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β -N-acetylglucosaminidase

β-N-acetylglucosaminidase (N-acetyl-β-D-glycosaminide N-acetylglucosaminohydrolase EC 3.2.1.30) cleaves all non-reducing terminal β-linked N-acetylglucosamine residues from complex carbohydrates and glycoproteins. The cleavage rates of different linkages of GlcNAc on bi-, tri- and tetraantennary oligosaccharides is greatly dependent on the steric hindrance by neighboring residues. The β(1-2)GlcNAc residue linked to the α(1-3)-linked mannose is cleaved at the highest rate and the β(1-2) GlcNAc residue linked to the α(1-6)-linked mannose at the lowest rate for all three oligosaccharides. The β(1-6) GlcNAc residue, when present, is removed at the second highest rate and the β(1-4) GlcNAc, third. On a triantennary structure, this residue is removed at the second highest rate (see Figure 1). A bisecting β(1-4) GlcNAc linked to the β-linked mannose severely hinders cleavage of other GlcNAc residues--high concentrations of enzymes and prolonged incubation times are required for cleavage.

β -N-acetylglucosaminidase is isolated from a clone of *Streptococcus pneumonia* (formerly *Diplococcus pneumonia*). The enzyme has been extensively characterized using oligosaccharide standards.

β-N-acetylglucosaminidase is useful for:

- Structural analysis of oligosaccharides
- Distinguishing different N-acetylglucosamine linkages
- Distinguishing between N-acetylglucosamine and N-acetylgalactosamine
- Removing heterogeneity from glycoproteins

Product Code: GE 31

Specifications

Activity: ≥ 80 U/mg, ≥ 50 U/mL

Storage: Store at 4°C. Do not freeze.

Formulation: The enzyme is provided as a sterile solution in 20 mM Tris pH 7.5, 25 mM NaCl.

Stability: Stable at least 12 months when stored properly. Several days exposure to ambient temperatures will not reduce activity.

Product Description

Molecular weight: ~140,000 Daltons

Purity: Each lot of β-N-acetylglucosaminidase is tested for contaminating protease as follows: 10 µg of denatured BSA is incubated for 24 hours with 2 µL of enzyme. SDS-PAGE analysis of the treated BSA shows no evidence of degradation.

The production host strain has been extensively tested and does not produce any detectable glycosidases.

Specificity: All non-reducing terminal β-linked N-acetylglucosamine. No activity on N-acetylgalactosamine. Bisecting GlcNAc hinders cleavage.

pH Range:	Optimum:	pH 5
	Range:	pH 5 – 7

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The supplied buffer concentrate provides the optimal pH for enzyme activity with the standard substrate. If glycosidase treatment is performed at suboptimal pH because of glycoprotein solubility or activity requirements, expect some diminution in enzyme activity.

Assay

One unit of β-N-acetylglucosaminidase is defined as the amount of enzyme required to produce 1 µmole of p-nitrophenol (pNP) in 1 minute at 37°C, pH 5.0 from p-nitrophenyl-β-D-N-acetylglucosaminide.

Reagents

- 5X Reaction buffer 5.0 – 250 mM $NaHPO_4$, pH 5.0

Suggestions For Use

Procedure for Deglycosylation

1. Add up to 100 µg of asialylagalactoglycoprotein or 1 nM of oligosaccharide to tube.
2. Add water to a total of 14 µL.
3. Add 4 µL of 5X Reaction Buffer 5.0.
4. Add 2 µL of β-N-acetylglucosaminidase
5. Incubate at 37°C for 18 hours for complete digestion. Much shorter incubation times are possible if GlcNAcβ(1-2)Man α(1-6) linkages or bisecting GlcNAc are not present.

Cleavage may be monitored by SDS-PAGE if the size differential between native and deglycosylated protein is sufficient for detection.

References

1. Clarke, V. A., N. Platt and T. D. Betters. Cloning and expression of the beta-N-acetylglucosaminidase gene from *Streptococcus pneumoniae*. Generation of truncated enzymes with modified aglycon specificity. **J Biol Chem 270:**8805-8814 (1995).
2. Dwek, R. A., C. J. Edge, D. J. Harvey, M. R. Wormald and R. B. Parekh. Analysis of glycoprotein-associated oligosaccharides. **Ann Rev Biochem 62:**65-100 (1993).
3. Glasgow, L. R., J. C. Paulson and R. L. Hill. Systematic purification of five glycosidases from *Streptococcus pneumonia*. **J. Biol Chem 252:**8615-8623 (1977).
4. Kobata, A. Use of endo- and exoglycosidases for structural studies of glycoconjugates. **Anal Biochem 100:**1-14 (1979).
5. Prime, S., J. Dearnley, A. M. Venton, R. B. Parekh and C. J. Edge. Oligosaccharide sequencing based on exo- and endoglycosidase digestion and liquid chromatographic analysis of the products**. J Chromatogr A 720:**263-274 (1996).

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This product is intended for in vitro research only.

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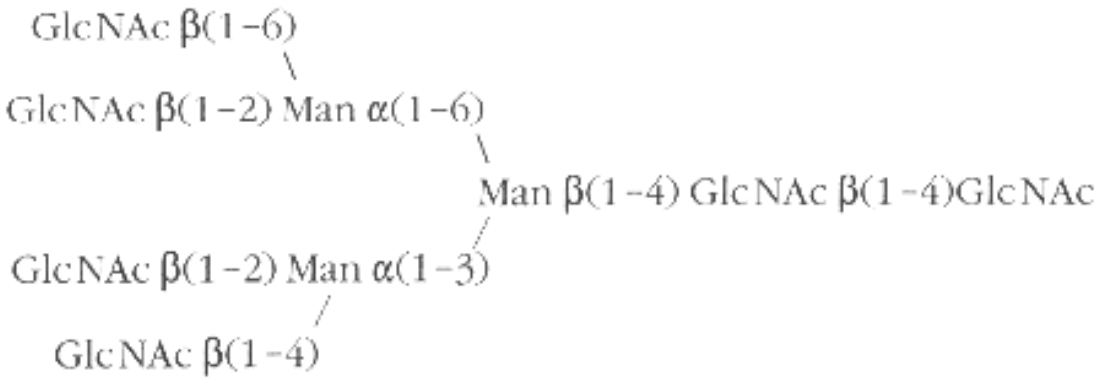
Figure 1 - Aslaloagalactotetraantennary Oligosaccharide
Man - Mannose; GlcNAc - N-acetylglucosamine



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