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ProductInformation

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

Product Number **A 0723** Storage Temperature –20 °C

Product Description

Molecular formula: $C_{24}H_{27}N_4O_8F_3$ Mol. wt.: 556.5

N-Acetyl-Val-Ala-Asp 7-amido-4-trifluoromethylcoumarin (Ac-VAD-AFC) is a fluorometric substrate designed for the determination of caspase activity.

When Ac-VAD-AFC is hydrolyzed, the free AFC produced can be quantified by fluorometric detection (excitation 400 nm, emission 505 nm) 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).

Ac-VAD-AFC is a pan caspase substrate, able to be hydrolysed by most, if not all, caspases. Caspases are a group of cysteine aspartate proteases that play a role in apoptosis. Caspase activity can lead to morphological features such as membrane blebbing, cytoplasmic and nuclear condensation, DNA fragmentation, and the formation of apoptotic bodies.

Preparation Instructions

Prepare stock 20mM solutions in DMSO.

Storage/Stability

Store at -20 °C. Material stable for at least one year, if stored as recommended.

Store stock solutions in frozen aliquots at -20 °C. Allow the material to warm to room temperature before use to ensure stability.

References

- Thornberry, N.A. et al., A novel heterodimeric cysteine protease is required for interleukin-1 beta processing in monocytes. Nature, **356**, 768-774 (1992).
- Nicholson, D.W., and Thornberry, N.A., Caspases: killer proteases. Trends. Biochem. Sci., 22, 299-306 (1997).
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- Lojda, Z., The use of substrates with 7-amino-3trifluoromethylcoumarine (AFC) leaving group in the localization of protease activities *in situ*. Acta Histochem., **98**, 215-228 (1996).
- 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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