Product Information

Mitochondria/Cytosol Fractionation Kit

I. Kit Contents:

Components	K2103-25 25 assays	K2103-100 100 assays	Part Number
Mitochondria Extraction Buffer	2.5 ml	10 ml	K2103-C-1
5X Cytosol Extraction Buffer	5.0 ml	20 ml	K2103-C-2
DTT (1 M)	110 µl	110 µl	K2103-C-3
Protease Inhibitor Cocktail	1 vial	1 vial	K2103-C-4

II. Introduction:

The Mitochondria/Cytosol Fractionation Kit utilize a special formulation of reagents to isolate enriched mitochondrial fraction from cytosolic fraction of both apoptotic and nonapoptotic mammalian cells. The enriched mitochondrial and cytosolic fraction can be used to detect translocation of factors in apoptosis and signaling pathways with Western blotting, ELISA and other assays etc. The protocol is simple to perform without unltracentrifugations and toxic chemicals.

III. Reagent Preparation:

Read the entire protocol before beginning the procedure.

After opening the kit, store buffers at 4℃. Store Protease Inhibitor Cocktail and DTT at -20℃.

Make 1X Cytosolic Extraction Buffer by mixing the 5 ml/20 ml of 5X buffer with 20 ml/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.

IV. Cell Fractionation Protocol:

1. Treat 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℃.
3. Wash cells with 10 ml of ice-cold PBS. Centrifuge at 600 x g for 5 minutes at 4℃. Remove supernatant.
4. Resuspend cells with 1.0 ml of 1X Cytosol Extraction Buffer Mix containing DTT and Protease Inhibitors.
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 (~3000 rpm) in a microcentrifuge for 10 minutes at 4℃. 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℃. 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.

10. 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℃).

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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