

Cytosol&Particulate&Cytoskeleton&Nuclear Isolation Kit

Cat. No. CPS-004K **Lot. No.** (See product label)

SPECIFICATION

Product Overview	This 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.
Applications	1-D or 2-D gel, enzyme activity assays, gel shift assay, and Western blotting.
Storage	Store kit at -20centigrade. Briefly spin small vials prior to opening.
Kit Components	• Cytosol Extraction Buffer (CEB) • Membrane Extraction Buffer-A (MEB-A) • Membrane Extraction Buffer-B (MEB-B) • Nuclear Extraction Buffer (NEB) • DTT (1 M) • Protease Inhibitor Cocktail (lyophilized) *Add 150 l of DMSO, and mix well before use.
Features & Benefits	• Simple procedure; takes less than 1 hour • Fast and convenient • The kit is designed for serial sample preparation of four distinct protein fractions including cytosol/particulate/cytoskeleton/nuclear fractions, from one sample.
Preparation	1. After opening the kit, you may store buffers at +4centigrade or –20centigrade. Store Protease Inhibitor Cocktail and DTT at –20centigrade. 2. Before starting the procedure, prepare sufficient Extraction Buffer Mix (EB-Mix) for

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your experiment: Add 2 ul Protease Inhibitor Cocktail and 2 ul 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 4centigrade.

4. The following protocol is described for fractionation of $4 - 8 \times 10^6$ cells. If more cells are used for fractionation, scale up the volumes proportionally.

Separation Protocol

1. Collect cells ($4 - 8 \times 10^6$) by centrifugation at $700 \times g$ for 5 min. Wash cells with 5 - 10 ml of ice-cold PBS and centrifuge at $700 \times g$ 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 $500 \times g$ 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 $700 \times g$ and remove supernatant.

3. Resuspend the pellet in 400 l 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 $700 \times g$ for 10 min. Collect supernatant (This is Cytosolic Fraction). Keep on ice.

5. Resuspend the pellet in 400 ul 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 ul of Membrane Extraction Buffer-B, vortex for 5 seconds. Incubate on ice for 1min.

7. Vortex for 5 seconds again and centrifuge for 5 min at $1000 \times g$ (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 ul 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 ul of 0.2 % SDS containing 10 mM DTT or dissolve directly in SDS-PAGE sample buffer.
12. Store all fractions at –80centigrade for future use.

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