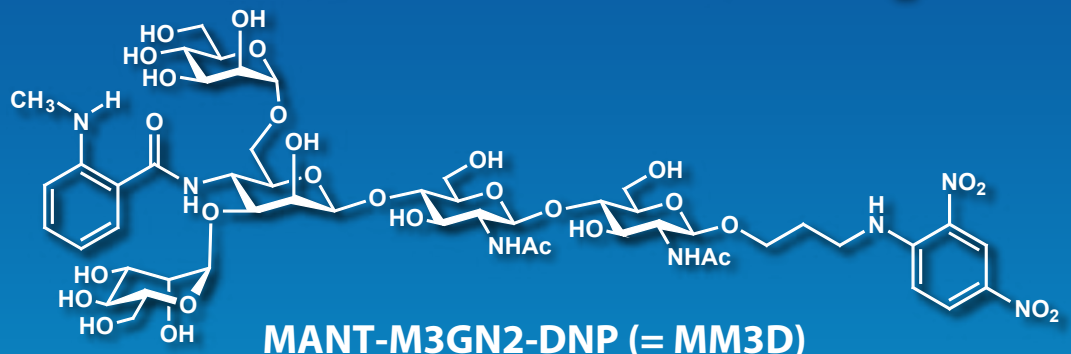
LIFE SCIENCE

MM3D-Fluorogenic Probe for Real-Time Measurement of ENGase Activity



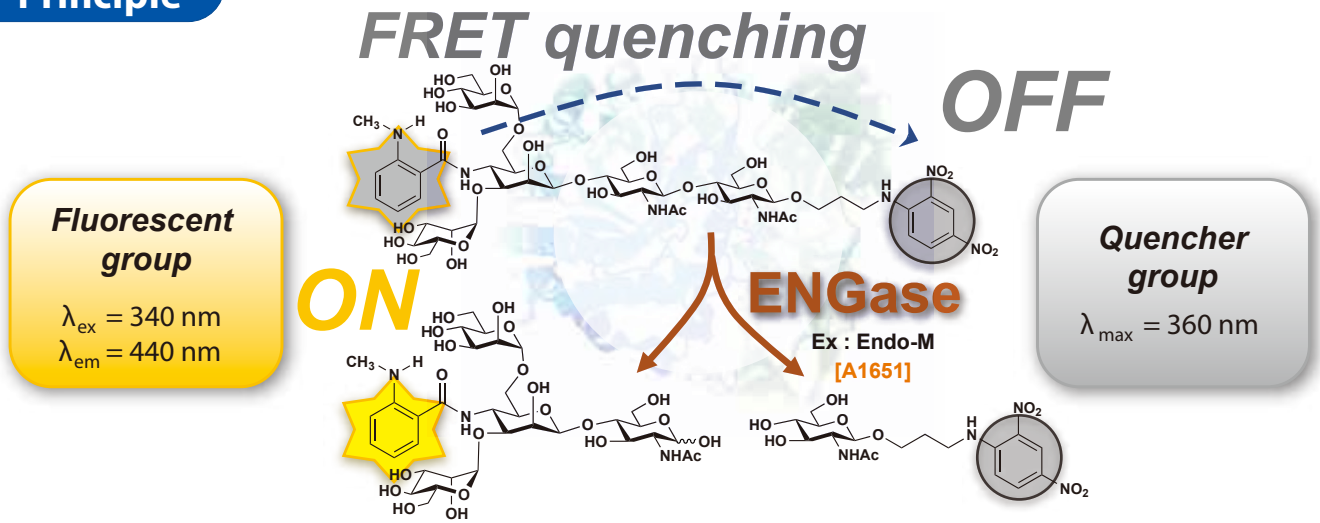
MANT-M3GN2-DNP (= MM3D)

1mg

[M3174]

MM3D is hydrolyzed by *endo*-β-*N*-acetylglucosaminidase (ENGase) to generate fluorescence. MM3D is a useful tool for real-time measurement of the enzymatic activity of ENGase.

Principle



Endo-β-*N*-acetylglucosaminidase (ENGase) first hydrolyzes the chitobiose moiety of *N*-glycan. MM3D, a *N*-glycan which features both a fluorescent group (*N*-methyl anthraniloyl, MANT) and a quencher group (dinitrophenyl, DNP) in the structure. Cleavage of MM3D by ENGase removes the self-quenching effect, leading to an increase in fluorescence intensity. MM3D has shown to be useful for high throughput analysis with plate readers, and in the search for new types of ENGase or inhibitor of ENGase. These inhibitors can be served as therapeutic agents for NGLY1-deficiency, a very rare genetic disease.

