

Thermal shifts (DSF) using the Bio-Rad CFX Connect

Preparing the plate:

Prepare your plate using the supplied plates and plate seals in the cupboard above the instrument. Recommended total well volume = 25 μL (5 μL min – 50 μL max; better results are achieved with $\geq 20 \mu\text{L}$).

Suggested protein concentration is $\sim 5 \mu\text{M}$ ($> 1 \mu\text{M}$ for a 20 kDa protein).

Suggested Sypro Orange dilution 1: \sim 2000, e.g. prepare a fresh 1:400 stock of dye (usually supplied as a 5000X stock in DMSO) in buffer, and add 5 μL to each well.

Pipette with care to avoid bubbles, or use a plate-spinner prior to the run.

Press the seal down thoroughly to avoid evaporation losses.

Always load duplicate/triplicate wells.

Katherine Stott, 6th January 2017