

Agilent Seahorse XFe96 Analyzer

Standard Operation Protocol

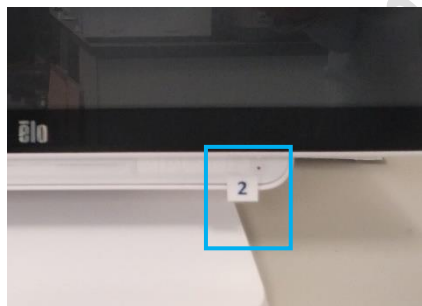
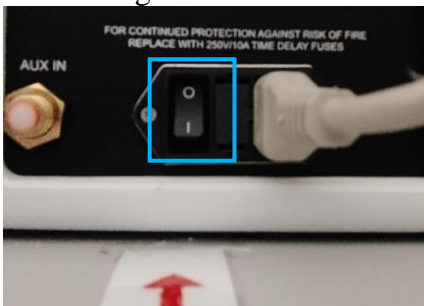
Please refer to individual assay kit's manual for detail steps/modifications

Day 1 before experiment

I. Switching ON Seahorse XFe96 Analyzer and Incubator

The software, machine & non-CO₂ incubator should **always be ON**. Please come to check one day before, if either is off, follow the step below to turn on the machines.

1. Log in PPMS tracker.
2. Power ON Seahorse Analyzer with switch **1** at right lower back of the Analyzer.
3. Power ON controller (all-in one) computer with switch **2** at right bottom of the screen.
4. Launch controller software **Wave**. Wait until **Heater ON** and status **Ready**.
5. Power ON Binder **non-CO₂** incubator by turning switch to "I" position. Refill water pan with autoclaved Milli-Q if necessary.
6. Log out PPMS tracker.



II. Hydrating XFe96 sensor cartridge

The sensor cartridge is spotted with a solid-state sensor material that detects changes in both pH and O₂ concentration over time to calculate rates of various metabolism. It must be thoroughly hydrated (at least overnight) before use. Refer to the kit's manual for cartridge hydration.

III. Prepare Seahorse 96-well XF Cell Culture Microplate

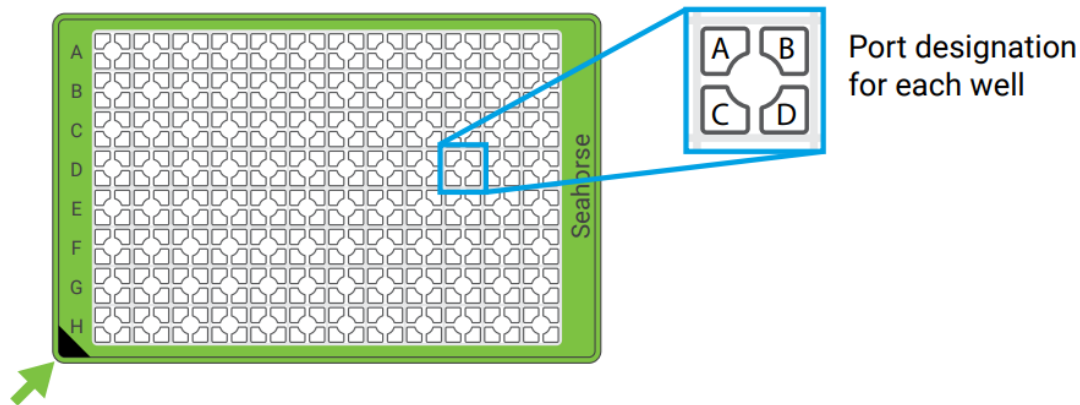
Refer to the kit's manual for cell seeding and equilibration procedures.

