**β-N-Acetylglucosaminidase**

**Certification of Analysis**

100 units 4,000 U/ml Lot: 0051406

**RECOMBINANT** Store at 4°C Exp: 6/15

**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*

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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

- GlcNAcβ(1–6)
- GlcNAcβ(1–2)
- GlcNAcβ(1–4)
- GlcNAcβ(1–3)
- GlcNAcβ(1–2)

B) 0.1 nm/µl substrate, 4 hour incubation

- GlcNAcβ(1–6)
- GlcNAcβ(1–3)
- Galβ(1–4)Glc

C) 0.1 nm/µl substrate, 18 hour incubation

- GlcNAcβ(1–2)Manβ(1–6)
- GlcNAcβ(1–4)Manβ(1–3)Manβ(1–4)GlcNAc
- GlcNAcβ(1–2)

D) 0.1 nm/µl substrate, 24 hour incubation

- GlcNAcβ(1–2)Manβ(1–6)
- GlcNAcβ(1–4)Manβ(1–3)GlcNAcβ(1–4)GlcNAc
- GlcNAcβ(1–2)Manβ(1–3)

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**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*

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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

- GlcNAcβ(1–6)
- GlcNAcβ(1–2)
- GlcNAcβ(1–4)
- GlcNAcβ(1–3)
- GlcNAcβ(1–2)

B) 0.1 nm/µl substrate, 4 hour incubation

- GlcNAcβ(1–6)
- GlcNAcβ(1–3)
- Galβ(1–4)Glc

C) 0.1 nm/µl substrate, 18 hour incubation

- GlcNAcβ(1–2)Manβ(1–6)
- GlcNAcβ(1–4)Manβ(1–3)Manβ(1–4)GlcNAc
- GlcNAcβ(1–2)

D) 0.1 nm/µl substrate, 24 hour incubation

- GlcNAcβ(1–2)Manβ(1–6)
- GlcNAcβ(1–4)Manβ(1–3)GlcNAcβ(1–4)GlcNAc
- GlcNAcβ(1–2)Manβ(1–3)

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**Specific Activity:** 20,000 units/mg

(See other side)

**Certificate of Analysis**

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.

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 GlcNAcβ1-4GlcNAcβ1-4GlcNAcβ7-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

(See other side)

**Certificate of Analysis**
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:**
  - GalNAcβ1-3Galβ1-4Glc-AMC ND
  - GalNAcβ1-3(Fucα1-2)Galβ1-4Glc-AMC ND

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

- **-Fucosidase:**
  - Fucx1-2Galβ1-4Glc-AMC ND
  - Galβ1-4(Fucα1-3)GalNAcβ1-3Galβ1-4Glc-AMC ND

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

- **α-Galactosidase:**
  - Galα1-3Galβ1-4Glc-AMC ND
  - Galα1-6Galα1-6Glcα1-2Fru-AMC ND

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

- **α-Mannosidase:**
  - Manα1-3Manβ1-4GlcNAc-AMC ND
  - Manα1-6Manβα1-6(Manβ1-3)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 Fα, Fβ, H:
  - Dansylated invertase high mannose. ND

- Endo Fα, Fβ:
  - Dansylated fibrinogen biantennary. ND

| **PNGase F:** | Fluoresceinated fetuin triantennary. ND |
| **Protease Assay:** | After incubation of 28 units of β-N-Acetylgalactosaminidase 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. |
| **Heat Inactivation:** | 65°C for 10 minutes. |

**References:**

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**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:**
  - GalNAcβ1-4Galβ1-4Glc-AMC ND

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

- **-Fucosidase:**
  - Fucα1-2Galβ1-4Glc-AMC ND
  - Galβ1-4(Fucα1-3)GalNAcβ1-3Galβ1-4Glc-AMC ND

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

- **α-Galactosidase:**
  - Galα1-3Galβ1-4Glc-AMC ND
  - Galα1-6Galα1-6Glcα1-2Fru-AMC ND

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

- **α-Mannosidase:**
  - Manα1-3Manβ1-4GlcNAc-AMC ND
  - Manα1-6Manβα1-6(Manβ1-3)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 Fα, Fβ, H:
  - Dansylated invertase high mannose. ND

- Endo Fα, Fβ:
  - Dansylated fibrinogen biantennary. ND

**Unit Definition Assay:** Two fold serial dilutions of β-N-Acetylgalactosaminidase 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-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.

**Heat Inactivation:** 65°C for 10 minutes.

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  - GalNAcβ1-4Galβ1-4Glc-AMC ND

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

- **-Fucosidase:**
  - Fucα1-2Galβ1-4Glc-AMC ND
  - Galβ1-4(Fucα1-3)GalNAcβ1-3Galβ1-4Glc-AMC ND

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

- **α-Galactosidase:**
  - Galα1-3Galβ1-4Glc-AMC ND
  - Galα1-6Galα1-6Glcα1-2Fru-AMC ND

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