BIO BASIC
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Product information

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Membrane and Cytoplasmic Protein Extraction Kit

Catalog #: BSP005
Size: 50 preps
Storage: Mixed components storage*

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

Product Description:

Membrane and Cytoplasmic Protein Extraction Kit is designed for effective extraction of membrane and cytoplasmic proteins from mammalian tissues and culture cells. Treatment using different mild, non-denaturing detergent based buffers allows selective separation of membrane and membrane-associated proteins from the cytoplasmic fraction. The kit is simple and rapid and can efficiently isolate high quality membrane and cytoplasmic protein extracts without cross-contamination between the two fractions. Both fractions contain high quality and purity, non-denatured functional proteins which can be utilized in a variety of applications, such as Western blotting, and enzyme analyses, SDS-PAGE, IP, EMSA, Pull down and so on. The procedure is based on stepwise differential protein solubilization. The kit can be used for 50 sample preparations (from 50 x 100 mg tissue or 50 x 10^7 culture cell per sample).

Storage and Transportation:

Transportation at ambient temperature. Upon receipt, store Solution A and B at 4°C. Protease inhibitor and Phosphatase inhibitor, DTT solution and PMSF solution should be stored at -20°C.

Composition:

Solution A	50 ml
Solution B	30 ml
Protease Inhibitor	80 µl
Phosphatase Inhibitor	400 µl
DTT	80 µl
PMSF	800 µl

Procedures:

1. Collect ~ 1 x 10^7 cells from cell suspension or Monolayer (Adherent) Cultures. Wash the cells once with ice cold PBS. Repeat washing steps three times. For tissue samples, weigh ~100 mg. Use a sharp scalp and remove fat and nerve tissue first. Cut tissue sample into small pieces, and then wash with pre-cold PBS for three times.
2. Add 1 ml ice cold Solution A (Before use, add 1 µl Protease inhibitor and 5 µl Phosphatase inhibitor, 1 µl DTT, 10 µl PMSF into 1 ml Solution A). Vortex, and homogenize mixture with a glass homogenizer for 30-50 strokes or sonicate mixture for 30 seconds with 1 minute interval. Repeat operation three times. Make sure no cells clumps left and ensure more than 90 percent cells have been homogenized.
3. Transfer the above homogenized solution into a new pre-cold 1.5 ml centrifuge tube, then centrifuge at 600 g at 4°C for 10 minutes. Discard pellet and keep supernatant.

   NOTE: The resulting pellet is the nuclei, cellular debris and intact cells. Alternatively, one can centrifuge the supernatant at 600 g at 4°C for additional 10 minutes to further remove impurities in the supernatant.

4. Transfer the above supernatant into a new pre-cold centrifuge tube. Centrifuge at 23,200 g at 4°C for 60 minutes. Resulting supernatant is the cytoplasmic protein. Transfer supernatant into a new centrifuge and store at -80°C till further research use.

   NOTE: The pellet contains membrane proteins. Ensure to remove all liquid which contains cytoplasmic proteins.

5. Add 0.5 ml ice cold Solution B to the precipitates from step 4 (Before use, add 1 µl Protease inhibitor and 5 µl Phosphatase inhibitor, 1 µl DTT, 10 µl PMSF into 1 ml Solution B). Vortex at high speed for 10 seconds, and then incubate mixture at 4°C for 30 minutes. Occasionally take out and vortex 3-5 times. Centrifuge at 18,330 g for 10 minutes and keep supernatant as Membrane Protein Fraction. Store at -80°C till further use.

Notes:

1. All of reagents and instruments must be pre-cold; this is to preserve protein activity and integrity.

2. Dialysis is optional depending on specifications of each experiment.

3. For Membrane and Cytoplasmic Protein quantitation, one can use Non-Interfering Protein Concentration Determination Kit (SK3071) or Better BCA Protein Assay Kit (SK3051).

4. If low protein concentration is observed in cytosolic fraction, cell lysis may not be efficient. Please increase the number of strokes performed with the homogenizer.

5. If low protein concentration is observed in Membrane and Cytoplasmic Protein fractions, please add 1 µl Protease inhibitor and 5 µl Phosphatase inhibitor, 1 µl DTT, 10 µl PMSF into 1 ml Solution A, B.

6. Tested using the extraction protocols for adherent, suspension cells and tissues. Extracted protein fractions are separated on a 12% SDS-PAGE, transferred onto PVDF membrane and blotted for the presence of Calnexin (membrane protein) and Tubulin (cytoplasmic protein). Kit should yield at least 100 µg of membrane proteins per prep. Cross-contamination of cytoplasmic proteins in the membrane fraction should not exceed 10%.