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## Product Information

### N-Acetyl-Leu-Glu-Val-Asp 7-Amido-4-trifluoromethylcoumarin

Product Number **A 2099**

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

#### Product Description

Molecular formula:  $\text{C}_{32}\text{H}_{40}\text{F}_3\text{N}_5\text{O}_{11}$

Mol. wt.: 727.7

N-Acetyl-Leu-Glu-Val-Asp 7-Amido-4-trifluoromethylcoumarin (Ac-LEVD-AFC) is a fluorometric substrate suitable for the determination of caspase 4 activity.

When Ac-LEVD-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).

Caspases (cysteine aspartate proteases) are responsible for specific protein cleavage in apoptotic cells. Caspase 4, a member of the caspase-1 subfamily, has a close homology to caspase 5. It is found in most tissues with the exception of brain tissue. The preferred substrate sequence of caspase 4 is LEVD/GW.

#### Preparation Instructions

Prepare stock 20 mM solutions in DMSO.

#### Storage/Stability

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

Store stock solutions in frozen aliquots at  $-20^{\circ}\text{C}$ . Allow the material to warm to room temperature before use to ensure stability.

#### References

1. Talanian, R.V. et al., Substrate specificities of caspase family proteases. *J. Biol. Chem.*, **272**, 9677-9682 (1997).
2. Cohen, G. M., Caspases: the executioners of apoptosis. *Biochem. J.*, **326**, 1-16 (1997).
3. Lojda, Z., The use of substrates with 7-amino-3-trifluoromethylcoumarine (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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