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We use this protocol and it's working

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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

Keywords: ASAPCRN

Day 1

1 Seed cells in 10 cm plates aiming for a confluency of ~80-90% at the time of treatment

2 Add 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

3 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

5 Aliquot out 15 µg of mitochondria per sample, diluting each aliquot to a final volume of 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

8 To the equilibrated cartridge plate, load 20 µL of 20 mM ADP, 22 µL 50 ug/uL oligomycin, 24 µL of 10 uM FCCP, 26 µL of 40 uM 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 12 (takes ~ 00:30:00). 1h 15m

10 During step 8, add a 25 µL aliquot of mitochondria to each corresponding well of the prechilled 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 analysing 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 controls each day.