Caspase Inhibitor Control
Z-FA-FMK

Catalog Number: FMKC01

Reagent Specifications

Inhibitor Sequence: Z-F-A-FMK
Molecular Weight: 386 Da
Quantity: 5 mg

Storage

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

Instructions for Use

Reconstitution: Reconstitute using highly pure (ACS grade) DMSO. A pellet may not be visible at the bottom of the vial. Add 648 µL of DMSO to the vial to yield a 20 mM stock solution. Make sure the reagent is thoroughly in solution before use.

Cell Cultures: Cells may be cultured as required to induce apoptosis. Caspase inhibitors or their controls are typically added at the beginning of the cell culture process. Concentrations of DMSO above 1.0% may cause cellular toxicity thus masking the effect of the caspase inhibitor. Therefore, immediately prior to cell culture addition, the required amount of 20 mM stock solution should be diluted 1:10 (2 mM) or 1:20 (1 mM) in a protein-containing buffer such as PBS + 1% BSA or tissue culture media supplemented with 5 - 10% fetal bovine serum. The inhibitor or control is then added as a fraction of the total cell culture volume to achieve the desired final concentration. The following table may be used as a guide for dilutions. If the assay requires a greater concentration of inhibitor, we recommend running a solvent control to monitor any DMSO-related effect(s). Caspase inhibitors have been successfully used in tissue cultures to inhibit apoptosis at final working concentrations of 50 nM to 100 µM. This variability is largely dependent on cell type, apoptotic signal and length of culture. The investigator must establish the most effective inhibitor concentration for their particular assay. FMK-controls are typically added at the same concentration as the caspase inhibitor. Cells can then be harvested and tested for evidence of apoptosis using standard apoptosis assays.

Final Concentration of Inhibitor	Dilution into Cell Culture: 2mM stock	1mM stock
200 µM	1:10	1:5
100 µM	1:20	1:10
10 µM	1:200	1:100
1 µM	1:2000	1:1000

Intended Use

Designed for *in vitro* use as a cell permeable control for the effects of fluoromethyl-ketone derivatives on caspase activity.

Principle of the Test

Cells induced to undergo apoptosis can be cultured in the presence or absence of caspase inhibitors or their controls. Treated cells can then be assayed for evidence of apoptosis inhibition by examining either whole cells or cell lysates using standard apoptosis assays.

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).
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. The compound Z-FA-FMK, an inhibitor of cathepsins B and L but not caspases, has been used in several systems (4 - 7) as a negative control for the peptide inhibitors of caspases. 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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