



# Laboratory protocol: isolation of rat liver mitochondria

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## 1. Preparation

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

### 1.1. Anesthesia

Rats are anesthetised by intraperitoneal injection of Thiopental (0.1 g/kg) or by CO<sub>2</sub> narcosis.

### 1.2. Isolation procedure

1. Kill rat, dissect out liver (take weight) and put liver in ice-cold isolation medium.
2. Determine wet weight, take 1.5 g of the tissue for isolation.
3. Transfer the tissue to a pre-cooled glass beaker (20 ml) with ice-cold isolation medium, discard all medium.
4. Mince the tissue into small pieces using a pair of sharp scissors (should become a mash), add drops of medium while cutting.
5. Suspend with ~ 5 - 10 volumes of ice-cold isolation medium and transfer to a pre-cooled glass/Teflon potter.
6. Homogenize the tissue with 8 - 10 strokes at 1,000 rpm, add more media.
7. Transfer to the 50 ml Falcon tube, bring the volume to get <10% homogenate (1 g tissue to 15 - 20 ml homogenate).
8. Centrifuge at 1,000 *g* for 10 min at 4 °C.
9. Filter the supernatant through two layers of gauze (optional), transfer it into a new tube and centrifuge at 6,200 *g* for 10 min at 4 °C.
10. Discard the supernatant and re-suspend the mitochondria (sediment) in 15-20 ml of isolation medium 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 medium (the volume of mitochondrial suspension from 1.5 g tissue ~ 1.5 ml).
12. Store mitochondria on ice, use within 3-4 h.

13. Transfer subsamples (20  $\mu$ l) into Eppendorf tubes and store at -20 °C for further analysis (protein concentration, citrate synthase).

## 2. Media

### 2.1. Isolation buffer

Chemical	Final conc. [mM]	Add to 1000 ml [g]
Mannitol	225.0	40.99
Succrose	75.0	25.67
EDTA	0.2	0.0744

Adjust pH to 7.4 with Tris, HCl.

## 3. References

Isolation protocol modified after Kucharska et al 2000:

Kucharská J, Braunová Z, Ulicná O, Zlatos L, Gvozdjáčková A (2000) Deficit of coenzyme Q in heart and liver mitochondria of rats with streptozotocin-induced diabetes. *Physiol Res* 49:411-8. »[Bioblast link](#)«



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