



O2k-Fluorometry

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Laboratory Protocol: Isolation of mouse heart mitochondria

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high-resolution respirometry

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An isolation protocol modified after Mela and Seitz, 1979 (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).

Euthanasia: mice are killed by cervical dislocation.

Isolation procedure:

1. Kill the mouse and dissect the heart w/o blood vessels, atria and fat. Immediately transfer the tissue into ice cold BIOPS (2).
2. Transfer heart to Petri dish on cooling plate, excise left ventricle if required or take whole heart and carefully eliminate blood clots. Transfer heart into 10 ml glass beaker on ice with 1 ml of ice cold BIOPS and cut tissue into small pieces with cooled scissors.
3. Transfer tissue paste into 10 ml potter glass, add 2ml isolation buffer B and dounce 6-8 times (middle speed).
4. Transfer tissue homogenate to 50 ml Falcon tube and add 3 ml isolation buffer B.
5. Centrifuge homogenate at 800 x g for 10 minutes at 4°C .
6. Transfer supernatant to new 50 ml Falcon tube.
7. Centrifuge the supernatant at 10,000 x g for 10 minutes at 4°C.
8. Remove the supernatant.
9. Carefully re-suspend the mitochondrial pellet in 500 µl isolation buffer A and then fill volume up to 2 ml of isolation buffer A.
10. Centrifuge at 10,000 x g for 10 minutes at 4°C.
11. Carefully re-suspend the final mitochondrial pellet in 200 µl suspension buffer (exact volume depends on the amount of tissue homogenized).
12. For respiration measurements add 5 µl of mitochondrial suspension into a 2 ml chamber.
13. transfer subsamples (20 µl) into Eppendorf tubes and store at -20°C for further analysis (protein concentration, citrate synthase)

Media:**BIOPS:**

Biopsy preservation solution, as described in (2).

Isolation buffer A:

Stock (4°C): 0.5 M Mannitol; 0.1 M EGTA pH 7.4 (Tris buffered),

Sucrose 0.5 M

Mix fresh daily:

Chemical	Final conc.	Add for 25 ml final volume
Mannitol	225mM	11.25 ml
Sucrose	75 mM	3.75 ml
EGTA	1 mM	0.25 ml
BSA	2.5 mg / ml	62.5 mg

Isolation buffer B:

Add 5 mg Subtilisin to 10 ml of Buffer A.

Suspension buffer

Isolation buffer A without BSA

References

1. Mela L, Seitz S (1979) Isolation of mitochondria with emphasis on heart mitochondria from small amounts of tissue. *Methods Enzymol* 55: 39-46. [»Bioblast Abstract«](#)
2. Fontana-Ayoub M, Fasching M, Gnaiger E (2014) Selected media and chemicals for respirometry with mitochondrial preparations. *Mitochondr Physiol Network* 03.02(17): 1-9. [»Bioblast Abstract«](#)