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MANUSCRIPT CITATION:
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).

Protocol status: Working
We use this protocol and it's working

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Mitochondrial isolation protocol

Elias Adriaenssens[1]

[1]Sascha Martens lab, University of Vienna, Max Perutz Labs - Vienna (AT)

ASAP Collaborative Research Network

Sascha Martens lab, University of Vienna, Max Perutz Labs - Vienna

Elias Adriaenssens
Sascha Martens lab, University of Vienna, Max Perutz Labs - ...

ABSTRACT

This protocol describes how to isolate crude mitochondrial fractions from HeLa cells.

ATTACHMENTS

752-1920.pdf

MATERIALS

Mitochondrial isolation buffer:

	A	B
	HEPES (pH 7.4)	5 mM
	mannitol	250 mM
	EGTA	0.5 mM

make fresh on the day itself!

Oligomycin/Antimycin A cocktail:

	A	B
	Oligomycin	10 µM
	Antimycin A	4 µM

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:

	A	B
	Tris-HCl pH 8.0	50 mM
	NaCl	150 mM
	sodium deoxycholate	0.50%
	SDS	0.10%
	NP-40	1%

supplemented by cOmplete EDTA-free protease inhibitors (11836170001, Roche) and phosphatase inhibitors (PhosphoSTOP, 4906837001, Roche).

Oligomycin A **ApexBio Technology Catalog #A5588**

Antimycin A from Streptomyces sp. **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A8674**

Q-VD-OPh **ApexBio Technology Catalog #A1901**

cOmplete™, Mini, EDTA-free (Protease Inhibitor) **Roche Catalog ##11836170001)**

Roche PhosSTOP™ **Merck MilliporeSigma (Sigma-Aldrich) Catalog #4906837001**

Mitochondrial isolation

1h 15m

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 300 x g, 4°C, 00:10:00. 10m

2.1 Resuspend the cell pellet in 1 mL of ice-cold PBS (1x) and spin again for 300 x g, 4°C, 00:05:00. 5m

3 Remove the PBS and resuspend the cell pellet in 1 mL mitochondrial isolation buffer, which was cooled to 4 °C.

Note

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.

Note

This should lyse the plasma membrane but leave organelles intact.

5 Spin the suspension down at 600 x g, 4°C, 00:10:00. 10m

5.1 Keep the supernatant and centrifugation 600 x g, 4°C, 00:10:00. 10m

Note

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 600 x g, subject the supernatant to 7000 x g, 4°C, 00:10:00. 10m

6.1 The pellet contains the mitochondria, so remove the supernatant and resuspend the pellet in 1 mL mitochondrial isolation buffer.

6.2 Then, centrifugation 7000 x g, 4°C, 00:10:00. 10m

Note

The supernatant from the first spin at 7000 x g can be stored as a cytosolic fraction.

7 After two spins at 7000 x g, subject the resuspended pellet to 10000 x g, 4°C, 00:10:00. 10m

7.1 The pellet contains the mitochondria, so remove the supernatant and resuspend the pellet in 1 mL mitochondrial isolation buffer.

7.2 Then, centrifugation 10000 x g, 4°C, 00:10:00. 10m

8 After two spins at 10000 x 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.