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Laboratory Protocol: Isolation of rat brain mitochondria

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Preparation: Switch on centrifuge and let it cool down to 4 ºC. Keep all buffers, dissection gear, homogenization tools and centrifuge rotor at 4 ºC (or on ice).

Anesthesia: Rats are anesthetised by intraperitoneal injection of Thiopental (0.1 g/kg) or by CO_2 narcosis.

Isolation procedure:

1. kill rat, dissect brain from the skull and place immediately in ice-cold isolation medium A
2. determine wet weight
3. transfer brain to a pre-cooled glass beaker (20 ml) with ice-cold isolation medium A, discard all medium
4. mince the tissue into small pieces using a pair of sharp scissors (tissue should become a mash), add drops of medium while cutting
5. suspend with 5 – 10 volumes of ice-cold isolation medium A and transfer to a pre-cooled glass/Teflon potter.
6. homogenize the tissue with 8 - 10 strokes at 1,000 rpm, add more medium
7. transfer to a 50 ml Falcon tube, adjust the volume to get ~ 5 % homogenate (1 g tissue per 20 – 30 ml homogenate)
8. centrifuge at 1000 g for 10 min at 4 °C
9. transfer the supernatant into new tube and centrifuge at 6,200 g for 10 min at 4 °C
10. discard the supernatant and re-suspend mitochondria in a small volume of the islation medium B (the volume of mitochondrial suspension from 1 g tissue ~ 1 ml)
11. store mitochondria on ice, use within 3-4 h
12. transfer 20 µl into an Eppendorf tube and store at -20°C for further analysis

Isolation buffer A:

Chemical	Final conc.	Required for 1000 ml buffer
Succrose	320 mM	109.54 g
Tris-Cl	10 mM	1.211 g
K^+EDTA	1 mM	0.372 g
BSA	2.5 g/l	2.5 g

Adjust pH to 7.4 with Tris, HCl

Isolation buffer B:

Isolation buffer A without BSA

References:

Sumbalová Z, Kucharská J, Kristek F. (2010) Losartan improved respiratory function and coenzyme Q content in brain mitochondria of young spontaneously hypertensive rats. Cell Mol Neurobiol. 30:751-8. »Bioblast Abstract«