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Striatal Mitochondria Isolation Protocol

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Livia Hecke Morais[1]

[1]California Institute of Technology

Livia Hecke Morais
California Institute of Technology

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We use this protocol and it's working

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Abstract

Striatal Mitochondria Isolation Protocol used in the Mazmanian lab for Seahorse experiments and others

Reagents

1 **Reagents**

500 mL of MSHE (pH 7.2) for isolation

- 210 mM mannitol (Sigma, Cat #M1902) (19.13 g)
- 70 mM sucrose (Sigma, Cat #S0389) (11.98 g)
- 5 mM HEPES (Sigma, Cat #H3537) (2.5 mL of 1M stock)
- 1 mM EGTA (Sigma, Cat #E3889) (2 mL of 250 mM stock)
- 0.2% fatty acid-free (Fraction V) Bovine-serum albumin (BSA) (1 g, or 10 mL of 10% stock) (Sigma, Cat #A3803)

1. Measure all the powders into a 500 mL bottle.
2. Fill the bottle to about 400 mL with tissue culture grade water.
3. pH to 7.2 at 37˚C with 1M KOH solution while stirring.
4. Pour contents of 500 mL bottle into a 500 mL graduated cylinder. Fill up to 500 mL with tissue culture grade water.
5. Filter through 0.22 µm vacuum filter into a 500 mL receiver.
6. Store at 4˚C.
7. Prepare it fresh the day before experiment. Or freeze it for future use (maximum of 3 months). Once you defrost it, adjust the pH.
8. Also prepare, 250 mL of MSHE without BSA

10% Digitonin (in TC-grade water). (Sigma, Cat #D5628)

- Bring to 95˚C in the heat block to dissolve digitonin.
- Store at 4˚C.

Isolation protocol

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1. Pre-chill a 15 mL glass Dounce homogenizers and pestles to 4˚C on ice. Wash the glass tubes with MSHE.
2. After dissection transfer tissue to the 15 mL dounce homogenizer containing MSHE.
3. Homogenize with 10 strokes of the pestle.
4. Transfer the tissue homogenate back into the 2 mL Eppendorf tube.
5. Centrifuge at 2000g for 3 minutes at 4˚C.
6. Aliquot the filtered supernatant into chilled 2 mL Eppendorf tubes.

7. Centrifuge the 2 mL Eppendorf tubes at 12,000g for 10 min at 4˚C.
8. While waiting for the spin to complete, place 10% digitonin in the heat block to bring it back into solution. Once dissolved, remove it from the heat block to cool before using.
9. Add 40 µL of 10% digitonin into 20 mL of MSHE (for 0.02% w/v). Scale down if you have less than 10 tubes.
10. Aspirate supernatant (leave the white layer). Resuspend pellets in 700 µL of the digitonin + MSHE solution (400 µL first then 300 µL).
11. Centrifuge again at 12,000g for 10 min at 4˚C.
12. Aspirate most of the white layer (the light fluffly layer that sits atop the dark brown mitochondria) of the pellet. Resuspend in 700 µL of MSHE.
13. Centrifuge again at 12,000g for 10 min at 4˚C.
14. Aspirate the supernatant and remaining white layer of the pellet.
15. Resuspend the pellet in 30 uL of MSHE (change final volume or buffer as needed).