Oroboros **O2k-Procedures**
mt-Preparations

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Laboratory protocol: isolation of beef 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. Beef heart

A chunk of left ventricle from beef heart is obtained from a local slaughterhouse within one hour after killing of the animal. The heart sample is immediately transferred into ice cold BIOPS and transported into the laboratory.

1.2. Isolation procedure

1. Wash the left ventricle with ice-cold BIOPS, remove a 2 g piece and dissected free of pericard tissue.
2. Transfer the heart sample to a 10 ml glass beaker on ice with 1 ml of ice cold BIOPS and cut into small pieces with cooled scissors.
3. Transfer tissue into 10 ml potter, add 8 ml isolation buffer B (containing subtilisin) and dounce 6-8 times (middle speed)
4. Transfer tissue suspension to a 50 ml Falcon tube and add 12 ml isolation buffer B.
5. Suspend sample by carefully inverting the tube a few times and then centrifuge at 800 *g* for 10 min at 4 °C.
6. Transfer supernatant to new 50 ml Falcon tube.
7. Centrifuge the supernatant at 10,000 *g* for 10 min at 4 °C.
8. Remove the supernatant and carefully re-suspend the mitochondrial pellet in 500 µl of isolation buffer A, then add up to 20 ml.
9. Centrifuge at 10,000 *g* for 10 min at 4 °C.
10. Discard supernatant and carefully re-suspend mitochondria with 500 µl suspension buffer (w/o BSA).
11. Keep mitochondrial suspension on ice until use.
12. For respiration measurements add ≥ 20 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).

2. Media

2.1. BIOPS

Biopsy preservation solution [2].

2.2. 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. [mM]	Add for 50ml final volume [ml]
Mannitol	225	22.5
Sucrose	75	7.5
EGTA	1	0.5
Remove 1 ml of medium to serve as suspension buffer, then add:		
BSA	2.5 mg / ml	125 mg

~ 50 ml buffer are needed for 2 g of tissue.

2.3. Isolation buffer B

Add 10 mg subtilisin to 20 ml of buffer A.

2.4. Suspension buffer

Isolation buffer A without BSA.

3. References

This isolation protocol was modified after Mela and Seitz 1979 [1].

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 link«
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 link«

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