Mammalian Membrane Protein Extraction Kit

Catalog No.: abx098855

Size: 50 rxns

Storage: Store the 100X EDTA-free Protease Inhibitor Cocktail at -20°C and the Membrane Protein Extraction Buffers at 2-8°C for up to one year.

Introduction: Abbexa's Mammalian Total Protein Extraction Kit is a simple and efficient way to extract membrane proteins from mammalian cells and tissues without ultracentrifugation in 70 minutes, with up to 90% efficiency for membrane proteins with 1-2 transmembrane domains. The extracted proteins can be analyzed by SDS-PAGE, ELISA, WB and other functional assays.

Kit components

1. Membrane Protein Extraction Buffer 1 (MPEB1): 50 ml
2. Membrane Protein Extraction Buffer 2 (MPEB2): 7.5 ml
3. Membrane Protein Extraction Buffer 3 (MPEB3): 15 ml
4. 100X EDTA-free Protease Inhibitor Cocktail: 1 ml

Materials Required But Not Provided

1. Phosphate-Buffered Saline (PBS)
2. Phenylmethanesulfonyl Fluoride (PMSF)
3. Distilled water
4. High-precision pipette and sterile pipette tips
5. Centrifuge and centrifuge tubes
6. Timer
7. Ice
8. Vortexer
9. Homogenizer

Notes

- Before use, add Protease Inhibitor Cocktail and PMSF (not provided in this kit) to Membrane Protein Extraction Buffers 1, 2 and 3 (MPEB1, MPEB2 and MPEB3).
- All steps should be carried out on ice or at 2-8 °C.
- A BCA assay is recommended for protein quantification.

Procedure

A. Cultured cells

1. Harvest 0.5-1 × 10^7 cells. Wash the cells with 1 ml of pre-chilled PBS and centrifuge at 1000 × g for 3 minutes. Carefully discard the supernatant and repeat the wash one more time.
2. Add 750 µl of MPEB1 to the cell pellet. Mix thoroughly by vortexing for 15 seconds, and allow to stand on ice for 10 minutes, vortexing every 2 minutes.
3. Centrifuge at 16,000 × g at 2-8 °C for 15 minutes.
4. Carefully transfer the supernatant (which contains cytoplasmic proteins) to a new 1.5 ml microcentrifuge tube. The isolated cytoplasmic proteins can be analyzed immediately or stored at -80°C.
5. Add 150 µl of MPEB2 to the cell pellet. Resuspend the pellet by vortexing for 15 seconds, and allow to stand on ice for 30

minutes, vortexing every 5 minutes.

6. Centrifuge at 16,000 × g at 2-8 °C for 15 minutes.
7. Carefully transfer the supernatant (which contains membrane proteins) to a new 1.5 ml microcentrifuge tube. The isolated cytoplasmic proteins can be analyzed immediately or stored at -80°C.

B. Tissues

1. Wash 20-60 mg of tissue with 2 ml of pre-chilled PBS and vortex briefly. Carefully discard the supernatant.
2. Add 1 ml of PBS. Cut and mince the tissue into small pieces. Centrifuge at 5,000 × g for 3 minutes, then carefully discard the supernatant.
3. Add 750 µl of MPEB1 to the tissue. Mix thoroughly by vortexing. Transfer the suspension to a pre-chilled glass homogenizer and homogenize the tissue with 6-10 strokes.
4. Allow to stand on ice for 10 minutes, vortexing every 2 minutes.
5. Centrifuge at 16,000 × g at 2-8 °C for 15 minutes.
6. Carefully transfer the supernatant (which contains cytoplasmic proteins) to a new 1.5 ml microcentrifuge tube. The isolated cytoplasmic proteins can be analyzed immediately or stored at -80°C.
7. Add 150 µl of MPEB2 to the cell pellet. Resuspend the pellet by vortexing for 15 seconds, and allow to stand on ice for 30 minutes, vortexing every 5 minutes.
8. Centrifuge at 16,000 × g at 2-8 °C for 15 minutes.
9. Carefully transfer the supernatant (which contains membrane proteins) to a new 1.5 ml microcentrifuge tube. The isolated cytoplasmic proteins can be analyzed immediately or stored at -80°C.