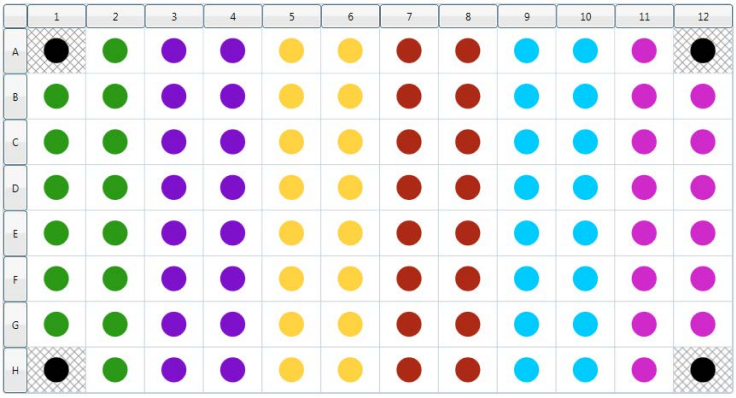
FCCP Optimization with the XF Cell Mito Stress Test

(Note: For this assay, seed cells at the optimal cell number and use the optimal oligomycin concentration that was determined in Training Assay 1.)

The XF Cell Mito Stress Test is run with six different concentrations of FCCP to determine the optimal FCCP concentration to use in your XF assays. In a typical FCCP optimization assay, it is not necessary to inject Rotenone and Antimycin A. However, for the purposes of providing richer data for discussion, we will run the XF Cell Mito Stress Test and inject (A) Oligomycin (optimal concentration), (B) FCCP (six different concentrations) and (C) Rotenone/Antimycin A.

Plate Layout:

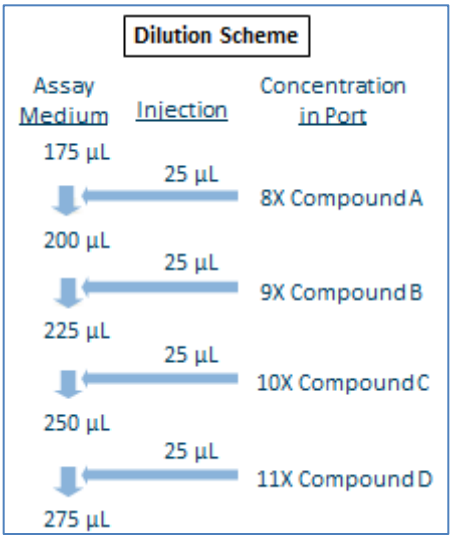
[FCCP] 0 µM 0.125 µM 0.25 µM 0.5 µM 1.0 µM 2.0 µM



Injections:

Port A: Oligomycin: _____ µM (Optimal) final concentration in the well (8x stock)

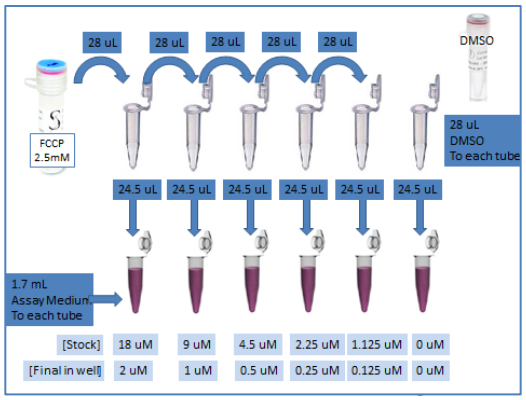
Port B: FCCP
Columns 1-2: 0 µM final concentration in the well (0 µM stock)
Columns 3-4: 0.125 µM final concentration in the well (1.125 µM stock)
Columns 5-6: 0.25 µM final concentration in the well (2.25 µM stock)
Columns 7-8: 0.50 µM final concentration in the well (4.5 µM stock)
Columns 9-10: 1.0 µM final concentration in the well (9 µM stock)
Columns 11-12: 2.0 µM final concentration in the well (18 µM stock)



Port C: Rotenone/Antimycin A: 1 µM final concentration in the well (10 µM stock)

Protocol:

1. Warm the pre-made XF Cell Mito Stress Test Assay Medium to 37°C. Adjust pH to 7.4 ± 0.1 at 37°C.
2. Thaw 1 set of vials from previously reconstituted XF Cell Mito Stress Test kit (oligomycin, FCCP, rotenone and antimycin A).
3. Retrieve your cell plate from the CO_2 incubator. Note the time.
4. Look at cells under the microscope to:
    a. Confirm cell health, morphology, seeding uniformity and purity (no contamination).
    b. Ensure cells are adhered, and no gaps are present.
    c. Make sure no cells were plated in the background correction wells.
5. Wash cells with XF Cell Mito Stress Test Assay Medium
    a. Using a XF Prep Station
        i. Attach bottle of XF Cell Mito Stress Test Medium to XF Prep Station. Open the Seahorse XF Prep Station software. On the "Media Change" tab, select "Do Prime", set final volume to 175 µL of assay medium, and unselect "Do Rinse".
        ii. Place the cell plate vertically onto the tray and remove the lid.
        iii. Press "Start".
    b. Without using a XF Prep Station
        i. Remove all but 20 µL of the culture medium from each well.
        ii. Rinse cells two times with 200 µL of assay medium.
        iii. Add 155 µL of assay medium to each well for a final volume of 175 µL/well.
6. Place the plate in a 37°C incubator **without CO_2** for one hour prior to the assay.
7. Prepare your stock compounds that you will load into the cartridge ports.
    a. Pipette the appropriate amount of oligomycin into a 3 mL aliquot of assay medium.
    b. Prepare serial dilutions of FCCP in DMSO, as shown below.
        i. Pipette 28 µL of DMSO into each tube.
        ii. Pipette 28 µL of FCCP into the first tube. Mix.
        iii. Perform four more serial dilutions as shown.
        iv. Pipette 24.5 µL of each dilution into 1.7 mL aliquots of assay medium.
    c. Pipette 12 µL of 2.5 mM rotenone and 12 µL of 2.5 mM antimycin A into a 3 mL aliquot of assay medium.



8. Get a hydrated cartridge from the non-CO_2 incubator. Load the cartridge with 25 µL in each port as outlined below. **Note the layout!**
    a. Port A – ____ µM oligomycin final concentration in the well (8x stock)
    b. Port B – FCCP dilutions: **Note the layout!**
        i. Columns 1-2: 0 µM final concentration in the well (0 µM stock)
        ii. Columns 3-4: 0.125 µM final concentration in the well (1.125 µM stock)
        iii. Columns 5-6: 0.25 µM final concentration in the well (2.25 µM stock)
        iv. Columns 7-8: 0.50 µM final concentration in the well (4.5 µM stock)
        v. Columns 9-10: 1.0 µM final concentration in the well (9 µM stock)
        vi. Columns 11-12: 2.0 µM final concentration in the well (18 µM stock)
    c. Port C – 1 µM Rot/AA final concentration in the well (10 µM stock)
9. Create or load your assay template on the XF Controller. Default Mix-Wait-Measure times are 3 min – 0 min – 3 min. Usually 3 basal rate measurements are taken prior to the first injection; then 3 rate measurements after each injection.
10. On the Run Screen, Press Start and load the cartridge.
11. When prompted by the software, replace the Utility Plate with the Cell plate. Press Continue.