Mitochondria/Cytosol Fractionation Kit

PromoKine

Instruction Manual

Catalog Number	PK-CA577-K256-25
Description	The Mitochondria/Cytosol Fractionation Kit provides unique formulations of reagents for effective isolation of a highly enriched mitochondrial fraction from the cytosolic fraction of mammalian cells including both apoptotic and non-apoptotic cells. The enriched mitochondrial and cytosolic fractions can be used for studying apoptotic and signal transduction pathways and to detect translocation of factors between the two fractions by applying Western blotting, ELISA or other assays. Procedures are simple and easy to perform, no ultracentrifugations and toxic chemicals are involved.
Quantity	25 assays
Kit Components	Components — Quantity Mitochondria Extraction Buffer — 2.5 ml 5X Cytosol Extraction Buffer — 5 ml DTT (1 M) — 110 µl Protease Inhibitor Cocktail — 1 vial* *Add 250 µl of DMSO, and mix well before use.
Applications / Assay Protocol	A. General Consideration and Reagent Preparation: Read the entire protocol before beginning the procedure. After opening the kit, store buffers at 4°C. Store Protease Inhibitor Cocktail and DTT at –20°C. Make 1X Cytosolic Extraction Buffer by mixing the 5 ml of 5X buffer with 20 ml ml ddH_2O. Prepare enough Mitochondria Extraction Buffer Mix and Cytosol Extraction Buffer Mix for your experiment: Add 2 µl Protease Inhibitor Cocktail and 1 µl DTT to 1 ml of Mitochondria Extraction Buffer and 1 ml of 1X Cytosol Extraction Buffer, respectively, before use. Be sure to keep all buffers on ice at all times during the experiment. B. Cell Fractionation Protocol: 1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction. 2. Collect cells (5×10^7) by centrifugation at 600 x g for 5 minutes at 4°C. 3. Wash cells with 10 ml of ice-cold PBS. Centrifuge at 600 x g for 5 minutes at 4°C. Remove supernatant. 4. Resuspend cells with 1.0 ml of 1X Cytosol Extraction Buffer Mix containing DTT and Protease Inhibitors (prepared as in Section A). 5. Incubate on ice for 10 minutes. 6. Homogenize cells in an ice-cold dounce tissue grinder. Perform the task with the grinder on ice. We recommend 30-50 passes with the grinder; however, efficient homogenization may depend on the cell type. Note: To check the efficiency of homogenization, pipette 2-3 µl of the homogenized suspension onto a coverslip and observe under a microscope. A shiny ring around the cells indicates that cells are still intact. If 70-80% of the cells do not have the shiny ring, proceed to step 7. Otherwise, perform 10-20 additional passes using the dounce tissue grinder. Excessive homogenization should also be avoided, as it can cause damage to the mitochondrial membrane which triggers release of mitochondrial components. 7. Transfer homogenate to a 1.5 ml microcentrifuge tube, and centrifuge at 700 x g (~3,000 rpm) in a microcentrifuge for 10 minutes at 4°C. Collect the supernatant carefully and discard the pellet. 8. Transfer the supernatant to a fresh 1.5 ml tube, and centrifuge at 10,000 x g (~13,000 rpm) in a microcentrifuge for 30 minutes at 4°C. Collect supernatant and save the pellet. 9. Collect the supernatant from Step 8 as Cytosolic Fraction (Store at –80°C). 10. If intact mitochondria are desired, resuspend the pellet from Step 8 in 0.1 ml 1X PBS (Not provided). These are the intact mitochondria. If mitochondrial protein lysate is desired, resuspend the pellet from Step 8 with 100 µl of the Mitochondrial Extraction Buffer Mix containing DTT and protease inhibitors (as prepared in Section A), vortex for 10 seconds and save as Mitochondrial Fraction (Store at –80°C).

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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