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Laboratory Protocol: Isolation of Rat Liver Mitochondria

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Starve the rat over night and put the medium into a cold room for de-freezing. All steps are performed at 4 °C. The rotor used is a.HFA 21.94. Rinse the liver with **Medium A1** [MiPNet03.02], remove vessels and other tissue, and slice it with a pair of scissors into small pieces. Wash it carefully. After washing away the blood with medium A pass the tissue through a sieve (0.8 mm pore diameter) for homogenization. Move the homogenizer tube (inner diameter of the tube: 3 cm with a rough surface, space between tube and pestle: 1.5 - 2 mm) gently up and down past the slowly rotating pestle (~200 - 300 rpm).

1st centrifugation: 660x*g* (2,200 rpm, 4 °C, 10 min), Pr.Nr. 7

Filtration of the supernatant. If necessary remove lipid layers.

2nd centrifugation: 10,000x*g* (9,200 rpm, 4 °C, 10 min), Pr.Nr. 8

Rinse away the light fraction (uppermost layer) if necessary. Resuspend the pellet with a 5 ml pipette by using **Medium B1** [MiPNet03.02], and homogenize in a small potter (inner diameter of the tube: 1.9 cm, space

between tube and pestle: 0.5 - 1 mm). Continue with one centrifugation tube.

3rd centrifugation: 10,000x*g* (9,200 rpm, 4 °C, 10 min)
Add some Medium B1, wash away the uppermost fluffy layer which at least partly consists of broken mitochondria. Resuspend the pellet with a pipette by using Medium B1 and homogenize (inner diameter of the tube: 1.9 cm, space between tube and pestle: 0.5 - 1 mm).

4th centrifugation: 10,000x*g* (9,200 rpm, 4 °C, 10 min)
Add some Medium B1, wash away the uppermost fluffy layer. Homogenize (inner diameter of the tube: 0.9 cm, space between tube and pestle: 0.5 - 0.75 mm) the pellet in a small potter with ca. 1 ml mitochondrial preservation medium (**MiP03)**; [MiPNet14.13]. Most frequently, mitochondria are resuspended in Medium B1, but MiP03 was shown to yield higher and more stable respiration rates in isolated heart mitochondria (Gnaiger et al. 2000).

Store the mitochondrial suspension on ice for one hour before starting measurements to permit the rearrangement of the membranes.

References

Gnaiger E, Kuznetsov AV, Schneeberger S, Seiler R, Brandacher G, Steurer W, Margreiter R (2000) Mitochondria in the cold. In: Life in the Cold. (Heldmaier G, Klingenspor M, eds) Springer, Heidelberg, Berlin, New York: pp 431-42.

Protocols

MiPNet03.02	Selected media and chemicals.
MiPNet14.13	Mitochondrial respiration medium - MiR06.