Instruction Sheet

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FCCP Optimization Using the Agilent Seahorse XF Cell Mito Stress Test with the Agilent Seahorse XF Pro Analyzer

Introduction

FCCP is a potent uncoupler that disrupts ATP synthesis by transporting protons across cell membranes in mitochondria. This stimulates respiration or oxygen consumption rate (OCR). FCCP is a good tool chemical for estimation of the maximal respiratory capacity of the cell. The FCCP concentration used to estimate maximal respiration must be optimized by titration experiments because FCCP can inhibit OCR at high concentrations. Performing an optimization experiment for each cell type is recommended because the optimal FCCP concentration is cell type-dependent. Some assay conditions can also alter FCCP optimal concentration, such as the inclusion of BSA or serum in assay medium. Changing buffer conditions requires caution. This instruction sheet describes a basic procedure for optimizing FCCP concentration using the Agilent Seahorse XF Cell Mito Stress Test with XF Pro analyzer.

The XF Cell Mito Stress Test is run with five different FCCP concentrations to determine the optimal concentration for use in XF assays, as indicated in Figure 1A. The test can be combined with cell density optimizations (Figure 1B). Visit the XF Learning Center to learn how to create an XF assay template.

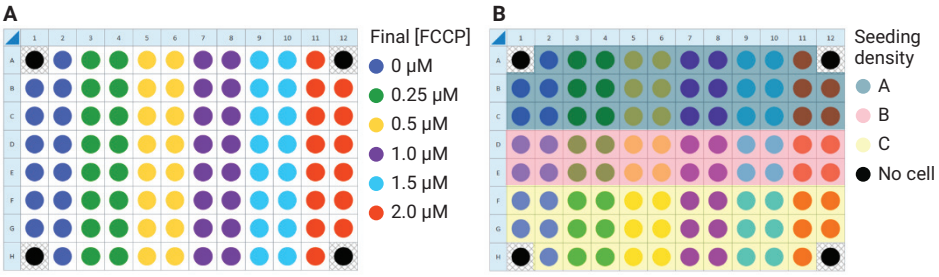

Figure 1. Example plate layout for FCCP optimization experiment (A). To achieve cell density optimization, three cell densities can be accommodated on the plate map (B).

Procedure

1. On the day before the assay, hydrate an Agilent Seahorse XF sensor cartridge by filling a utility plate with 200 µL/well of calibrant. Place the sensor cartridge/hydrobooster/utility plate assembly in a 37 °C, non-CO_2 incubator overnight.
2. Also on the day before the assay, seed cells onto an Agilent Seahorse XF Pro M cell culture plate at a desirable density (Figure 1B) and culture the cells in a 37 °C, CO_2 incubator overnight.
3. On the day of the assay, prepare the assay medium (Agilent Seahorse XF DMEM medium, pH 7.4), supplemented with 1 mM pyruvate, 2 mM glutamine, and 10 mM glucose. Warm up to 37 °C.
4. Remove the cell plate from the CO_2 incubator.
5. Examine the cells under a microscope to confirm cell health and seeding uniformity. Make sure no cells were plated in the background correction wells (A1, H1, A12, and H12).
6. Wash the cells twice with warm assay medium. Visit the XF Learning Center for best practices on cell washing.
7. Examine the cells again under the microscope to ensure that the cells were not disturbed or washed away.
8. Place the plate in a 37 °C non-CO_2 incubator for 45 to 60 minutes before performing the assay.
9. Remove one foil pouch from the XF Cell Mito Stress Test Kit box and prepare stock compound solution according to Table 1. Vortex tubes with the cap on to solubilize the compounds.

Table 1. Stock solution preparation.

Compound	Volume of Assay Medium	Stock Concentration
Oligomycin	465 µL	135 µM
FCCP	720 µL	100 µM
Rot/AA	490 µL	55 µM

10. Prepare injection solutions according to Table 2. Visit the XF Learning Center for the best loading techniques.

Table 2. Compound injection strategy in the FCCP optimization experiment. Starting well volume is 200 µL.

Compound	Final Conc. (µM)	Port Conc. (Fold)	Stock Volume (µL)	Medium Volume (µL)	Loading Volume (µL)
Port A Oligomycin	1.5	9x	300	2,700	25
Port B FCCP	0.25	10x	25	975	25
	0.5		50	950	
	1.0		100	900	
	1.5		150	850	
	2.0		200	800	
Port C Rotenone/Antimycin A	0.5	11x	300	2,700	25

11. Remove the hydrated cartridge from the incubator. Load the injection solutions according to Table 2.
12. Use the default XF Cell Mito Stress Test template to create an assay template for FCCP optimization (see Figure 1 for groups and plate map).
13. Save the template if desired or click **Run Assay** to perform the assay.
14. Follow the screen prompts to initiate the cartridge calibration and load the cartridge/utility plate. Ensure that the cartridge lid is removed.
15. After cartridge calibration is completed, follow the software prompts to replace the utility plate with the cell plate and start the assay.
16. After the assay run is completed, upload the result file to Seahorse Analytics and use the Max Respiration widget in the XF Cell Mito Stress Test to analyze the maximal OCR and determine the optimal FCCP concentration.

Additional information

Agilent XF Learning Center:
www.agilent.com/en/products/cell-analysis/how-to-run-an-assay

Technical support:
cellanalysis.support@agilent.com

www.agilent.com

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