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Quick Start Guide

XF Substrate Oxidation Stress Test Kits: Standard Assay

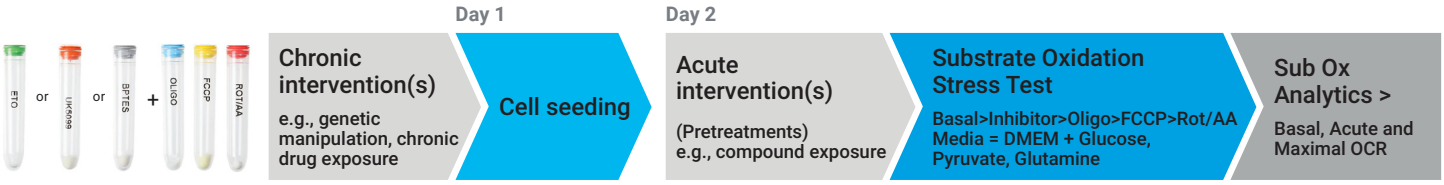

Figure 1. Standard assay design for the XF Long Chain Fatty Acid Oxidation Stress Test Kit (p/n 103672-100), XF Glucose/Pyruvate Oxidation Stress Test Kit (p/n 103673-100), and XF Glutamine Oxidation Stress Test Kit (p/n 103674-100)

One day prior to the assay

1. Ensure the XF Analyzer is powered on and thermally equilibrated to 37 °C (minimum of 5 hours).
2. Hydrate a sensor cartridge in sterile or distilled water at 37 °C in a non-CO_2 incubator overnight.
3. For adherent cells, plate cells at a predetermined density in cell culture growth medium.
4. Use the XF Substrate Oxidation Stress Test – Standard Assay template to design the experiment in Wave and make any necessary modifications to the template to suit experimental design.

Day of assay

1. Complete sensor cartridge hydration: replace water with XF calibrant (200 µL/well for XF96 or 500 µL/well for XF24, and incubate at 37 °C, no CO_2, for 1 hr.
2. Prepare 100 mL Assay Media: Supplement XF DMEM or XF RPMI with XF Agilent substrates (Table 1).
3. Aspirate media from cell plate and replace with Assay Media: 180 µL for 96-well plates, 500 µL for 24-well plates.
4. Place cell plate in non-CO_2, 37 °C incubator for 60 min, or place in Biotek instrument for normalization.
5. Prepare stock solutions: resuspend dry compounds in assay media and vortex for ~1 min (Table 2, back page).
6. Using stock solutions, prepare 10X working solutions by mixing stock solutions with the appropriate amount of assay media (Table 2, back page).

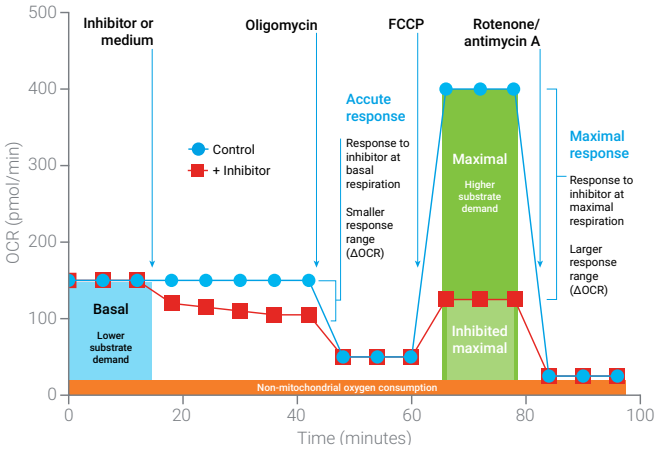

Figure 2. Standard assay output

Assay media component	Volume (mL)	Final concentration (mM)
Seahorse XF DMEM or RPMI Medium, pH 7.4	97	-
XF Glucose (1 M)	1.0	10
XF Pyruvate (100 mM)	1.0	1.0
XF Glutamine (200 mM)	1.0	2.0

Table 1. Standard Substrate Oxidation Stress Test assay media.

Port	Compound	Stock solution	10X working solutions for injection ports		Volume added to port (µL)	
		Volume of assay medium (µL)	Stock volume (µL)	Volume of assay medium (µL)	XFe96/XFe24	Final well concentration (µM)
A	Etomoxir or	700	500	1500	20/56	4.0
	UK5099 or	700	500	1500	20/56	2.0
	BPTES	700	500	1500	20/56	3.0
B	Oligomycin	420	300	2700	22/62	1.5
C	FCCP (use optimal concentration determined prior to assay)	720	75	2925	25/69	0.25
			150	2850	25/69	0.5
			300	2700	25/69	1.0
			600	2400	25/69	2
D	Rotenone + antimycin A	540	300	2700	27/75	0.5

Table 2. Standard Substrate Oxidation Stress Tests: Stock and Working solutions.

7. Pipette the 10X working solutions into the each of the four injector ports (Table 2). *Note: Use assay media in Port A for the control (i.e., – inhibitor) wells.*
8. Open Wave and the designed assay template. Click **Start Run** when you are ready.
9. When prompted, place the loaded sensor cartridge into the analyzer and click **I'm Ready**.
10. Following calibration, Wave will display Load Cell Plate. Click **Open Tray**, then replace the Utility Plate with the Cell Plate.
11. Ensure the lid is removed from the Cell Plate, then click **Load Cell Plate** to start the assay.
12. Optional: Perform post-assay cell normalization using the Biotek instrument.

Ordering Information

Description	Part Number
XF Long Chain Fatty Acid Oxidation Stress Test Kit	103672-100
XF Glucose/Pyruvate Oxidation Stress Test Kit	103673-100
XF Glutamine Oxidation Stress Test Kit	103674-100
Seahorse XF DMEM Medium, pH 7.4	103575-100
Seahorse XF RPMI Medium, pH 7.4	103576-100
Seahorse XF 1.0 M Glucose	103577-100
Seahorse XF 100 mM Pyruvate	103578-100
Seahorse XF 200 mM Glutamine	103579-100

Additional information

XF Substrate Oxidation Stress Test Kits User Manual:
www.agilent.com/chem/subox-usermanual

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

Technical assistance:
cellanalysis.support@agilent.com

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