Agilent
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Quick Start Guide

XF Palmitate Oxidation Stress Test Kit: Advanced Assay

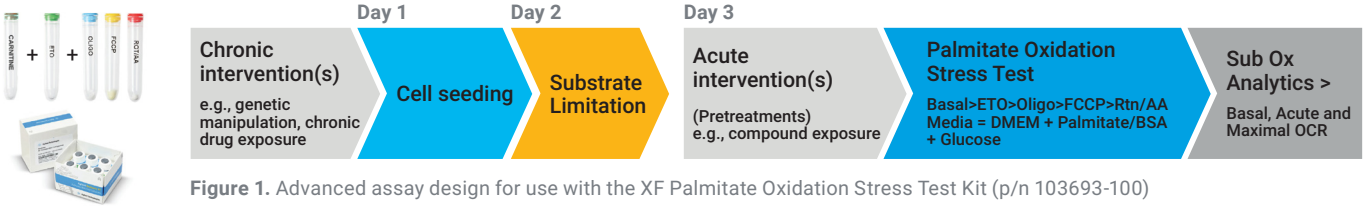

Figure 1. Advanced assay design for use with the XF Palmitate Oxidation Stress Test Kit (p/n 103693-100)

Two day prior to the assay (Day 1)

1. For adherent cells, plate cells at a predetermined density in cell culture growth medium.
2. Use the XF Substrate Oxidation Stress Test- Advanced assay template to design experiment in Wave and make any necessary modifications to the template to suit experimental design.

One day prior to the assay (Day 2)

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. Prepare appropriate volume of Substrate-Limited Growth Media (~15 mL, Table 1).
4. Aspirate growth media from cell plate and replace with Substrate-Limited Growth Media (100 µL for 96-well plates, 250 µL for 24-well plates); incubate overnight.

Day of assay (Day 3)

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 75 mL Assay Media: Supplement XF DMEM or XF RPMI with XF Agilent substrates (Table 2).
3. Aspirate media from cell plate and replace with Substrate-Limited Assay Media: 180 µL for 96-well plates, 500 µL for 24-well plates (Note: Palmitate and BSA will be added just prior to the assay, step10).
4. Place cell plate in non-CO_2, 37 °C incubator for 60 min, or place in Biotek instrument for normalization.

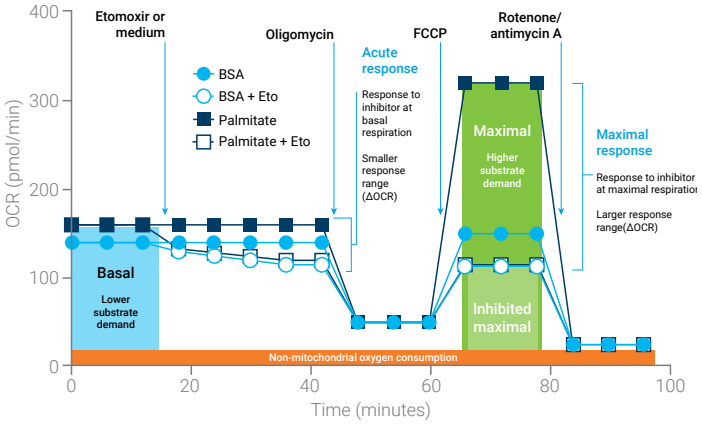

Figure 2. Advanced assay output

Base growth media	Growth media supplement	Suggested initial concentration in Substrate-Limited Growth Media
DMEM or RPMI without glucose, pyruvate, glutamine, or GlutaMAX	Glucose	0.5 mM
	Glutamine or GlutaMAX	1.0 mM
	Serum (e.g., FBS)	1.0 %
	XF L-Carnitine	0.5 mM

Table 1. Suggested initial Substrate-Limited Growth Media composition.*

** Optimal limited substrate concentrations and optimal time of incubation are cell dependent and should be empirically determined for the cell type of interest.*

5. Prepare stock solutions: resuspend dry compounds in assay media and vortex for ~1 min (Table 3).
6. Using stock solutions, prepare 10X working solutions by mixing stock solutions with the appropriate amount of assay media (Table 3).
7. Pipette the 10X working solutions into the each of the four injector ports (Table 3). *Note: Use assay media in Port A for the control (i.e., – etomoxir) wells.*
8. Open Wave and the designed assay template. Click **Start Run** when you are ready.
9. When sensor cartridge calibration is complete, aspirate media from cell plate and replace with Substrate-Limited Assay Media: 150 µL for 96-well plates, 415 µL for 24-well plates
10. Add Palmitate-BSA or BSA control to appropriate cell plate wells: 30 µL for XFe96, 85 µL for XFe24.
11. When prompted, place the loaded sensor cartridge into the analyzer and click **I'm Ready**.
12. Following calibration, Wave will display **Load Cell Plate**. Click **Open Tray**, then replace the Utility Plate with the Cell Plate.
13. Ensure the lid is removed from the Cell Plate, then click **Load Cell Plate** to start the assay.
14. Optional: Perform post-assay cell normalization using the Biotek instrument.

Assay media component	Volume	Final concentration (mM)
Seahorse XF DMEM or RPMI Medium, pH 7.4	75 mL	-
XF Glucose (1 M)	150 µL	2.0
XF L-Carnitine	75 µL	0.5

Table 2. Suggested initial Substrate-Limited Assay Media. Note that Palmitate-BSA and BSA control are added just prior to the assay, see Step 10.

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	700	500	1500	20/56	4.0
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 3. Standard Substrate Oxidation Stress Tests: Stock and Working solutions.

Ordering Information

Description	Part Number
XF Palmitate Oxidation Stress Test Kit	103693-100
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

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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