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Quick Start Guide

Agilent Seahorse XF Mito-rOCR Assay Kit

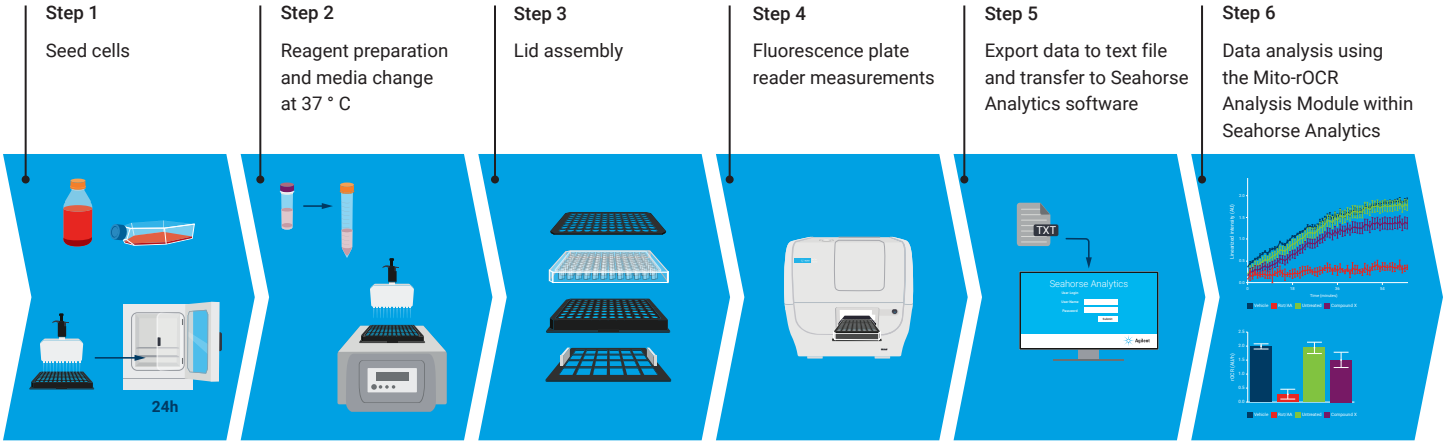


Figure 1. Agilent Mito-rOCR assay workflow for respiration measurements using a compatible fluorescence plate reader.

One day before the assay (Day 1)

Step 1. Seed cells and obtain Gen5 files

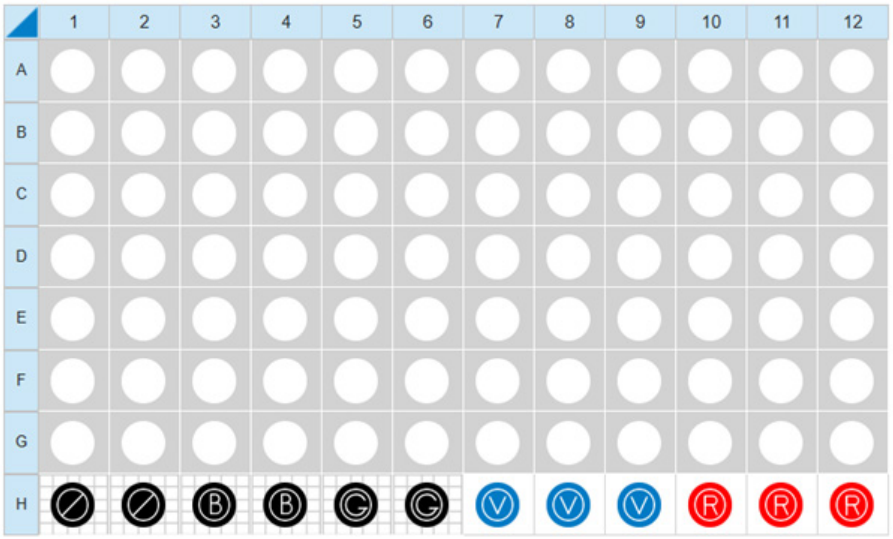
1. Seed cells in the Agilent Mito-rOCR microplate and allow the cells to grow overnight. Do not seed cells in Background, Blank or GOx control wells (H1 through H6, by default).
2. Download a Mito-rOCR Gen5 protocol file and the Mito-rOCR plate definition file. Import into Gen5 v3.16 or higher.

