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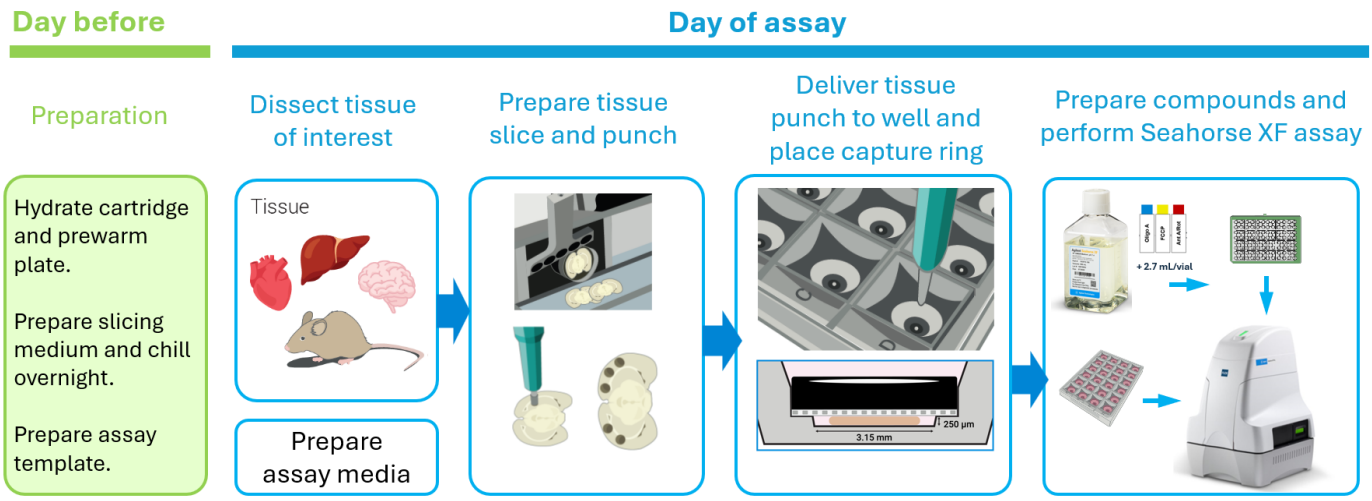
Quick Start Guide

Agilent Seahorse XF 3D Mito Stress Test Tissue Workflow

For use with the Agilent Seahorse XF Flex Analyzer and the Agilent Seahorse XF Flex 3D Capture Microplate-L



One day before the assay (Day 1)

1. Power up the Seahorse XF Flex analyzer to allow the temperature to stabilize overnight.
2. Hydrate the 24-well sensor cartridge with 1 mL of Agilent Seahorse XF calibrant solution per well.
3. Place sensor cartridge, Seahorse XF Flex 3D Capture Microplate-L, and 3D capture rings at 37 °C in a non-CO_2 incubator overnight.

Day of the assay (Day 2)

1. Prepare assay medium and warm to 37 °C. Pipette 100 µL of assay medium into each well of the 3D capture plate.
2. Prepare live tissue slices using a vibrating microtome (100 to 200 µm thickness) and use a biopsy tool for punching (1 to 2 mm diameter). The specimen sizes should be smaller than the sample chamber (3.15 mm diameter, 250 µm height).
3. Transfer each punch to the 3D capture plate, ensuring to center the tissue within the sample chamber.

4. Prewet 3D capture screens and insert into each well, mesh side downward.
5. Add additional assay medium to each well to bring the total volume up to 600 µL.
6. Place in a 37 °C non-CO_2 incubator for 45 to 60 minutes. Continue to next steps during this incubation period.
7. Resuspend Agilent Seahorse XF 3D Mito Stress Test kit compounds with assay medium in the volumes indicated in Table 1. Further dilute based on Table 2 and previous optimization assays.
8. Take the sensor cartridge out of the incubator. Remove the Seahorse XF Hydrobooster.
9. Dispense 75 µL of each compound injection solution into each set of ports.
10. Select the assay template on the controller software and follow the instrument prompts to perform the assay. Enter the XF 3D Mito Stress Test kit part number, lot number, and software code. Click "Start Run".
11. When prompted, remove the cartridge lid and place the loaded sensor cartridge with the utility plate on the thermal tray. Click "I'm Ready" to start the calibration process (~ 15 to 20 minutes). Reminder: The XF Hydrobooster must be removed before this step.
12. After completing the calibration, load the 3D capture plate containing tissue samples (without a lid) to start measurement.
13. After the assay is completed, import the assay result file to Agilent Seahorse Analytics. Open the assay result file and select the XF 3D Mito Stress Test analysis view.

Table 1. Preparation of stock solutions.

Compounds	Volume to Add (mL)	Stock Concentration (µM)
Oligomycin A	2.7	270
FCCP	2.7	200
Rotenone/Antimycin A	2.7	110

Table 2. Preparation of injection solutions for the Agilent Seahorse 3D Mito Stress Test without an acute injection and a starting well volume of 600 µL.

Injection Solution	Stock Solution (µL)	Assay Medium (µL)	Loading Port and Volume	Final Well Conc. (µM)
Oligomycin A (9x)	1000	2000	Port A: 75 µL	10
	2000	1000		20
	2700	0		30
FCCP (10x)	750	2250	Port B: 75 µL	5
	1500	1500		10
	2250	750		15
	2700	0		20
Rot/AA (11x)	2700	0	Port C: 75 µL	10

Ordering information

Part Number	Product Description
S7851A or S7851AN	Seahorse XF Flex Analyzer
103864-100	Seahorse XF Flex 3D Capture FluxPak-L
103016-100	Seahorse XF 3D Mito Stress Test kit

Additional information

User guide

https://www.agilent.com/lifesciences/user-manual-seahorse-xf-3d-mito-stress-test-kit

www.agilent.com/lifesciences/discoverXF

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