Product Information

Nuclei Isolation Kit: (Catalog: NI-03)

Description:
This kit is designed for isolating the intact nuclei 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
Detergents (D207050 Yellow cap)	1.5 mL	2-8°C
Nuclei storage buffer (N207080, Red cap)	10.0 mL	2-8°C

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

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 10-50mg 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.
  Tissues 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.

2. Add 30ul of detergents (yellow cap), vortex vigorously at highest speed for 10 seconds.
3. Centrifuge at 5,000 xg for 30 seconds at 4°C, immediately transfer the supernatant **(cytoplasmic protein fractions)** into a pre-chilled micocentrifuge tube.
4. Add 300ul cytoplasmic washing buffer to resuspend the pellet. Centrifuge at 5,000 xg for 30 seconds at 4°C. Aspirate liquids. (The remained cytoplasmic fractions were washed out).
5. Resuspend the pellet in 200ul nuclei storage buffer and store all the aliquots at -20°C.

Flow Chart of Nuclei isolation: