
Mammalian Mitochondria Isolation Kit for Cultured Cells**Catalog No.:** abx098856**Revision date:** 10-Feb-20**Size:** 50 rxns**Storage:** Store the 100X EDTA-free Protease Inhibitor Cocktail and Mitochondria Storage Buffer at -20°C for up to one year. Store the other reagents at 2-8°C for up to one year.**Introduction**

Abbexa's Mammalian Mitochondria Isolation Kit for Cultured Cells is 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 components

1. Mitochondria Isolation Buffer 1 (MIB1): 50 ml
2. Mitochondria Isolation Buffer 2 (MIB2): 500 µl
3. Mitochondria Isolation Buffer 3 (MIB3): 65 ml
4. Mitochondria Storage Buffer (MSB): 4 ml
5. 100X EDTA-free Protease Inhibitor Cocktail: 1.2 ml

Material required but not provided

1. Phosphate-Buffered Saline (PBS)
2. Phenylmethanesulfonyl Fluoride (PMSF)
3. Trypan Blue

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.

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Procedure

A. Reagent Method

1. Harvest 2×10^7 cells, then wash the cells with 1 ml of cold PBS. Centrifuge at $1000 \times g$ for 3 minutes. Discard the supernatant. Repeat the wash one more time.
2. Add 800 μ l 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 μ l 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 μ l of Mitochondria Isolation Buffer 3. Invert the tube 5-6 times to mix (do not vortex).
6. Centrifuge at $700 \times g$ at 2-8 °C for 10 minutes.
7. Gently transfer the supernatant to a new 2 ml microcentrifuge tube and centrifuge at $12,000 \times g$ at 2-8 °C for 15 minutes. For higher purity, the supernatant can be centrifuged at $3000 \times g$ 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 μ l of Mitochondria Isolation Buffer 3 and resuspend the pellet.
10. Centrifuge at $12,000 \times g$ 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 μ l/ 10^7 cells. Analyze within one hour after resuspension.

B. Homogenisation Method

1. Harvest 2×10^7 cells, then wash the cells with 1 ml of cold PBS. Centrifuge at $1000 \times g$ for 3 minutes. Discard the supernatant. Repeat the wash one more time.
2. Add 800 μ l 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.

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4. Transfer the supernatant to a new 2 ml microcentrifuge tube.
5. Add 800 μ l of Mitochondria Isolation Buffer 3. Invert the tube 5-6 times to mix (do not vortex).
6. Centrifuge at 700 \times g at 2-8 $^{\circ}$ C for 10 minutes.
7. Gently transfer the supernatant to a new 2 ml microcentrifuge tube and centrifuge at 12,000 \times g at 2-8 $^{\circ}$ C for 15 minutes. For higher purity, the supernatant can be centrifuged at 3000 \times g for 15 minutes at 2-8 $^{\circ}$ 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 $^{\circ}$ C.
9. Add 500 μ l of Mitochondria Isolation Buffer 3 and resuspend the pellet.
10. Centrifuge at 12,000 \times g at 2-8 $^{\circ}$ C for 15 minutes.
11. Carefully discard the supernatant. The resulting pellet contains mitochondria, which can be stored at -80 $^{\circ}$ 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 $^{\circ}$ C for future use.
 - Functional analysis: Mitochondria Storage Buffer can be added at a ratio of 40 μ l/ 10^7 cells. Analyze within one hour after resuspension.