Mitochondrial Apoptosis Detection Fluorometric Kit

(Catalog #BN00515 -25, -100; Store kit at –20˚C)

I. Introduction:

Disruption of the mitochondrial transmembrane potential is one of the earliest intracellular events that occur following induction of apoptosis. The Apoptosis Detection Kit provides a simple, fluorescent-based method for distinguishing between healthy and apoptotic cells by detecting the changes in the mitochondrial transmembrane potential. The kit utilizes a cationic dye that fluoresces differently in healthy vs apoptotic cells. In healthy cells, MitoCapture accumulates and aggregates in the mitochondria, giving off a bright red fluorescence. In apoptotic cells, MitoCapture cannot aggregate in the mitochondria due to the altered mitochondrial transmembrane potential, and thus it remains in the cytoplasm in its monomer form, fluorescing green. The fluorescent signals can be easily detected by fluorescence microscopy using a band-pass filter (detects FITC and rhodamine) or analyzed by flow cytometry using FITC channel for green monomers (Ex/Em = 488/530 ± 30 nm) and (optional) PI channel for red aggregates (Ex/Em = 488/590 ± 42 nm).

II. Kit Contents:

Component	BN00515 25 assays	BN00515 100 assays	Part Number
Reagent	25 µl	100 µl	BN00515-1
Incubation Buffer	50 ml	2 x 100 ml	BN00515-2

III. MitoCapture 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˚C 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 pre-warmed 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˚C 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˚C in a 5% CO_2 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.

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

FOR RESEARCH USE ONLY! Not to be used on humans.