Oroboros **O2k-Protocols**
mt-Preparations

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Laboratory protocol: isolation of rat brain mitochondria

Sumbalova Z[1], Fontana-Ayoub M[2], Krumschnabel G[2]

[1]Pharmacobiochem Lab, Fac Medicine,
Comenius Univ, Bratislava, Slovak Republic

[2]**Oroboros Instruments**
O2k High-Resolution FluoRespirometry (HRFR)
Schöpfstr 18, A-6020 Innsbruck, Austria
Email: instruments@oroboros.at
www.oroboros.at

1. 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).

1.1. Anesthesia

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

1.2. 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 1,000 *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 (sediment) in 20-30 ml of isolation medium B per g tissue, centrifuge at 6,200 g for 10 min at 4°C.
11. Discard the supernatant and re-suspend mitochondria in a small volume of the isolation medium B (the volume of mitochondrial suspension from 1 g tissue ~ 1 ml)

12. Store mitochondria on ice, use within 3-4 h.
13. Transfer 20 µl into an Eppendorf tube and store at -20°C for further analysis (protein concentration, citrate synthase).

2. Media

2.1. Isolation buffer A

Chemical	Final conc.	Required for 1,000 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

2.2 Isolation buffer B

Isolation buffer A without BSA.

3. 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 link«

http://wiki.oroboros.at/index.php/O2k-mitochondrial_preparations

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www.mitofit.org

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