Mitochondrial isolation protocol

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ABSTRACT

This protocol describes how to isolate crude mitochondrial fractions from HeLa cells. The methodology is based on Wieckowski et al. 2009 Nat Protocols. Wieckowski, M., Giorgi, C., Lebiedzinska, M.*et al.*Isolation of mitochondria-associated membranes and mitochondria from animal tissues and cells.*Nat Protoc***4**, 1582–1590 (2009)

MATERIALS

Mitochondrial isolation buffer: 5 mM HEPES (pH 7.4), 250 mM mannitol, 0.5 mM EGTA (make fresh on the day itself!)
Oligomycin/Antimycin A cocktail: 10 µM Oligomycin (A5588, ApexBio) and 4 µM Antimycin A (A8674, Sigma). In case cells were treated for more than 8 hours, we also added 10 µM Q-VD-OPh (A1901, ApexBio) to suppress apoptosis.
RIPA buffer: 50 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.5% sodium deoxycholate, 0.1% SDS, 1% NP-40, supplemented by cOmplete EDTA-free protease inhibitors (11836170001, Roche) and phosphatase inhibitors (Phospho-STOP, 4906837001, Roche).

Mitochondrial isolation

1 Seed HeLa cells in 15 cm dishes and grow until confluence. Treat the cells with DMSO or the mitophagy-inducing cocktail Oligomycin/Antimycin A (O/A) if needed.

2 Collect HeLa cells by trypsinization and resuspension in DMEM medium. Centrifuge the cells at 300x*g* for 10 min at 4 degrees. Resuspend the cell pellet in 1 ml of ice-cold PBS (1x) and spin again for 5 min at 300x*g* at 4 degrees.

3 Remove the PBS and resuspend the cell pellet in 1 ml mitochondrial isolation buffer, which was cooled to 4 degrees. Note that the mitochondrial isolation buffer is prepared fresh on the day itself.

4 To lyse cells without damaging the mitochondria, pipet the 1 ml cell suspension up and down (15-20x) with a 26.5G needle. This should lyse the plasma membrane but leave organelles intact.

5 Spin the suspension down at 600x*g* for 10 min at 4 degrees. Keep the supernatant and repeat this centrifugation step once more. The mitochondria are located in the supernatant at this speed, the pellet contains intact cells, cell nuclei, and other large cell debris.

6 After two spins at 600x*g*, subject the supernatant to 7000x*g* for 10 min at 4 degrees. The pellet contains the mitochondria, so remove the supernatant and resuspend the pellet in 1 ml mitochondrial isolation buffer. Then repeat this centrifugation step once more. The supernatant from the first spin at 7000xg can be stored as a cytosolic fraction.

7 After two spins at 7000x*g*, subject the resuspended pellet to 10,000x*g* for 10 min at 4 degrees. The pellet contains the mitochondria, so remove the supernatant and resuspend the pellet in 1 ml mitochondrial isolation buffer. Then repeat this centrifugation step once more.

8 After two spins at 10,000x*g,* the pellet can be resuspended in RIPA lysis buffer or used for further procedures such as mitochondrial import assays, proteinase K protection assays, etc.