β-*N*-Acetyl glucosaminidase

NEW ENGLAND BioLabs Inc.
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P0732S 005121013101

P0732S

100 units **4,000 U/ml** **Lot: 0051210**

RECOMBINANT **Store at 4°C** **Exp: 10/13**

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

Specificity:

↓
GlcNAc β 1–2, 3, 4, 6 R

Detailed Specificity: Specificity can vary depending on incubation time and branching structure.

Source: Cloned from *Xanthomonas manihotis* and expressed in *E. coli* (1).

Supplied in: 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C) and 1 mM Na_2EDTA.

Reagents Supplied with Enzyme:
10X G1 Reaction Buffer
100X BSA

Reaction Conditions:
1X G1 Reaction Buffer
50 mM Sodium Citrate (pH 6.0 @ 25°C). Supplement with 100 µg/ml BSA. Incubate at 37°C.

Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate.

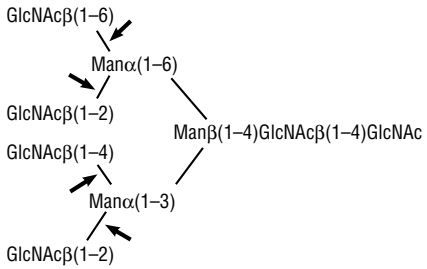
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal, non-reducing β-*N*-Acetylglucosamine from 1 nmol GlcNAcβ1-4GlcNAcβ1-4GlcNAc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

Specific Activity: 20,000 units/mg

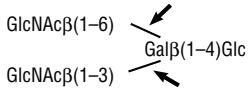
(See other side)

CERTIFICATE OF ANALYSIS

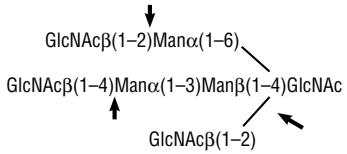
A) 0.1 nm/µl substrate, 4 hour incubation



B) 0.1 nm/µl substrate, 4 hour incubation



C) 0.1 nm/µl substrate, 18 hour incubation



D) 0.1 nm/µl substrate, 24 hour incubation

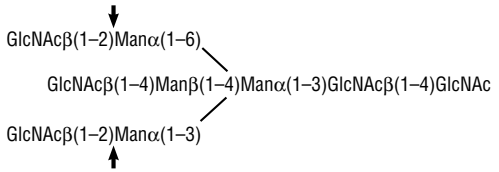


Figure 1: *Detailed specificity of β-N-Acetylglucosaminidase. All reactions contained 4 units of β-N-Acetylglucosaminidase, 1X G1 Reaction Buffer and 1X BSA in a total reaction volume of 10 µl. Reactions (B), (C) and (D) were treated with 8 units of β1-4 Galactosidase prior to treatment with β-N-Acetylglucosaminidase to form the above substrates. Reactions were incubated at 37°C.*

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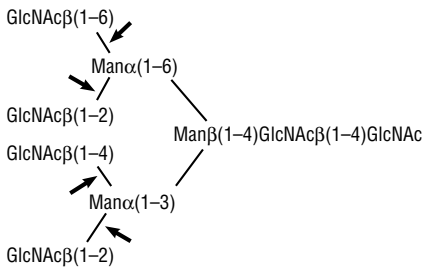

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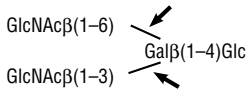
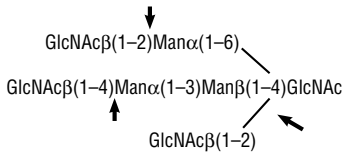


C) 0.1 nm/µl substrate, 18 hour incubation



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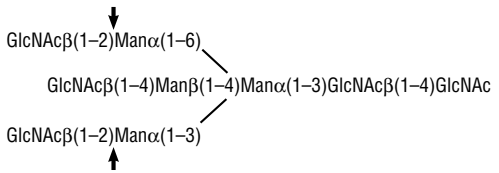

Figure 1: *Detailed specificity of β-N-Acetylglucosaminidase. All reactions contained 4 units of β-N-Acetylglucosaminidase, 1X G1 Reaction Buffer and 1X BSA in a total reaction volume of 10 µl. Reactions (B), (C) and (D) were treated with 8 units of β1-4 Galactosidase prior to treatment with β-N-Acetylglucosaminidase to form the above substrates. Reactions were incubated at 37°C.*

Unit Definition Assay: Two fold serial dilutions of β-*N*-Acetylglucosaminidase are incubated with 1 nmol AMC-labeled substrate in 1X G1 Reaction Buffer, supplemented with 100 µg/ml BSA, in a 10 µl reaction. The reaction mix is incubated for 1 hour at 37°C. Separation of reaction products are visualized via thin layer chromatography (2).

Specific Activity: 34,000 units/mg

Molecular Weight: 71,000 daltons.

Quality Assurance: No contaminating exoglycosidase or proteolytic activity could be detected.

Quality Controls

Glycosidase Assays:
16 units of β-*N*-Acetylglucosaminidase 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.

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

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:
Fucα1-2Galβ1-4Glc-AMC ND

Galβ1-4 (Fucα1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND

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

Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC ND

α-Galactosidase:
Galα1-3Galβ1-4Gal-AMC ND

Galα1-6Galα1-6Glcα1-2Fru-AMC ND

α-Neuraminidase:
Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC ND

α-Mannosidase:
Manα1-3Manβ1-4GlcNAc-AMC ND

Manα1-6Manα1-6(Manα1-3)Man-AMC ND

β-Glucosidase:
Glcβ1-4Glcβ1-4Glc-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

PNGase F:
Fluoresceinated fetuin triantennary. ND

Protease Assay: After incubation of 28 units of β-*N*-Acetylglucosaminidase 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.

Note: Recommended storage temperature is 4°C. Avoid repeated freeze/thaw cycles.

Heat Inactivation: 65°C for 10 minutes.

References:
1. Guthrie, E.P., Shimer, E.P., New England Biolabs, Inc. unpublished results.
2. Wong-Madden, S.T. and Landry, D. (1995) *Glycobiology* 5, 19–28.

U.S. Patent No. 5,770,405

Unit Definition Assay: Two fold serial dilutions of β-*N*-Acetylglucosaminidase are incubated with 1 nmol AMC-labeled substrate in 1X G1 Reaction Buffer, supplemented with 100 µg/ml BSA, in a 10 µl reaction. The reaction mix is incubated for 1 hour at 37°C. Separation of reaction products are visualized via thin layer chromatography (2).

Specific Activity: 34,000 units/mg

Molecular Weight: 71,000 daltons.

Quality Assurance: No contaminating exoglycosidase or proteolytic activity could be detected.

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GalNAcα1-3(Fucα1-2)Galβ1-4Glc-AMC ND

α-Fucosidase:
Fucα1-2Galβ1-4Glc-AMC ND

Galβ1-4 (Fucα1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND

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

Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC ND

α-Galactosidase:
Galα1-3Galβ1-4Gal-AMC ND

Galα1-6Galα1-6Glcα1-2Fru-AMC ND

α-Neuraminidase:
Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC ND

α-Mannosidase:
Manα1-3Manβ1-4GlcNAc-AMC ND

Manα1-6Manα1-6(Manα1-3)Man-AMC ND

β-Glucosidase:
Glcβ1-4Glcβ1-4Glc-AMC ND

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Glcα1-6Glcα1-4Glc-AMC ND

β-Xylosidase:
Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC ND

β-Mannosidase:
Manβ1-4Manβ1-4Man-AMC ND

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Dansylated invertase high mannose. ND

Endo F_2, F_3:
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