Mammalian Mitochondria Isolation Kit for Cultured Cells

Catalogue No.:abx098856

Mammalian Mitochondria Isolation Kit for Cultured Cells provides a fast and efficient way to isolate mitochondria from cultured mammalian cells. This kit provides two options for the separation of mitochondria from cytosolic components: a reagent-based method or homogenization base method. Reagent-based method allows multiple samples to be processed concurrently with a mild procedure. The isolated mitochondria are suitable for a variety of downstream applications, including protein analysis, apoptosis, signal transduction and metabolic studies.

Kit contents:

Component	**50 rxns**
Mitochondria Isolation Buffer 1	50 ml
Mitochondria Isolation Buffer 2	500 µl
Mitochondria Isolation Buffer 3	65 ml
Mitochondria Storage Buffer	4 ml
100X EDTA-free Protease Inhibitor Cocktail	1.2 ml

Reagents required but not provided:

- Phosphate-Buffered Saline (PBS)
- Phenylmethylsulfonyl Fluoride (PMSF)
- Trypan Blue

Target: Mammalian Mitochondria Isolation Kit for Cultured Cells

Storage: Store the 100X EDTA-free Protease Inhibitor Cocktail and Mitochondria Storage Buffer at -20 °C, and the other reagents at 2-8 °C. Stable for 12 months from date of receipt.

Note: THIS PRODUCT IS FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC, THERAPEUTIC OR COSMETIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION.
This product is shipped with dry ice.

Abbexa LTD, Cambridge, UK · Phone: +44 (0) 1223 755950 · Fax: +44 (0) 1223 755951
Abbexa LLC, Houston, TX USA · Phone: +1 832 327 7413
Abbexa BV, Leiden, NL
Website: www.abbexa.com · Email: info@abbexa.com

Directions for use:

Notes:

• Before use, add Protease Inhibitor Cocktail and PMSF (not provided in this kit) to Mitochondria Isolation Buffers 1, 2 and 3.
• All steps should be carried out on ice or at 2-8 °C.
• It is recommended to use fresh cultured cells for mitochondria isolation if the isolated mitochondria will be used in functional assays.

Option A: Reagent Method

1. Harvest 2 × 10^7 cells, then wash the cells with 1 ml of cold PBS. Centrifuge at 1000 × g for 3 minutes. Discard the supernatant. Repeat the wash one more time.
2. Add 800 µl of Mitochondria Isolation Buffer 1 to the cell pellet. Vortex for 5 seconds, and allow to stand on ice for 2 minutes.
3. Add 10 µl of Mitochondria Isolation Buffer 2, then vortex for 5 seconds.
4. Allow to stand on ice for 5 minutes. Briefly vortex every minute.
5. Add 800 µl of Mirochondria Isolation Buffer 3. Invert the tube 5-6 times to mix (do not vortex).
6. Centrifuge at 700 × g at 2-8 °C for 10 minutes.
7. Gently transfer the supernatant to a new 2 ml microcentrifuge tube and centrifuge at 12,000 × g at 2-8 °C for 15 minutes. For higher purity, the supernatant can be centrifuged at 3000 × g for 15 minutes at 2-8 °C, but this may result in lower yield.
8. Carefully collect the supernatant (cytoplasmic protein). The isolated cytoplasmic proteins can be used for downstream applications or stored at -80 °C.
9. Add 500 µl of Mitochondria Isolation Buffer 3 and resuspend the pellet.
10. Centrifuge at 12,000 × g at 2-8 °C for 15 minutes.
11. Carefully discard the supernatant. The resulting pellet contains mitochondria, which can be stored at -80 °C or immediately processed:

- Protein analysis: The pellet can be dissolved and lysed with protein lysis buffer. Our Mammalian Total Protein Extraction Kit (abx098853) can be used for protein extraction. Mitochondria or mitochondrial lysate can be stored at -80 °C for future use.

- Functional analysis: Mitochondria Storage Buffer can be added at a ratio of 40 µl/10^7 cells. Analyze within one hour after resuspension.

Option B: Homogenization Method

1. Harvest 2 × 10^7 cells, then wash the cells with 1 ml of cold PBS. Centrifuge at 1000 × g for 3 minutes. Discard the supernatant. Repeat the wash one more time.
2. Add 800 µl of Mitochondria Isolation Buffer 1 to the cell pellet. Vortex for 5 seconds, and allow to stand on ice for 2 minutes.
3. Transfer the suspension to a glass homogenizer and homogenize the cells (30-50 strokes).

Note: To check cell lysis efficiency, stain the cells with Trypan Blue and view under a microscope. If more than 50% cells are stained, the homogenization process can be stopped. Under-homogenization may result in a lower mitochondrial yield, while over-homogenization may damage the mitochondria.

4. Transfer the supernatant to a new 2 ml microcentrifuge tube.
5. Add 800 µl of Mirochondria Isolation Buffer 3. Invert the tube 5-6 times to mix (do not vortex).
6. Centrifuge at 700 × g at 2-8 °C for 10 minutes.
7. Gently transfer the supernatant to a new 2 ml microcentrifuge tube and centrifuge at 12,000 × g at 2-8 °C for 15 minutes. For higher purity, the supernatant can be centrifuged at 3000 × g for 15 minutes at 2-8 °C, but this may result in lower yield.
8. Carefully collect the supernatant (cytoplasmic protein). The isolated cytoplasmic proteins can be used for downstream applications or stored at -80 °C.
9. Add 500 µl of Mitochondria Isolation Buffer 3 and resuspend the pellet.
10. Centrifuge at 12,000 × g at 2-8 °C for 15 minutes.
11. Carefully discard the supernatant. The resulting pellet contains mitochondria, which can be stored at -80 °C or immediately processed:

- Protein analysis: The pellet can be dissolved and lysed with protein lysis buffer. Our Mammalian Total Protein Extraction Kit (abx098853) can be used for protein extraction. Mitochondria or mitochondrial lysate can be stored at -80 °C for future use.

- Functional analysis: Mitochondria Storage Buffer can be added at a ratio of 40 µl/10^7 cells. Analyze within one hour after resuspension.

Abbexa LTD, Cambridge, UK · Phone: +44 (0) 1223 755950 · Fax: +44 (0) 1223 755951
Abbexa LLC, Houston, TX USA · Phone: +1 832 327 7413
Abbexa BV, Leiden, NL
Website: www.abbexa.com · Email: info@abbexa.com