PRODUCT DATA SHEET

Product: **Ac-LEHD-AFC**

Cat. No.: **AC-010 (5 mg)**

Chemical Name:
Acetyl-Leu-Glu-His-Asp-AFC•TFA

Molecular Weight:
765 (not including TFA salt)
879 (including TFA salt)

Form:
White solid

Purity:
>95% by HPLC

Description:
Trifluoroacetic acid salt of a peptide substrate labeled at the carboxy end with AFC (7-amino-4-trifluoromethyl coumarin). Designed to measure Caspase-4, Caspase-5, or Caspase-9 activity *in vitro*.

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. Caspases-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)jEXD. Since Caspase-9 has a strict requirement for His in the P4 position, it is not unexpected 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.

Principal:
A synthetic peptide substrate, Ac-LEHD, has been labeled with AFC at the carboxy end. AFC is a fluorescent molecule whose release from the substrate can be used to measure Caspase-4, -5, or -9 activity. Caspase-4, -5, or -9 activity in the sample is proportional to the amount of free AFC produced.

When AFC is attached to the peptide substrate, it produces a blue fluorescence upon exposure to UV light (400 nm). Caspase-4, -5, or -9 enzymatically cleaves the AFC-substrate and releases free AFC, which produces a yellow-green fluorescence at 505 nm when exposed to UV light.

AFC has two advantages over other fluorogenic labels. The wide Stokes shift between bound and free AFC enables the substrate to be both chromogenic (yellow-green color visible to the naked eye) and fluorogenic (emission at 505 nm). The wide Stokes shift also makes the assay more sensitive.

Specificity:
Serves equally well as a substrate for Caspases-4, -5, and -9. Can also serve as a weak substrate for Caspases-1, -2, -6, and -8 at an efficiency of 30 - 40% .

Applications:
For *in vitro* assays of Caspases-4, -5, and -9 activity. Can be used with purified or partially purified enzymes, or possibly with crude cell lysates (if the Caspase-4, -5, -9 Inhibitor is included to determine background protease activity).

Protocol:
Dissolve the caspase inhibitor in high purity DMSO (>99.9%) before use.

Fluorometer calibration:
The fluorometer is calibrated using known concentrations of free AFC (Excitation = 400 nm, Emission = 505 nm) to generate a calibration curve of fluorescence versus µmoles AFC.

Samples:
Can be either purified or partially purified caspase preparations. Crude cell lysates can be assayed if the non-specific protease background is determined using our Caspase-4, -5, -9 Inhibitor (Z-LEHD-FMK, Cat. No. AB-010).

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General Fluorometric Assay Procedure:
CAUTION: The following procedure is provided only as an example for reference purposes. The user should determine the optimal conditions for their system.

Materials:

- Buffer: 0.1 M HEPES buffer, pH 7.5 with 20% (v/v) glycerol, 5 mM DTT, 0.5 mM EDTA.
- Substrate: 20 mM stock solution of Ac-LEHD-AFC in high purity (>99.9%) DMSO.
- Enzyme: Cell lysate or purified enzyme solution (~15 nanograms enzyme).
- Fluorescence Calibrator: 80 µM free AFC in DMSO.

Method:

1. Add 10 µL of enzyme to 490 µL buffer. Mix. Incubate at 30°C for 30 minutes.
2. With fluorometer adjusted to 400 nm excitation and 505 nm emission, add 20 µL of substrate to enzyme solution.
3. Record increase in fluorescence from T_0 to T_{end} where fluorescence generated at T_{end} are significantly different from those at T_0.
4. Record fluorescence units generated by 10, 20 and 30 µL free AFC in 490, 480 and 470 µL buffer solution, respectively.
5. Graph fluorescence units vs. micromole AFC. Use slope to convert fluorescence units generated by enzyme to activity.

The number of assays that can be run with the substrate provided depends upon the reaction volumes.

Storage:
Store Ac-LEHD-AFC in a desiccator at room temperature or 4°C. For long term, 4°C is recommended. Ac-LEHD-AFC has a shelf-life of at least two years if stored at 4°C. DMSO solutions have a shelf-life of one year if stored at -20°C.

Limitations:
For *in vitro* 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.