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Isolation of mitochondria from heart (mouse)

Two distinct populations of mitochondria are discerned in the heart, *subsarcolemmal* and *interfibrillar* [1]. When isolating the mitochondria from the heart it is important to use protease (Nagarse) to digest the tissue and ensure that both populations are yielded. However, the exposure time needs to be limited to avoid the effect of the enzyme on mitochondria [2].

The isolation buffers

MSE solution A:

Compound	M_w	[C], mM	Amount per g/L
Mannitol	182	225	41.0
Sucrose	342	75	25.65
EGTA	380	1	380
MOPS	209	20	4.18
DTT	154,2	0.5	77

pH 7.2
The presence of 0.1% BSA is not necessary for the heart mitochondria.

Use 0.5% Nagarse solution (5 mg Nagarse in10 ml MSE-A)

MSE solution B:

Compound	M_w	[C], mM	Amount per g/L
KCl	74.6	75	5.6
Sucrose	342	150	51.3
EGTA	380	0.1	38
MOPS	209	20	4.18

pH 7.2

Procedure:

1. Remove and immediately cut the heart of a mouse euthanized by exposure to CO_2, in two pieces and put in ice-cold MSE-A solution and shake to wash out the blood.

2. Mince the tissue with scissors in a small volume of MSE-A containing 0.5% Nagarse (per 1g heart tissue about 5-10 ml MSE-A with 0.5% Nagarse). The time spent for tissue mincing

should be enough for enzyme to digest the tissue pieces, about 5 min. Rinse the tissue pieces with about 10ml of MSE-A w/o Nagarse 2-3 times! 4^{o}C

3. Homogenize manually in glass-Teflon homogenizer (10-25 ml tubes are suggested).

4. Thereafter, add 20-30 ml of ice-cold MSE-A w/o Nagarse and centrifuge the homogenate at 900 g for 5 min. Discard the pellet containing tissue debris.

5. Filter the supernatant through a cheesecloth, centrifuge at 9000g for 10 min, 4^{o}C and collect the pellet.

6. Resuspend the pellet in 15% Percoll, prepared in MSE-A, and purify using 15-23-40% Percoll gradient by centrifuging for 20 min at 30,000 g, 4^{o}C.

7. Collect the mitochondria layer between 23 and 40% Percoll. Re-suspend the mitochondria in a large volume (up to 10ml) of MSE-A buffer and centrifuge for 10 min at 17,000 g, 4^{o}C.

8. The pellet will be loose! Don't pour supernatant tilting the tube, remove it with the pipette. Resuspend the pellet in MSE-B, bring the final volume to ~10 ml leaving 1-1.5 cm space from the top. The large volume helps to significantly dilute the remaining Percoll in mitochondria suspension. Centrifuge for 10 min at 17,000 g, 4^{o}C. If needed, rinse the mitochondria with MSE-B one more time to ensure that all Percoll is removed.

9. Resuspend the final pellet in 100-200 µl MSE-B.

References

1. Palmer JW, Tandler B, Hoppel CL (1977) Biochemical properties of subsarcolemmal and interfibrillar mitochondria isolated from rat cardiac muscle. J Biol Chem 252: 8731–8739.

2. Panov A. Practical Mitochondriology. Pitfalls and problems in studies of mitochondria. ISBN-10: 1483963853