β-N-Acetyl-hexosaminidase$_f$

NEW ENGLAND BioLabs Inc.
1-800-632-7799
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www.neb.com

P0721S 0011210 14101

P0721S

500 units **5,000 U/ml** **Lot: 0011210**

RECOMBINANT **Store at –20°C** **Exp: 10/14**

Description: β-N-Acetyl-hexosaminidase$_f$ is a recombinant protein fusion of β-N-Acetyl-hexosaminidase (1) and maltose binding protein. It has identical activity to β-N-Acetyl-hexosaminidase. β-N-Acetyl-hexosaminidase$_f$ catalyzes the hydrolysis of terminal β-D-N-acetyl-galactosamine and glucosamine residues from oligosaccharides.

***Note: Specificity Change**

***Specificity:**

GlcNAc } β1 ↓ = 3 R, β1 – 4 R, β1 – 6 R

GalNAc } β1 ↓ = 4 R

Source: Cloned from *Streptomyces plicatus* (1) and overexpressed in *E. coli* (2).

Supplied in: 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C) and 5 mM Na_2EDTA.

Reagents Supplied with Enzyme:
10X G2 Reaction Buffer

Reaction Conditions:
1X G2 Reaction Buffer:
50 mM Sodium Citrate (pH 4.5 @ 25°C).
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 β-D-N-acetyl-galactosamine from 1 nmol of GalNAcβ1-4Galβ1-4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

Unit Definition Assay: Two fold dilutions of β-N-Acetyl-hexosaminidase$_f$ are incubated with 1 nmol AMC-labeled substrate in 1X G2 Reaction Buffer 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 (3).

Specific Activity: ~ 10,000 units/mg

Molecular Weight: 100,000 daltons

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

Quality Controls

Glycosidase Assays:
50 units of β-N-Acetyl-hexosaminidase$_f$ 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.

No other glycosidase activities were detected (ND) with the following substrates:

α-Fucosidase:
Fucα1-2Galβ1-4Glc-AMC Galβ1-4 (Fucα1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND

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

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

α-Neuraminidase:
Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ 1-4Glc-AMC ND

α-Mannosidase:
Manα1-3Manβ1-4GlcNAc-AMC
Manα1-6Manα1-6(Manα1-3)Man-AMC ND

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

(See other side)

CERTIFICATE OF ANALYSIS

β-N-Acetyl-hexosaminidase$_f$

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

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

α-Neuraminidase:
Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ 1-4Glc-AMC ND

α-Mannosidase:
Manα1-3Manβ1-4GlcNAc-AMC
Manα1-6Manα1-6(Manα1-3)Man-AMC ND

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

(See other side)

CERTIFICATE OF ANALYSIS

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 50 units of β-N-Acetyl-hexosaminidase$_f$ 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:** Non-branched oligosaccharides only.

References:

1. Robbins, P. et al. (1992) *Gene* 111, 69–76.
2. Guan, C. and Wong, S. New England Biolabs Inc., unpublished results.
3. Wong-Madden, S.T. and Landry, D. (1995) *Glycobiology* 5, 19–28.

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

β-Mannosidase:
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