Mitochondrial Physiology Network 20.09:1-2 (2015)

Laboratory Protocol: Isolation of Beef Heart Mitochondria

Mona Fontana-Ayoub, Alexia Gomez Rodriguez, Gerhard Krumschnabel

OROBOROS INSTRUMENTS Corp
high-resolution respirometry
Schöpfstr 18, A-6020 Innsbruck, Austria
Email: Mona.Fontana@oroboros.at, Gerhard.Krumschnabel@oroboros.at
www.oroboros.at

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

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.

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 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 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 x g for 10 minutes 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).

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 50ml final volume
Mannitol	225mM	22.5 ml
Sucrose	75 mM	7.5 ml
EGTA	1 mM	0.5 ml

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 2g of tissue

Isolation buffer B:

Add 10 mg Subtilisin to 20 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.
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.