Product Information

Lysosome and Nuclei Isolation Kit

Catalog Number: V**LN-03**

Description:
This kit is designed for extracting intact nuclei and lysosome organelles 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, clear sticker) 25 mL 2-8°C
Cytoplasmic Washing Buffer (C207030 purple sticker) 12.5 mL 2-8°C
Lysosome Cleanup Buffer (L21010, red cap) 250 uL 2-8°C
Lysosome Precipitation Buffer (L21020, blue cap) 250 uL 2-8°C
Lysosome storage buffer (L207080, yellow sticker) 10 mL 2-8°C
Nuclei storage buffer (N207080, red sticker) 10 mL 2-8°C

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

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 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:

- 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 300 xg for 10 minutes at 4°C. Transfer the supernatant to a new 1.5ml microcentrifuge tube. Discard the pellet.
4. Centrifuge the homogenization at 1,000 xg for 10 minutes at 4°C. Transfer the supernatant (containing the cytoplasmic proteins and intact Lysosome organelles) to a new 1.5ml microcentrifuge tube. Resuspend the pellet (nuclei) with 200ul Nuclei storage buffer. **(*Note)**
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 5ul lysosome precipitation buffer (Blue 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. Discard the supernatant and resuspend the pellet (Lysosome) with 200ul Lysosome storage buffer.
7. Store all the aliquots at -80°C.

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

Cells (10e7)/ Tissues (50mg)
Add cytoplasmic lysis buffer (500ul)
Homogenization, spin 20 minutes
Supernatant----------Pellet
(Cytoplasmic proteins & Lysosome) (Nuclei)

Add lysosome cleanup buffer (5ul) Add washing buffer (250ul) Add lysosome precipitation buffer (5ul) Spin, 10 minutes Spin 10minutes

Supernatant -------- Pellet Pellet
(**Cytoplasmic proteins)** (Lysosome) (Nuclei)

Add Lysosome storage buffer (200ul) Add Nuclei storage buffer (200ul)

Suspension Suspension
(lysosome) (Nuclei)

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.
• ***Note:**
Nuclei cleanup procedure:

1. Add 250ul cytoplasmic washing buffer to the Nuclei pellet (step.4) prior to resuspend in Nuclei storage buffer.
2. Mix thoroughly by pipette up and down for several times.
3. Centrifuge at 1,000 xg for 10 minutes at 4°C to remove the remained mitochondria and other small organelles.
4. Discard the supernatant and resuspend the Nuclei pellet with 200ul Nuclei storage buffer.