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ProductInformation

4-Methylumbelliferyl β-D-N,N′,N′-triacetylchitotrioside hydrate

Product Number **M 5639**
Storage Temperature 2-8 °C

Product Description

Molecular Formula: $C_{34}H_{47}N_3O_{18}$
Molecular Weight: 785.8
CAS Number: 53643-13-3
Extinction Coefficient: E^{mM} = 12.3 (316 nm, water)[1]

This product has some fluorescent properties as the uncleaved molecule (λ_{ex} = 330 nm, λ_{emm} = 375 nm) and has been used to monitor saccharide binding with lysozyme.[1] This product has also been used as a fluorogenic substrate for lysozyme[1] and in the detection of endo-chitinases, together with the mono- and disaccharide-analogs.[2]

Cleavage of this substrate produces a fluorescent product, 4-methylumbelliferone (λ_{ex} = 360 nm, λ_{emm}= 455 nm). The released methylumbelliferone can be reliably be measured at concentrations of 10^{-10} M.

The anionic form of methylumbelliferone is responsible for the observed fluorescence and equilibrium does not favor anion formation at the acid pH values used for the assay of many hydrolases. Enzyme-catalyzed assays based on the release of this compound are usually conducted at low pH. The reaction is then stopped and the fluorescent anion formed by the addition of a strong alkaline buffer.[3] The fluoresence at pH 10.4 is 36% greater than the intensity at pH 7.4 and 90% greater than at pH 4.6.[4]

Precautions and Disclaimer

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

Preparation Instructions

This product is soluble in pyridine:water (1:1) (5 mg/ml) and DMF:water (1:1) (10 mg/ml) with heat, yielding a colorless solution. It has also been reported soluble in 20 mM sodium actate, pH 5.0, containing 0.1 M NaCl (3 μM).[4]

References

1. Yang, Y., and Hamaguchi, K., Hydrolysis of 4-methylumbelliferyl N-acetyl-chitotrioside catalyzed by hen and turkey lysozymes. pH dependence of the kinetics constants. J. Biochem. (Tokyo), **87(4)**, 1003-1014 (1980).
2. Tronsmo, A., and Harman, G. E., Detection and quantification of N-acetyl-beta-D-glucosaminidase, chitobiosidase, and endochitinase in solutions and on gels. Anal. Biochem., **208(1)**, 74-79 (1993).
3. John, R. A., in Photometric assays. Enzyme Assays A Practical Approach, Eisenthal, R., and Danson, M. J., eds., IRL Press (New York, NY: 1992), pp. 59-92.
4. Jacks, T. J., and Kircher, H. W., Fluorometric assay for the hydrolytic activity of lipase using fatty acyl esters of 4-methylumbelliferone. Anal. Biochem., **21(2)**, 279-285 (1967).

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