ELISA PRODUCT INFORMATION & MANUAL

Mitochondrial Apoptosis Detection Assay Kit (Fluorometric)

NBP2-54877

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.

Not for diagnostic or therapeutic procedures.

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1. Overview

Disruption of the mitochondrial transmembrane potential is one of the earliest intracellular events that occur following induction of apoptosis. Novus Biologicals's Mitochondrial Apoptosis Detection Assay Kit (Fluorometric) 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 MitoCapture™, a cationic dye that fluoresces differently in healthy vs apoptotic cells. In healthy cells, MitoCapture accumulates and aggregates in the mitocondria, 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 (Em = 488/590 ± 42 nm).

2. Protocol Summary

Induce Apoptosis in Sample Cells

Add MitoCapture Reagent

Quantify Using Flow Cytometry

OR

Detect Using Fluorescence Microscopy

3. Components and Storage

A. Kit Components

Item	Quantity (25 assays)	Quantity (100 assays)
MitoCapture Reagent	25 µL	100 µL
Incubation Buffer	50 mL	2 x 100 mL

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

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Flow Cytometer or Fluorescence Microscope
- Glass slides and coverslips

4. Assay Protocol

1. Incubation of cells with MitoCapture Reagent:

a) Induce apoptosis by desired methods. Concurrently incubate a control culture *without* induction.

b) Collect ~1 x 10^6 cells per sample by centrifugation at 500 x g for 5 minutes.

c) 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. Dilute MitoCapture Reagent immediately prior to use: Dilute 1 µl MitoCapture to 1 ml in 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.

d) Re-suspend cells in 1 ml of the diluted MitoCapture solution.

e) Incubate at 37°C in a 5% CO_2 incubator for 15-20 min.

f) Centrifuge cells at 500 x g and discard supernatant.

g) Re-suspend in 1 ml of the pre-warmed Incubation Buffer.

Proceed to Step 2 or 3 below depending on method of analysis.

2. Quantification by Flow Cytometry:

Analyze cells immediately following step 1.g 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.

3. Detection by Fluorescence Microscopy:

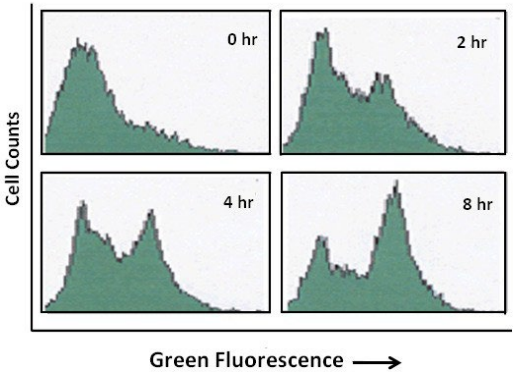
a) Place the cell suspension from Step 1.g on a glass slide. Cover the cells with a glass coverslip.

For analyzing ***adherent cells***, grow cells on a coverslip and perform the entire procedure directly on the coverslip in culture dish. Following incubation (1.e), invert coverslip on a glass slide.

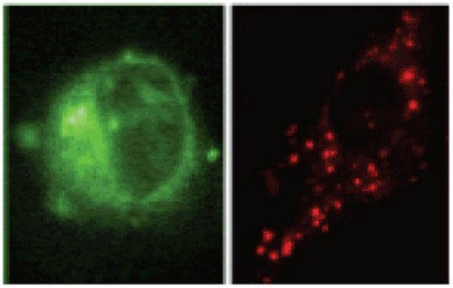
b) Observe cells immediately under a fluorescence microscope using a band-pass filter (detects fluorescein and rhodamine).

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.

5. Data Analysis



Apoptosis was induced in Jurkat cells by 2 μM camptothecin for various hours as indicated. Cells were incubated with MitoCapture for 15 minutes. Results were analyzed by Flow Cytometry using the FITC channel.



Detection of change in Mitochondrial Transmembrane Potential by MitoCapture and fluorescence Microscopy: The photograph shows examples of cells scored for intact and apoptotic cells using MitoCapture probe. The apoptotic cell (left panel) shows diffused green fluorescence, whereas the normal control cell (right panel) shows punctuate red fluorescence.

6. Troubleshooting

Problem	Reason	Solution
Lower signal levels	Washing cells with PBS before/after fixation (adherent cells)	Always use binding buffer for washing cells
	Cells did not initiate apoptosis	Determine the time-point for initiation of apoptosis after induction (time-course experiment)
	Very few cells used for analysis	Refer to data sheet for appropriate cell number
	Incorrect setting of the equipment used to read samples	Refer to datasheet and use the recommended filter setting
	Use of expired kit or improperly stored reagents	Always check the expiry date and store the components appropriately
Problem	**Reason**	**Solution**
Erratic results	Uneven number of cells seeded	Seed only healthy cells (correct passage number)
	Adherent cells dislodged at the time of experiment	Perform experiment gently and in duplicates or triplicates for each treatment
	Incorrect incubation times or temperatures	Refer to datasheet & verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly

Problem	Reason	Solution
High Background	Cell density is higher than recommended	Refer to datasheet and use the suggested cell number
	Increased volumes of components added	Use calibrated pipettes accurately
	Incubation of cell samples for extended periods	Refer to datasheets and incubate for exact times
	Use of extremely confluent cells	Perform assay when cells are at 80-95% confluency
	Contaminated cells	Check for bacteria/ yeast/ mycoplasma contamination