

Product Information

N-Acetyl-Ala-Glu-Val-Asp 7-Amido-4-trifluoromethylcoumarin

Product Number **A 0973**

Storage Temperature $-20\text{ }^{\circ}\text{C}$

Product Description

Molecular formula: $\text{C}_{29}\text{H}_{34}\text{N}_5\text{O}_{11}\text{F}_3$
Mol. wt.: 685.6

N-Acetyl-Ala-Glu-Val-Asp 7-amido-4-trifluoromethylcoumarin (Ac-AEVD-AFC) is a fluorometric substrate suitable for the determination of caspase 8 and caspase 9 activity.

When Ac-AEVD-AFC is hydrolyzed, the free AFC produced in the reaction can be quantified by fluorometric detection (excitation 400 nm, emission 505nm) or by spectrophotometric detection at 380 nm (extinction coefficient = 12,600 at pH 7.2). When used in an enzyme assay with fluorescence detection, AFC has higher sensitivity than 4-methoxy-2-naphthylamide (MNA).

Caspases (cysteine aspartate proteases) are responsible for specific protein cleavage in apoptotic cells. Caspase 8 plays a central role in apoptosis because it can activate all known caspases. Caspase 9 is a member of the CED-3 subfamily and has high homology with caspase 3.

Preparation Instructions

Prepare stock 20 mM solutions in dry DMSO.

Storage/Stability

Store at $-20\text{ }^{\circ}\text{C}$. Material stable for at least one year, if stored as recommended.

Store stock solutions in frozen aliquots at $-20\text{ }^{\circ}\text{C}$. Stock solutions are stable 6-8 months under these conditions. Allow the material to warm to room temperature before use to ensure stability.

References

1. Duan, H. et al., ICE-LAP6, a novel member of the ICE/Ced-3 gene family, is activated by the cytotoxic T cell protease granzyme B. *J. Biol. Chem.*, **271**, 16720-16724 (1996).
2. Srinivasula, S.M. et al., The Ced-3/interleukin 1beta converting enzyme-like homolog Mch6 and the lamin-cleaving enzyme Mch2alpha are substrates for the apoptotic mediator CPP32. *J. Biol. Chem.*, **271**, 27099-27106 (1996).
3. Lojda, Z., The use of substrates with 7-amino-3-trifluoromethylcoumarin (AFC) leaving group in the localization of protease activities *in situ*. *Acta Histochem.*, **98**, 215-228 (1996).
4. Johansen, H. T., et al., Colorimetric and fluorimetric microplate assays for legumain and a staining reaction for detection of the enzyme after electrophoresis. *Anal. Biochem.*, **273**, 278-283 (1999).

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