Product Information

Mitochondrial Apoptosis Detection Fluorometric Kit

I. Kit Contents:

Components	K2097-25 25 assays	K2097-100 100 assays	Part Number
MitoCapture™ Reagent	25 µl	100 µl	K2097-C-1
Incubation Buffer	50 ml	2 x 100 ml	K2097-C-2

II. Introduction:

The first intracellular action that happens after the activation of apoptosis is the disruption of the mitochondrial transmembrane potential. By detecting the fluctuation in the mitochondrial transmebrane potential, this kit is an easy and fluorescent-based way for differentiate between healthy and apoptotic cells. It applies MitoCapture, a cationic dye that show different fluorescent- red and green in healthy and apoptotic cells, respectively. MitoCapture accumulates and aggregates in the mitochondria, emitting a bright red fluorescence in healthy cells. However, in apoptotic cells, MitoCapture lose its ability of accumulation and aggregation due to the altered mitochondrial transmembrane potential, and remain in its monomer form that emits fluorescing green.

III. Assay Protocol:

A. General Considerations

Aliquot enough Incubation Buffer for the number of assays to be performed (total 2 ml for each assay) and pre-warm to 37℃ before use.

B. Incubation of Cells with MitoCapture Reagent

1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction.

2. Count cells and pellet ~ 1 x 10^6 cells per sample at 500 X g for 5 minutes.

3. Dilute MitoCapture Reagent just prior to use: Dilute 1 µl MitoCapture to 1 ml prewarmed Incubation Buffer for each assay. Vortex the solution immediately.

Note: MitoCapture is poorly soluble in aqueous solutions. It is critical to warm the Incubation Buffer to 37℃ before dilute, and vortex the solution immediately after adding into the buffer. To remove precipitated particles (optional), centrifuge the dye solution for 1 minute at 13,000 x g and carefully transfer the supernatant without disturbing pelleted debris.

4. Resuspend cells in 1 ml of the diluted MitoCapture solution.

5. Incubate at 37℃ in a 5% CO2 incubator for 15 - 20 min.

6. Centrifuge cells at 500 x g and discard supernatant.

7. Resuspend in 1 ml of the pre-warmed Incubation Buffer.

C. Quantification by Flow Cytometry Analyze cells immediately following step B.7 by flow cytometry. MitoCapture monomers in apoptotic cells are detectable in the FITC channel (usually FL1) showing diffused green fluorescence. MitoCapture aggregates in healthy cells are detectable in the PI channel (usually FL2) showing punctate red fluorescence. (You can logon to www.apexbt.com to see the image examples.).

D. Detection by Fluorescence Microscopy

1. Place the cell suspension from B.7 on a glass slide. Cover the cells with a glass coverslip.

For analyzing adherent cells, grow cells on a coverslip and perform the entire procedure directly on the coverslip in culture dish. Following incubation (B.7), invert coverslip on a glass slide.

2. Observe cells immediately under a fluorescence microscope using a band-pass filter (detects fluorescein and rhodamine). MitoCapture that has aggregated in the mitochondria of healthy cells fluoresces red. In apoptotic cells, MitoCapture cannot accumulate in mitochondria, it remains as monomers in the cytoplasm, and fluoresces green.

IV. Storage and Stability:

Store MitoCapture at -20℃. Avoid freeze-thaw. Protect from light. Store Incubation Buffer at 4℃ after opening. All reagents are stable for 1 year.

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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