Complete Cell Fractionation Kit

PromoKine

Instruction Manual

Catalog Number	PK-CA577-K270
Description	The Complete Cell Fractionation Kit has been designed to provide parallel, 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, taking only 2 hours and no ultracentrifugation step is involved. All four protein fractions obtained are suitable for many downstream applications such as 1-D or 2-D gel electrophoresis, enzyme activity assays, gel shift assays and Western blotting.
Quantity	50 assays
Kit Components	Components / Quantity / Cap code Cytosol Extraction Buffer (CEB) / 20 ml / WM Membrane Extraction Buffer-A (MEB-A) / 20 ml / WM Membrane Extraction Buffer-B (MEB-B) / 1.2 ml / Green Nuclear Extraction Buffer (NEB) / 10 ml / NM DTT (1 M) / 150 µl / Blue Protease Inhibitor Cocktail (lyophilized) / 1 vial* / Red *Add 150 µl of DMSO, and mix well before use.
Applications / Assay 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, respectively. 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 accordingly. B. Fractionation Protocol: 1. Collect cells (4-8 x 10^6) by centrifugation at 700xg for 5 minutes. Wash cells with 5-10 ml of ice-cold PBS and centrifuge at 700 x g for 5 minutes. 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 Eppendorf tube. Spin for 5 minutes 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 minutes with gentle tapping (3-4 times) every 5 minutes. 4.Centrifuge the sample at 700 x g for 10 minutes. Collect supernatant (This is the 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 minute. 7. Vortex for 5 seconds once again and centrifuge for 5 minutes at 1000 x g (3400 rpm). 8. Immediately transfer the supernatant to a clean pre-chilled tube (This is the 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 vortexing 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 the 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 dissolved directly in SDS-PAGE sample buffer. 12. Store all fractions at –80°C for future use.

Intended Use	For in vitro research use only. Not for diagnostic or therapeutic procedures.
Storage & Stability	Store kit at –20°C upon arrival. Store individual reagents as indicated on the respective labels.

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