Laboratory protocol: isolation of beef heart mitochondria

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This isolation protocol was 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 g for 10 min at 4 ºC.
6. Transfer supernatant to new 50 ml Falcon tube.
7. Centrifuge the supernatant at 10,000 g for 10 min at 4 ºC.
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 ºC.
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 ºC for further analysis (protein concentration, citrate synthase).
Media

**BIOPS**

Biopsy preservation solution [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.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Final conc.</th>
<th>Add for 50ml final volume</th>
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</thead>
<tbody>
<tr>
<td>Mannitol</td>
<td>225 mM</td>
<td>22.5 ml</td>
</tr>
<tr>
<td>Sucrose</td>
<td>75 mM</td>
<td>7.5 ml</td>
</tr>
<tr>
<td>EGTA</td>
<td>1 mM</td>
<td>0.5 ml</td>
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</tbody>
</table>

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.

**Isolation buffer B**

Add 10 mg subtilisin to 20 ml of buffer A.

**Suspension buffer**

Isolation buffer A without BSA.

**References**