



PRODUCT DATA SHEET

Product: Z-IETD-FMK (Caspase-8(FLICE) Inhibitor)

Cat. No.: AB-004 (3 mg)

Chemical Name:

Z-Ile-Glu(OMe)-Thr-Asp(OMe)-FMK

Formula:

C₃₀H₄₃N₄O₁₁F

Molecular Weight:

654

Description:

Peptide-fluoromethyl ketone inhibitor of caspase-8(FLICE).

The CH₂F or FMK (fluoromethyl ketone) inhibitor has several advantages over other types of derivatives: Penetrates cell membranes, is non-toxic to cells, irreversible inhibition.

Introduction:

Caspase-8 (also known as FLICE, MACH or Mch5) is a member of the caspase family of cysteine proteases. Caspases play an important role in apoptosis signaling and effector mechanisms. Caspase-8 is most similar to caspase-10, both of which have "death domain" motifs. Caspase-8 appears to be physically associated with the signaling mechanism during Fas-mediated cell death and its association with Fas or tumor necrosis factor receptors via an interaction with FADD suggests it functions as an initiator rather than an effector of the cell death pathway. Thus, caspase-8 is an upstream activator in the protease cascade that proteolytically matures other caspases.

Form:

Off white solid

Specificity:

Highly specific inhibitor of caspase-8. May have some inhibitory activity towards caspase-6.

Applications:

For caspase-8 fluorometric assays using the Caspase-8(FLICE) Fluorogenic Substrate, Ac-IETD-AFC (Cat. No. AC-004), Caspase-8(FLICE) Inhibitor can be used to assess the contribution of contaminating proteases to the overall rate of proteolysis.

Protocol:

Dissolve the Caspase-8(FLICE) Inhibitor in high purity (>99.9%) DMSO before use.

For use on intact cells:

1. Prepare desired concentrated stock solutions as follows: 3 mg Z-IETD-FMK in 459 μ L DMSO = 10 mM
2. Adding 2 μ L of a 10 mM stock solution to 1 mL of culture medium gives a final Z-IETD-FMK concentration of 20 μ M. Effective final concentrations are estimated to be 5-20 μ M.
Note: Levels of DMSO above 0.2% may cause some cellular toxicity, thus masking the effect of the protease inhibitor.

For extended use *in vivo* and *in vitro*:

For experiments extending 12 to 48 hours, fresh inhibitor may have to be added (injected) due to inactivation of the inhibitor by endogenous cysteine proteases.

IMPORTANT NOTE for *in vitro* use: Our peptide inhibitors are synthesized as methyl esters to enhance cell permeability. In intact cells, the methyl groups are removed by endogenous enzymes. For *in vitro* experiments with purified enzymes, however, the methyl groups must first be removed by treating the inhibitor with esterase. A procedure is available upon request.

Storage:

Solid product is stable for at least 3 years when stored in a desiccator at -20°C. We recommend 4°C storage of DMSO solution. DMSO stock solutions have a shelf-life of 6-8 months when stored at -20°C. Keep sealed after removing from the freezer until vial temperature equilibrates with room temperature.

Limitations:

For research use only. Not for use in diagnostics or in humans.

Warranty:

No warranties, expressed or implied, are made regarding the use of this product. KAMIYA BIOMEDICAL COMPANY is not liable for any damage, personal injury, or economic loss caused by this product.