Product Information

Cytoplasmic, Mitochondrial and Nuclear Protein Extraction Kit

Catalog Number: V**MCN-002**

Description:
This kit is designed for extracting intact nuclear proteins, mitochondrial 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 extractions) Storage
Cytoplasmic Lysis Buffer (C207020, Blue sticker) 25.0 mL 2-8°C
Cytoplasmic Washing Buffer (C207030 Purple sticker) 15.0 mL 2-8°C
Mitochondrial Lysis Buffer (M207060, Yellow cap) 1.5 mL 2-8°C
Nuclear Lysis Buffer (N207040, Green sticker) 2.5 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: (10e7 cells or 50 mg tissues/Extraction)
Cytoplasmic Lysis Buffer (5ml) add 5.0ul (1M DTT) and 100ul Protease/phosphatase Inhibitors (I208052)
Cytoplasmic Washing Buffer (3.0ml) add 2.0ul (1M DTT) and 30ul Protease/phosphatase Inhibitors (I208052)
Mitochondria Lysis Buffer (0.3ml) add 0.5ul (1M DTT) and 20ul Protease/phosphatase Inhibitors (I208052)
Nuclear Lysis Buffer (0.5ml) add 0.5ul (1M DTT) and 20ul Protease/phosphatase Inhibitors (I208052)

1. **Preparation of samples from culturing/frozen cells:**

- 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.

Preparation of samples from tissues:

- Weigh 50mg 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:

1) Using a clean pre-chilled Teflon pestle homogenizer to homogenize the tissues for 10-20 strokes on ice, simply spin down the cells/tissue suspension and continue to homogenize tissues another 10-20 strokes.

2) (**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.

3) (**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 2500 rpm for 20 minutes at 4°C. Transfer the supernatant (containing the cytosolic proteins and intact mitochondria organelles) to a clean pre-chilled 1.5ml microcentrifuge tube. Keep the pellet on ice for nuclear proteins extraction.
4. Centrifuge the supernatant at 3000 rpm for 8 minutes at 4°C to remove the remained cellular debris. Discard the pellet.
5. Transfer the supernatant to a new 1.5ml pre-chilled tube and centrifuge at 14,000 xg for 15 minutes at 4°C to separate the soluble cytoplasmic proteins and the mitochondria pellet.

6. Immediately transfer the supernatant (**cytoplasmic/cytosolic protein fractions**) into a clean prechilled microcentrifuge tube and place the tube on ice. This is cytosolic protein fractions.
7. Add 30ul of mitochondrial lysis buffer (yellow cap) to resuspend the mitochondria pellet and incubate on ice for 15 minutes, vortex vigorously at highest speed for 10 seconds every 5 minutes. This is the mitochondrial protein fractions.
8. Add 300ul of cytoplasmic washing buffer to resuspend the nuclear pellet from **step 3.** 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 of nuclear lysis buffer and vortex vigorously for 10 seconds. Incubate the suspension for 30 minutes on ice (vortex 10 seconds every 10 minutes).
10. Centrifuge at 14000xg for 10 minutes at 4°C. Transfer the supernatant (**nuclear protein fractions)** to a clean pre-chilled microcentrifuge tube.
11. Determine the protein concentrations of cytoplasmic (cytosolic), mitochondrial and nuclear with spectrometers, by Bradford or by BCA Assay. Store all the extracts aliquots at -80°C.

Flow Chart of Protein Extraction:

Cells (10e7)/ Tissues (50mg)

Add cytoplasmic lysis buffer (500ul)
Homogenization, spin, 20 minutes

Supernatant----------Pellet
(Cytoplasmic proteins and mitochondria) (Nuclei)

Spin, 15 minutes washing buffer (300ul)

spin, 30 seconds
Supernatant----------Pellet Pellet
(Cytoplasmic proteins) (mitochondria) (Nuclei)

Add mitochondria lysis buffer (30ul) Add nuclear lysis buffer (50ul) Incubate, 15 minutes Incubate 30 minutes, spin 10 minutes
Suspension Supernatant
(Mitochondria proteins) (Nuclear proteins)

Additional information:
- The nuclear protein markers: Lamin B (68kDa), LaminA/C (70 KDa), HDAC, Histone H1 (33KDa), Histon 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.