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Analysing mitochondrial oxygen consumption using the Seahorse XFe96 analyzer

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ABSTRACT

Protocol for analysing mitochondrial oxygen consumption using the Seahorse XFe96 analyzer





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protocols.io

https://protocols.io/view/anal ysing-mitochondrial-oxygenconsumption-using-tcyewxtfe

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

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Day 1

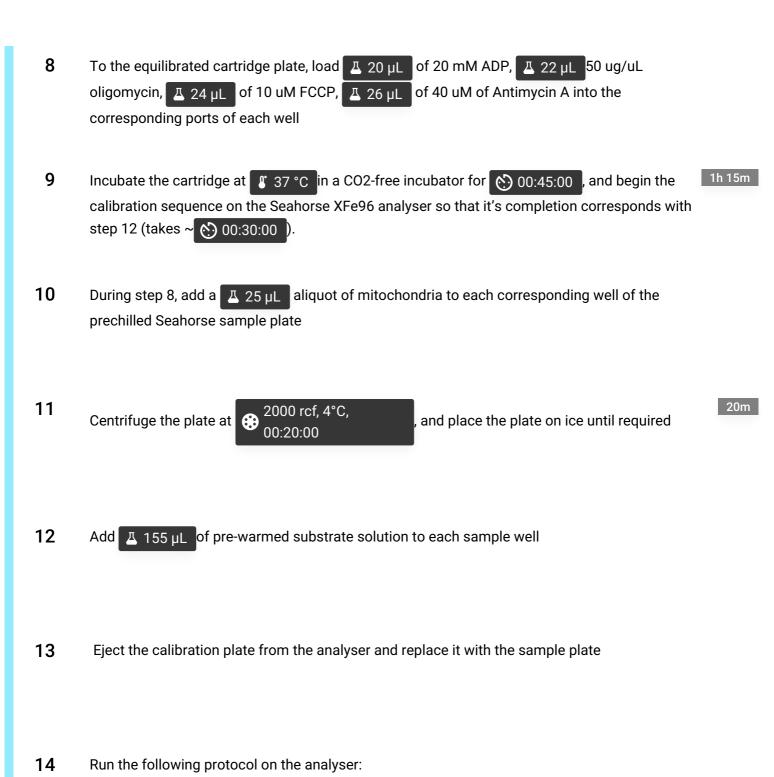
- 1 Seed cells in 10 cm plates aiming for a confluency of ~80-90% at the time of treatment
- Add Δ 200 μL of Seahorse XFe calibrant solution to each well of a Seahorse (Agilent) cartridge plate, and incubate Overnight at 37 °C in a CO2-free incubator

Day 2

2h 35m

- Isolate mitochondria (see previously published protocol) with the following modifications: cells were scraped 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), cell pellets were stored on ice prior to homogenization, and mitochondrial samples were stored on ice and immediately assayed after quantification
- 4 Quantify mitochondria by bicinchoninic assay
- Aliquot out Δ 15 μg of mitochondria per sample, diluting each aliquot to a final volume of Δ 25 μL in mitochondrial assay solution (MAS: 70 mM sucrose, 220 mM mannitol, 10 mM KH2PO4, 5 mM MgCl26H2O, 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



Basal (3 min mix, 3 min measure, 3 min mix, 3 min measure)

ADP (injection, 30 sec mix, 3 min measure)