PROzyme®

NOTICE: ProZyme was purchased by Agilent in July 2018. Documents for products and product lots manufactured before August 2019 will contain references to ProZyme. For more information about these products and support, go to: **www.agilent.com/en/contact-us.**

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α(1-2,3,6) MANNOSIDASE

SPECIFICATIONS

Product Code: GKX-5010
Activity: ≥150 U/mL
Storage: Store at 2-8°C
Shipped with cold pack for next day delivery.
Formulation: A sterile-filtered solution in 20 m*M* Tris-HCl, 20 m*M* NaCl, pH 7.5.

Glyko® sequencing-grade α(1-2,3,6) Mannosidase (α-D-mannoside mannohydrolase, EC 3.2.1.24) cleaves all α(1-2,3,6)-linked mannose. The enzyme is prepared from jack beans.

Glyko α(1-2,3,6) Mannosidase is useful for:

- Glycan structure analysis of glycoproteins and determination of glycosidic linkage configuration[1,2,3,4]
- Transglycosylation synthesis of α-mannose containing glycans[5]

CHARACTERISTICS

Specificity: The enzyme has a broad substrate specificity, cleaving α(1-2, 3, and 6)-linked mannose residues from oligosaccharides and glycoproteins. However, the enzyme exhibits some kinetic preference for 1-2, 3>6-linked residues, Figure 1. By using enzyme concentrations at about 50 U/ml and extended incubation times (up to 18 hours) at 37°C, complete removal of all α-linked mannose units from complex-type high mannose glycans can be achieved, giving rise to the core trisaccharide, Man β(1,4)-GlcNAc β(1,4)-GlcNAc as the end product.

In order to expedite glycan sequencing studies, the sluggish activity of the Jack Bean enzyme toward α(1-6)-linked mannose residues can be overcome by using the enzyme in combination with the alpha mannosidase from *Xanthomonas mannihotis* (ProZyme product code GK80070) which rapidly cleaves the 1-6 linkages.

The mechanism of the enzyme has been investigated and has been shown to cleave the glycosidic bond between the two carbohydrate residues, forming a stable enzyme substrate intermediate. The enzyme-bound mannose residue can be transferred to other carbohydrate acceptors, with reasonable efficiency. In this manner the enzyme can be utilized for synthesis of novel mannose-containing glycans with a defined anomeric configuration.

Interestingly, the jack bean mannosidase is a glycoprotein containing high-mannose type structures. Apparently, these glycan side chains are not accessible to the enzyme because they may be shielded from the catalytic site by the polypeptide. The carbohydrate side chains are required for proper protein folding and maintaining catalytic activity.

The enzyme requires Zn^{2+} ions for activity and optimal stability, but the addition of Zn^{2+} to the incubation buffer is not usually required.

Molecular Weight: ~190,000 daltons.

pH Optimum: pH 4.0 - 4.5

Stability: The enzyme is stable at 2 - 8°C and -20°C. The enzyme is unstable below pH 5.5 unless Zn^{2+} ions are present. It is stable between 6.0 - 8.5 for 17 hours at 37°C. Ag^{+} and Hg^{2+} are potent inhibitors of enzyme activity.

Purity: Contaminating glycosidase activities are determined using p-nitrophenyl glycoside substrates and are reported when they are greater than 0.001% of the enzyme activity. Protease contamination is determined using the method of Twining (1984)[13].

Applications: The enzyme has been found to be of extensive value in the structural analysis of glyco-conjugates, particularly when exploiting its fine specificity.

ASSAY

One unit of ProZyme α(1-2,3,6) Mannosidase is defined as the amount of enzyme required to hydrolyze 1 µmole of *p*-nitrophenyl-α-D-mannoside to *p*-nitrophenol in 1 minute at pH 5.0 and 37°C.

Supplied Reagents (research pack only)

- 5x Reaction Buffer (100 m*M* sodium acetate, 2 m*M* Zn^{2+} pH 5.0)

Additional Reagents (not supplied)

- 250 µ*M* *p*-nitrophenyl-α-D-mannopyranoside
- (Sigma N2127) in 1x Reaction Buffer 5.0 (50 m*M* sodium phosphate, pH 5.0)
- 1 *M* sodium carbonate

Procedure

1. Adjust spectrophotometer to read at 400 nm.
2. Add 0.4 mL of substrate solution to two tubes and warm to 37°C.
3. Add 5 µL of enzyme to one tube and mix.
4. After 5 minutes, add 0.6 mL of 1 *M* sodium carbonate to both tubes.
5. Blank spectrophotometer to the control tube without the enzyme.
6. Read absorbance at 400 nm.

Calculation:

$$Units/ml = \frac{A}{18.3 \times T \times V} = 2.2 \times A$$

where:

A = Absorbance of sample at 400 nm
T = Time in minutes (5)
V = Volume of enzyme in mL (0.005)
18.3 = Millimolar absorption coefficient of *p*NP at pH >10

SUGGESTIONS FOR USE

Procedure for De-mannosylation

1. Add up to 1 nmole of oligosaccharide to the tube.
2. Add de-ionized water to a total volume of 15 µL.
3. Add 4 µL of 5x Reaction Buffer 5.0.
4. Add 1 µL (0.15 U) α(1-2,3,6) Mannosidase (final concentration ~8 U/mL).
5. Incubate 10 minutes at 37°C.

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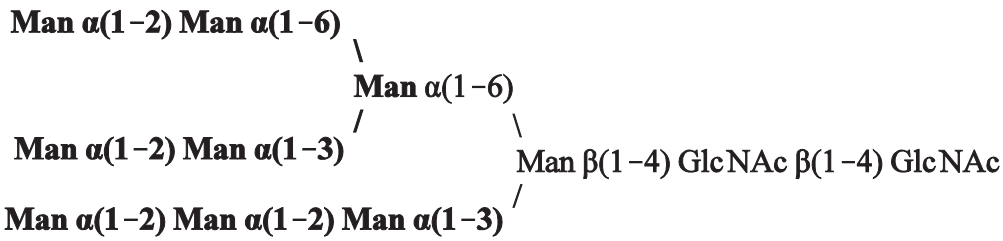
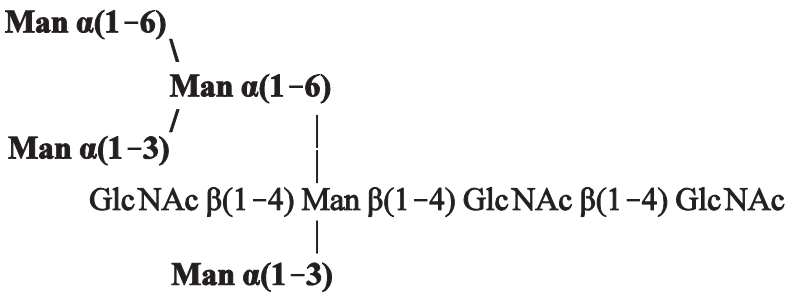
Mannose residues cleaved by GKX-5010 α(1-2,3,6) Mannosidase (shown in bold)
Man = mannose, GlcNAc = N-acetylglucosamine





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