****Please come to L6-01 within office hours. ****

Day 2

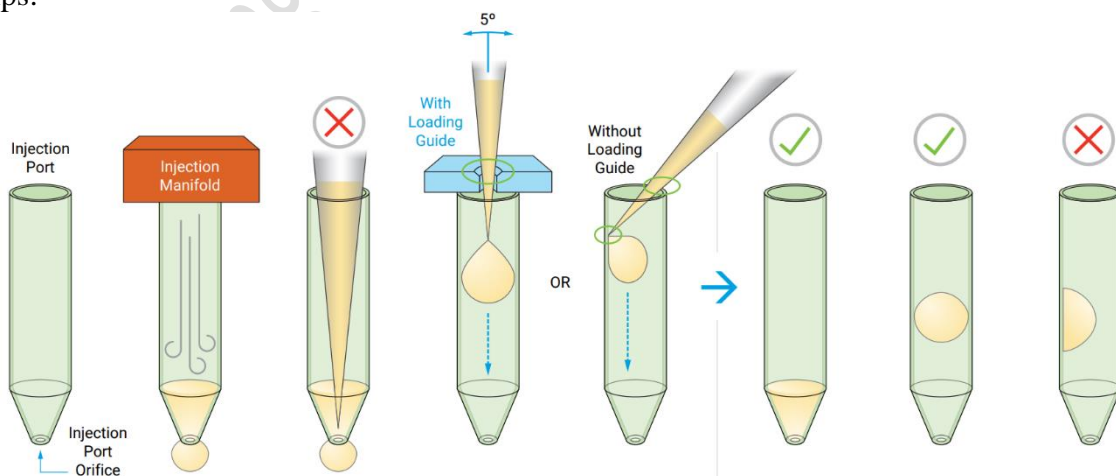
IV. Prepare drugs in the injection port

- A. Equilibrate cartridge and replace culture medium with XF Assay medium according to kit's manual.
- B. Loading compounds in injection port
 1. Compounds (warmed to room temperature) should be diluted with the appropriate prewarmed 37°C XF Medium. Gently pipette up and down or vortex to solubilize the compounds. Prepare appropriate volume of 10x dilution of compounds for loading in injection port.



2. **All** injection ports of same series (e.g. All A, All B etc...), including those of Background Correction or control wells, need to be loaded with the same volume of solution (either with compound or control) (e.g. all A ports filled with the 20 μ L solution; all B ports filled with the 22 μ L...) for proper injection. The solution will only be injected when all same ports of all well are loaded.
3. Use multichannel pipette and Cartridge Loading Guide when appropriate. When using the Guide, orient the pipette tips at a very slight angle ($<5^\circ$). Insert the tips until there is slight resistance and then dispense the compound to the ports.

Tips:



- Do not fully insert the pipet tips into the port as this may result in accidentally forcing injection solutions through the port orifice.
- The hydrated XF Sensor Cartridge must remain in the XF Utility Plate, flat on the work surface throughout the loading procedure. Hold the base of the XF Utility Plate when

handling the XF Sensor Cartridge to avoid triggering any leaking of solutions from the injection ports.

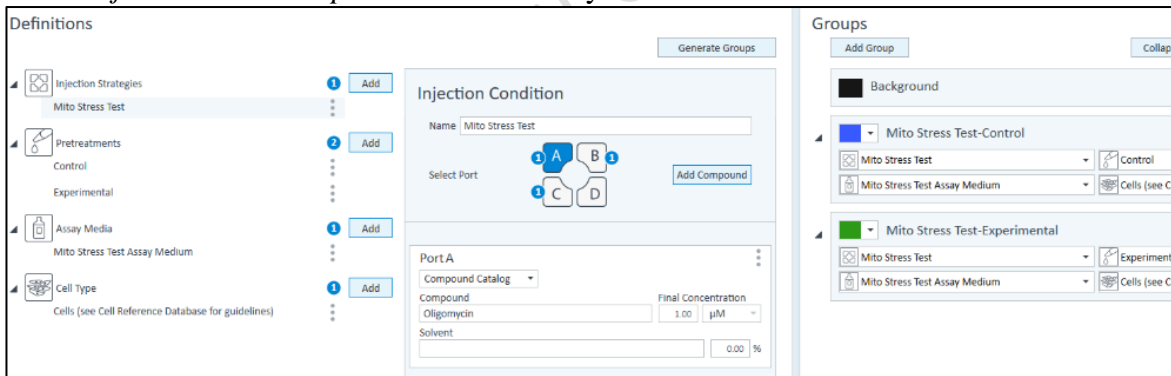
- Avoid creating air bubbles. Do NOT tap any portion of the cartridge or use centrifugation which may cause compound leakage from the injection port.
 - Handle the XFe96 cartridge very carefully. Hold the base of the utility plate when transporting a cartridge. Consider loading drug in workspace provided by the Core in close proximity to the XFe96 Analyzer.
4. Inspect all injection ports for even loading while the cartridge remains flat of working surface. The solution should be at the bottom of the port.

