

## Mitochondria/Cytosol Fractionation Kit (Catalog #K256-25, -100) Store at $-20^{\circ}\text{C}$

### I. Introduction:

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 blotting, ELISA, or other assays. Procedures are simple and easy to perform, no ultracentrifugations and toxic chemicals are involved.

### II. Kit Contents:

Component	K256-25	K256-100
	25 assays	100 assays
Mitochondria Extraction Buffer	2.5 ml	10 ml
5X Cytosol Extraction Buffer	5.0 ml	20 ml
DTT (1 M)	110 $\mu\text{l}$	110 $\mu\text{l}$
Protease Inhibitor Cocktail*	1 vial*	1 vial*

\*Add 250  $\mu\text{l}$  of DMSO, and mix well before use.

### III. Mitochondria/Cytosol Fractionation Protocol:

#### A. General Consideration and Reagent Preparation:

- Read the entire protocol before beginning the procedure.
- After opening the kit, store buffers at  $4^{\circ}\text{C}$ . Store Protease Inhibitor Cocktail and DTT at  $-20^{\circ}\text{C}$ .
- Make 1X Cytosolic Extraction Buffer by mixing the 5 ml/20 ml of 5X buffer with 20 ml/80 ml ddH<sub>2</sub>O.
- Prepare enough Mitochondria Extraction Buffer Mix and Cytosol Extraction Buffer Mix for your experiment: Add 2  $\mu\text{l}$  Protease Inhibitor cocktail and 1  $\mu\text{l}$  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 ( $5 \times 10^7$ ) by centrifugation at  $600 \times g$  for 5 minutes at  $4^{\circ}\text{C}$ .

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 (BioVision, Cat.# 1998-1). 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  $\mu$ l 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 (~3000 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 (~13000 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  $\mu$ l 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).

#### **IV. Related Products:**

1. Nuclear/Cytosol Fractionation Kit
2. Cytosol/Particulate Rapid Separation Kit
3. Membrane Protein Extraction Kit
4. Mammalian Cell Extraction Kit
5. FractionPREP Cell Fractionation System
6. Apoptosis Assay Kits and Reagents
7. Annexin V Apoptosis Kits & Reagents
8. Caspase Assay Kits & Reagents
9. Mitochondrial-Mediated Apoptosis Kits & Reagents
10. Nuclear-Mediated Apoptosis Kits & Reagents
11. Akt and JNK Activity Assay Kits
12. Histone Deacetylase Activity Assay Kits (Fluorometric & Colorimetric)
13. HAT Activity Colorimetric Assay Kit
14. Calpain Activity Assay Kit
15. Cell Proliferation & Cytotoxicity Assay Kits
16. Senescence Detection Kit
17. Glutathione Fluorometric & Colorimetric Detection Kits
18. Nitric Oxide Fluorometric & Colorimetric Detection Kits
19. cAMP & cGMP Assay Kits
20. InsertFinder™ Quick Insert Screening Kit
21. 5 minutes DNA Ligation Kit