Product Information

Membrane Protein Extraction Kit

Catalog Number: MeP-02

Description:

This kit is designed for extracting the native, non-denatured membrane proteins from various cell types or tissues, prepared for EMSA, ELISA, 1D and 2D electrophoresis, Western blotting, TF-TF interaction arrays and other protein/DNA assays.

Kit contains:

Components Quantity (50 extracts) Storage
Cytoplasmic Lysis Buffer (C207020, blue sticker) 25.0 mL 2-8°C
Cytoplasmic Washing Buffer (C207030 purple sticker) 5.0 mL 2-8°C
Nuclear Lysis Buffer (N207040, green sticker) 2.5 mL 2-8°C
Membrane Lysis Buffer (M207060, yellow sticker) 5.0 mL 2-8°C
DTT, 1M (Dissolved in 0.1 ml ddH_2O) 1 vial –20°C
Protease/Phosphatase Inhibitors (I208052) 1 vial –20°C supplied in DMSO, contains optimized AEBSF, Aprotinin, E64, Leupeptin, Pepstatin A, Sodium fluoride, Sodium orthovanadate and Sodium pyrophosphate.

Protocol: (Keep all buffers and cell/tissue samples on ice)

*Prepare working reagents prior to proceeding.

For 10 Extractions: (50-100mg tissues or 10e7 cells/Extraction)
Cytoplasmic Lysis Buffer (5ml) add 5.0ul (1M DTT) and 100ul Protease/phosphatase Inhibitors (I208052)
Cytoplasmic Washing Buffer (1ml) add 1.0ul (1M DTT) and 20ul Protease/phosphatase Inhibitors (I208052)
Nuclear Lysis Buffer (0.5ml) add 1.0ul (1M DTT) and 10ul Protease/phosphatase Inhibitors (I208052)
Membrane Lysis Buffer (1ml) add 1.0ul (1M DTT) and 20ul Protease/phosphatase Inhibitors (I208052)

1. **Preparation of samples from culturing cells or tissues:**

- Harvest cells (1x 10e7 cells) as usual and wash cells once with 1.0ml 1x ice-cold PBS/DPBS, centrifuge at 1,600 rpm for 8 minutes, aspirate liquids. Add 500ul cytoplasmic lysis buffer to resuspend cell pellet. Gently pipette up and down several times and incubate on ice for 10 minutes.
- Weigh 50-100mg frozen/ fresh tissues and chop tissues into small pieces using a clean razor blade. Immediately transfer into a 2.0ml microcentrifuge tube contained 500ul cytoplasmic lysis buffer. Vortex at mid-speed for 20 seconds and incubate on ice for 10 minutes.

2. Homogenization:

- Using a clean pre-chilled Teflon pestle homogenizer to homogenize the cells/tissues for 10-20 strokes on ice, simply spin down the cells/tissue suspension and continue to homogenize cells/tissues another 10-20 strokes.
- (**Alternative-1**): Prepare a syringe with a needle gauged between 23 and 25. Pass cells/tissues through needle about 20 times to disrupt the cell membrane and release the intact nuclei and organelles.
- (**Alternative-2**): Using a pre-chilled, clean Dounce homogenizer to homogenize the cells/tissues

twice at speed 4 (moderate) speed for 20 seconds.

3. Centrifuge the homogenate at 3000 rpm for 10 minutes at 4°C. Keep the pellet on ice, prepared for nuclear membrane protein extraction at **step7**.
4. Transfer the supernatant (containing the cytosolic, plasma membranes) to a new 1.5ml microcentrifuge. Centrifuge at 14,000xg for 30 seconds at 4°C. Aspirate liquids.
5. Add 50ul membrane lysis buffer and incubate on ice for 15 minutes, Vortex vigorously 10 seconds every 5 minutes.
6. Centrifuge at 14,000 xg for 10 minutes at 4°C. Transfer the supernatant to a new 1.5ml microcentrifuge tube and keep on ice. This is the plasma membrane protein fractions.

7. Resuspend the pellet from **step 3** with 50ul nuclear lysis buffer and vortex vigorously for 10 seconds. Incubate the suspension for 10 minutes on ice (vortex 10 seconds every 5 minutes).
8. Centrifuge at 14000 xg for 30 seconds at 4°C. Aspirate liquids.
9. Add 100ul cytoplasmic washing buffer to resuspend the pellet. Centrifuge at 14,000 xg for 30 seconds at 4°C. Aspirate liquids. (The remained nuclear fractions were washed out).
10. Resuspend the pellet in 50ul membrane lysis buffer and incubate suspension for 20 minutes on ice (vortex 10 seconds every 5 minutes).
11. Centrifuge at 14000 xg for 15 minutes at 4°C. Transfer the supernatant to a new 1.5ml microcentrifuge tube. This is the nuclear membrane protein fractions.
12. Combine nuclear membrane proteins from **step 11** and plasma membrane proteins from **step 6** to obtain total membrane protein fractions if need.
13. Determine the protein concentrations of plasma membrane and nuclear membrane by Bradford or BCA Assay. Stored all proteins at -80°C.

Flow Chart of Protein Extraction:

Cells (10e7)/ Tissues (50mg)
Add cytoplasmic lysis buffer (500ul)
Homogenization, spin 10 minutes
Supernatant----------Pellet
(Cytoplasmic proteins & membranes) (Nuclei)

Add nuclear lysis buffer (50ul) Add washing buffer (100ul)
Spin, 30 seconds

Supernatant -------- Pellet Supernatant -------- Pellet (**Cytoplasmic proteins)** (Plasma membranes)
(Nuclear proteins) (Nuclear membranes)

Add membrane lysis buffer (50ul) Incubate 15 minutes Spin, 10 minutes

Supernatant Supernatant **(Plasma membrane proteins) (Nuclear membrane proteins)**

Additional information:
• The nuclear protein markers: Lamin B (68kDa), LaminA/C (70 KDa), HDAC, Histone H1 (33KDa), Histone H4(43KDa); • The cytoplasmic protein markers: GAPDH, anti-b-actin;
• The membrane protein markers: EGFR, Na+/K+ ATPase, anti-Sp1;
• The cytoskeleton protein markers: Vimentin.
• The lysosome protein markers: LAMP1/2/3. Capthepsin D.
• The peroxisome protein markers: PMP70.
• The protease/phosphatase Inhibitors (I208052) supplied in DMSO, contains optimized AEBSF, Aprotinin, E64, Leupeptin, Pepstatin A, Sodium fluoride, Sodium Orthovanadate and Sodium pyrophosphate.