β-*N*-Acetyl-glucosaminidase S

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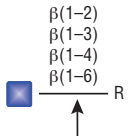
P0720S 0151407160711

P0744S

100 units **4,000 U/ml** **Lot: 0011407**
RECOMBINANT **Store at 4°C** **Exp: 7/15**

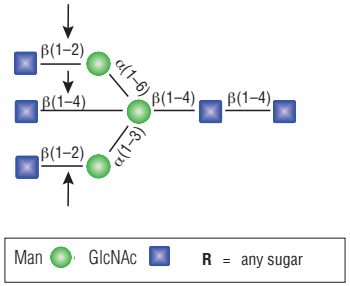
Description: β-*N*-Acetylglucosaminidase S is a highly specific exoglycosidase that catalyzes the hydrolysis of terminal, non-reducing β-*N*-Acetylglucosamine residues from oligosaccharides.

Specificity:



Detailed Specificity:

β-*N*-Acetylglucosaminidase S is able to efficiently cleave bisecting β-*N*-Acetylglucosaminidase residues.



Source: Cloned from *Streptococcus pneumoniae* and expressed in *E. coli* (1).

Supplied in: 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C) and 1 mM EDTA.

Reagents Supplied with Enzyme:
10X GlycoBuffer 1
(0.5 M Sodium Acetate, pH 5.5 @ 25°C and 50 mM $CaCl_2$)

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal, non-reducing β-*N*-Acetylglucosamine from 1 nmol GlcNAcβ1-4GlcNAcβ1-4GlcNAc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

Unit Definition Assay: Two fold dilutions of β-*N*-Acetylglucosaminidase S are incubated with 1 nmol AMC-labeled substrate and 1X GlycoBuffer 1 in a 10 µl reaction. The reaction mix is incubated at 37°C for 1 hour. Separation of reaction products are visualized via thin layer chromatography (2).

Specific Activity: ~2,700 units/mg.

Molecular Weight: 125,000 daltons.

Quality Assurance: No contaminating exoglycosidase or endoglycosidase F1, F2 or F3 activity could be detected. No contaminating proteolytic activity could be detected.

Quality Controls

Glycosidase Assays: 16 units of β-*N*-Acetyl-glucosaminidase S were incubated with 0.1 mM of flourescently-labeled oligosaccharides and glycopeptides, in a 10 µl reaction for 20 hours at 37°C. The reaction products were analyzed by TLC for digestion of substrate.

No other glycosidase activities were detected (ND) with the following substrates:

β-*N*-Acetylgalactosaminidase:
GalNAcβ1-4Galβ1-4Glc-AMC ND

α-N-Acetylgalactosaminidase:
GalNAcα1-3(Fucα1-2)Galβ1-4Glc-AMC ND

α-Fucosidase:
Galβ1-4 (Fucα1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND
Fucα1-2Galβ1-4Glc-AMC ND

β-Galactosidase:
Galβ1-3GlcNAcβ1-4Galβ1-4Glc -AMC ND
Galβ1-4GlcNAcβ1-3Galβ1-4Glc -AMC ND

(see other side)

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CERTIFICATE OF ANALYSIS

α-Galactosidase:

Galα1-3Galβ1-4Gal-AMC	ND
Galα1-6Galα1-6Glcα1-2Fru-AMC	ND

α-Neuraminidase:

Neu5Aca2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC	ND

α-Mannosidase:

Manα1-3Manβ1-4GlcNAc-AMC	ND
Manα1-6Manα1-6(Manα1-3)Man-AMC	ND

α-Glucosidase:

Glcα1-6Glcα1-4Glc-AMC	ND

β-Xylosidase:

Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC	ND

β-Mannosidase:

Manβ1-4Manβ1-4Man-AMC	ND

Endo F_1, F_2, H:

Dansylated invertase high mannose.	ND

Endo F_2, F_3:

Dansylated fibrinogen biantennary.	ND

Protease Assay: After incubation of 24 units of β-*N*-Acetylglucosaminidase S with 0.2 nmol of a standard mixture of proteins in a 20 µl reaction, for 20 hours at 37°C, no proteolytic activity could be detected by SDS-PAGE.

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

Reaction Conditions: Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate. Typical reaction conditions are as follows:

1. Combine 1 µg of glycoprotein or 100 nM of oligosaccharide and H_2O (if necessary) to make a 9 µl total reaction volume.
2. Add 1 µl of 10X GlycoBuffer 1 to make a 10 µl total reaction volume.
3. Add 1 µl of β-*N*-Acetylglucosaminidase S.
4. Incubate at 37°C for 1 hour.

Notes on Use:

- Reactions may be scaled-up linearly to accommodate larger reaction volumes.
- The amount of exoglycosidase enzyme required varies when different substrates are used. Start with 1–2 µl for 1 µg of glycoprotein or 100 nM of oligosaccharide for one hour in a 10–25 µl reaction. If there is still undigested material, let the reaction go overnight.
- β-*N*-Acetylglucosaminidase S cannot be heat inactivated.

References:

1. Chen, M. New England Biolabs, Inc., unpublished results.
2. Wong-Madden, S. T. and Landry, D. (1995) *Glycobiology* 5, 19–28.