

Saint Louis, Missouri 63103 USA
Telephone (800) 325-5832 (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

## **ProductInformation**

N-Acetyl-Lys-Pro-Arg 7-Amino-4-Trifluoromethylcoumarin

Product Number **C6608** Storage Temperature –20 °C

**Product Description** 

Molecular formula: C<sub>29</sub>H<sub>39</sub>F<sub>3</sub>N<sub>8</sub>O<sub>6</sub>

Mol. wt.: 652.7

N-Acetyl-Lys-Pro-Arg 7-Amino-4-trifluoromethylcoumarin (Ac-KPR-AFC) is a fluorogenic substrate suitable for the assay of tryptase.<sup>1</sup>

When Ac-KPR-AFC is hydrolyzed, the free AFC produced in the reaction 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).<sup>2</sup>

Tryptases are a group of trypsin-related serine proteases expressed in mast cells and bas ophils. They are generally secreted as active, heparin-bound oligomers that are resistant to inactivation by plasma antiproteases. Human tryptases have been divided into three groups,  $\alpha, \, \beta,$  and  $\gamma. \, \alpha\text{-Tryptase}$  is the circulating isoform and is expressed by basophils. The  $\beta\text{-isoforms}$  are stored in secretory granules and are found in lung and skin mast cells.  $\gamma\text{-Tryptases}$  are referred to as novel tryptases and are distinct from  $\alpha, \, \beta,$  and other human tryptases.  $^{3,4}$ 

 $\beta$ -Tryptases are approximately 99% homologous with a single glycosylation the difference between  $\beta$ I- and  $\beta$ II-tryptase. Both  $\beta$ I- and  $\beta$ II-tryptase have a cleavage preference after Lys or Arg. Preference for extended specificity is Asp in P2, Arg or Lys in P3, and Pro at P4.  $^5$ 

Z-KPR-AFC is supplied as a trifluoroacetate salt.

## **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

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- 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. Pallaoro, M., et al., Characterization of Genes encoding known and novel human mast cell tryptases on chromosome 16p13.3. J. Biol Chem., **274**, 3355 (1999).
- Caughey, G. H., et al., Characterization of human γ-tryptases, novel members of the chrosome 16p mast cell tryptase and prostatin gene families.
   J. Immunol., 164, 6566-75 (2000).
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JXU,JWM,RBG,MAM 12/05-1