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ProductInformation

N-Acetyl-Arg-Gly-Phe-Phe-Pro
7-Amido-4-Trifluoromethylcoumarin

Product Number: **C 6983**
Storage Temperature: -20 °C

Product Description

Molecular Formula: $C_{49}H_{52}N_9O_9F_3$
Molecular Wt.: 968.00 Da

N-Acetyl-Arg-Gly-Phe-Phe-Pro 7-Amido-4-trifluoromethylcoumarin (Ac-RGFFP-AFC) is a fluorogenic substrate suitable for the assay of cathepsin D.

Because the preferential cleavage site for cathepsin D is Phe-Phe, the first reaction product is FP-AFC. For detection, the reaction mixture using must include dipeptidyl dipeptidase IV (DPP-IV). When FP-AFC is hydrolyzed by DPP-IV, the free AFC produced 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). Used in an enzyme assay with fluorescence detection, AFC has higher sensitivity than 4-methoxy-2-naphthylamide (MNA).[2]

Cathepsin D, a member of the mammalian aspartic proteinase family, is a lysosomal enzyme widely distributed in almost all cells. It has a high sequesnce homology with cathepsin E. Both cathepsins have broad substrate specificities, digesting proteins at the recognition sequence -Phe-Phe-. In addition to degradation of intracellular and extracellular proteins, they may play a role in the generation of biologically active peptides and processing exogenous antigens.[3]

Preparation Instructions

Prepare stock 10 mM 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. 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. Smith, R. E., et al., The evolution of proteinase substrates with special reference to dipeptidylpeptidase IV. Histochem. J., **24,** 637-47 (1992).
2. 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).
3. Yasuda, Y. et al. Characterization of new fluorogenic substrates for the rapid and sensitive assay opf cathepsin E and cathepsin D. J. Biochem. (Tokyo), **125,** 1137-1143 (1999).
4. Pimenta, D.C., et al., Substrate specificity of human cathepsin D using internally quenched fluorescent peptides derived from reactive site loop of kallistatin. Biochim. Biophys. Acta, **1544,** 113-122 (2001).

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