Flx800T Top/Bottom Read Fluorometer – Notes (from Manish)

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This machine quantifies samples relative to a standard curve; the software will determine unknowns relative to this curve.

The machine is driven by the software, NOT using the touch panel. The Wizard icon takes you through all the set-up

Plate Type and Top/Bottom Reading

Use top read, for cell extracts --- use solid black plates (black on bottom)
Use bottom read for intact cells ---- use clear-bottom, black plate
Uses up to 384 well plates

Turning on/off

1. At beginning, turn on switch, right side, 3min warm-up.
2. Turn off manually at end.
3. Machine incubates and shakes
4. Tungsten halogen lamp
5. DO NOT use panel, just computer-driven software.

KC4 Software Set-up

1. If already saved protocol, then 'Go to Protocol' and Open.
2. KC4 software – use Wizard icon to write protocol
3. Use Wizard Help as needed
4. Plate settings – Lot#/User – write anything

A.**Wizard Icon -- Reading Parameters (this is the most important section)**

1.choose endpoint or Kinetic (for most, Endpoint would be used)
2.choose filters Ex: 360nm Bandwidth Em460 Bandwidth
485nm Em528nm
440nm Em600nm
540nm Em508nm
--can also select multiple filter sets

3. Select Optics position – select top or bottom read
4.Temperature – heating only -- select
5.Select Lag time (if reaction proceeding)
5. Define first well and last well
6. Set shaking (orbital) to 1(low) --- 3 (vigorous)
7. Set Sensitivity – RLU – Relative Light Units –0 to 99,9999 – want strongest sample to read near maximum; probably set highest standard to 99,999 to expand linear range using gain.
8. Options – Auto – this scales to high wells – select high well eg. A5, then type in the high value (eg. 80,000) – can change to lower value in case a sample exceeds the highest standard – DO NOT adjust STARTING SENSITIVITY

B. Wizard Icon – Plate Layout

1. Select standards and unknowns (unknowns = SPL samples to quantify)
2. If label as SPL1, then Press Next ID to give sequential numbers (SPL2, SPL3, etc)
3. Empty button – used to erase label
4. Replicates – choose Horizontal or Vertical (the software will calculate mean, CV and standard deviation automatically) – can also do replicates of standard curve
5. Blanks – this would be buffer only or cells without expressing GFP, etc. The software will automatically take the mean of the "blanks" and subtract this from the standard curve and samples --- therefore, label these wells

C. Wizard Icon -- Basic Analysis

1. Curves – set to raw or Correction (this subtracts blanks)
2. Advanced Analysis – unlikely ever to use this:
   --Single plante –allows one to do a mathematical transformation (for example all samples x3.5)
   --Multiplate – if using 2 filters – can enter formula X1-X2 to normalize two different reads
   --Cut-offs – for clinical (to remove all data below a certain threshold)

D. Wizard Icon – Outputs – choose how data should be exported for report

1. In Excel, the plate layout will be imported
2. Wizard – Output – Export – Configure to Excel
3. Use 5 buttons on left in Excel
4. Press X to delete.
5. select plate properties and wavelength – this is the key step which will prepare template and embed Excel into Protocol
6. Press 'close", NOT 'Esc.'

General Notes

1. Top icons (buttons) are just short-cuts to within Wizard set-up protocol steps
2. Usually two files can be saved, the Protocol file and the Data file