ELISA PRODUCT INFORMATION & MANUAL

Subcellular Protein Fractionation Kit

NBP2-47659

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

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Subcellular Protein Fractionation Kit

For serial sample preparation of four distinct protein fractions including cytosol/particulate/cytoskeleton/nuclear fractions, from one sample
For research use only - not intended for diagnostic use.

Storage and Stability

On receipt entire assay kit can be stored at -20°C. After opening the kit, you may store buffers at +4°C or –20°C. Store Protease Inhibitor Cocktail and DTT at –20°C. Use kit within 12 months.

Materials Supplied

Item	Quantity	Storage Condition
Cytosol Extraction Buffer (CEB)	20 ml	-20°C
Membrane Extraction Buffer-A (MEB-A)	20 ml	-20°C
Membrane Extraction Buffer-B (MEB-B)	1.2 ml	-20°C
Nuclear Extraction Buffer (NEB)	10 ml	-20°C
DTT (1 M)	150 µl	-20°C
Protease Inhibitor Cocktail	1 vial*	-20°C

*Add 150 µl of DMSO, and mix well before use.

General Consideration and Reagent Preparation:

- After opening the kit, you may store buffers at +4°C or –20°C. Store Protease Inhibitor Cocktail and DTT at –20°C.
- Before starting the procedure, prepare sufficient Extraction Buffer Mix (EB-Mix) for your experiment: Add 2 µl Protease Inhibitor Cocktail and 2 µl DTT to 1 ml of CEB, MEB-A, and NEB, individually.
- Be sure to keep all buffers on ice at all times during the experiment. All centrifugation procedures are recommended to be performed at 4°C.
- The following protocol is described for fractionation of 4 - 8 x 10^6 cells. If more cells are used for fractionation, scale up the volumes proportionally.

Fractionation Protocol:

1. Collect cells (4 - 8 x 10^6) by centrifugation at 700 x g for 5 min. Wash cells with 5 - 10 ml of ice-cold PBS and centrifuge at 700 x g for 5 min. If using fresh tissue, cut the tissue (~400 mg) into small pieces, add ice cold PBS (1 - 2 ml), and homogenize in a manual tissue homogenizer. Pellet the cells by centrifugation at 500 x g for 5 minutes and remove the supernatant.
2. Resuspend the cell pellet in 1 ml of ice-cold PBS and transfer cells to a microfuge tube. Spin for 5 min at 700 x g and remove supernatant.
3. Resuspend the pellet in 400 µl of Cytosol Extraction Buffer-Mix (CEB-Mix containing DTT and Protease Inhibitor cocktail). Pipette several times to mix well with cells. Incubate sample on ice for 20 min with gentle tapping 3 - 4 times every 5 minutes.
4. Centrifuge the sample at 700 x g for 10 min. Collect supernatant (This is Cytosolic Fraction). Keep on ice.
5. Resuspend the pellet in 400 µl of ice-cold Membrane Extraction Buffer-A Mix (MEB-A Mix containing DTT and Protease Inhibitor Cocktail). Pipette several times and vortex the sample for 10 - 15 seconds to mix well.
6. Add 22 µl of Membrane Extraction Buffer-B, vortex for 5 seconds. Incubate on ice for 1 min.
7. Vortex for 5 seconds again and centrifuge for 5 min at 1000 x g (3400 rpm).
8. Immediately transfer the supernatant to a clean pre-chilled tube (This is Membrane/Particulate Fraction). Keep on ice.
9. Resuspend the pellet in 200 µl of ice-cold Nuclear Extraction Buffer Mix (NEB-Mix containing DTT and Protease Inhibitor Cocktail), vortex for 15 seconds, keep on ice for 40 minutes with constant vortex for 15 seconds every 10 minutes.
10. Centrifuge the sample at top speed in a microcentrifuge for 10 minutes.
11. Transfer the supernatant to a clean pre-chilled tube (This is Nuclear Fraction). The pellet is the Cytoskeletal Fraction. The Cytoskeletal fraction can be dissolved in 100 µl of 0.2 % SDS containing 10 mM DTT or dissolve directly in SDS-PAGE sample buffer.
12. Store all fractions at –80°C for future use.