PRODUCT DATA SHEET

Product: Ac-IETD-AFC (Fluorogenic caspase-8/FLICE substrate)

Cat. No.: **AC-004 (5 mg)**

Chemical Name:
Ac-Ile-Glu-Thr-Asp-7-AFC

Molecular Weight:
729

Formula:
$C_{31}H_{38}N_5O_{12}F_3$

Form:
White lyophilized powder

Purity:
>98% by HPLC

Description:
Synthetic peptide substrate labeled at the carboxy end with AFC (7-amino-4-trifluoromethyl coumaride). Designed to measure caspase-8 (FLICE) activity *in vitro*.

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.

Principal:
A synthetic peptide substrate, Ac-Ile-Glu-Thr-Asp, 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-8 activity. Caspase-8 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-8 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:
Highly specific substrate for caspase-8. May be weak substrate for caspase-6.

Applications:
For *in vitro* assays of caspase-8 activity. Can be used with purified or partially purified enzymes (~15 ng enzyme), or possibly with crude cell lysates (if the caspase-8 Inhibitor is included to determine background protease activity).

Protocol:
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 enzyme preparations. Application to crude cell lysates has not been confirmed. If crude cell lysates are to be assayed, the non-specific protease background must be determined using our Caspase-8/FLICE Inhibitor, Z-IETD-FMK (Cat. No. AB-004).

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.

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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-IETD-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 at 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 5 mg of substrate provided depends upon the reaction volumes.

Storage:

Store Caspase-8 Substrate in a desiccator at 4°C for long term. DMSO/DMF stock solutions have a shelf-life of at least 1 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.