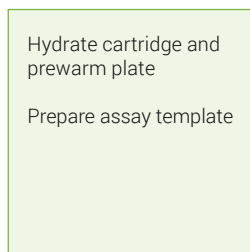
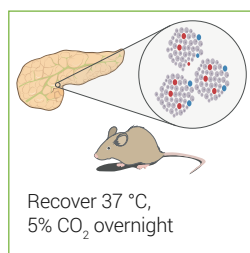


Agilent Seahorse XF Mito Stress Test Islet Workflow

For use with the Agilent Seahorse XF Flex analyzer and the Agilent Seahorse XF Flex 3D capture microplate-S

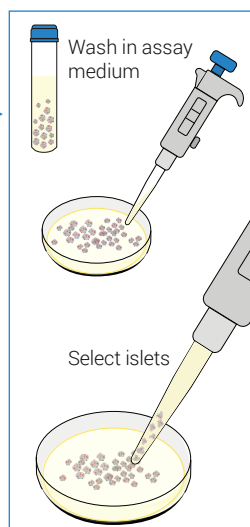
Day before assay

Isolate islets and prepare assay

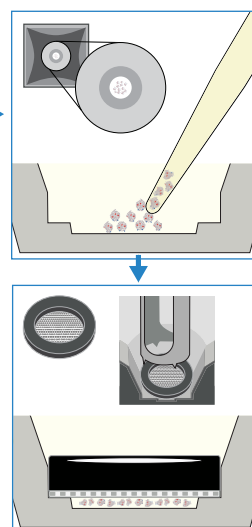


Day of assay

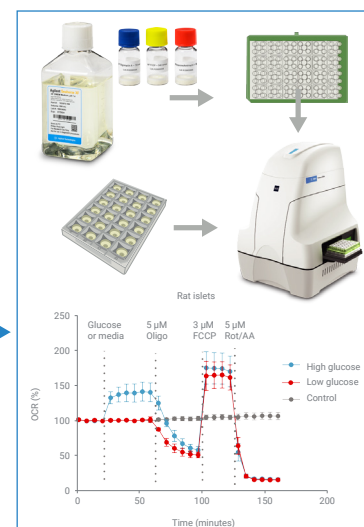
Prepare media and collect islets



Deliver islets to well and install capture screen



Prepare compounds and perform XF assay



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-S, and 3D capture rings at 37 °C in a non-CO₂ incubator overnight.
4. Isolate whole islets by the standard protocol(s) used in your laboratory and recover overnight under conditions for islet culture.

Day of the assay (Day 2)

1. Prepare assay medium and warm to 37 °C (Table 1 and 2). Pipette 525 µL of assay medium with 0.2% bovine serum albumin (BSA) into each well of the capture plate.
2. Islets are collected from the source culture plate, washed in assay medium with 0.2% BSA, and transferred to a sterile, noncoated petri dish.
3. While viewing the petri dish under a microscope, manually pick-up the required number of islets using prewetted P10 tip, in a volume of 4 to 6 µL (typically 10 to 20 islets).
4. Under the microscope, place tip containing islets into the bottom center of the sample chamber of a well of the XF 3D capture microplate. Slowly dispense Islets so they collect in the center. Repeat for each well. **Do not load islets into wells A1 and D6**, these are background wells and they contain media only.
5. Prewet 3D capture screens in assay media and insert gently into each well, mesh side downward.
6. Place the XF 3D capture microplate in a 37 °C non-CO₂ 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 using assay medium without BSA in the volumes indicated in Table 3.
8. Prepare further dilutions for compound injections, including glucose, and adjust according to previous optimization assays, using the example in Table 4.
9. 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.
10. Select the Islet MST (Acute) 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. (Reminder: The XF hydrobooster must be removed before this step.)

12. After completing the calibration, load the 3D capture plate containing Islets (without a lid) to start measurements.
13. 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 Islet XF assay media (No BSA).

Reagent	Final (mM)	Stock Solution (nM)	Volume of Stock Solution (mL)
XF DMEM medium, pH 7.4			100
XF 1.0 M glucose solution	2.5	1000	0.25
XF 200 mM glutamine solution	2	200	1
XF 100 mM pyruvate solution	1	100	1

Table 2. Preparation of Islet XF assay media with BSA.

Reagent	Final (%)	Stock Solution (%)	Volume of Stock Solution
Islet XF Assay media (No BSA)			50
100 mg/mL BSA (FA free)	0.2	10	1.0

Table 3. Preparation of 3D Mito Stress Test 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 4. Example preparation of injection solutions for the Agilent Seahorse 3D Mito Stress Test with acute glucose injection and a starting well volume of 525 µL.

Injection Solution	Stock Solution (µL)	Assay Medium (µL)	Loading Port and Volume (µM)	Final Well Concentration (uM)
Glucose (8x)	341 (1M stock)	2659	Port A: 75 µL	16700 (+14200)
Oligo A (9x)	500	2500	Port B: 75 µL	5
FCCP (10x)	450	2550	Port C: 75 µL	3
Rot/AA (11x)	1500	1500	Port D: 75 µL	5

Ordering information

Part Number	Product Description
S7851A or S7851AN	Seahorse XF Flex analyzer
103874-100	Seahorse XF Flex 3D capture FluxPak-S
103875-100	Seahorse XF Flex 3D capture microplate-S
103016-100	Seahorse XF 3D Mito Stress Test kit

www.agilent.com/lifesciences/discoverXF

DE-010522

This information is subject to change without notice.