Oroboros **O2k-Procedures**
mt-Preparations

Mitochondrial Physiology Network 20.15(02):1-2 (2016)
Version 02: 2016-03-25 ©2015-2016 Oroboros
Updates: http://wiki.oroboros.at/index.php/MiPNet20.15_IsolationRatHeart-mt

Laboratory protocol: isolation of rat heart mitochondria

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

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

[2]**Oroboros Instruments**
High-Resolution Respirometry
Schöpfstrasse 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 out heart (take weight) and put it into ice-cold isolation medium A, wash to remove blood, discard all medium.
2. Cut the heart into small pieces (should become a mash), add drops of isolation buffer A while cutting.
3. Add isolation buffer B (10 ml/g tissue), transfer to pre-cooled glass/Teflon potter and homogenize with 8-12 strokes at medium speed (1,000 rpm).
4. Transfer the homogenate to a 50 ml beaker, bring volume up to ~ 15 ml/g tissue with isolation buffer A, place it on a magnetic stirrer, stirr slowly for 20 min in an ice bath.
5. Re-homogenize the homogenate briefly in the potter, bring the volume up to ~ 20–30 ml with isolation buffer A.
6. Centrifuge: 1,000 *g*, 10 min, 4 °C.
7. Transfer the supernatant to a new 50 ml Falcon tube.
8. Centrifuge: 6,200 *g*, 10 min, 4 °C.
9. Discard the supernatant, carefully re-suspend the mitochondrial pellet in a small volume of isolation buffer C and fill up to a volume of 20-30 ml (for 1 g tissue) with isolation buffer C.
10. Centrifuge: 6,200 *g*, 10 min, 4 °C.
11. Discard supernatant and carefully re-suspend mitochondria with small volume of isolation buffer C. The volume of mitochondrial suspension for 1 g tissue is ~ 1.2 ml.

12. Store mitochondria on ice; perform functional studies within 3-4 h.
13. Transfer subsamples (20 µl) into Eppendorf tubes and store at −20°C for further analysis (protein concentration, citrate synthase activity).
14. For respiratory measurements add 2.5 µl of mitochondrial suspension into the 2 ml O2k-Chamber.

2. Media

2.1. Isolation buffer A

Chemical	Final conc.	Required for 500 ml buffer
KCl	180 mM	6.71 g
EDTA	4 mM	0.745 g
BSA	1 g/l	0.5 g

Adjust pH to 7.4 with Tris, HCl

2.2. Isolation buffer B

Isolation buffer A with 0.25 mg/ml Subtilisin. Add 2.5 mg Subtilisin to 10 ml of Buffer A.

2.3. Isolation buffer C

Isolation buffer A without BSA.

2.4. Preparation of buffers

Prepare 500 ml of buffer C for 3 - 4 isolations. Add BSA to 250 ml of buffer C to obtain buffer A. A and C can be stored at -20 °C. Prepare buffer B fresh.

Acknowledgements

Sumbalova Z was supported by Action Austria – Slovakia (2015). O2k-Protocol development supported in part by K-Regio project MitoFit. www.mitofit.org

This project is funded by the Tyrolean Government and the European Regional Development Fund (ERDF) and is subject to the regulations of EU law as well as to the Directive of the Tyrolean Government on the Funding of Science, Research and Development.

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