**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.

**Reaction Conditions:**
- **10X G1 Reaction Buffer**: 100 mM Tris-Cl pH 8.0, 100 mM NaCl, 1 mM MgCl₂, 10 mM CaCl₂, 15 mM MgSO₄, 100 mM Na₂EDTA (pH 7.5 @ 25°C) and 1 mM Na₂EDTA.
- **1X G1 Reaction Buffer**: 10X G1 Reaction Buffer diluted 1:10 with water, 1X G1 Reaction Buffer diluted 1:100, 100X BSA

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

**Unit Definition:** One unit is defined as the amount of enzyme required to cleave >95% of the terminal, non-reducing β-N-Acetylglucosaminase from 1 nmol GlcNAcβ1-4GlcNAcβ1-4GlcNAcβ7-aminor-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)

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

(See other side)
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.

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: GalNacea1-3(Fucxx1-2)Galβ1-4Glc-AMC ND

α-Fucosidase: Fucxx1-2Galβ1-4Glc-AMC ND

Galβ1-4(Fucxx1-3)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

Manα1-6Manα1-6(Manα1-3)Man-AMC ND

β-Glucosidase: Glcβ1-1Glcβ1-4Glc-AMC ND

α-Glucosidase: Glcα1-6Glcβ1-4Glc-AMC ND

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

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

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

Endo Fα, Fβ: Donsylated fibrinogen biantennary. ND

PNGase F: Fluronscinated 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.

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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