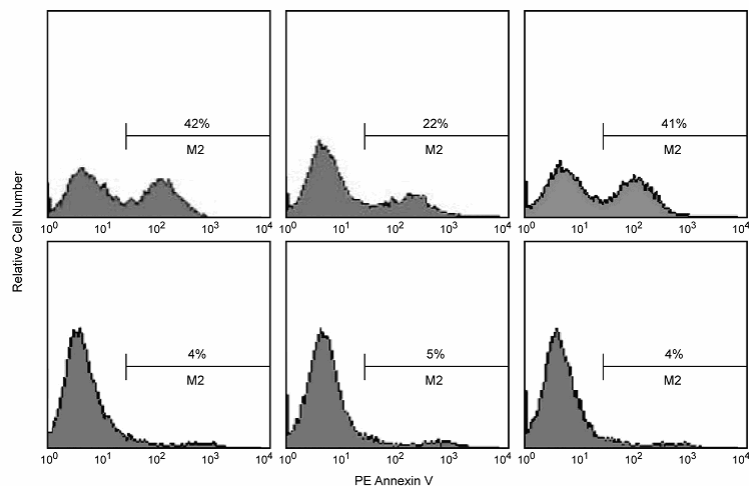


Technical Data Sheet

Z-LEHD-FMK, Caspase-9 Inhibitor**Product Information****Material Number:** 550381**Size:** 1.0 mg**Description**

Members of the caspase family play key roles in inflammation and mammalian apoptosis. Z-LEHD-FMK is an irreversible and cell permeable inhibitor of caspase-9. The peptide is O-methylated in the P1 position on aspartic acid providing enhanced stability and increased cell permeability. Z-LEHD-FMK can be used to inhibit primarily caspase-9 activity and to study events downstream of caspase-9 activation. Z-LEHD-FMK has been reported to have a molecular weight of 804 Daltons.



Flow cytometric analysis of apoptosis in Jurkat cells. Jurkat cells (Human T-cell leukemia; ATCC TIB-152) were preincubated with the following: no inhibitor (top and bottom left panels), 20 μ M Z-LEHD-FMK (top and bottom center panels) or 20 μ M control inhibitor, Z-FA-FMK (top and bottom right panels) for 30 min, and then either left untreated (bottom row) or treated with 4 μ M of camptothecin for 3 hr (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-9 inhibitor Z-LEHD-FMK reduced the level of apoptosis to half of that level observed in treated 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.

Z-LEHD-FMK, Caspase-9 Inhibitor is provided as a lyophilized powder. Store the lyophilized inhibitor at -20°C . Reconstitute the inhibitor in DMSO before use. The reconstituted inhibitor may be stored in small aliquots at -20°C .

Application Notes**Application**

Flow cytometry

Routinely Tested

Recommended Assay Procedure:

The Z-LEHD-FMK inhibitor can be used for both in vivo and in vitro cell based assays to measure the inhibition of apoptosis. While there are numerous methods to induce and measure apoptosis, Annexin V staining can be used on Jurkat cells (Human T-cell leukemia; ATCC TIB-152) for evaluating apoptosis (see figure).

Reconstitute 1.0 mg of Z-LEHD-FMK in DMSO. A 10 mM stock solution may be prepared by dissolving 1.0 mg of Z-LEHD-FMK in 124 μ l DMSO. When using the Z-LEHD-FMK inhibitor, the optimal concentration needed may vary with the experimental system being used and investigators are highly encouraged to titrate the reagent. As a precautionary note, please do not exceed a final DMSO concentration of 0.2% as higher levels may increase the risk for cellular toxicity which may mask the effect of the caspase inhibitor.

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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/pharmingen/protocols for technical protocols.

References

Maccarrone M, Lorenzon T, Bari M, Melino G, Finazzi-Agro A. Anandamide induces apoptosis in human cells via vanilloid receptors. Evidence for a protective role of cannabinoid receptors. *J Biol Chem.* 2000; 275(41):31938-31945.(Biology)

Thornberry NA, Lazebnik Y. Caspases: enemies within. *Science.* 1998; 281(5381):1312-1316.(Biology)