Product Information

Cytoplasmic, Membrane and Nuclear Protein Extraction Kit

Catalog Number: MCN-003

Description:
This kit is designed for extracting intact nuclear proteins, membrane proteins and native, non-denatured cytoplasmic 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)	25.0 mL	2-8°C
Nuclear Lysis Buffer (N207040, green sticker)	2.5 mL	2-8°C
Membrane Lysis Buffer (M207060, yellow sticker)	4.0 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 (5ml)	add 3.0ul (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 (0.8ml)	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.

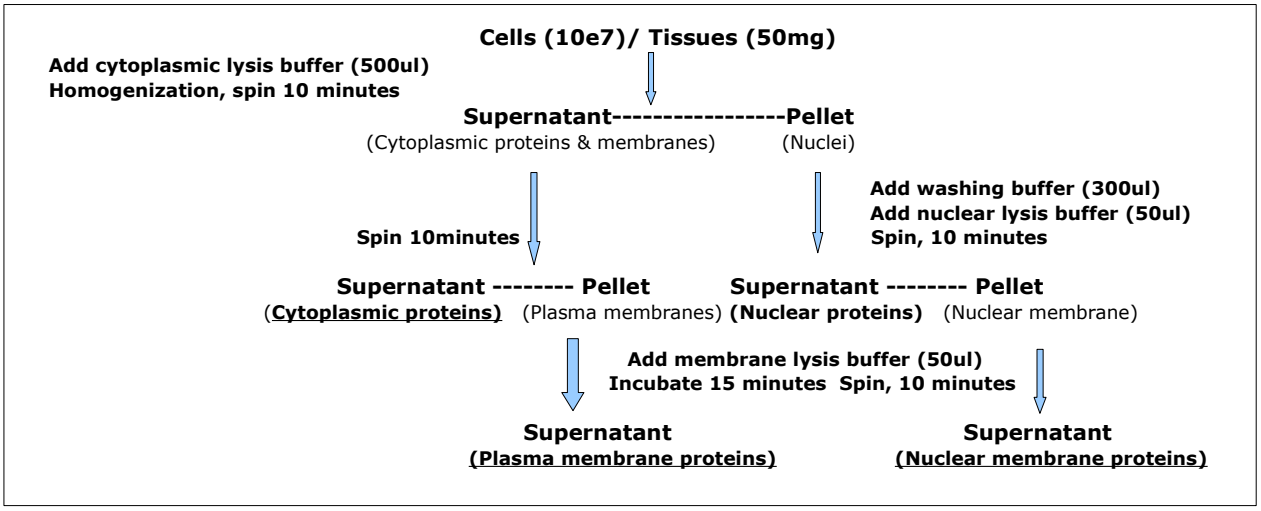
2. **Homogenization:**

- 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 homogenization at 300xg for 10 minutes at 4°C. Transfer the supernatant to a new 1.5ml microcentrifuge tube. Discard the pellet.
4. Centrifuge the homogenization at 1,000xg for 10 minutes at 4°C. Transfer the supernatant (containing the cytoplasmic proteins and plasma membranes) to a new 1.5ml microcentrifuge tube. Keep the pellet on ice for nuclear protein extraction.
5. Centrifuge the supernatant at 14,000xg for 10 minutes at 4°C. Transfer the supernatant (**the cytoplasmic protein fractions)** to a clean pre-chilled 1.5ml microcentrifuge tube. Keep the pellet on ice for plasma membrane protein extraction.

6. Add 50ul of membrane lysis buffer to the pellet from **step 5** and incubate on ice for 15 minutes. Vortex at highest speed for 10 seconds every 5 minutes.
7. Centrifuge at 14,000 xg for 10 minutes at 4°C. Transfer the supernatant to a new 1.5ml microcentrifuge tube. This is **the plasma membrane protein fractions**.
8. Add 300ul cytoplasmic washing buffer to resuspend the pellet from **step 4.** Centrifuge at 14,000 xg for 30 seconds at 4°C. Aspirate liquids. (The remained cytoplasmic fractions were washed out).
9. Resuspend the pellet in 50ul nuclear lysis buffer and vortex vigorously for 10 seconds. Incubate the suspension for 15 minutes on ice (vortex 10 seconds every 5 minutes).
10. Centrifuge at 14000 xg for 10 minutes at 4°C. Transfer the supernatant (**the nuclear protein fractions**) to a clean pre-chilled 1.5ml microcentrifuge tube.
    Note, stop here if not interest the nuclear membrane protein fractions.
11. Add 200ul 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).
12. Resuspend the pellet in 30ul membrane lysis buffer and incubate the suspension for 15 minutes on ice (vortex 10 seconds every 5 minutes).
13. Centrifuge at 14,000 xg for 10 minutes at 4°C. Transfer the supernatant to a new 1.5ml microcentrifuge tube. This is **the nuclear membrane protein fractions.**
14. Combine the nuclear membrane proteins form **step 13** and the plasma membrane proteins from **step 7** to obtain the total membrane protein fractions if need.
15. Determine the protein concentration of cytoplasmic, membrane and nuclear with spectrometers or by BCA Assay. Store all the extracts aliquots at -80°C.

Flow Chart of Protein Extraction:



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 Zmtech protease/phosphatase Inhibitors (I208052) supplied in DMSO, contains optimized AEBSF, Aprotinin, E64, Leupeptin, Pepstatin A, Sodium fluoride, Sodium Orthovanadate and Sodium pyrophosphate.