V. Starting Assay in Seahorse XFe96 Analyzer

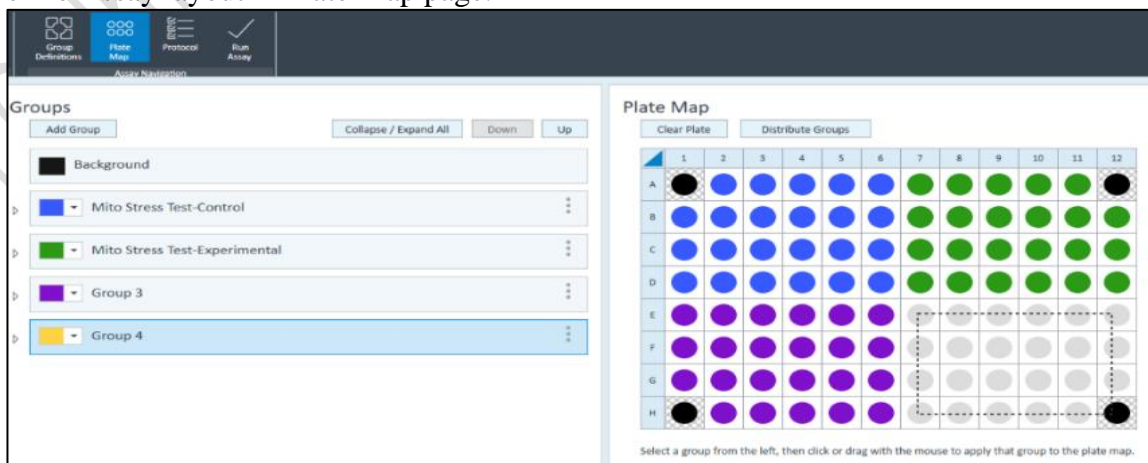
1. Log in PPMS tracker.
2. Set up the protocol in XFe96 Analyzer in advance.
3. Use protocol template (blue) in the XF Controller or modify / load your assay protocol (green).



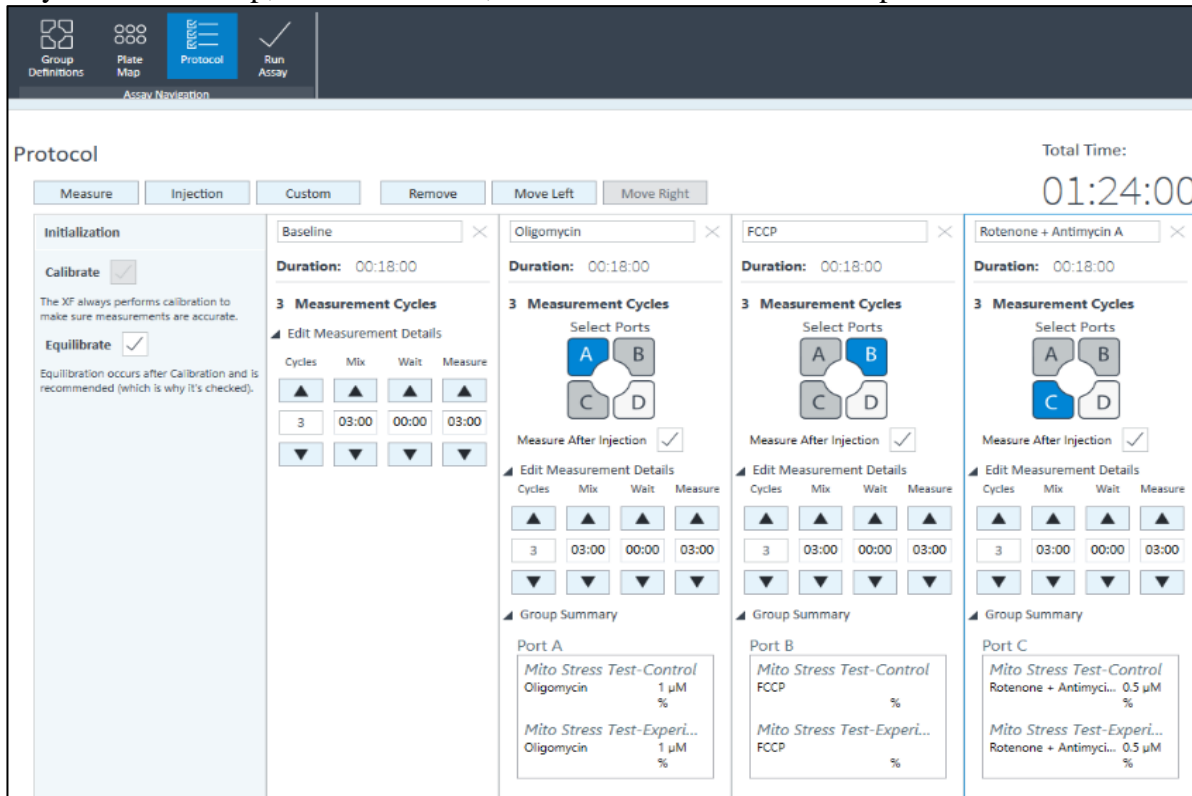
4. Define *Injection and Groups* detail is necessary.



