

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	Color Code	Part Number
	50 assays		
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	1 vial*	Red	K270-50-6

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

III. 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 10⁶ cells. If more cells are used for fractionation, scale up the volumes proportionally.

IV. FractionPREP Fractionation Protocol:

- Collect cells (4 - 8 x 10⁶) 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.
- Resuspend the cell pellet in 1 ml of ice-cold PBS and transfer cells to an microfuge tube. Spin for 5 min at 700 x g and remove supernatant.
- 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.
- Centrifuge the sample at 700 x g for 10 min. Collect supernatant (This is Cytosolic Fraction). Keep on ice.
- 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.

- Add 22 µl of Membrane Extraction Buffer-B, vortex for 5 seconds. Incubate on ice for 1 min.
- Vortex for 5 seconds again and centrifuge for 5 min at 1000 x g (3400 rpm).
- Immediately transfer the supernatant to a clean pre-chilled tube (This is Membrane/Particulate Fraction). Keep on ice.
- 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.
- Centrifuge the sample at top speed in a microcentrifuge for 10 minutes.
- 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).
- Store all fractions at -80° C for future use.

RELATED PRODUCTS:

- 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
- Apoptosis Products
- Cell Proliferation & Senescence
- Cell Damage & Repair
- Metabolism Assay kits
- Cholesterol, HDL and LDL Assay Kits
- cAMP & cGMP Assay Kits
- Growth Factors & Cytokines
- Antibodies for Cytokines & Cell signaling Molecules

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