

Intracellular Caspase Detection ApoStat

Catalog Number: FMK012
100 tests

Reagent Information

Fluorescein-conjugated VAD-FMK

Quantity: 1.0 mL at a concentration of 50 µg/mL

Molecular Weight: 722 Da

Stability & Storage: Store reagent at ≤ -20 °C in the dark upon arrival. Product is stable for 12 months from the date of receipt at ≤ -20 °C as supplied. Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

Intended Use

Designed for flow cytometric applications intended to identify and quantitate caspase activity in cells undergoing apoptosis.

Principle of the Test

Cells induced to undergo apoptosis are labeled with a cell-permeable, FITC-conjugated, pan-caspase inhibitor (ApoStat). Any unbound reagent diffuses out of the cell and is washed away. The cells are then assayed by flow cytometry for detection of the bound reagent. The increased fluorescence is a measure of caspase activity within individual cells of the treated population.

Background Information

Members of the caspase gene family (cysteine proteases with aspartate specificity) play significant roles in both inflammation and apoptosis. Caspases exhibit catalytic and substrate-recognition motifs that have been highly conserved (1). These characteristic amino acid sequences allow caspases to interact with both positive and negative regulators of their activity (1). The substrate preferences or specificities of individual caspases have been exploited for the development of peptides that successfully compete for caspase binding (1-3) while maintaining their distinctive aspartate cleavage sites at the P1 position.

It is possible to generate reversible or irreversible inhibitors of caspase activation by coupling caspase-specific peptides to certain aldehyde, nitrile or ketone compounds. Fluoromethyl ketone (FMK)-derivatized peptides act as effective irreversible inhibitors with no added cytotoxic effects. Inhibitors synthesized with a benzyloxycarbonyl group (also known as BOC or Z) at the N-terminus and O-methyl side chains exhibit enhanced cellular permeability. Conjugation of the inhibitor to FITC allows for intracellular detection of the reagent upon binding to the active caspase enzymes. The detection of active caspases by flow cytometry is a rapid method for identifying cells undergoing apoptosis.

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Instructions for Use

Cell Cultures: Culture cells as required to induce apoptosis. The density of the cultured cells should not exceed 10⁶ cells/mL. Typically, 10⁶ cells are aliquoted into wells of a 24 well culture plate in a final volume of 1.0 mL. Investigators must optimize the conditions and kinetics for induction of apoptosis in their system. A control group of cells not induced to undergo apoptosis should also be cultured. It is recommended that induced and non-induced cultures be set up for each labeling condition.

Sample Staining: Cells can be stained directly during the last 30 minutes of the apoptosis induction period. Add 10 µL of ApoStat per 1.0 mL culture volume. The staining is performed at 37 °C. After the staining period, harvest the cells into 5.0 mL tubes, centrifuge at 500 x g for 5 minutes and wash once with 4.0 mL of PBS to remove unbound reagent. Resuspend the cells in 500 µL of PBS for flow cytometric analysis.

Flow Cytometric Analysis: Observe induced and non-induced cells on a SS versus FS linear dot plot to identify and gate on cells of interest. Note that apoptotic cells have been described to undergo changes in their light scattering properties. Make sure that the scatter gate used to identify cells of interest will accommodate these changes in the light scattering properties of the cells.

Fluorescein detection is usually collected on the FL1 channel (employing an argon laser at 488 nm). Generate histograms depicting FL1 (x-axis) versus cell count (y-axis). Monitor the fluorescence of the non-induced cell culture. Voltage adjustment of the photomultiplier tubes may be desirable to optimally position the fluorescence distribution around the first log decade of the histogram display. Mark the regions of the histogram that identify positive and negative events. Cells from other culture conditions may now be tested for the presence of apoptotic cells. The caspase positive cell population will appear as a separate peak or as a shoulder of the first peak demonstrating increased fluorescence intensity. See the reverse side for typical sample data.

Technical Notes

Sample staining with ApoStat can be achieved during the last 15 to 60 minutes of the apoptosis induction culture period. Longer incubation times with ApoStat may inhibit activation of caspases and the apoptotic cascade. This could result in reduced ApoStat binding. Shorter incubation times with ApoStat during the staining process may not allow for the ApoStat binding reaction to go to completion.

ApoStat staining may also be carried out following cell harvest. Collect the cells in 5.0 mL (12 x 75 mm) tubes and wash once as described in Sample Staining. Resuspend the cells in 1.0 mL of PBS. Add 10 μ L of ApoStat to each tube and incubate for 30 minutes at 37 $^{\circ}$ C. Wash cells once in PBS to remove unbound reagent. Resuspend the cells in 500 μ L PBS for flow cytometric analysis.

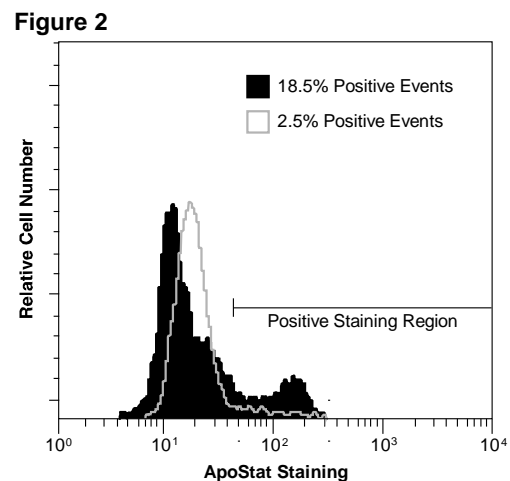
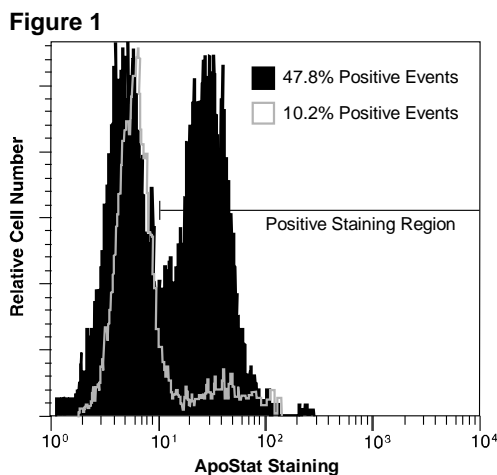


Figure 1: Jurkat T leukemic cells were cultured for 4 hours in 24 well culture plates in the absence or presence of 1 μ M staurosporine. ApoStat (10 μ L) was added to the culture wells for the last 30 minutes of the culture period. The cells were harvested, washed, and assayed by flow cytometry. The histogram demonstrates that nearly 50% of the cells express active caspases following activation.

Figure 2: U937 myeloid lymphoma cells were cultured for 4 hours in 24 well culture plates in the absence or presence of 2.0 μ g/mL camptothecin. ApoStat (10 μ L) was added to the culture wells for the last 30 minutes of the culture period. The cells were harvested, washed, and assayed by flow cytometry. The histogram demonstrates that 18% of the cells express active caspases following apoptosis induction.

References

1. Cryns, V. and J. Yuan (1998) *Genes Dev.* **12**:1551.
2. Talanian, R.V. *et al.* (1997) *J. Biol. Chem.* **272**:9677.
3. Garcia-Calvo, M. *et al.* (1998) *J. Biol. Chem.* **273**:32608.