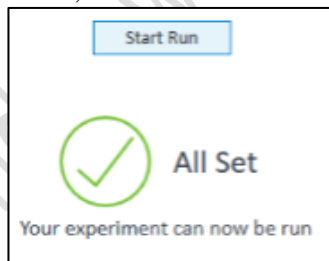
5. Define Assay layout in Plate Map page.



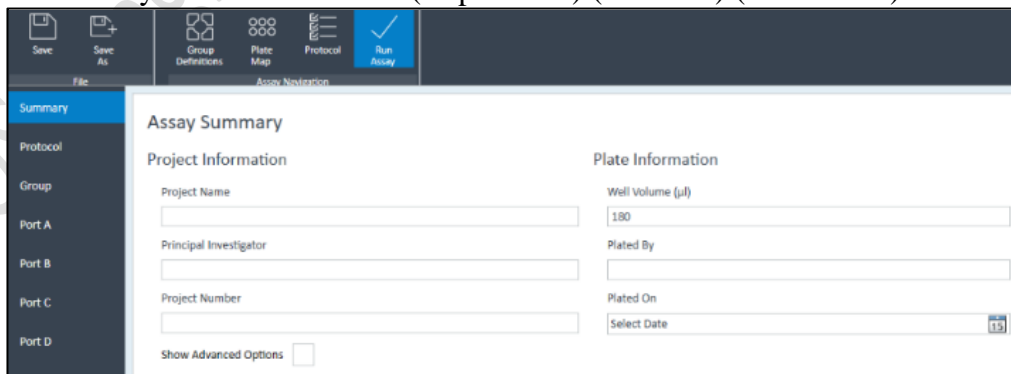
- Modified the Protocol by editing Number and Sequence of injection; number of measurement cycles in each step; duration of Mix, Wait and Measure in each step.



- When protocol is finalized and saved, Click Start on the Run Screen.

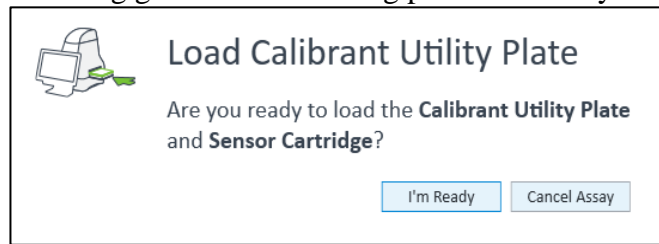


- Input Experiment information in Assay Summary and check the setting in Protocol and Ports tabs. Save Assay Data at C:!\DATA/(Department)/(PI Name)/(User Name).



- Carefully transfer the cartridge together with the utility plate and load in A1 in left-top orientation.

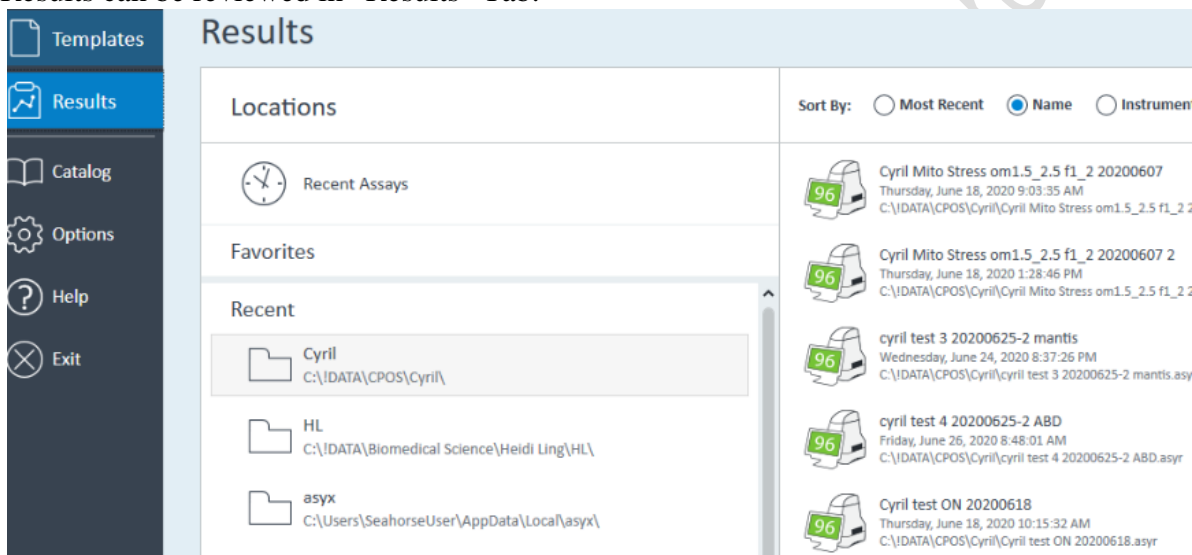
10. *Remove all lid and loading guide before loading plate into Analyzer.*



11. When calibration is completed, replace the Utility Plate with the Cell culture plate according to the software instruction.

