Mitochondria/Cytosol Isolation Kit

Cat. No. MCI-011K **Lot. No.** (See product label)

SPECIFICATION

Product Overview	
Applications	Effective isolation of a highly enriched mitochondrial fraction from cytosolic fraction of mammalian cells including both apoptotic and nonapoptotic cells.
Storage	Store kit at -20centigrade. Briefly spin small vials prior to opening.
Kit Components	• Mitochondria Extraction Buffer • 5X Cytosol Extraction Buffer • DTT (1 M) • Protease Inhibitor Cocktail(Add 250 ul of DMSO, and mix well before use)
Target Species	Mammalian
Features & Benefits	• Simple procedure; takes only 3-4 hours • Fast and convenient • The fractionation procedure is simple and staightforward. No ultracentrifugations are required. No toxic chemicals are involved.
Separation Protocol	A. General Consideration and Reagent Preparation: Read the entire protocol before beginning the procedure. After opening the kit, store buffers at 4centigrade. Store Protease Inhibitor Cocktail and DTT at –20centigrade. Make 1X Cytosolic Extraction Buffer by mixing the 5 ml/20 ml of 5X buffer with 20 ml/80 ml ddH2O.

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Prepare enough Mitochondria Extraction Buffer Mix and Cytosol Extraction Buffer. Mix for your experiment: Add 2 ul Protease Inhibitor cocktail and 1 ul DTT to 1 ml of Mitochondria Extraction Buffer and 1 ml of 1X Cytosol Extraction Buffer, individually, before use.
Be sure to keep all buffers on ice at all times during the experiment.

B. Cell Fractionation Protocol:
1. Treat apoptosis in cells by desired method. Concurrently incubate a control culture without induction.
2. Collect cells (5 x 10^7) by centrifugation at 600 x g for 5 minutes at 4centigrade.
3. Wash cells with 10 ml of ice-cold PBS. Centrifuge at 600 x g for 5 minutes at 4centigrade. 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 ul 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 (~3000rpm) in a microcentrifuge for 10 minutes at 4centigrade. Collect the supernatant carefully and discard the pellet.

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8. Transfer the supernatant to a fresh 1.5-ml tube, and centrifuge at 10,000 x g (~13000rpm) in a microcentrifuge for 30 minutes at 4centigrade. Collect Supernatant and save the pellet.

9. Collect the supernatant from Step 8 as Cytosolic Fraction (Store at –80centigrade).

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 ul 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 –80centigrade).

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