*Note: MM3D is not hydrolyzed by Endo-H which belongs to GH Family 18, and therefore is not suitable for measurement of Endo-H activity.

References

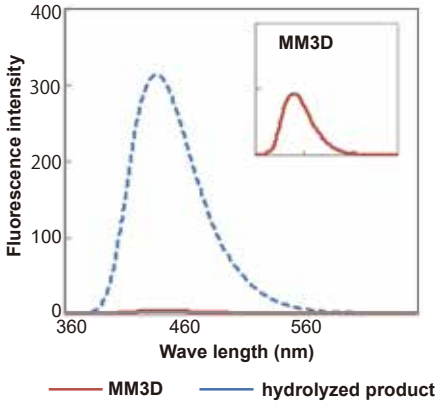
1) N. Ishii, C. Sunaga, K. Sano, C. Huang, K. Iino, Y. Matsuzaki, T. Suzuki , I. Matsuo, *ChemBioChem* **2018**, *19*, 660.
2) H. Fujihira, Y. Masahara-Negishi, M. Tamura, C. Huang, Y. Harada, S. Wakana, D. Takakura, N. Kawasaki, N. Taniguchi, G. Kondoh, T. Yamashita, Y. Funakoshi, T. Suzuki, *PLoS Genet.* **2017**, *13*, e1006696.
3) C. Huang, Y. Harada, A. Hosomi, Y. Masahara-Negishi, J. Seino, H. Fujihira, Y. Funakoshi, T. Suzuki, N. Dohmae, T. Suzuki, *Proc. Natl. Acad. Sci. U.S.A.* **2015**, *112*, 1398.

This product has been commercialized under the instruction of Prof. Ichiro Matsuo.

MM3D-Fluorogenic Probe for Real-Time Measurement of ENGase Activity

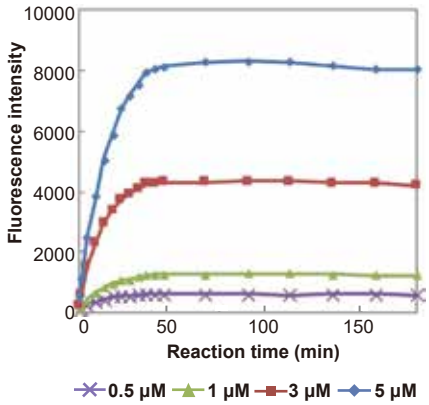
Measurement of Endo-M [A1651] activity using MM3D[1)]

(a) Fluorescence emission spectra of MM3D and the hydrolyzed product



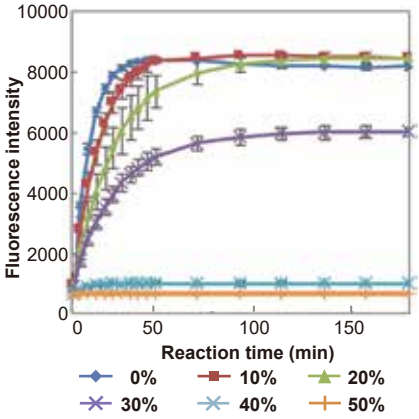
【conditions】
MM3D (solid line) and the hydrolyzed product (dotted line) in 40 μM aqueous solutions. Excitation: 340 nm.

(b) Assays of hydrolysis activity with various concentrations of the MM3D



【conditions】
A solution of the MM3D (0.5, 1, 3, or 5 μM) in a phosphate buffer (40 mM, pH6, 50 μL) was incubated in the presence of Endo-M (20 ng) at 37 °C

(c) Measurement of Endo-M activity containing DMSO



【conditions】
A solution of the probe MM3D (5 μM) in phosphate buffer (40 mM, pH6, 50 μL) containing 0, 10, 20, 30, 40, or 50% DMSO was incubated in the presence of Endo-M (20 ng) at 37 °C

(Data are provided by Prof. Ichiro Matsuo.)

Comparing the fluorescence intensity of MM3D with that of the hydrolyzed product, the fluorescence of MANT is decreased by 98% due to the quenching effect of the DNP group (a). However, since the cleaved product of MM3D is highly sensitive, the concentration of MM3D is sufficient in 0.5 μM to measure Endo-M [A1651] activity (b). Next, addition of DMSO to hinder the enzymatic reaction suppresses enzyme activity in a concentration-dependent manner (c). These results indicate that MM3D is suitable for screening for potential inhibitors of ENGase.

Related Product

***endo*-β-*N*-Acetylglucosaminidase (= Endo-M)**
Recombinant: from *Mucor hiemalis* expressed in *Candida boidinii*

1vial [A1651]

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