KAMIYA BIOMEDICAL COMPANY

Cytochrome c Releasing Apoptosis Assay

For the detection of cytochrome c translocation from mitochondria into cytosol during apoptosis.

Cat. No. KT-147

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

PRODUCT INFORMATION

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Cat. No. KT-147
100 tests

PRODUCT
The **K-*ASSAY*®** Cytochrome c Releasing Apoptosis Assay is for the detection of cytochrome c translocation from mitochondria into cytosol during apoptosis.

PRINCIPLE
Cytochrome c plays an important role in apoptosis. The protein is located in the space between the inner and outer mitochondrial membranes. An apoptotic stimulus triggers the release of cytochrome c from the mitochondria into cytosol where it binds to Apaf-1. The cytochrome c/Apaf-1 complex activates caspase-9, which then activates caspase-3 and other downstream caspases. The **K-*ASSAY*®** Cytochrome c Releasing Apoptosis Assay provides an effective means for detecting cytochrome c translocation from mitochondria into cytosol during apoptosis. The kit provides unique formulations of reagents to isolate a highly enriched mitochondria fraction from cytosol. The procedure is so simple and easy to perform, no ultracentrifugation is required and no toxic chemicals are involved. Cytochrome c releasing from mitochondria into cytosol is then determined by Western blotting using the cytochrome c antibody provided in the kit.

COMPONENT

• Mitochondria Extraction Buffer	10 ml	
• 5X Cytosol Extraction Buffer	20 ml	
• DDT (1 M)	110 µl	(Blue cap)
• 500X Protease Inhibitor Cocktail (Lyophilized)	1 vial	(Red cap)
• Anti-Cytochrome c mouse mAb (0.2 mg/ml)	0.5 ml	(Green cap)

PROTOCOLS
A. General Consideration and Reagent Preparation
- Read the entire protocol before beginning the procedure.
- After opening the kit, store buffers at 4°C. Store antibody, Protease Inhibitor Cocktail, and DTT at –20°C.
- 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.

B. 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°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 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.

7. Transfer homogenate to a 1.5-ml microcentrifuge tube, and centrifuge at 700 x g for 10 minutes at 4°C.
8. Collect supernatant into a fresh 1.5-ml tube, and centrifuge at 10,000 x g for 30 minutes at 4°C.
9. Collect supernatant as Cytosolic Fraction.
10. Resuspend the pellet in 0.1-ml Mitochondrial Extraction Buffer Mix containing DTT and protease inhibitors (prepared as in section A), vortex for 10 seconds and save as Mitochondrial Fraction.
11. Load 10 µg of each cytosolic and mitochondrial fraction isolated from uninduced and induced cells on a 12% SDS-PAGE. Then proceed with standard Western blot procedure and probe with anti-Cytochrome c antibody (1 µg/ml is recommended).

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

STORAGE

Stable at -20°C for 1 year. After opening the kit, store buffers at 4°C. Store antibody, Protease Inhibitor Cocktail, and DTT at –20°C.

FOR RESEARCH USE ONLY

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