O2k-Procedures: Heart mitochondria

Mitochondrial Physiology Network 20.06(02):1-4 (2021)
Version 02: 2021-08-09 ©2021 Oroboros
Updates: http://wiki.oroboros.at/index.php/MiPNet20.06_IsolationMouseHeart-mt

Laboratory protocol: isolation of mouse heart mitochondria

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1. General Information

Mitochondrial preparations (mtprep) include isolated mitochondria, tissue homogenate, and mechanically or chemically permeabilized fibers or chemically permeabilized cells. **Isolated mitochondria (imt)** are mitochondria separated from a tissue or cells by breaking the plasma membranes and attachments to the cytoskeleton, followed by centrifugation steps to separate the mitochondria from other components. Harmonization of isolation protocols yielding intact and enzymatically active mitochondria are critical to assess mitochondrial respiratory function.

The isolation protocol described here was modified after Lai *et al* 2019 and Palmer *et al* 1977.

2. Animals

After cervical dislocation, remove the mouse heart, and place it immediately in ice-cold BIOPS.

3. Media and chemicals

3.1. BIOPS

10 mM Ca-EGTA buffer, 0.1 µM free calcium, 20 mM imidazole, 20 mM taurine, 50 mM K-MES, 0.5 mM DTT, 6.56 mM $MgCl_2$, 5.77 mM ATP, 15 mM phosphocreatine, pH 7.1. For preparation see: MiPNet03.02

3.2. Isolation buffer CP1

The buffer composition is based on the paper of Chappell and Perry 1954, and Tomec and Hoppel 1975. Isolation buffer CP1 can be stored for longer periods in 50-mL Falcons at -20 °C. The pH is set to 7.4 with KOH or HCl.

Component	Concentration [mM]	Molar mass [g/mol]	Mass for 250 mL [g]
KCl	100	74.5	1.863
MOPS	50	209.3	2.62
$MgSO_4 \cdot 7H_2O$	5	246.5	0.308
EGTA	1	380.4	0.095
ATP disodium salt	1	551.1	0.138

3.3. Isolation buffer CP2

The CP2 buffer has the same composition of the CP1 buffer plus 0.2 % BSA. Add 10 mg of BSA (Sigma Aldrich A6003) to 5 mL CP1 buffer directly before the isolation.

3.4. KME buffer

KME buffer can be stored for longer time periods at -20 °C in 2-mL Eppendorf tubes. The pH is set to 7.4 with KOH or HCl.

Component	Concentration [mM]	Molar mass [g/mol]	Mass for 50 mL [g]
KCl	100	74.5	0.3725
MOPS	50	209.3	0.523
EGTA	0.5	380.4	0.0095

4. Isolation procedure

Before starting the isolation, switch on the 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 bath). This protocol is described for tissue from one heart. If more than one heart is used, the amounts and volumes need to be adjusted.

1. Dissect the heart by removing blood, connecting vessels, and fat.
2. Determine wet mass (after removing the excess amount of liquid adhering externally to the sample using blotting paper).
3. Transfer the heart to a pre-cooled 10 mL glass beaker with ice-cold CP1 buffer (~ 5 mL).

4. Mince the tissue into small pieces using a pair of sharp scissors until the tissue becomes a mash.
5. Rinse the tissue 3 times with CP1 buffer (~5 mL x 3) to remove remaining blood.
6. Preparation of the trypsin type IX-S (T0303; Sigma Aldrich; 9 mg of 13000-20000 U trypsin/1 g wet mass) solution for digestion: weigh five times more trypsin than required for the tissue (45 mg/1 g wet mass). Wash the trypsin from the weighing paper with 5 mL CP1 buffer into a separate beaker and stir it for 1-2 min.
7. Using a pipette remove the excess CP1 buffer from the tissue leaving ~ 1 mL in the beaker. Place a small stirrer bar (8 mm x 3 mm) into the pre-chilled 10-mL beaker containing the tissue. Place the beaker to an ice bath with stirring. Digest the tissue with 1 mL trypsin solution for 2.5 min (the timer must be started immediately after trypsin addition) while continuous stirring. If you take one heart, the tissue is digested in ~2 mL solution (1 mL remaining CP1 buffer and 1 mL trypsin solution).
8. Immediately after the 2.5 min of digestion, add 2 mL of CP2 buffer to the tissue, allowing the protease to also work on the BSA, and homogenize with a Potter-Elvehjem homogenizer (1000 rpm) with a loose-fitting pestle (0.15–0.25 mm, glass-PTFE) five times on ice bath.
9. Transfer the homogenate into a 15-mL Falcon tube and centrifuge it for 10 min at 800 g, 4 °C (acceleration: 9, deceleration: 9).
10. Discard the pellet and centrifuge the supernatant in a 15 mL Falcon tube for 10 min at 3000 g, 4 °C (acceleration: 9, deceleration: 5).
11. Discard the supernatant (remove all the remaining buffer with a pipette) and resuspend the pellet with CP2 buffer (20 mL/g wet mass), transfer to a new 15 mL Falcon tube. Centrifuge the pellet for 10 min at 3000 g, 4 °C (acceleration: 9, deceleration: 5).
12. Discard the supernatant (remove all the remaining buffer with a pipette) and then resuspend the pellet in KME buffer (100 µL/ 0.1 g wet tissue mass).
13. For respirometry measurements using a 2 mL O2k-Chamber, use 5–20 µL of mitochondrial suspension.
14. Transfer subsamples (10–20 µL) into Eppendorf tubes and store at -20 °C for further analysis (protein concentration, citrate synthase).

5. References

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6. Acknowledgements

Diana Antunes as a former member of the Oroboros Instruments contributed to the project and to this MiPNet. Supported by the NextGen-O2k project.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 859770.