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# Laboratory protocol: isolation of rat heart mitochondria

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## 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.

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