

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

Cat. No. DE501

**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 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 <sup>™</sup> 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 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<sup>®</sup> 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<sup>7</sup> 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  $\mu$ l of trypsin (0.25%) on ice for 3 minutes. Centrifuge at 1,000 $\times$ g for 3 minutes, gently discard the supernatant.
3. Add 750  $\mu$ l 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 $\times$ 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  $\mu$ l of PBS/BSA (PBS with 4 mg/ml BSA), mix thoroughly. Centrifuge at 1,000 $\times$ 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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