FractionPREP™ Cell Fractionation kit

(Catalog #K270-50; 50 fractionations; Store at –20°C)

I. Introduction:

This FractionPREP Cell Fractionation system is designed to provide reproducible extraction of four subcellular protein fractions (Cytosol, nucleus, membrane/particulate, and cytoskeletal fractions) from a single mammalian sample. The method is fast and simple, needing only 2 hours and no ultracentrifugation involved. All four protein fractions obtained are suitable for many downstream applications such as 1-D or 2-D gel, enzyme activity assays, gel shift assay, and Western blotting.

II. Kit Contents:

Component	K270-50	Bottle	Part. No.
	50 assays	Cap Color	Component
Cytosol Extraction Buffer (CEB)	20 ml	WM	K270-50-1
Membrane Extraction Buffer-A (MEB-A)	20 ml	WM	K270-50-2
Membrane Extraction Buffer-B (MEB-B)	1.2 ml	Green	K270-50-3
Nuclear Extraction Buffer (NEB)	10 ml	NM	K270-50-4
DTT (1 M)	150 µl	Blue	K270-50-5
Protease Inhibitor Cocktail (lyophilized)	1 vial*	Red	K270-50-6

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

III. FractionPREP Fractionation Protocol:

A. 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.

B. Fractionation Protocol:

1. Collect cells (4-8 x 10^6) by centrifugation at 700xg for 5 min. Wash cells with 5-10 ml of ice-cold PBS and centrifuge at 700xg 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 500xg for 5 minutes and remove the supernatant.

2. Resuspend the cell pellet in 1 ml of ice-cold PBS and transfer cells to an Eppendorf tube. Spin for 5 min at 700xg 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 700xg 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 vertex the sample for 10-15 seconds to mix well.
6. Add 22 µl of Membrane Extraction Buffer-B, vertex for 5 seconds. Incubate on ice for 1 min.
7. Vertex for 5 seconds again and centrifuge for 5 min at 1000xg (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), vertex for 15 seconds, keep on ice for 40 minutes with constant vertex 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 (BioVision Cat.# 2108-10).
12. Store all fractions at –80°C for future use.

FOR RESEARCH USE ONLY! Not to be used in human.

IV. Related Products:

1. Cell Fractionation Products:
   - Mammalian Cell Extraction Kit
   - Mitochondria/Cytosol Fractionation Kit
   - Nuclear/Cytosol Extraction Kit
   - Cytosol/Particulate Rapid Separation Kit
   - Membrane Protein Extraction Kit
   - Mitochondrial DNA Isolation Kit
2. Apoptosis Products
3. Cell Proliferation & Senescence
4. Cell Damage & Repair
5. Metabolism Assay kits
6. Cholesterol, HDL and LDL Assay Kits
7. cAMP & cGMP Assay Kits
8. Growth Factors & Cytokines
9. Antibodies for Cytokines & Cell signaling Molecules

BioVision Research Products
980 Linda Vista Avenue, Mountain View, CA 94043 USA

Tel: 650-428-0236 • Fax: 650-428-0336
www.biovision.com • Email: tech@biovision.com