

Technical Data Sheet

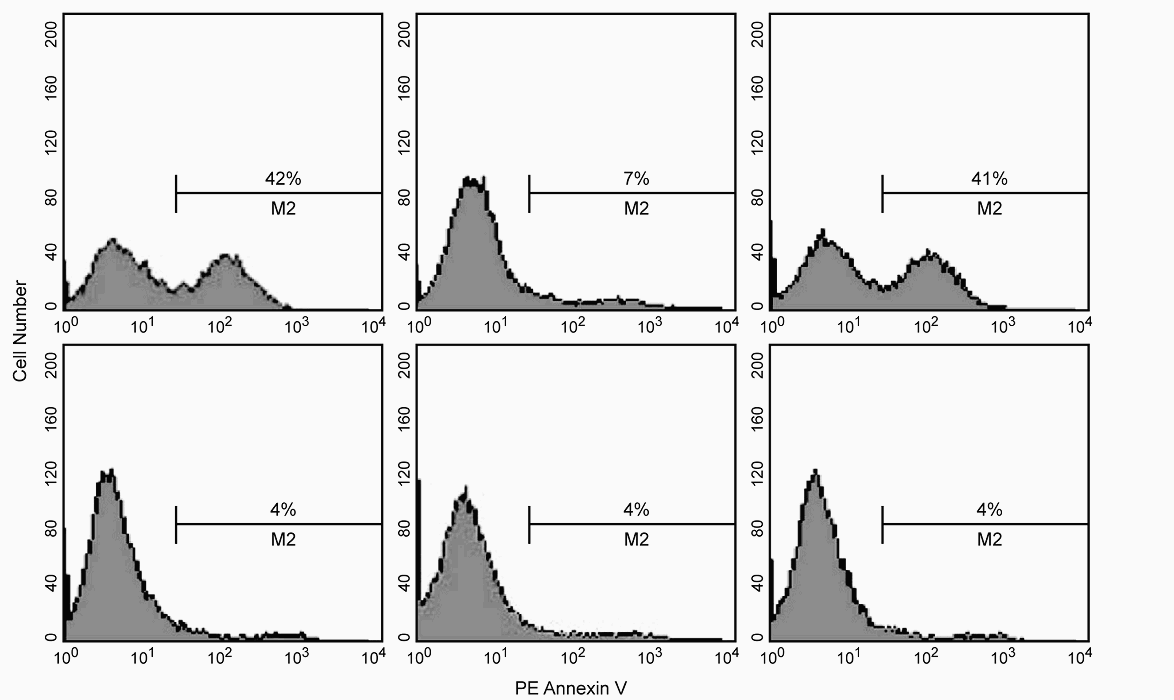
Z-IETD-FMK, Caspase-8 Inhibitor

Product Information

Material Number: 550380
Size: 1.0 mg
Storage Buffer: Lyophilized in dimethyl sulfoxide (DMSO).

Description

Members of the caspase family play key roles in inflammation and mammalian apoptosis. Z-IETD-FMK is an irreversible and cell permeable inhibitor of caspase-8. The peptide is O-methylated in the P1 position on aspartic acid providing enhanced stability and increased cell permeability. This inhibitor can be used to inhibit caspase-8 activity and to study events downstream of caspase-8 activation. Z-IETD-FMK has a molecular weight of 654 Daltons.



Flow cytometric analysis of Jurkat cells (Human T-cell leukemia; ATCC TIB-152). Jurkat cells were preincubated with the following: no inhibitor (upper left and bottom left panels), 20 μM Z-IETD-FMK (upper center and bottom center panels) or 20 μM of a negative control inhibitor Z-FA-FMK (upper right and bottom right panels) for 30 minutes, and then either left untreated (bottom row) or treated with 4 μM of camptothecin for 3 hours (top row). Following incubation, cells were collected and stained with PE Annexin V (Cat. No. 559763) to identify cells undergoing apoptosis. The results indicate that in camptothecin treated cells, approximately 42% of the cells were induced to undergo apoptosis and the use of the caspase-8 inhibitor Z-IETD-FMK reduced the level of apoptosis to that observed in untreated controls. Cells treated with Z-FA-FMK (Cat. No. 550411) showed similar results to the treated cells without inhibitor, indicating that the control inhibitor did not attenuate apoptosis.

Preparation and Storage

Avoid multiple freeze-thaws of product.
Store the lyophilized Z-IETD-FMK inhibitor at -20°C. Reconstitute the Z-IETD-FMK inhibitor in DMSO before use. The reconstituted Z-IETD-FMK inhibitor may be stored in small aliquots at -20°C.

Application Notes

Application

Fluorescence quantitation	Routinely Tested
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Recommended Assay Procedure:

The Z-IETD-FMK inhibitor is designed to be used in both *in vivo* and *in vitro* cell based assays to measure the inhibition of apoptosis. Reconstitute 1.0 mg of the Z-IETD-FMK inhibitor in DMSO. A 10 mM stock solution may be made by dissolving 1.0 mg of Z-IETD-FMK in 150

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μl DMSO. The final concentration of inhibitor may vary between experimental systems and investigators are encouraged to titrate the inhibitor for optimal performance. As a precautionary note, do not exceed a final DMSO concentration of 0.2% as higher levels may cause cellular toxicity and mask the effects of the caspase inhibitor.

Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
559763	PE Annexin V Apoptosis Detection Kit I	100 tests	(none)
550411	Z-FA-FMK, Negative Control for Caspase Inhibitors	1.0 mg	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharming/protocols for technical protocols.

References

Gregoli PA, Bondurant MC. Function of caspases in regulating apoptosis caused by erythropoietin deprivation in erythroid progenitors. *J Cell Physiol.* 1999; 178(2):133-143.(Biology)
Thornberry NA, Lazebnik Y. Caspases: enemies within. *Science.* 1998; 281(5381):1312-1316.(Biology)
Wang J, Zhen L, Klug MG, Wood D, Wu X, Mizrahi J. Involvement of caspase 3- and 8-like proteases in ceramide-induced apoptosis of cardiomyocytes. *J Card Fail.* 2000; 6(3):243-249.(Biology)