ProteinExt® Mammalian Mitochondria Isolation Kit for Cultured Cells

Cat. No. DE401

Storage: *ProteinSafe*™ Protease Inhibitor Cocktail, EDTA-free (100×) and MSB at -20°C for one year, others at 2-8°C for one year

Description

ProteinExt® 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	DE401-01 (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)	4 ml
ProteinSafe™ Protease Inhibitor Cocktail, EDTA-free (100×)	1.2 ml

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, 2-8°C for 10 minutes.
7. Gently transfer the supernatant to a new 2 ml microcentrifuge tube and centrifuge at 12,000×g, 2-8°C for 15 minutes (for higher purity, suggest to centrifuge the supernatant at 3000×g for 15 minutes at 2-8°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, 2-8°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. We recommend to use TransGen *ProteinExt*® Mammalian Total Protein Extraction Kit, Cat. No. DE101. 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.

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

• Prior to use, Proteinase Inhibitor Cocktail and PMSF (not provided in the kit) should be added into MIB I and II and III.
• All steps should be carried out on ice or at 2-8°C.
• Use fresh cultured cells for mitochondria isolation if the isolated mitochondria will be used for functional assays.

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