Product Information

Cytochrome c Apoptosis Assay Kit

I. Kit Contents:

Components	K2104-100 100 assays	Cap Color	Part Number
Mitochondria Extraction Buffer	10 ml	NM	K2104-C-1
5X Cytosol Extraction Buffer	20 ml	WM	K2104-C-2
DTT (1 M)	110 µl	Blue	K2104-C-3
500X Protease Inhibitor Cocktail	1 vial	Red	K2104-C-4
Anti-Cytochrome c mouse mAb	0.5 ml	Green	K2104-C-5

II. Introduction:

Cytochrome c is located between the inner and the outer mitochondrial membranes. Once activated by the apoptosis stimulus, cytochrome c is released from mitochondria into cytosol where it couples to Apaf-1. The cytochrome c/Apaf-1 complex further activates the downstream caspases. The Cytochrome c Releasing Apoptosis Assay Kit gives an efficient way for sensing cytochrome c releasing from mitochondria into cytosol under apoptosis. The kit utilizes a special formulation of reagents to isolate enriched mitochondrial fraction from cytosol. The protocol is simple to perform without unltracentrifugations and toxic chemicals. The cytochrome c antibody provided in the kit is then used to determine the Cytochrome c releasing from mitochondria into cytosol by Western blotting.

III. Reagent Preparation:

Read the entire protocol before beginning the procedure.

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

Add 250 µl DMSO to dissolve the 500X Protease Inhibitor Cocktail before use.

Before use, prepare just enough Mitochondria Extraction Buffer Mix for your experiment: Add 2 µl Protease Inhibitor cocktail and 1 µl DTT to 1 ml of Mitochondria Extraction Buffer.

Dilute the 5X Cytosol Extraction Buffer to 1X buffer with ddH_2O. Before use, prepare just enough Cytosol Extraction Buffer Mix for your experiment: Add 2 µl Protease Inhibitor cocktail and 1 µl DTT to 1 ml of 1X Cytosol Extraction Buffer.

Be sure to keep all buffers on ice at all times during the experiment.

IV. Assay 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℃.
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 (as prepared in Section A). Incubate on ice for 10 minutes.
5. 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 nuclei indicates that cells are still intact. If 70 - 80% of the nuclei 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.

6. Transfer homogenate to a 1.5 ml microcentrifuge tube, and centrifuge at 700 x g for 10 minutes at 4℃.

7. Collect supernatant into a fresh 1.5 ml tube, and centrifuge at 10,000 x g for 30 minutes at 4℃. Collect supernatant as Cytosolic Fraction.

8. Resuspend the pellet in 0.1 ml Mitochondrial Extraction Buffer Mix containing DTT and protease inhibitors (as prepared in section A), vertex for 10 seconds and save as Mitochondrial Fraction.

9. Load 10 µg each of the cytosolic and mitochondrial fractions isolated from uninduced and induced cells on a 12% SDS-PAGE. Then proceed with standard Western blot procedure and probe with cytochrome c antibody (1:200 dilution is recommended).

Note: The anti-Cytochrome c antibody is a mouse monoclonal antibody that reacts with denatured human, mouse, and rat cytochrome c.

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Our promise

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