PRODUCT DATA SHEET

Product: **Ac-DEVD-AFC (Fluorogenic caspase-3(CPP32) substrate)**

Cat. No.: **AC-003 (10 mg)**

Chemical Name:
Ac-Asp-Glu-Val-Asp-AFC

Molecular Weight:
729

Form:
White lyophilized powder

Purity:
>95% by HPLC

Description:
Peptide substrate labeled at the carboxy end with AFC (7-amino-4-trifluoromethyl coumarin). Designed to measure caspase-3(CPP32) activity or caspase-1, -4, -7, -8, or -10 activities *in vitro*.

Introduction:
Caspase-3 is a member of the caspase family of proteases involved in apoptosis induction. All apoptotic pathways studied to date involve proteolytic activation of caspase-3 as a central event in the progression of cell death. Although the death-inducing consequences of caspase-3 activation have not been conclusively established, several crucial substrates for the protease have been identified *in vitro*, including DNA-dependent protein kinase, Poly(ADP-ribose) Polymerase (PARP), Replication factor C, and gelsolin. These substrates are involved in the later stages of apoptosis, strongly suggesting that caspase-3 has a key role in promoting the final processes leading to cell death.

Principal:
A synthetic peptide substrate, Ac-Asp-Glu-Val-Asp, has been labeled with AFC, a fluorescent molecule whose release from the substrate can be used to measure caspase-3 activity. When AFC is attached to the peptide substrate, it produces a blue fluorescence upon exposure to UV light (400 nm). Caspase-3 enzymatically cleaves the AFC-substrate and releases free AFC, which produces a yellow-green fluorescence at 505 nm when exposed to UV light. Caspase-3 activity in the sample is proportional to the amount of free AFC produced.

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 as a substrate for caspase-3, and can be used for caspase-1, -4, -7, -8, and -10. Can be a very weak substrate for caspase-6. Is not a substrate for caspase-2.

Applications:
For *in vitro* assays of caspase-3 and caspase-7 activities. Can also be used to assay caspase-1, -4, -8, and -10 activities. Can be used with purified or partially purified enzymes, or possibly with crude cell lysates (if the Caspase-3/CPP32 Inhibitor is included to determine background protease activity).

Solubility:
Soluble in DMSO and DMF.

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-3/CPP32 Inhibitor (Cat. No. AB-003).

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 7.4 with 2 mM DTT, 0.1% CHAPS and 1% sucrose.
- Substrate: 20 mM stock solution of Ac-DEVD-AFC in high purity (>99.9%) DMSO or DMF.
- Enzyme: Cell lysate or enzyme solution in PBS.
- Fluorescence calibrator: 80 µM free AFC in DMSO.

Method:

1. To buffer add ~15 nanograms of purified enzyme or 50 µL of cell lysate in a total volume of 500 µL.
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 10 mg of substrate provided depends upon the reaction volumes.

Storage and Stability:

Store Ac-DEVD-AFC in a desiccator at room temperature or 4°C. Store DMSO/DMF stock solutions at –20°C. DMSO stock solutions have a shelf-life of 1 year if stored as recommended.

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.