KAMIYA BIOMEDICAL COMPANY

Mitochondria/Cytosol Fractionation Kit

For isolation of highly enriched mitochondrial and cytosolic fractions

Cat. No. KT-148

For research use only, not for use in diagnostic procedures.

PRODUCT INFORMATION

Mitochondria/Cytosol Fractionation Kit

Cat. No. KT-148
100 tests

PRODUCT

The **K-ASSAY®** Mitochondria/Cytosol Fractionation kit is for isolation of highly enriched mitochondrial and cytosolic fractions.

PRINCIPLE

The Mitochondria/Cytosol Fractionation Kit provides unique formulations of reagents for effective isolation of a highly enriched mitochondrial fraction from cytosolic fraction of mammalian cells including both apoptotic and nonapoptotic cells. The enriched mitochondrial and cytosolic fractions can be used for studying apoptotic and signal transduction pathways to detect translocation of factors interested between the two fractions by Western blot, ELISA, or other assays. The cell fractionation procedure is simple and easy to perform, no ultracentrifugations and no toxic chemicals are involved.

COMPONENT

• Mitochondria Extraction Buffer	10 mL
• 5X Cytosol Extraction Buffer	20 mL
• DTT (1 M)	110 µL
• Protease Inhibitor Cocktail (500X)*	1 vial*

*Add 250 µL of DMSO, and mix well before use.

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 20 mL of 5X buffer with 80 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, individually, 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 x 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℃. Collect 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℃. Collect Supernatant and save the pellet.
9. Collect the supernatant from Step 8 as Cytosolic Fraction (Store at –80℃).
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), vertex for 10 seconds and save as Mitochondrial Fraction (Store at -80℃).

STORAGE

Store at -20℃.

FOR RESEARCH USE ONLY

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