Beta-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. N-acetyl-galactosamine residues are not cleaved. 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 slows 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*). It is purified free of contaminating exo- and endoglycosidases and proteases by chromatographic methods. 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

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 at 37°C 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-acetyl-glucosamine. No activity on N-acetylgalactosamine. Bisecting GlcNAc slows the reaction.

pH Range:
Optimum: pH 5 Range: pH 5 - 7

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 (*p*NP) in 1 minute at 37°C, pH 5.0 from *p*-nitrophenyl-β-D-acetylglucosaminidase.

Reagents

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

Suggestions for Use

Procedure for Degalactocosylation

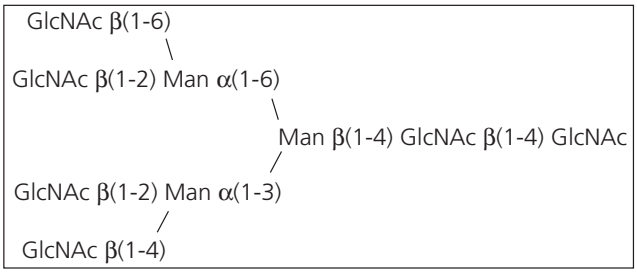
1. Add up to 100 µg of asialgalactoglycoprotein or 1 nmol of oligosaccharide to a tube.
2. Add water to a total of 14 µL.
3. Add 4 µL 5X Reaction Buffer 5.0.
4. Add 2 µL of β-N-acetylglucosaminidase.
5. Incubate at 37°C for 18 hours for complete digestion. Much shorter incubation times are necessary if GlcNAcβ(1-2)Manα(1-6) linkages or bisecting GlcNAc is 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).

Figure 1 - Asialoagalactotetraantennary Oligosaccharide



Man = Mannose; GlcNAc = N-acetylglucosamine

Order Information

Catalog No.	Product Description	Package Size	Temp. °C
120389-1	**β-N-acetylglucosaminidase** *(Streptococcus pneumonia* recombinant*)*	60 µL	+4

AMSBIO | www.amsbio.com | info@amsbio.com

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85