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Laboratory Protocol: Isolation of rat liver mitochondria

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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 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 1000 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 in a small volume of the medium (the volume of mitochondrial suspension from 1.5 g tissue ~ 1.5 ml).
11. store mitochondria on ice, use within 3-4 h.
12. transfer subsamples (20 µl) into Eppendorf tubes and store at -20°C for further analysis (protein concentration, citrate synthase)

Isolation buffer:

Chemical	Final conc.	Required for 1000 ml buffer
Mannitol	225 mM	40.99 g
Succrose	75 mM	25.67 g
EDTA	0.2 mM	0.0744 g

Adjust pH to 7.4 with Tris, HCl