BIO BASIC
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Product information

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Nuclear and Cytoplasmic Protein Extraction Kit

Catalog #: BSP001
Size: 50 preps
Storage: Mixed components storage*

*: Product will be shipped with ice pack. Check storage conditions.

Product Description:

The kit is designed for efficient separation of nuclear and cytoplamic fractions from animal tissue and culture cells. Extracted nuclear and cytoplasmic proteins are functional and compatible with down stream assays such as transcriptional activity, gel shift assay, reporter assays, enzyme activity assays, and Western blotting. The kit is sufficient for 50 x 10^7 cells or 50 x 200 mg tissue samples.

Storage:

Store DTT, protease inhibitor and PMSF at -20°C. Store other reagents at 2-8°C

Composition:

Buffer A	25 ml
Buffer B	1.5 ml
Buffer C	12.5 ml
DTT	50 µl
Protease Inhibitor	50 µl
PMSF	500 µl

Materials provided by end-user(s):

- PBS (pre-cold at –20°C)
- Water Bath
- Microcentrifuge

Procedures:

1. Collect cell from $5\times10^6 \sim 1\times10^7$ cell culture by centrifuging at 500 g for 3 minutes at 4°C. Discard media and keep cell precipitates.
2. Wash cell pellet with pre-cold PBS for two times. Spin at 500 g for 3 minutes during each wash. Drain completely to remove PBS and keep cell pellets. Evaluate volume of cell pellets.
3. If tissue sample is used, cut the tissue sample into small pieces. Add sufficient volume of pre-cold PBS so that tissue sample is completely immersed. Homogenize tissue sample 10-15 times and keep on ice for 5 minutes. Transfer cell suspension into a new centrifuge tube. Do not transfer fat tissue, cell debris or extracellular matrix. Centrifuge at 500 g for 3 minutes and discard supernatant. Keep cell pellets on ice and evaluate volume of cell pellets in the tube.
4. Add 200 µl pre-cold Buffer A per 20 µl cell pellet. Vortex at high speed for 15 seconds, then place them on ice for 10-15 minutes.

   NOTE: Before use, add 1 µl DTT, 10 µl PMSF and 1 µl protease inhibitor into 1 ml buffer A.
5. Add 11 µl pre-cold Buffer B, then vortex at high speed for 5 seconds and keep at ice for one minute.
6. Vortex at high speed for 5 minutes, then centrifuge at 10,000 g for 5 minutes at 4°C. The mixture will form three layers, the below layer is transparent, middle layer is white cell nucleolus, the top layer is transparent.
7. Transfer the top layer supernatant into a new centrifuge and keep on ice. This is the cytoplasmic protein components. Store at -80°C.

8. Transfer white cell nucleolus into a new centrifuge, then add 100 µl Buffer C. Vortex at high speed for 10 minutes, and keep in ice bath beaker. Shake for 30 minutes at 150 rpm on rotator, vortex at high speed for 30 seconds.

NOTE: Before use, add 1 µl DTT, 10 µl PMSF and 1 µl protease inhibitor into 1 ml buffer C.

9. Centrifuge at 10,000 g at 4°C for 5 minutes. Transfer supernatant into a new centrifuge. This is the nuclear protein components. Store at -80°C. Avoid freeze-thaw procedure.

Notes:

1. All reagents and instruments must be treated pre-cold, which preserves protein activity and integrity.

2. The following chart illustrates the relationship between Buffer A, B, C and volumes of cell pellets.

Volume of Cell Paste	Buffer A	Buffer B	Buffer C
10 µl	100 µl	5.5 µl	50 µl
20 µl	200 µl	11 µl	100 µl
50 µl	500 µl	27.5 µl	250 µl
100 µl	1 ml	55 µl	500 µl

3. Dialysis is optional depending on the requirements of each experiment.