

## Reagent Specifications

**Inhibitor Sequence:** Z-A-T-A-D(OMe)-FMK  
**Molecular Weight:** 540 Da  
**Quantity:** 1 mg

## Storage

Store reagent at  $\leq -20^{\circ}\text{C}$  upon arrival. Lyophilized, samples are stable for 1 year at  $-20^{\circ}$  --  $-70^{\circ}\text{C}$ . Upon reconstitution in DMSO, compound is stable for 6 months at  $\leq -20^{\circ}\text{C}$ .

## Instructions for Use

**Reconstitution:** Inhibitor must be reconstituted using highly pure (ACS grade) DMSO. A pellet may not be visible at the bottom of the vial. Add 92.6  $\mu\text{L}$  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 should be cultured as required to induce apoptosis. Caspase inhibitors are typically added at the beginning of the cell culture process. A total level of DMSO above 1.0% may cause cellular toxicity thus masking the effect of the caspase inhibitor. Immediately prior to adding to the cell culture, 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 caspase inhibitor is then added as a fraction of the total volume of the cell culture to achieve the desired final concentration. The following table may be used as a guide. If the assay requires a greater concentration of inhibitor, we recommend running a solvent control to monitor any DMSO-related effect(s).

Final Concentration of Inhibitor	Dilution into Cell Culture:	
	2mM stock	1mM stock
200 $\mu\text{M}$	1:10	1:5
100 $\mu\text{M}$	1:20	1:10
10 $\mu\text{M}$	1:200	1:100
1 $\mu\text{M}$	1:2000	1:1000

## Intended Use

Designed for *in vitro* use as a cell-permeable, irreversible inhibitor of caspase activity.

## Principle of the Test

Cells that are induced to undergo apoptosis can be cultured in the presence or absence of the caspase inhibitor. The caspase inhibitor binds to the active site of the protease. Caspase inhibitors have been successfully used in tissue cultures to inhibit apoptosis at final working concentrations of 50 nM to 100  $\mu\text{M}$ . This variability is largely dependent on cell type, apoptotic signal and length of culture. The investigator must establish the most effective concentration for their particular assay. 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). WEHD, VDVA, 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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