**β-N-Acetyl glucosaminidase**

**P0732S**

100 units 4,000 U/ml Lot: 0051508

RECOMBINANT Store at 4°C Exp: 8/16

**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

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

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

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

\[
\text{GlcNAc(1-6)} \rightarrow \text{Man(1-6)}
\]

\[
\text{GlcNAc(1-2)} \rightarrow \text{Man(1-4)GlcNAc(1-4)GlcNAc}
\]

\[
\text{GlcNAc(1-4)} \rightarrow \text{Man(1-3)}
\]

\[
\text{GlcNAc(1-2)} \rightarrow \text{GlcNAc(1-2)}
\]

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

\[
\text{GlcNAc(1-6)} \rightarrow \text{Gal(1-4)Glc}
\]

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

\[
\text{GlcNAc(1-2)Man(1-6)}
\]

\[
\text{GlcNAc(1-4)Man(1-3)Man(1-4)GlcNAc}
\]

\[
\text{GlcNAc(1-2)GlcNAc(1-2)}
\]

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

\[
\text{GlcNAc(1-2)Man(1-6)}
\]

\[
\text{GlcNAc(1-4)Man(1-4)Man(1-3)GlcNAc(1-4)GlcNAc}
\]

\[
\text{GlcNAc(1-2)Man(1-3)}
\]

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**Figure 1:** Detailed specificity of β-N-Acetylglucosaminidase. All reactions contained 4 units of β-N-Acetylglucosaminidase. 1X GlycoBuffer 1 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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\]

\[
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\]

\[
\text{GlcNAc(1-2)GlcNAc(1-2)}
\]

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\[
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\]

\[
\text{GlcNAc(1-4)Man(1-4)Man(1-3)GlcNAc(1-4)GlcNAc}
\]

\[
\text{GlcNAc(1-2)Man(1-3)}
\]

---

**Figure 1:** Detailed specificity of β-N-Acetylglucosaminidase. All reactions contained 4 units of β-N-Acetylglucosaminidase. 1X GlycoBuffer 1 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-Acetylgalactosaminidase are incubated with 1 nmol AMC-labeled substrate in 1X GlycoBuffer 1, 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-Acetylgalactosaminidase 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-Acetylgalactosaminidase:** GaINac1-4Galα1-4Glc-AMC
- **α-N-Acetylgalactosaminidase:** GaINAc1-3(Fucα1-2)Galβ1-4Glc-AMC
- **Fucosidase:** Fucα1-2Galβ1-4Glc-AMC
- **Galβ1-4(Fucα1-3)GalNAcβ1-3Galβ1-4Glc-AMC
- **β-Galactosidase:** Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC
- **α-Galactosidase:** Galα1-3Galβ1-4Gal-AMC
- **Galα1-6Galα1-6Glcα1-2Fru-AMC

**Neuraminidase:**

- **α-Neuraminidase:** Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC
- **β-Neuraminidase:** Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC

**Mannosidase:**

- **α-Mannosidase:** Manα1-3Manβ1-4GlcNAc-AMC
- **β-Mannosidase:** Manβ1-4Manβ1-4Man-AMC

**Galactosidase:**

- **β-Galactosidase:** Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC
- **α-Galactosidase:** Galα1-3Galβ1-4Gal-AMC
- **Galα1-6Galα1-6Glcα1-2Fru-AMC

**Fucosidase:**

- **β-Fucosidase:** Fucβ1-4Galβ1-4Glc-AMC
- **α-Fucosidase:** Fucα1-2Galβ1-4Glc-AMC
- **Galβ1-4(Fucα1-3)GalNAcβ1-3Galβ1-4Glc-AMC

**Glucosidase:**

- **β-Glucosidase:** Glcβ1-4Glcβ1-4Glc-AMC
- **α-Glucosidase:** Glcα1-6Glcβ1-4Glc-AMC

**Xylosidase:**

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

**Endo F1, F3, H:**

- **Endo F1, F3:** Danychylated invertase high mannose.

**PNGase F:**

- **PNGase F:** Danychylated fibroigamin biantennary.

**Protease Assay:** After incubation of 28 units of β-N-Acetylgalactosaminidase with 0.2 nmol of a standard mixture of proteins in a 10 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:**