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ProductInformation

4-Methylumbelliferyl 4-guanidinobenzoate hydrochloride monohydrate

Product Number **M 4149**
Storage Temperature -0 °C

Product Description

Molecular Formula: $C_{18}H_{15}N_3O_4 \bullet HCl$
Molecular Weight: 373.8
CAS Number: 34197-46-1
Melting Point: 219-221 °C[1]
Synonyms: MUGB

4-Methylumbelliferyl 4-guanidinobenzoate hydrochloride is a sensitive probe for the measurement of minute amounts of serine proteases involved in clotting, clot removal, and complement action. The ester is non fluorescent, but the released methylumbelliferone fluoresces and provides an increase in sensitivity of about two orders of magnitude over p-nitrophenyl 4-guanidinobenzoate.[1] 4-Methylumbelliferyl 4-guanidinobenzoate is subject to base catalyzed hydrolysis, therefore the rate of this reaction must be determined in the absence of enzyme. The concentration of trypsin can be determined in two independent ways from the end-point titration: from the fluorescence yield at the end point or the known initial concentration of the titrant.[1]

The optimal excitation and emission wavelengths for the measurement of methylumbelliferone fluorescence must be determined for the selected combination of buffer and pH used with the enzyme. In the case of trypsin, in 0.1 M sodium phosphate buffer, pH 6.0, the excitation and emission wavelengths were 323 and 446 nm, respectively.[1] The substrate solution and enzyme solutions are successively added to the requisite buffer in a cuvette. The change in fluorescence intensity with time was recorded and compared with that of a control containing no enzyme. In the first examination of a system, the substrate concentration to enzyme concentration ratios ranged from 1.3 to 80. Typically, the enzyme and substrate concentrations in the cuvette were 0.1-0.2 μM and 1.0 μM respectively.[2]

MUGB was initially tested on α-chymotrypsin, trypsin, thrombin, and Factor X_A.[2,3] It has also been used to assess the molar quantity of active sites generated nonproteolytically by streptokinase in human plasminogen[4] and in the study of recombinant human mast cell tryptase, a trypsin-like serine protease.[5,6] Subunit concentration of enzymatically active human mast cell tryptases was determined by burst titration with MUGB.[6]

Precautions and Disclaimer

For Laboratory Use Only. Not for drug, household or other uses.

Preparation Instructions

This product is soluble in dimethylformamide (50 mg/ml) with heat, yielding a clear, faint yellow solution. MUBG can be prepared in N-methylpyrrolid-2-one (10 mM, 3.7 mg/ml)[2]

Storage/Stability

Solutions of MUBG (10 mM) were prepared in N-methylpyrrolid-2-one, then diluted to 0.1 to 0.2 mM with 1 mM HCl before use. Frozen dilute solutions can be stored for several weeks without deterioration.[2]

References

1. Coleman, H. G., et al., Some sensitive methods for the assay of trypsinlike enzymes. Methods Enzymol., **45**, 12-28 (1978).
2. Jameson, G. W., et al., Determination of the operational molarity of solutions of bovine alpha-chymotrypsin, trypsin, thrombin and Factor X_A by spectrofluorimetric titration. Biochem J., **131(1)**, 107-117 (1973).
3. Sealock, R. W., and Laskowski, M., Jr., Thermodynamics and kinetics of the reactive site peptide-bond hydrolysis in bovine pancreatic secretory trypsin inhibitor (Kazal). Biochemistry, **12(17)**, 3139-3146 (1973).

4. Gladysheva, I. P., et al., Chimerism reveals a role for the streptokinase B-domain in nonproteolytic active site formation, substrate, and inhibitor interactions. J. Biol. Chem., **277(30)**, 26846-26851 (2002).
5. Niles, A. L., et al., Recombinant human mast cell tryptase: stable expression in *Pichia pastoris* and purification of fully active enzyme. Biotechnol. Appl. Biochem., **28(Pt. 2)**, 125–131 (1998).
6. Selwood, T., et al, Diverse stability and catalytic properties of human tryptase α and β isoforms are mediated by residue differences at the S1 pocket. Biochemistry, **41(10)**, 3329-3340 (2002).

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