

SEP 23, 2023

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dx.doi.org/10.17504/protocol s.io.kqdg3x4zzg25/v1

Protocol Citation: Elias Adriaenssens 2023. Mitochondrial isolation protocol. **protocols.io** https://dx.doi.org/10.17504/p rotocols.io.kqdg3x4zzg25/v1

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).

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

Created: Jun 26, 2023

Last Modified: Sep 23,

2023

PROTOCOL integer ID:

Keywords: Mitochondrial isolation, HeLa cells

Mitochondrial isolation protocol

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ABSTRACT

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

ATTACHMENTS

752-1920.pdf

MATERIALS

MATERIALS

Mitochondrial isolation buffer:

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

make fresh on the day itself!

Oligomycin/Antimycin A cocktail:

A	В
Oligomycin	10 μΜ
Antimycin A	4 μΜ

In case cells were treated for more than 8 hours, we also added 10 μ M Q-VD-OPh (A1901, ApexBio) to suppress apoptosis.

RIPA buffer:

A	В
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 (Phospho-STOP, 4906837001, Roche).

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

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

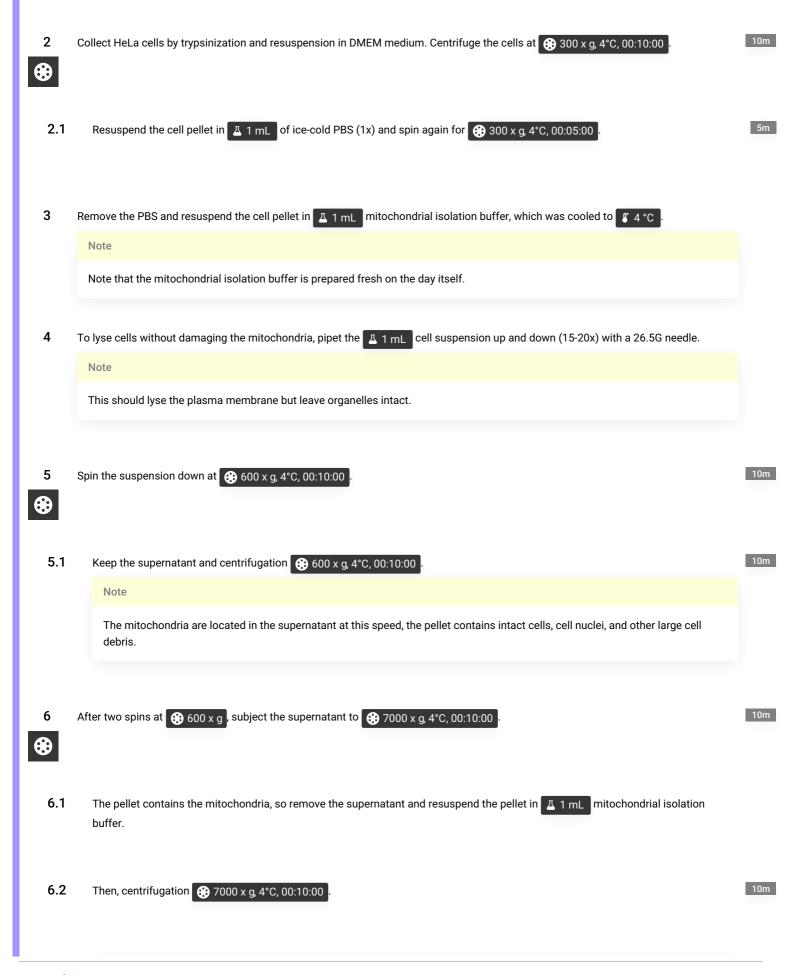
8 COmplete™, Mini, EDTA-free (Protease Inhibitor) Roche Catalog ##11836170001)

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.



Note

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

7 After two spins at 3 7000 x g , subject the resuspended pellet to 3 10000 x g, 4°C, 00:10:00



- **(*)**
 - 7.1 The pellet contains the mitochondria, so remove the supernatant and resuspend the pellet in L 1 mL mitochondrial isolation buffer.
 - 7.2 Then, centrifugation 10000 x g, 4°C, 00:10:00

- 10m
- 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.