ProteinExt® Mammalian Mitochondria Isolation Kit for Tissue

Cat. No. DE501

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 Tissue provides a fast and efficient isolation of mitochondria from tissues with simple procedure. 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	DE501-01 (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
Bovine Serum Albumin (BSA)	500 mg
ProteinSafe™ Protease Inhibitor Cocktail, EDTA-free (100×)	1.2 ml

Isolation of Mitochondria from Soft Tissues

Option A: Reagent-based Method

1. Wash 50-200 mg of tissue with 1 ml of pre-chilled PBS, cut tissue into small pieces.
2. Transfer the minced tissue to a glass homogenizer and homogenize the tissues (3-5 strokes avoid over homogenization).
3. Resuspend the pellet in 2 ml of PBS. Centrifuge at 1,000×g for 3 minutes and gently discard the supernatant.
4. Add 800 µl of MIB I/BSA, vortex for 5 seconds and incubate on ice for 2 minutes.
5. Add 10 µl of MIB II to the pellet. Vortex for 5 seconds.
6. Incubate on ice for 5 minutes. Briefly vortex every minute.
7. Add 800 µl of MIB III. Invert tube 5-6 times to mix (do not vortex).
8. Centrifuge at 700×g, 2-8°C for 10 minutes.
9. 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).
10. Gently collect the supernatant (cytoplasmic protein). The The isolated cytoplasmic proteins can be used for downstream applications or stored at -80°C.
11. Add 500 µl of MIB III to the pellet and resuspend it by vortexing.
12. Centrifuge at 12,000×g, 2-8°C for 15 minutes.
13. Gently discard the supernatant, the pellet is mitochondria, which can be stored at -80°C or processed as following.
14. (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 lysate can be stored at -80°C for future use.
15. (Option 2) For mitochondria will be 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. Same as Step 1 in “Reagent-based Method” for soft tissue.
2. Same as Step 2 in “Reagent-based Method” for soft tissue.
3. Same as Step 3 in “Reagent-based Method” for soft tissue
4. Add 1 ml of MIB I/BSA, vortex for 5 seconds and incubate on ice for 2 minutes.
5. Transfer the suspension to a glass homogenizer and homogenize the cells by 30-50 strokes (note: to check the cell lysis efficiency, stain the cells with Trypan Blue and view under a microscope. When more than 50% cells are stained, homogenization can be stopped. Under homogenization may result in lower mitochondria yield. Over homogenization may damage mitochondria).
6. Transfer the supernatant to a new 2 ml microcentrifuge tube.
7. Following steps are the same as the steps 7-15 described in “Reagent-based Method” for soft tissue.

Isolation of Mitochondria from Hard Tissues

Option A: Reagent-based Method

1. Wash 50-200 mg of tissue with 2-4 ml of pre-chilled PBS, gently discard the PBS and cut tissue into small pieces.
2. (Optional) For trypsin pre-treatment, incubate tissue in 750 µl of trypsin (0.25%) on ice for 3 minutes. Centrifuge at 1,000×g for 3 minutes, gently discard the supernatant.
3. Add 750 µl of PBS/BSA (PBS with 4 mg/ml BSA), transfer the tissue to a glass homogenizer and homogenize the tissues (3-5 strokes, avoid over homogenization). Centrifuge at 1,000×g for 3 minutes, gently discard the supernatant.
4. Following steps are the same as the steps 4-15 described in “Reagent-based Method” for soft tissue.

Option B: Homogenization

1. Same as Step 1 in “Reagent-based Method” for hard tissue.
2. (Optional) Same as Step 2 in “Reagent-based Method” for hard tissue.
3. Add 750 µl of PBS/BSA (PBS with 4 mg/ml BSA), mix thoroughly. Centrifuge at 1,000×g for 3 minutes, gently discard the supernatant.
4. Add 1 ml of MIB I/BSA (MIB I with 4 mg/ml BSA), vortex for 5 seconds and incubate on ice for 2 minutes.
5. Transfer the suspension to a glass homogenizer and homogenize the cells by 30-50 strokes (note: to check the cell lysis efficiency, stain the cells with Trypan Blue and view under a microscope. When more than 50% cells are stained, homogenization can be stopped. Under homogenization may result in lower mitochondria yield. Over homogenization may damage mitochondria.)
6. Transfer the supernatant to a new 2 ml microcentrifuge tube.
7. Following steps are the same as the steps 7-15 described in “Reagent-based Method” for soft tissue.

Notes

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

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