Product Information

FractionPREP™ Cell Fractionation Kit

I. Kit Contents:

Components	K2115-50 50 assays	Cap Color	Part Number
Cytosol Extraction Buffer (CEB)	20 ml	WM	K2115-C-1
Membrane Extraction Buffer-A (MEB-A)	20 ml	WM	K2115-C-2
Membrane Extraction Buffer-B (MEB-B)	1.2 ml	Green	K2115-C-3
Nuclear Extraction Buffer (NEB)	10 ml	NM	K2115-C-4
DTT (1 M)	150 µl	Blue	K2115-C-5
Protease Inhibitor Cocktail	1 vial	Red	K2115-C-6

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

II. Introduction:

The FractionPREP™ Cell Fractionation system is a fast and simple way of extracting four subcellular protein factions (Cytosol, nucleus, membrane/particulate, and cytoskeletal fractions) from a single mammalian sample. The method takes only 2 hours without ultracentrifugation. The four protein extracted can be utilized for various downstream applications (e.g. western blotting, 1-D or 2-D gel and enzyme activity assays etc.)

III. General Consideration and Reagent Preparation:

1. After opening the kit, you may store buffers at +4℃ or –20℃. Store Protease Inhibitor Cocktail and DTT at –20℃.
2. 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.
3. Be sure to keep all buffers on ice at all times during the experiment. All centrifugation procedures are recommended to be performed at 4℃.
4. 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.

IV. FractionPREP 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 an 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 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 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), 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.

12. Store all fractions at –80℃ for future use.

Frequently Asked Questions:

1. What components does each of the isolated fractions contains?

Nuclear Fraction: Total nucleus soluble proteins, including the nuclear membrane proteins.

Cytosol Fraction: Total cellular soluble proteins from cytoplasm.

Membrane/Particulate: Total cellular membrane proteins including cellular organelles and organelles membrane proteins (but excluding the nuclear membrane proteins).

Cytoskeletal Fraction: Total cellular insoluble proteins, genomic DNA.

2. In western analysis, a membrane protein appeared mainly in his nuclear fraction. Why?

The membrane extraction step may not be sufficient. First, make sure to add Membrane Extraction Buffer B into the extraction, which contain NP-40 detergent. If it still shows the same result, increase incubation time from 1 min to 5 min after adding MEB-B. NP-40 should extract membrane from most of cells.

3. Does the last obtained fraction contain DNA? Should DNase be added in the sample?

DNA will be in the last fraction, and small amount DNA can be in nuclear extract fraction. If you really concern about the presence of DNA, you can certainly add DNase. However, for most applications, it might be fine without DNase treatment.

4. How much are the concentration of the salts in the fractions?

The nuclear fraction has high salt concentration. The other ones have medium level similar to the physiological buffers.

5. What is the exact volume of sample required for this assay?

There is no specific volume we can recommend for the amount any sample to be used since it is completely sample concentration and quality based. You have to do a pilot expt with multiple sample volumes to determine the optimal volume which gives a reading within the linear range of the standard curve. Please refer to the citations for this product to see what other clients have used with similar sample types.

6. What is the shelf life of this kit?

This kit is good for 12 months from the date of shipment in the unopened form when stored at the appropriate temperature and appropriate conditions. After opening and reconstitution, some of the components in this kit are good for 2 months at -20℃. Please refer to the data sheet for storage information and shelf life of each of the components.

7. Why are my standard curve values lower than those shown on the datasheet?

There are multiple factors which influence the signals like the incubation times, room temperature, handling etc. In general, to increase the value of the standards, you can increase the incubation time. As long as the standard curve is linear, it should be fine to use, since all of your samples will also be measured under the same conditions on this curve.

8. How do I normalize my samples against protein concentration?

You can use a protein quantitation assay on the supernatants you get from cell/tissue lysates or with any other liquid sample in the assay buffer.

9. Can we use an alternate buffer for sample preparation (cell lysis, sample dilutions etc)?

Our assay buffers are optimized for the reactions they are designed for. They not only contain some detergents for efficient lysis of your cells/tissue, but also contain some proprietary components required for the further reactions. Therefore, we highly recommend using the buffers provided in the kit for the best results.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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