Biotin-Conjugated Caspase Inhibitor Z-VKD-biotin-FMK

Catalog Number: FMK011

Reagent Specifications

Inhibitor Sequence: Z-V-K(biotin) -D(OMe)-FMK
Molecular Weight: 863 Da
Quantity: 0.5 mg

Storage

Store reagent at -20°C upon arrival.
Lyophilized, samples are stable for 1 year at -20 to -70° C.
Upon reconstitution in DMSO, compound is stable for 6 months at -20° C.

Reconstitution: Inhibitor must be reconstituted using highly pure (ACS grade) DMSO. A pellet may not be visible at the bottom of the vial. Add 58 µL of DMSO to the vial to yield a 10 mM stock solution. Make sure the reagent is thoroughly in solution before use.

Instructions for Use

1) Cells (10^5 to 10^6) may be cultured as required to induce apoptosis.

2) The caspase inhibitor should be added to the cells during the last 1 hour of culture. Add 1/1,000 of the total culture volume such that the final concentration of inhibitor is 10 µM.

3) Detection of the biotinylated inhibitor is achieved through the use of a secondary reagent that has been conjugated to a reporter fluorochrome or enzyme (*e.g.* avidin-fluorescein, avidin-horse-radish peroxidase, etc.). Collect the cultured cells and wash the cells 2 times to remove excess inhibitor. Resuspend the cell pellet in 0.5 mL of 4% paraformaldehyde PBS fixative solution for 10 minutes at 18 - 24° C.

4) The fixed cells are washed 1 time in PBS and then resupended in 2.0 mL of permeabilization buffer (PBS supplemented with 0.1% saponin, pH 7.4).

5) The cells are centrifuged 1 time and resuspended in 200 µL of permeabilization buffer and the secondary developing reagent is added (10 µL of avidin-FITC at 10 µg/mL).

6) Cells are incubated at 18 - 24° C for 45 minutes and then washed 2 times in permeabilization buffer.

7) Cells can be resupended in 0.4 mL of PBS for flow cytometric analysis.

Apoptotic cells should exhibit greater fluorescent staining intensity when compared to non-apoptotic cells.

Intended Use

Designed for *in vitro* use as an intracellular detector of apoptotic cells through its properties as a cell-permeable, irreversible inhibitor of caspase activity.

Principle of the Test

Cells that are induced to undergo apoptosis often exhibit activation of a variety of intracellular caspases. Cells may be cultured in the presence of a biotinylated caspase inhibitor which can enter the cell and irreversibly bind the active site of activated proteases. Treated cells can then be assayed for evidence of apoptosis by examining the intracellular presence of the inhibitor using avidin or streptavidin-FITC conjugates as developing reagents following a cell fixation and permeabilization step. Alternatively, cell extracts may be analyzed by western blotting techniques, which can be developed with avidin-HRP.

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). In addition to their distinctive aspartate cleavage sites at the P1 position, the catalytic domains of the caspases require at least four amino acids to the left of the cleavage site with P4 as the prominent specificity-determining residue (3). WEHD, VDVAD, and DEVD are examples of peptides that preferentially bind caspase-1, caspase-2 and caspase-3, respectively.
It is possible to generate reversible or irreversible inhibitors of caspase activation by coupling caspase-specific peptides to certain aldehyde, nitrile or ketone compounds. These caspase inhibitors can successfully inhibit the induction of apoptosis in various tumor cell lines (4 - 8) as well as normal cells (9, 10). 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 thus facilitating their use in both *in vitro* cell culture as well as *in vivo* animal studies. Caspase inhibitors are important tools in the investigation of many biologic processes utilizing whole cells, cell lysates, and *in vivo* systems.

References

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