



MitoCapture™ Mitochondrial ApoptosisDetection Kit

Manufactured by BioVision.

ALX-850-232-KI01: ~25 tests
ALX-850-232-KI02: ~100 tests

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For laboratory use only. Not for human or diagnostic use.

I. Introduction:

Disruption of the mitochondrial transmembrane potential is one of the earliest intracellular events that occur following induction of apoptosis. The **MitoCapture™ Apoptosis Detection Kit** provides a simple, fluorescent-based method for distinguishing between healthy and apoptotic cells by detecting the changes in the mitochondrial membrane potential. The kit utilizes MitoCapture™, a cationic dye that fluoresces differently in healthy cells and in 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 membrane 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:

Components	850-232-KI01	850-232-KI02
		25 tests
MitoCapture™ Reagent	25 µl	100 µl
Incubation Buffer	50 ml	2 x 100 ml

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 10⁶ cells per sample by centrifugation at 500 x g for 5 minutes.
3. Dilute MitoCapture™ Reagent immediately prior to use: Dilute 1 µl MitoCapture™ to 1 ml pre-warmed Incubation Buffer for each assay. Vortex the solution.
Note: MitoCapture™ is poorly soluble in aqueous solutions. To remove 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% CO₂ incubator for 15-20 minutes.
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. Therefore, the cells fluorescing green represent apoptotic cells. 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 the mitochondria, it remains as monomers in the cytoplasm, and fluoresces green.

IV. Storage:

- Store MitoCapture™ at -20°C . Avoid freeze-thaw cycles. Protect from light!
- Store Incubation Buffer at 4°C after opening.