

# ProteinExt<sup>®</sup> Mammalian Mitochondria Isolation Kit for Cultured Cells

Cat. No. DE401

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

## Description

ProteinExt<sup>®</sup> 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 <sup>™</sup> Protease Inhibitor Cocktail, EDTA-free (100×)	1.2 ml

## Procedures

### Option A: Reagent-based Method

1. Harvest  $2 \times 10^7$  cells and wash the cells with 1 ml of pre-chilled PBS. Centrifuge at  $1,000 \times g$  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  $700 \times g$ , 2-8°C for 10 minutes.
7. Gently transfer the supernatant to a new 2 ml microcentrifuge tube and centrifuge at  $12,000 \times g$ , 2-8°C for 15 minutes (for higher purity, suggest to centrifuge the supernatant at  $3000 \times g$  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  $12,000 \times g$ , 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<sup>®</sup> 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  $\sim 40 \mu\text{l}/1 \times 10^7$  cells. Analyze within one hour after resuspension.



#### Option B: Homogenization

1. Harvest  $2 \times 10^7$  cells and wash the cells with 1 ml of pre-chilled PBS. Centrifuge at  $1,000 \times g$  for 3 minutes. Discard the supernatant. Repeat the wash once.
2. Add 800  $\mu$ l 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.

**For research use only, not for clinical diagnosis.**

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