PRODUCT DATA SHEET

Product: Z-LEHD-FMK (Caspase-4, -5, -9 Inhibitor) TFA salt

Cat. No.: AB-010 (1 mg)

Chemical Name:
Z-Leu-Glu(OMe)-His-Asp(OMe)-CH_2F (TFA salt)

Formula:
$C_{32}H_{43}N_6O_{10}F$

Molecular Weight:
690 (not including TFA salt)

Description:
Trifluoroacetic acid salt of the fluoromethyl ketone peptide inhibitor of caspase-4, -5, and -9.

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

Introduction:
Caspase-4 (also known as ICErel-II, TX, or ICH-2), caspase-5 (also known as ICErel-III or TY), and caspase-9 (also known as ICE-LAP6 or Mch6) are members of the caspase family of cysteine proteases involved in apoptosis. Caspase-4 and -5 belong to Group I (along with caspase-1), which prefer the tetrapeptide substrate sequence WEHD, and are thought to be involved in inflammation through the maturation of pro-IL-1ß. Their role in apoptosis, however, is unclear. Caspase-9 is a member of Group III, which prefer the substrate sequence (L/V)EXD. Since caspase-9 has a strict requirement for His in the P4 position, it is expected that the LEHD inhibitor sequence would work well on this caspase. The Group III caspases optimal recognition sequence resembles the activation sites within several effector caspase proenzymes, implicating the Group III enzymes as upstream components in the proteolytic cascade that amplifies the death signal.

Specificity:
Inhibitor of several caspase activities. Strong inhibition of caspase-4, -5, and -9 activities. Weak inhibition of caspase-1, -2, -6, and -8 activities.

Form:
Tan solid

Solubility:
Soluble in DMSO.

Protocol:
Dissolve Caspase-4, -5, -9 Inhibitor in high purity DMSO (>99.9%) before use to make a stock solution of 20 mM.

For use on intact cells:

1. Prepare desired concentrated stock solutions as follows: 1 mg Z-LEHD-FMK•TFA
   in 62 µL DMSO = 20 mM
   in 124 µL DMSO = 10 mM
   in 248 µL DMSO = 5 mM, etc.
2. Adding 2 µL of above stock solutions to 1 mL of culture medium containing cells gives a final DMSO concentration of 0.2%. Levels of DMSO above 0.2% may cause some cellular toxicity in culture medium, thus masking the effect of the protease inhibitor. Adding 2 µL of a 10 mM stock solution to 1 mL of culture medium gives a final Z-LEHD-FMK•TFA concentration of 20 µM.

For extended use *in vivo* or *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:
Z-LEHD-FMK•TFA is stable for 3 years when stored desiccated at -20°C. (Because this product is very hygroscopic, it is suggested that it be keep well desiccated and under nitrogen.) DMSO stock solutions have a shelf-life of 6-8 months when stored at –20°C.

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Keep sealed after removing from the freezer until the temperature of the vial equilibrates to 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.