



AUG 09, 2023

Analyzing oxygen consumption of isolated mitochondria using the Seahorse XFe96 analyzer

Louise Uoselis¹

¹Lazarou Lab, WEHI



Louise Uoselis
WEHI

ABSTRACT

Protocol for analyzing oxygen consumption of isolated mitochondria using the Seahorse XFe96 analyzer.

OPEN  ACCESS



DOI:

[dx.doi.org/10.17504/protocol
s.io.3byl4qj6zvo5/v1](https://dx.doi.org/10.17504/protocols.io.3byl4qj6zvo5/v1)




Protocol Citation: Louise Uoselis 2023. Analyzing oxygen consumption of isolated mitochondria using the Seahorse XFe96 analyzer. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.3byl4qj6zvo5/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working



Created: Aug 09, 2023



Day 1








- 1 Seed cells in 10 cm plates aiming for a confluency of ~80-90% at the time of treatment.
- 2 Add  200 μ L of Seahorse XFe calibrant solution to each well of a Seahorse (Agilent) cartridge plate, and incubate  Overnight at  37 °C in a CO2-free incubator.




Day 2


2h 35m



- 3 Isolate mitochondria (see previously published protocol) with the following modifications: at harvesting, scrape cells into ice cold modified isolation buffer (70 mM sucrose, 210 mM mannitol, 1 mM EGTA, 0.5% w/v BSA (fatty acid free), 5 mM HEPES pH 7.2), store cell pellets on ice prior to homogenization, store mitochondrial samples on ice, and immediately assay mitochondrial samples after quantification (frozen mitochondrial stocks cannot be used for oxygen consumption analysis).
- 4 Quantify mitochondria by bicinchoninic assay
- 5 Aliquot out  15 μ g of mitochondria per sample, diluting each aliquot to a final volume of  25 μ L in mitochondrial assay solution (MAS: 70 mM sucrose, 220 mM mannitol, 10 mM KH₂PO₄, 5 mM MgCl₂·6H₂O, 1 mM EGTA, 0.1% w/v BSA (fatty acid free), 2 mM HEPES pH 7.2), and leave the samples on ice until needed.
- 6 Pre-chill a Seahorse sample plate on ice


- 7 Make up the substrate solution (10 mM Glutamate, 10 mM malate in MAS buffer) and place at  37 °C in a CO2 free incubator for at least  01:00:00 1h

- 8 To the equilibrated cartridge plate, load  20 µL of  20 millimolar (mM) ADP,  22 µL of 50 ug/uL oligomycin,  24 µL of  10 micromolar (µM) FCCP,  26 µL of  40 micromolar (µM) of Antimycin A into the corresponding ports of each well.

- 9 Incubate the cartridge at  37 °C in a CO2-free incubator for  00:45:00, and begin the calibration sequence on the Seahorse XFe96 analyser so that it's completion corresponds with step 13 (takes ~  00:30:00). 1h 15m

- 10 During step 9, add a  25 µL aliquot of mitochondria to each corresponding well of the pre-chilled Seahorse sample plate.

- 11 Centrifuge the plate at  2000 rcf, 4°C,  00:20:00, and place the plate on ice until required. 20m

- 12 Add  155 µL of pre-warmed substrate solution to each sample well.

- 13 Eject the calibration plate from the analyser and replace it with the sample plate.

- 14 Run the following protocol on the analyser:
 Basal (3 min mix, 3 min measure, 3 min mix, 3 min measure)
 ADP (injection, 30 sec mix, 3 min measure)
 Oligomycin (injection, 30 sec mix, 30 sec wait, 3 min measure)
 FCCP (injection, 20 sec mix, 3 min measure)

Antimycin A (injection, 30 sec mix, 3 min measure)

- 15** If analyzing mitochondrial respiration across multiple days, perform step 2 of day 1 the day before the time point analysis, and perform step 3 – 14 on each day of the time course with the appropriate vehicle-treated controls each day.