Product Information

ZmTech® Cytoplasmic, Lysosome and Nuclear Protein Extraction Kit

Catalog Number: LCN-001

Description:
This kit is designed for extracting intact nuclear proteins, lysosome 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 (100 extracts)	Storage
Zmtech Cytoplasmic Lysis Buffer (C207020, clear cap)	50.0 mL	2-8°C
Zmtech Cytoplasmic Washing Buffer (C207030 purple cap)	25.0 mL	2-8°C
Zmtech One-step Lysis Buffer (N207040, green cap)	10.0 mL	2-8°C
Zmtech Lysosome Cleanup Buffer (L21010, red cap)	500 uL	2-8°C
Zmtech Lysosome Precipitation Buffer (L21020, blue cap)	500 uL	2-8°C
DTT, 1M (Dissolved in 0.1 ml ddH_2O)	1 vial	–20°C
Protease/Phosphatase Inhibitors (I208052)	1.5 mL	–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 samples on ice)

*Prepare working reagents prior to proceeding.

For 10 Extractions: (50-100mg tissues/Extraction)	
Zmtech Cytoplasmic Lysis Buffer (5ml)	add 5.0ul (1M DTT) and 100ul Protease/phosphatase Inhibitors (I208052)
Zmtech Cytoplasmic Washing Buffer (2.5ml)	add 2.0ul (1M DTT) and 30ul Protease/phosphatase Inhibitors (I208052)
Zmtech One-step Lysis Buffer (1.0ml)	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 (Clear Cap) to resuspend cell pellet. Gently pipette up and down several times and incubate on ice for 10 minutes.
- 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 (Clear Cap). Vortex at mid-speed for 20 seconds and incubate on ice for 10 minutes.

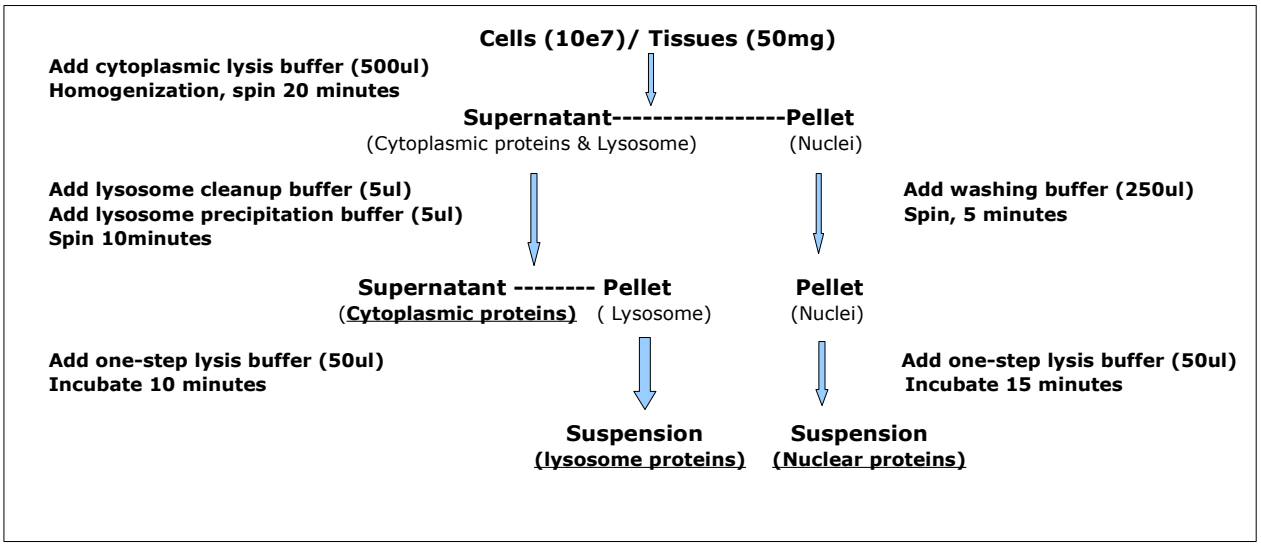
2. **Homogenization:**

- 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 intact Lysosome organelles) to a new 1.5ml microcentrifuge tube. Keep the pellet on ice for nuclear protein extraction.
5. Add 5ul lysosome cleanup buffer (Red cap) into the supernatant, mix thoroughly by pipette up and down and centrifuge at 1,000 xg for 10 minutes at 4°C to remove the remained mitochondria and endoplasmic reticulum. Transfer the supernatant into a new pre-chilled 1.5ml tube. Discard the pellet.

6. Add 5 ul lysosome precipitation buffer (Green cap) into the supernatant, mix thoroughly by pipette up and down several times and centrifuge at 10,000 xg for 10 minutes at 4°C to separate the soluble cytoplasmic proteins and the Lysosome pellet. Keep pellet on ice for Lysosome protein extraction.
7. Transfer the supernatant into a clean pre-chilled 1.5mL tube and centrifuge at 14,000 xg for 10 minutes at 4°C. Transfer the supernatant (cytoplasmic proteins) into a new 1.5ml tube and discard the pellet. Keep the supernatant (cytoplasmic proteins) tube on ice.
8. Add 50 ul One-step lysis buffer (Green Cap) to resuspend the pellet from **step 6** and incubate on ice for 10 minutes. Vortex at highest speed for 10 seconds every 5 minutes. This is the Lysosome protein fractions.
9. Add 250ul Zmtech washing buffer (Purple cap) to the pellet from **step 4.** Vortex for 10 seconds and centrifuge at 300 xg for 5 minutes at 4°C. Transfer the supernatant (Nuclei) into a new 1.5ml tube and discard the pellet. Continue to centrifuge the supernatant (Nuclei) at 10,000xg for 5 minutes. Aspirate liquids. (The remained cytoplasmic proteins were washed out).
10. Resuspend the pellet in 50 ul One-step lysis buffer (Green Cap) and vortex vigorously for 10 seconds. Incubate the suspension for 15 minutes on ice. Vortex vigorously for 10 seconds every 5 minutes.
11. Centrifuge at 14000xg for 10 minutes at 4°C. Transfer the supernatant (nuclear protein fractions) into a clean pre-chilled 1.5ml microcentrifuge tube.
12. Determine the protein concentration of cytoplasmic, lysosome and nuclear with spectrometers or by BCA Assay. Store all the extracts aliquots at -80°C.

Flow Chart of Protein Extraction: (an innovative lysosome cleanup/precipitation technology)



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.

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