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Product Information

Cytoplasmic, Cytoskeleton and Nuclear Protein Extraction Kit

Catalog Number: V**NCS-001**

Description:
This kit is designed for extracting intact nuclear proteins, cytoskeleton proteins and native, non-denatured cytoplasmic proteins from various cell types or tissues, prepared for EMSA, DNA footprinting, 1D and 2D electrophoresis, Western blotting, TF-TF interaction arrays and other protein/DNA assays.

Kit contains:

Component Quantity (50 extractions) Storage
Cytoplasmic Lysis Buffer (C207020, blue sticker) 25.0 mL 2-8°C
Cytoplasmic Washing Buffer (C207030, purple sticker) 30.0 mL 2-8°C
Cytoskeleton Lysis Buffer-1 (S207060, clear cap) 2.0 mL 2-8°C Cytoskeleton Lysis Buffer-2 (S207060, blue cap) 2.0 mL 2-8°C
Nuclear Lysis Buffer (N207040, green sticker) 2.5 mL 2-8°C
DTT, 1M (Dissolved in 0.5 ml ddH_2O) 1 vial –20°C
Protease/Phosphatase Inhibitors (I208052) 2 vials –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 samples on ice)

Prepare working reagents prior to proceeding

For 10 Extractions: (50mg tissues/Extraction) **add 1M DTT** and **Protease/phosphatase Inhibitors (I208052)**
Cytoplasmic Lysis Buffer (5ml) 5.0ul 100ul
Cytoplasmic Washing Buffer (6ml) 6.0ul 30ul
Nuclear Lysis Buffer (0.5ml) 0.5ul 10ul
Cytoskeleton Lysis Buffer-1 (0.4ml) 0.4ul 10ul Cytoskeleton Lysis Buffer-2 (0.4ml) 0.4ul 10ul

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 at 10,000 xg for 10 minutes at 4°C and transfer the supernatant (**cytoplasmic protein fractions**) into a clean pre-chilled microcentrifuge tube.

4. Add 300ul cytoplasmic washing buffer to resuspend the pellet. Centrifuge at 14,000 xg for 1 minute at 4°C. Aspirate liquids. (The remained cytoplasmic protein fractions were washed out).

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5. 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).

6. Centrifuge at 14000xg for 2 minutes at 4°C. Transfer the supernatant (**nuclear protein fractions**) to a clean pre-chilled microcentrifuge tube and place the tube on ice.

7. Add 300ul cytoplasmic washing buffer to resuspend the pellet. Centrifuge at 14,000 xg for 1 minute at 4°C. Aspirate liquids. (The remained nuclear protein fractions were washed out).

8. Resuspend the pellet in 40ul cytoskeleton lysis buffer-1 (Pre-warm the cytoskeleton lysis buffer-1 at room temperature for 10 minutes prior to use) and vortex vigorously for 10 seconds. Incubate suspension for 10 minutes at room temperature (vortex 10 seconds every 5 minutes).

9. Centrifuge at 14000 xg for 5 minutes at 4°C. Transfer the supernatant (**cytoskeleton protein fractions**) to a clean pre-chilled microcentrifuge tube.

10. Resuspend the pellet in 40ul cytoskeleton lysis buffer-2 and vortex vigorously for 10 seconds. Incubate the suspension for 15 minutes on ice (vortex 10 seconds every 5 minutes).

11. Centrifuge at 14000 xg for 10 minutes at 4°C. Transfer the supernatant (**cytoskeleton protein fractions**) to a clean pre-chilled microcentrifuge tube. Combine the supernatant from **step 9** and from **step 11.** The cytoskeleton proteins are in the mixed solution.

12. Determine the protein concentrations of cytoplasm, cytoskeleton 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) **Washing buffer (300ul) Spin, 1 minute Add nuclear lysis buffer (50ul), spin**

Pellet----------Supernatant (Nuclear proteins) **Washing buffer (300ul), spin, 1 minute**
Add cytoskeleton lysis buffer-1 (40ul)
Add cytoskeleton lysis buffer-2 (40ul)
Incubate 15 minutes, spin 10 minutes Supernatant
(Cytoskeleton 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.