Day of the assay (Day 2)

Caution: Assay is temperature sensitive, and it is critical to keep all components at 37 °C.

Step 2. Reagent preparation and media change

1. Place the magnetic holder and a packaged Seal Lid into a 37 °C incubator for at least one hour.
2. Prewarm media and any treatment solutions.
3. Set plate block heater and fluorescence plate reader to 37 °C.
4. Resuspend reagents as outlined in Table 1 and keep at 37 °C. Prepare 11x stock of all test compounds.
5. Add the ~ 1 mL stock Mito-rOCR reagent to 5.5 mL of assay media to make ~ 6.5 mL of Mito-rOCR assay media.
6. Carefully remove the culture media from all microplate wells.
7. Add 50 µL of Mito-rOCR assay media containing rOCR reagent to all wells, excluding the Blank wells (H1 and H2).
8. Add 50 µL of assay media without rOCR reagent to Blank wells, H1 and H2.
9. Add 5 µL/well of test compound.
10. Add 1 µL of Rot/AA to wells H10 through H12.
11. Cover the plate with the standard lid and incubate in the prewarmed plate reader for 10 minutes.
12. Add 5 µL of GOx to wells H5 and H6.



		Cells	rOCR Reagent	GOx
⊘	Blank	✗	✗	✗
Ⓑ	Background	✗	✓	✗
Ⓖ	GOx	✗	✓	✓
Ⓥ	Vehicle	✓	✓	✗
Ⓡ	Rot/AA	✓	✓	✗

Figure 2. Mito-rOCR plate map and controls.

Step 3. Lid assembly

1. On a plate warmer, place the magnetic holder base plate and the microplate.
2. Remove the microplate lid.
3. Align the prewarmed Mito-rOCR Seal lid with the plate and carefully insert the lid into the microplate.
4. Carefully add the prewarmed metal top plate on top of the seal lid.

Step 4. Fluorescence plate reader measurements

1. Read the plate immediately in the preheated fluorescence microplate reader (37 °C, 1 read/minute for 45 mins).
2. When prompted, select the GOx wells (default H5 and H6) for gain adjustment.
3. Upon completion, save the experiment file and export data as a text file (.txt).

Step 5. Data analysis using Seahorse Analytics software

1. Login to Seahorse analytics: https://seahorseanalytics.agilent.com/.
2. Upload the exported .txt file and define plate layout.
3. Select the Mito-rOCR View Widgets to display the report.

www.agilent.com/lifesciences/discoverXF

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This information is subject to change without notice.

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Table 1. Preparation of stock solutions.

Compound	Resuspend in:
Mito-rOCR reagent	1 mL assay media
GOx	100 µL ddH_2O
Rotenone/Antimycin A	106 µL assay media
Test compound (11x)	Assay media

Table 2. Example of test compound (11x) stock preparation using the Agilent Seahorse XFp Mito Stress Test kit (103010-100)

Compound	Amount (nmol/tube)	Assay Media (µL)	Final Well (µM)
Oligomycin	12.6	763	1.5
FCCP	14.4	654	2
Rot/AA	5.4 (both)	982	0.5

Ordering information

Order the Agilent Mito-rOCR Assay Kit (MO-300-4) for reagents, seal lids and plates for 4 additional assays

www.agilent.com/lifesciences/mito-rocr

Additional information

User guide

https://www.agilent.com/cs/library/usermanuals/public/user-guide-mito-rocr-5994-7821en-agilent.pdf

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