AllPure Mammalian Mitochondria Isolation Kit
(for Cultured Cells)

Cat. No. ABTGDE401

Storage:MSB at -20°C for one year, others at 2-8°C for one year

Description

AllPure Mammalian Mitochondria Isolation Kit for Cultured Cells provides a fast and efficient method 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-based method. Reagent-based method uses a mild procedure to process single or multiple samples. The isolated mitochondria is suitable for a variety of downstream applications, including protein analysis, apoptosis, signal transduction and metabolic assays.

Kit Contents

Component	50 rxns
Mitochondria Isolation Buffer I (MIB I)	50 ml
Mitochondria Isolation Buffer II (MIB II)	500 µl
Mitochondria Isolation Buffer III (MIB III)	65 ml
Mitochondria Storage Buffer (MSB) -20°C	4 ml
Protease Inhibitor Cocktail, EDTA-free (100X)	Not Provided

Prior to use, Proteinase Inhibitor Cocktail or PMSF (not provided in the kit) should be added into MIB I and II and III.

Procedures

Option A: Reagent-based Method

1. Harvest 2×10^7 cells and wash the cells with 1 ml of pre-chilled PBS. Centrifuge at 1,000×g for 3 minutes. Discard the supernatant. Repeat the wash once.
2. Add 800 µl of MIB I to cell pellet. Vortex for 5 seconds, and incubate on ice for 2 minutes.
3. Add 10 µl of MIB II. Vortex for 5 seconds.
4. Incubate on ice for 5 minutes. Briefly vortex every minute.
5. Add 800 µl of MIB III. Invert tube 5-6 times to mix (do not vortex).
6. Centrifuge at 700×g, 4°C for 10 minutes.
7. Gently transfer the supernatant to a new 2 ml microcentrifuge tube and centrifuge at 12,000×g, 4°C for 15 minutes (for higher purity, suggest to centrifuge the supernatant at 3000×g for 15 minutes at 4°C, but this may result in lower yield).
8. Gently 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 MIB III and resuspend the pellet.
10. Centrifuge at 12,000×g, 4°C for 15 minutes.
11. Gently discard the supernatant, the pellet is mitochondria, which can be stored at -80°C or processed as following.
12. (Option 1) For mitochondria will be used for protein analysis, the pellet can be dissolved and lysed with protein lysis buffer. Mitochondria or mitochondria lysate can be stored at -80°C for future use.
13. (Option 2) For mitochondria used for functional analysis, MSB can be added at the ratio ~40 µl/1×10^7 cells. Analyze within one hour after resuspension.

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Option B: Homogenization

1. Harvest 2×10^7 cells and wash the cells with 1 ml of pre-chilled PBS. Centrifuge at 1,000×g for 3 minutes. Discard the supernatant. Repeat the wash once.
2. Add 800 µl of MIB I to cell pellet. Vortex for 5 seconds, and incubate on ice for 2 minutes.
3. Transfer the suspension to a glass homogenizer and homogenize the cells by 30-50 strokes (to check the cell lysis efficiency, stain the cells with Trypan Blue and view under a microscope. If more than 50% cells are stained, homogenization can be stopped. Under homogenization may result in lower mitochondria yield. Over homogenization may damage mitochondria).
4. Transfer the supernatant to a new 2 ml microcentrifuge tube.
5. Following steps are the same as the steps 5-12 described in “Reagent-based Method”.

Notes

• All steps should be carried out on ice or at 4°C.

• Use fresh cultured cells for mitochondria isolation if the isolated mitochondria will be used for functional assays.