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Isolated Brain Mitochondria Respiration protocol

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

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Abstract

Brain Mitochondria Respiration protocol. Assay developed by Dr. Linsey Stiles at the UCLA Metabolomics Center, David Geffen School of Medicine at the University of California, Los Angeles, CA, USA

Preparation

1 The day prior to the Seahorse assay, a Seahorse cartridge needs to be hydrated overnight.

2 All isolated brain mitochondria samples are prepared in Mitochondrial Respiration Buffer (MAS) prepared with containing:
220 mM mannitol, 70 mM sucrose, 5 mM KH_2PO_4, 5 mM $MgCl_2$,
2 mM HEPES, 1 mM EGTA, and 0.1% (w/v) fatty acid-free BSA.

Load the Seahorse Cartridge

3 Prepare the compounds in MAS to be loaded into the Seahorse cartridge ports. Final concentrations in the well were:
a. 4 mM ADP
b. 3 µM oligomycin
c. 4 µM FCCP
d. 2 µM rotenone and antimycin A

4 Load 20 µL per port in the Seahorse cartridge with a multichannel pipet

Prepare the XFe96 Seahorse Instrument and Calibrate the Cartridge

5 The prepared Seahorse cartridge needs to be calibrated prior to loading the sample plate.

6
1. Prepare template with plate map and running program. An example of an isolated mitochondria running protocol:

Command	Time (minutes)	Port	Repeat
Calibrate	18		
Mix	2		2
Time Delay	2		
Mix	0.5		1

	Measure	2		
	Mix	1		
	Injec t A (ADP + substrates)			
	Mix	0.5		2
	Measure	2		
	Mix	1		
	Inject B (Oligomycin)			
	Mix	0.5		2
	Measure	2		
	Mix	1		
	Injec t C (FCCP)			
	Mix	0.5		2
	Measure	2		
	Mix	1		
	Inject D (Antimycin A)			
	Mix	0.5		2
	Measure	2		
	End Program			

7 2. Start the Seahorse Run

8 3. Load the cartridge for calibration

9 1. Prepare mitochondria sample dilutions based on the protein concentration of each sample

10 a. Samples preparation includes 10X substrate, isolated mitochondria, and MAS Buffer for the desired number of wells

11 2. Load 20µL of the mitochondria sample per well of the XF96 microplate

12 3. Centrifuge the plate at 2,100 x g for 5 minutes

13 4. Stop the centrifugation without a break

14 5. Add 130 of MAS per well after centrifugation

15 6. Load the Seahorse plate into the instrument