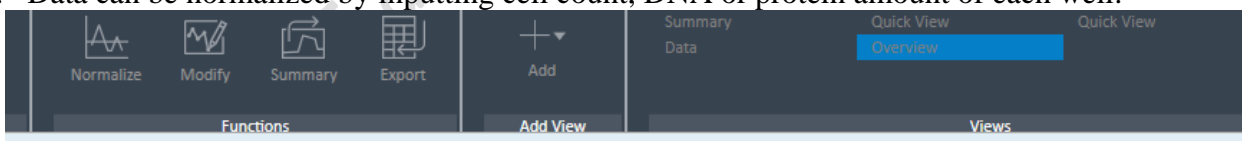
C. Data Processing

1. Results can be reviewed in “Results” Tab.



2. Injection and Groups information can be edited, if necessary, in “Groups Definition”

3. Data can be normalized by inputting cell count, DNA or protein amount of each well.



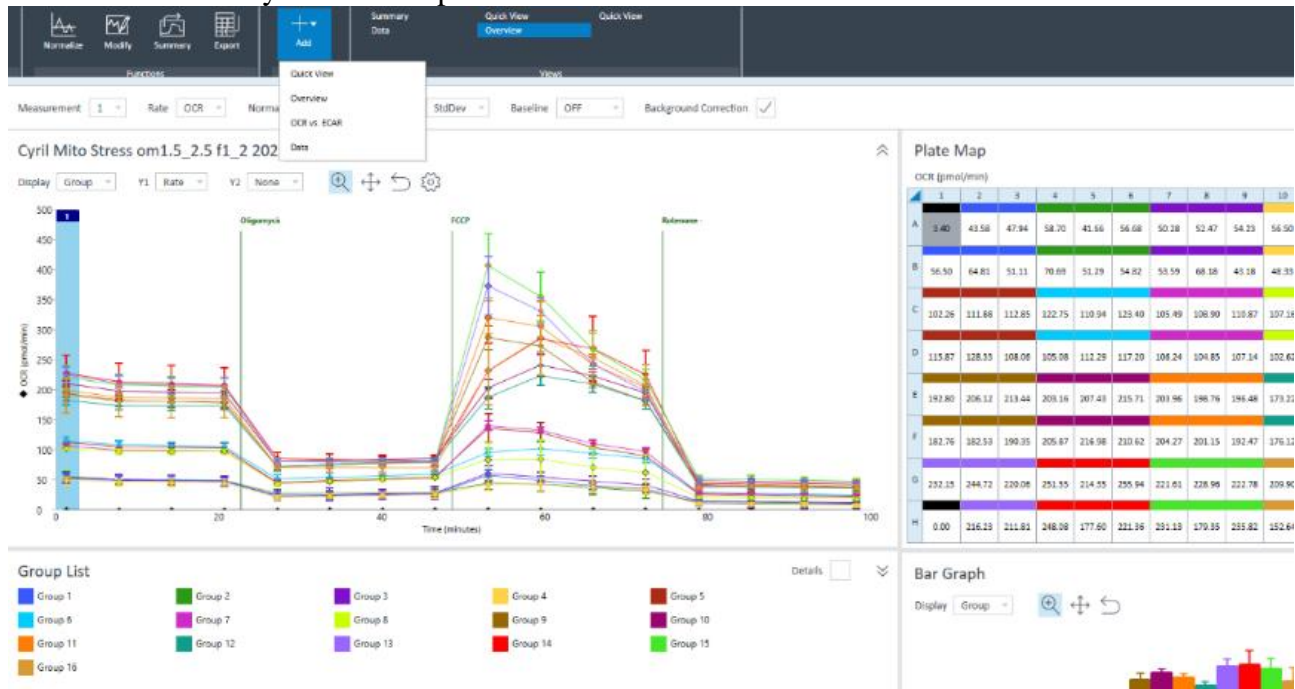
Edit Normalization Mode

Normalization Unit: Scale Factor:

Normalization Values

Select All		1	2	3	4	5	6	7	8
Paste	A								
Clear All Data	B								
	C								
	D								
	E								

4. Reviews of assay in different presentation can be “Add”



5. Results can be exported as excel, Prism and Report (.pdf) format.

