

β -N-Acetylglucosaminidase S



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www.neb.com



P0744S 001151116111

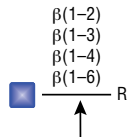
P0744S



100 units **4,000 U/ml** **Lot: 0011511**
RECOMBINANT **Store at 4°C** **Exp: 11/16**

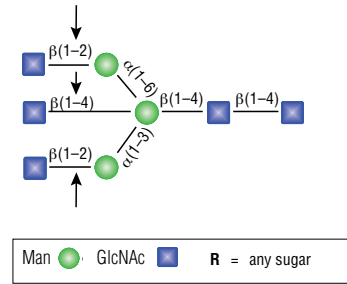
Description: β -N-Acetylglucosaminidase S is a highly specific exoglycosidase that catalyzes the hydrolysis of terminal, non-reducing β -N-Acetylglucosamine residues from oligosaccharides.

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 CaCl₂)

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 GlcNAc β 1-4GlcNAc β 1-4GlcNAc-7-amino-4-methylcoumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 μ l.

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 μ l 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-Acetylglucosaminidase S were incubated with 0.1 mM of fluorescently-labeled oligosaccharides and glycopeptides, in a 10 μ l 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:
GalNAc β 1-4Gal β 1-4Glc-AMC ND

α -N-Acetylgalactosaminidase:
GalNAc α 1-3(Fuc α 1-2)Gal β 1-4Glc-AMC ND

α -Fucosidase:
Gal β 1-4 (Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc-AMC ND
Fuc α 1-2Gal β 1-4Glc-AMC ND

β -Galactosidase:
Gal β 1-3GlcNAc β 1-4Gal β 1-4Glc-AMC ND
Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc-AMC ND

(see other side)

CERTIFICATE OF ANALYSIS

β -N-Acetylglucosaminidase S



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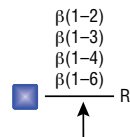
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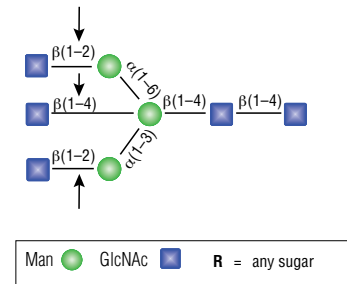
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α -N-Acetylgalactosaminidase:
GalNAc α 1-3(Fuc α 1-2)Gal β 1-4Glc-AMC ND

α -Fucosidase:
Gal β 1-4 (Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc-AMC ND
Fuc α 1-2Gal β 1-4Glc-AMC ND

β -Galactosidase:
Gal β 1-3GlcNAc β 1-4Gal β 1-4Glc-AMC ND
Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc-AMC ND

(see other side)

CERTIFICATE OF ANALYSIS

α -Galactosidase:
Gal α 1-3Gal β 1-4Gal-AMC ND
Gal α 1-6Gal α 1-6Glc α 1-2Fru-AMC ND

α -Neuraminidase:
Neu5Aca2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc-AMC ND

α -Mannosidase:
Man α 1-3Man β 1-4GlcNAc-AMC ND
Man α 1-6Man α 1-6(Man α 1-3)Man-AMC ND

α -Glucosidase:
Glc α 1-6Glc α 1-4Glc-AMC ND

β -Xylosidase:
Xyl β 1-4Xyl β 1-4Xyl β 1-4Xyl-AMC ND

β -Mannosidase:
Man β 1-4Man β 1-4Man-AMC ND

Endo F₁, F₂, H:
Dansylated invertase high mannose. ND

Endo F₂, F₃:
Dansylated fibrinogen biantennary. ND

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α -Galactosidase:
Gal α 1-3Gal β 1-4Gal-AMC ND
Gal α 1-6Gal α 1-6Glc α 1-2Fru-AMC ND

α -Neuraminidase:
Neu5Aca2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc-AMC ND

α -Mannosidase:
Man α 1-3Man β 1-4GlcNAc-AMC ND
Man α 1-6Man α 1-6(Man α 1-3)Man-AMC ND

α -Glucosidase:
Glc α 1-6Glc α 1-4Glc-AMC ND

β -Xylosidase:
Xyl β 1-4Xyl β 1-4Xyl β 1-4Xyl-AMC ND

β -Mannosidase:
Man β 1-4Man β 1-4Man-AMC ND

Endo F₁, F₂, H:
Dansylated invertase high mannose. ND

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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 μ l 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 μ g of glycoprotein or 100 nM of oligosaccharide and H₂O (if necessary) to make a 9 μ l total reaction volume.
2. Add 1 μ l of 10X GlycoBuffer 1 to make a 10 μ l total reaction volume.
3. Add 1 μ l of β -*N*-Acetylglucosaminidase S.
4. Incubate at 37°C for 1 hour.

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2. Add 1 μ l of 10X GlycoBuffer 1 to make a 10 μ l total reaction volume.
3. Add 1 μ l 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 μ l for 1 μ g of glycoprotein or 100 nM of oligosaccharide for one hour in a 10–25 μ l 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.



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