# *ProteinExt*™ Mammalian Membrane Protein Extraction Kit

Cat. No. DE301

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

## Description

*ProteinExt*™ Mammalian Membrane Extraction Kit provides a simple and efficient method to extract membrane proteins from mammalian cells and tissues. Native proteins can be obtained within 70 minutes without ultracentrifugation. Up to 90% efficiency for membrane proteins have at least 1-2 transmembrane domains. The extracted protein is suitable for a variety of downstream applications, including SDS-PAGE, Western Blot, ELISA, and enzyme-activity assays.

## Kit Contents

| Component | DE301-01 (50 rxns) |
|---|---|
| Membrane Protein Extraction Buffer I (MPEB I) | 50 ml |
| Membrane Protein Extraction Buffer II (MPEB II) | 7.5 ml |
| Membrane Protein Extraction Buffer III (MPEB III) | 15 ml |
| *ProteinSafe*™ Protease Inhibitor Cocktail, EDTA-free (100×) | 1 ml |

## Procedures

**a. Cultured Cells**

1. Harvest 0.5-1×$10^7$ 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 750 µl of MPEB I to cell pellet. Mix thoroughly by vortexing for 15 seconds. Incubate on ice for 10 minutes with vortexing at every 2 minutes.
3. Centrifuge at 16,000×g, 4°C for 15 minutes.
4. Gently transfer the supernatant (cytoplasmic protein) to a new 1.5 ml microcentrifuge tube. The isolated cytoplasmic proteins can be used for downstream applications or stored at -80°C.
5. Add 150 µl of MPEB II to the pellet and resuspend the pellet by vortexing for 15 seconds. Incubate on ice for 30 minutes and briefly vortex at every 5 minutes.
6. Add 300 µl of MPEB III to the pellet and vortexing for 5 seconds.
7. Centrifuge at 16,000×g, 4°C for 15 minutes.
8. Gently collect the supernatant (membrane proteins). The isolated membrane proteins can be used for downstream applications or stored at -80°C.

**b. Tissues**

1. Wash 20-60 mg of tissues with 2 ml of pre-chilled PBS and vortex briefly, gently discard the supernatant.
2. Add 1 ml of PBS. Cut 20-60 mg of tissues into small pieces. Centrifuge at 500×g for 3 minutes, gently discard the supernatant
3. Add 1 ml of MPEB I to the tissues and vortex thoroughly. Transfer the suspension to a pre-chilled glass homogenizer and homogenize the tissue normally by 6-10 strokes.
4. Incubate on ice for 10 minutes with vortexing at every 2 minutes.
5. Following steps are the same as the steps 3-8 described in “Cultured Cells” section.

Notes

• Prior to use, Proteinase Inhibitor Cocktail and PMSF (not provided in the kit) should be added into MPEB I and II and III.
• All steps should be carried out on ice or at 4°C.
• If protein quantification is needed, we suggest to use BCA method (*Easy* II Protein Quantitative Kit (BCA), Cat. No DQ111).

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