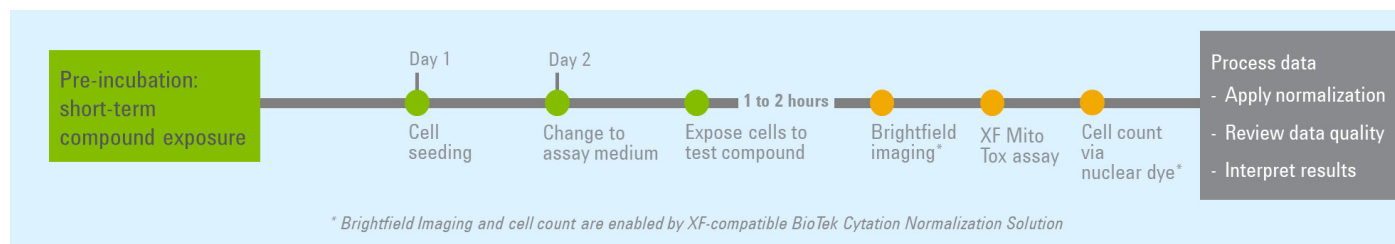


Agilent Seahorse XF Mito Tox Assay Kit



Day 1: one day before the assay

1. Ensure that the Agilent Seahorse XF Pro Analyzer is powered on and thermally equilibrated to 37 °C (for a minimum of two hours).
2. Hydrate a sensor cartridge using calibrant, following the detailed instructions in the XF Mito Tox Assay Kit user guide.
3. For adherent cells, seed cells in an Agilent Seahorse XF Pro M culture plate at a predetermined density in growth medium, 80 µL per well. Dispense 80 µL of culture medium into each background well. Do not seed cells in background wells.
4. Fill the four moat chambers around the wells with water using an eight-channel P1000 pipettor set to 250 µL. Place the plate in a 37 °C CO₂ incubator overnight to allow cells to attach.
5. Create or modify the assay template and upload it to the Agilent Seahorse XF Pro Controller.

Day 2: day of the assay

1. Prepare 100 mL of assay medium with XF supplements (Table 1). Warm up to 37 °C.
2. Wash the cell plate twice with warm assay medium. Leave a final volume of 100 µL/well.
3. Prepare test compound solutions at 2x of the final concentration using assay medium.

Table 1. Standard assay medium for XF Mito Tox assay.

Assay Media Component	Volume (mL)	Final Concentration (mM)
XF DMEM Medium, pH 7.4	97	–
XF Glucose (1 M)	1.0	10
XF Pyruvate (100 mM)	1.0	1
XF Glutamine (200 mM)	1.0	2

4. If test compound solutions contain DMSO or other solvents, prepare 5 mL of XF assay medium containing the same concentration of DMSO or other solvents. This is your vehicle control solution.
5. Prepare 1.0 µM of rotenone/antimycin A solution by adding 400 µL of assay medium to the vial. Vortex to dissolve the compounds. Then, transfer all 400 µL to a larger tube and add 5 mL of assay medium. This should give a total of 5,400 µL.
6. Add 100 µL of test compound solution to each well according to the predesigned plate map in the assay template.
7. Transfer 100 µL of rotenone/antimycin A solution to the eight wells containing cells in column 12 (B12 to G12).
8. Add 100 µL of vehicle control solution to the eight wells containing cells in column 1 (B1 to G1) and to the four corner background wells.
9. Incubate the cell plate in a non-CO₂ incubator at 37 °C for 45 to 60 minutes before the assay.

10. Prepare stock solutions for the kit compounds according to Table 2.

Table 2. Preparation of kit compound stock solutions.

Compounds	Assay medium to Add (µL)	Concentration (µM)
Oligomycin	465	135
FCCP	720	100

11. Prepare injection solutions and load them into injection ports according to Table 3.

Table 3. Prepare injection solutions and loading volume. The starting volume for each well is 200 µL

Injection Solution	Stock Solution (µL)	Assay Medium (µL)	Port Concentration		Final Well Conc. (µM)	Loading Port and Volume
			µM	Fold		
Oligomycin	300	2,700	13.5	9x	1.5	Port A: 25 µL
FCCP	150	2,850	5	10x	0.5	Port B: 25 µL
	300	2,700	10	10x	1.0	
	450	2,550	15	10x	1.5	
	600	2,400	20	10x	2.0	

- Open the assay template in XF Pro Controller. Click **Start Run** when you are ready.
- After calibration, the software will display Load Cell Plate. Click **Open Tray**, then replace the utility plate with the cell plate.
- Ensure that the lid is removed from the cell plate, then click **Load Cell Plate** to start the assay.
- Optional:** Perform postassay cell normalization using the Agilent BioTek Cytation reader.
- After completing an assay run, upload the result file to Seahorse Analytics and use **XF Mito Tox Screening View** or **XF Mito Tox Dose View** to process assay results.

Additional information

Agilent XF Learning Center

www.agilent.com/en/products/cell-analysis/how-to-run-an-assay

Technical support

cellanalysis.support@agilent.com

Ordering information

Part Number	Product Description	Compatible Analyzer
103595-100	Seahorse XF Mito Tox Assay Kit, 6 assays	XF Pro analyzer
Related Products		
103575-100	Seahorse XF DMEM Medium, pH 7.4, 500 mL*	All analyzers
103577-100	Seahorse XF 1.0 M Glucose Solution, 50 mL	All analyzers
103578-100	Seahorse XF 100 mM Pyruvate Solution, 50 mL	All analyzers
103579-100	Seahorse XF 200 mM Glutamine Solution, 50 mL	All analyzers
103775-100	Seahorse XF Pro M FluxPak, 18 assays	XF Pro analyzer
103777-100	Seahorse XF Pro M FluxPak mini, 6 assays	XF Pro analyzer
103774-100	Seahorse XF Pro M cell culture plates, 6 plates	XF Pro analyzer

* This medium can also be purchased together with the supplements listed in this table as a bundled product (part number 103680-100).

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RA44671.6334143519

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Printed in the USA, April 29, 2022
5994-4808EN

