Mitochondrial Physiology Network 19.22:1-2 (2014)

Version 1: 2014-12-18

Laboratory Protocol: Isolation of Rat Heart Mitochondria

Zuzana Sumbalova[1], Mona Fontana-Ayoub[2], Gerhard Krumschnabel[2]

[1] Pharmacobiochemical Laboratory of 3rd Department of Internal Medicine, Medical Faculty, Comenius University in Bratislava, Slovak Republic

[2]**OROBOROS INSTRUMENTS Corp**
high-resolution respirometry
Schöpfstr 18, A-6020 Innsbruck, Austria
Email: Gerhard.Krumschnabel@oroboros.at
www.oroboros.at

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 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 (1000 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, stir 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 C
6. centrifuge: 1000 x g, 10 min, 4°C
7. transfer the supernatant to a new 50 ml falcon tube
8. centrifuge: 6200 x g, 10 min, 4°C
9. discard the supernatant, carefully re-suspend the mitochondrial pellet in a small volume of isolation buffer C and bring the volume to 20-30 ml (for 1 g tissue) with isolation buffer C
10. centrifuge: 6200 x 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 ~ 1.2 ml)
12. store mitochondria in ice/water bath, use within 3-4 h
13. transfer subsamples (20 µl) into Eppendorf tubes and store at -20°C for further analysis (protein concentration, citrate synthase)

14. for respiration measurements add 2.5 µl of mitochondrial suspension into a 2 ml chamber

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

Isolation buffer B:

Isolation buffer A with 2.5 mg/ml Subtilisin. Add 25 mg Subtilisin to 10 ml of Buffer A.

Isolation buffer C:

Isolation buffer A without BSA

For 3 - 4 isolations 500 ml of media need to be prepared. Add BSA to 250 ml of medium and leave the residual 250 ml without BSA.