**β-N-Acetylglucosaminidase**

**Description:** β-N-Acetylglucosaminidase is a highly specific exoglycosidase that catalyzes the hydrolysis of terminal, non-reducing β-N-Acetylglucosamine residues from oligosaccharides. The specificity can vary depending on incubation time and branching structure.

**Source:** Cloned from Xanthomonas manihotis

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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.

**A) 0.1 nm/µl substrate, 4 hour incubation**

1. GlcNAc(1-6)
2. GlcNAc(1-2)
3. GlcNAc(1-3)
4. GlcNAc(1-4)

**B) 0.1 nm/µl substrate, 4 hour incubation**

1. GlcNAc(1-6)
2. GlcNAc(1-3)
3. Galβ(1-4)Glc

**C) 0.1 nm/µl substrate, 18 hour incubation**

1. GlcNAc(1-2)
2. GlcNAc(1-4)
3. GlcNAc(1-3)
4. GlcNAc(1-6)

**D) 0.1 nm/µl substrate, 24 hour incubation**

1. GlcNAc(1-2)
2. GlcNAc(1-4)
3. GlcNAc(1-3)
4. GlcNAc(1-6)

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**Notes:**

- One unit is defined as the amount of enzyme required to cleave > 95% of the terminal, non-reducing β-N-Acetylglucosamine from 1 nmol GlcNAcβ1-4GlcNAcβ1-4GlcNAcβ7-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
- 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 GlcNAcβ1-4GlcNAcβ1-4GlcNAcβ7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.
- Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate.

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**Supplied in:** 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C) and 1 mM Na2EDTA.

**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.

**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 GlcNAcβ1-4GlcNAcβ1-4GlcNAcβ7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.
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 fluorescently-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-Acetylglucosaminidase: GalNacβ1-4Galβ1-4Glc-AMC
α-N-Acetylagalactosaminidase: GalNacα1-3(Fucα1-2)Galβ1-4Glc-AMC
α-Fucosidase: Fucα1-2Galβ1-4Glc-AMC
Galβ1-4(Fucα1-3)GlnNacβ1-3Galβ1-4Glc-AMC
β-Galactosidase: Galβ1-3GlnNacβ1-4Galβ1-4Glc-AMC
Galβ1-4GlnNacβ1-3Galβ1-4Glc-AMC
α-Galactosidase: Galα1-3Galβ1-4Gal-AMC
Galα1-6Galα1-6Glcα1-2Fru-AMC

α-Neuraminidase: NeuSαα2-3Galβ1-3GlnNacβ1-3Galβ1-4Glc-AMC
α-Mannosidase: Manα1-3Manβ1-4GlcNAc-AMC
Manα1-6Manβ1-6(Manα1-3)Man-AMC
β-Glucosidase: Glicβ1-4Glicβ1-4Glc-AMC
α-Glucosidase: Glicα1-6Glicβ1-4Glc-AMC
β-Xylosidase: Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC
β-Mannosidase: Manβ1-4Manβ1-4Man-AMC
Endo Fα, Fβ, Fγ, H: Dansylated invertase high mannose.
Endo Fα, Fβ: Dansylated fibrinogen biantennary.

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

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