Product Information

Nuclear Protein Extraction Kit (Catalog Number: NP-002)

Description:
This kit is designed for rapidly extracting intact, non-denatured nuclear protein fragments from various cell types or tissues without freeze/thaw cycles or sonication, 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 extracts)	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**
Detergents (D207050, yellow cap)	**1.5 mL**	**2-8°C**
Nuclear Lysis Buffer (N207040, green sticker)	**2.5 mL**	**2-8°C**
DTT, 1M (Dissolved in ddH 2 O)	**1 vial**	**–20°C**
Protease/Phosphatase Inhibitors (I208052) supplied in DMSO, contains optimized AEBSF, Aprotinin, E64, Leupeptin, Pepstatin A, Sodium fluoride, Sodium orthovanadate and Sodium pyrophosphate.	**1 vial**	**–20°C**

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

Prepare working reagents by adding DTT and Protease inhibitors into the following buffers prior to proceeding.

For 10 Extractions: (50mg cells/Extraction)	**add 1M DTT** and **Protease/phosphatase Inhibitors (I208052)**	
Cytoplasmic Lysis Buffer (5ml)	5.0ul	100ul
Cytoplasmic Washing Buffer (3ml)	3.0ul	30ul
Nuclear Lysis Buffer (0.5ml)	0.5ul	10ul

1. Add 500ul ice-cold cytoplasmic lysis buffer into a clean 1.5ml microcentrifuge tube containing 50mg cells (approximately 50ul packed cells or 1x 10e7 cells) or 100mg frozen/ fresh tissues. Gently pipette up and down several times and incubate on ice for 10 minutes.

   Note 1. For preparation of cells, harvest cells from culturing wells/plates/flasks as usual and wash cells once with 1x ice-cold PBS/DPBS, centrifuge at 1,600 rpm for 8 minutes, aspirate liquids. For tissue preparation, chop tissues into small pieces using a clean razor blade. This step may help to completely lysate cells/tissues.
   Note 2. The buffer volumes used in all steps need to coincide with the weight of tissue/cell preparation.

2. Homogenization (**Optional**): using a pre-chilled, clean Dounce homogenizer to homogenize the tissues twice at speed 4 (moderate) speed for 20 seconds. **Or** using the pre-chilled Teflon pestle homogenizer to homogenize the tissues/cells 15-20 strokes on ice. **Or** using a syringe with a needle gauges between 25 and 28. Pass cells/tissues through needle about 30-50 times to disrupt the cell membrane and release the intact nuclei.

3. Add 30ul of detergents, vortex vigorously at highest speed for 10 seconds.
   Centrifuge at 12,000 xg for 30 seconds at 4°C. Aspirate liquids.

4. Add 300ul cytoplasmic washing buffer to resuspend the pellet. Centrifuge at 12,000 xg for 30 seconds at 4°C. Aspirate liquids. (The remained cytoplasmic fractions were washed out).

5. Resuspend the pellet in 50ul nuclear lysis buffer and vortex vigorously for 10 seconds.

6. Incubate suspension for 30 minutes on ice (vortex 10 seconds every 10 minutes).

7. Centrifuge at 12000xg for 10 minutes at 4°C. Transfer the supernatant (**the nuclear protein fractions**) to a clean prechilled microcentrifuge tube.

8. Determine the nuclear protein concentration by Bradford or BCA Assay.
   Store all the extracts aliquots at -80°C.

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