Z-LE(OMe)HD(OMe)-FMK (Caspase 9 Inhibitor)

Catalog No:	**NBP2-29398**
Sequence:	**Z-Leu-Glu(OMe)-His-Asp(OMe)-FMK.** **TFA Caspase Inhibitor** **Z-LE(OMe)HD(OMe)-FMK** **Z-LEHD-FMK**
Molecular Weight:	690 (not including TFA salt)
Formula:	$C_{32}H_{43}N_6O_{10}F$
Storage:	Store well desiccated and under nitrogen, very hygroscopic. The solid product is stable in the desiccator at room temperature or 4°C for 1 year. However, we recommended storing stable at -20°C. For extra precaution, store the solid product under a blanket of nitrogen because it is hygroscopic material.
Form:	Tan crystals, (crystalline gel)
Analytical Data:	Mass Spec: M+1=691.2 TLC: B:8, A:1, W:1, Rf:065 NMR: All functional groups are present

Background

Members of the caspase family play key roles in apoptosis and inflammation. Z-LE(OMe)HD(OMe)-FMK is a cellpermeable caspase peptide inhibitor that irreversibly binds to the catalytic site of caspases proteases, and inhibits caspase mediated apoptosis by preventing the processing of pro-caspases to their active forms (reviewed in 1-3). ZLEHD-FMK was first described is an irreversible and cell permeable inhibitor of Caspase 9.

The Z-LE(OMe)HD(OMe)-FMK peptide is O-methylated in the P1 and P3 positions providing enhanced stability and increased cell permeability. Z-LE(OMe)HD(OMe)-FMK (Z-LEHD-FMK) is typically used in assays to inhibit apoptosis. Z-LEHD-FMK has been used in many different types of apoptosis assays and published using a number of model system. Users may want to consult the literature for additional information regarding applications for Z-LEHDFMK. Z-LEHD-FMK is recommended as a search term for identifying references in PubMed using this peptide inhibitor.

Solubility

Make a stock solution of 5, 10 or 20 mM in high purity DMSO (>99.9%). The stock solution is stable at -20°C for 6-8 months. Avoid repeated freeze/thaw cycles of the stock solution. For multiple uses, we suggest aliquoting the stock solution prior to freezing. Bring the solution to room temperature before opening the vial cap.

Assay Method for the Z-LEHD-FMK Inhibitor:

Materials:

Dissolve 5 mg of Z-LEHD-FMK in DMSO to get appropriate concentration:

310 µl DMSO = 20 mM
620 µl DMSO = 10 mM
1240 µl DMSO = 5 mM

Method:

Add 2 µl of above stock solutions to 1 ml of culture medium containing cells to give final DMSO concentration of 0.2%. Levels of DMSO above this may cause some cellular toxicity thus masking the effect of the ICE-protease inhibitors. 2 µl of 10 mM stock in 1 ml medium = 20 µM final Z-LEHD-FMK concentration.

Notes:

1 *This inhibitor is designed as a methyl ester to facilitate cell permeability. If the intended use is on purified or recombinant enzymes, esterase should be added to generate the free carboxyl groups. Please contact us for more details.*
2. *For in-vivo or in-vitro experiments extending longer than 12 hrs, fresh inhibitor may have to be added to culture medium or injected into animals.*

Reference:

1. Thornberry, N.A., and Lazebnik, Y. 1998. *Science* 281:1312-1316
2. Gregoli, P.A., and M.C. Bondurant. 1999. *J. Cell Pysiol.* 178:133-143.
3. Schrantz, N., D.A. Blanchard, M.T. Auffredou, S. Sharma, G. Leca, and A. Vazquez. 1999. *Oncogene* 18:3511-3519.