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Quick Start Guide

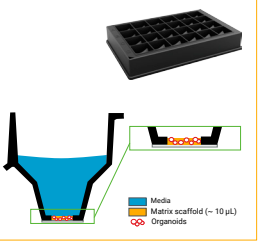
Agilent Seahorse XF 3D Mito Stress Test Organoid Workflow

For use with the Agilent Seahorse XF Flex analyzer and the Agilent Seahorse XF Flex organoid microplate

Days before assay

Prepare organoid cultures in XF Flex organoid microplate

Seed cell or organoid fragments in matrix scaffold placed within the sample reservoir (10 ± 2 µL per well)



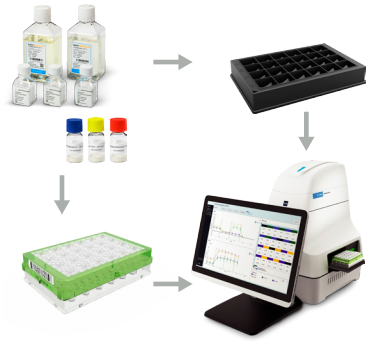
Day before assay

Prepare assay

Hydrate cartridge
Prepare the assay template

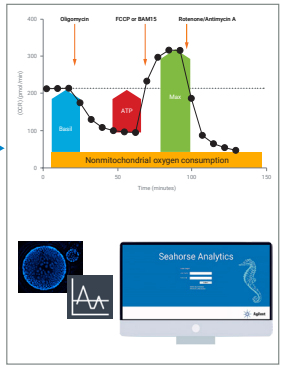
Day of assay

Prepare compounds and perform XF assay



Data analysis

Data/image processing and normalization



Organoid culture

Option one

1. Pipette 10 µL ice-cold diluted Matrigel per well in two background wells.
2. Resuspend cells or organoids in ice-cold diluted Matrigel and carefully pipette 10 µL suspension per well in all the other wells.
3. Incubate in a humified CO_2 incubator at 37 °C for 25 minutes.
4. Add 250 µL prewarmed growth medium to each well and maintain the organoid cultures as required.

Option two

1. Pipette 6 µL ice-cold diluted Matrigel per well, including background wells. Incubate in a humified CO_2 incubator at 37 °C for 25 minutes.
2. Resuspend cells or organoids in ice-cold diluted Matrigel. Carefully pipette 4 µL suspension on top of the polymerized Matrigel layer in all wells except the two background wells. Incubate in a humified CO_2 incubator at 37 °C for 25 minutes.
3. Add 250 µL prewarmed growth medium to each well and maintain the organoid cultures as required.

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 at 37 °C in a non-CO_2 incubator overnight.

Day of the assay (Day 2)

1. Prepare XF assay medium as indicated in Table 1 and warm to 37 °C.
2. Carefully remove growth medium and add 600 µL prewarmed XF assay medium into each well of the plate.
3. Remove 550 µL XF assay medium and replace with 550 µL fresh XF assay medium. Place the microplate in 37 °C non-CO_2 incubator for 45 to 60 minutes. Continue to next steps during this incubation period.
4. Resuspend Agilent Seahorse XF 3D Mito Stress Test kit compounds with assay medium in the volumes indicated in Table 2. Further dilute based on Table 3 and previous optimization assays.
5. Take the sensor cartridge out of the incubator. Remove the Seahorse XF hydrobooster. Dispense 75 µL of each compound injection solution into each set of ports.
6. Select the **XF Organoid Mito Stress Test assay template**, specifically designed for embedded organoids (3 min max, 0 min wait, 4 min measure). Follow the instrument prompts to perform the assay.
7. Enter the XF 3D Mito Stress Test kit part number, lot number, and software code. Click **Start Run**.
8. 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. (Reminder: The XF hydrobooster must be removed before this step.)
9. After completing the calibration, refresh the XF assay medium by removing and replacing 550 µL XF assay medium again. Load the organoid plate (without a lid) to start measurement.
10. After the assay is completed, import the assay result file to Agilent Seahorse Analytics. Open the assay results file and select the XF 3D Mito Stress Test analysis view.

Table 1. Preparation of Agilent Seahorse XF assay media.

Reagent	Volume (mL)	Final Concentration (mM)
XF DMEM medium, pH 7.4	97	-
XF 1.0 M glucose solution	1	10
XF 200 mM glutamine solution	1	1
XF 100 mM pyruvate solution	1	2

Table 2. Preparation of stock solutions.

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

Table 3. 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 Concentration (uM)
Oligo 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
	3000	0		20
Rot/AA (11x)	3000	0	Port C: 75 µL	10

Ordering information

Part Number	Product Description
S7851A or S7851AN	Seahorse XF Flex analyzer
103866-100	Seahorse XF Flex Organoid FluxPak
103016-100	Seahorse XF 3D Mito Stress Test kit
103860-100	Seahorse XF DMEM assay media pack

Additional information

– Technical overview

Note: Agilent Seahorse XF Cell Mito Stress Test kit (103595-100) or Agilent Seahorse XF T Cell Metabolic Profiling Assay kit (103772-100) may be preferred depending on the organoid type or source. The final volume of assay medium is 500 uL for those kits. Prepare the injection solutions according to the corresponding user guide.

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DE-012066

Printed in the USA, January 13, 2026
D0139619 Rev. A.01

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