
// Response:
//   "OK\r\n"
//   "Error: <reason>\r\n"

const char AUT_CMD_ANALYZE[] = "Analyze";
// Runs an analysis
// Args:
//   -p <path>      Path to parameters (INI file)
//   -x <path>      Path to XML information file (optional, can be multiple
//                  XML info files)
// Response:
//   "OK\r\n"

```



```
//      "Error: <reason>\r\n"

const char AUT_CMD_STATUS[] = "Status";
// Returns status: OK=ready, Busy=running, Error=Action was terminated by
// an error.
// Busy is followed by descriptive information on the progress of the
// experiment (% complete)
// Args: (none)
// Response:
//      "OK\r\n"
//      "Busy\r\n"
//      "Running (nn%)\r\n"
//      "Error: <reason>\r\n"

#endif // INC_ANALYSIS_AUTOMATIONAPI_H
```

