

β -N-Acetyl-hexosaminidase_f



1-800-632-7799
info@neb.com
www.neb.com



P0721S 001121014101

P0721S

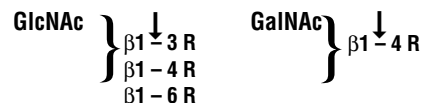


500 units 5,000 U/ml Lot: 0011210
RECOMBINANT Store at -20°C Exp: 10/14

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:



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 Na₂EDTA.

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 GalNAc β 1-4Gal β 1-4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 μ l.

Unit Definition Assay: Two fold dilutions of β -N-Acetyl-hexosaminidase, are incubated with 1 nmol AMC-labeled substrate in 1X G2 Reaction Buffer in a 10 μ l 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 μ l 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:
Fuc α 1-2Gal β 1-4Glc-AMC Gal β 1-4
(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc-AMC ND

β -Galactosidase:
Gal β 1-3GlcNAc β 1-4Gal β 1-4Glc-AMC ND

α -Galactosidase:
Gal α 1-3Gal β 1-4Gal α 1-3Gal-AMC ND

α -Neuraminidase:
Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β
1-4Glc-AMC ND

α -Mannosidase:
Man α 1-3Man β 1-4GlcNAc-AMC
Man α 1-6Man α 1-6(Man α 1-3)Man-AMC ND

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

(See other side)

CERTIFICATE OF ANALYSIS

β -N-Acetyl-hexosaminidase_f



1-800-632-7799
info@neb.com
www.neb.com



P0721S 001121014101

P0721S

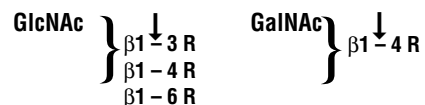


500 units 5,000 U/ml Lot: 0011210
RECOMBINANT Store at -20°C Exp: 10/14

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:



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 Na₂EDTA.

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 GalNAc β 1-4Gal β 1-4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 μ l.

Unit Definition Assay: Two fold dilutions of β -N-Acetyl-hexosaminidase, are incubated with 1 nmol AMC-labeled substrate in 1X G2 Reaction Buffer in a 10 μ l 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 μ l 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:
Fuc α 1-2Gal β 1-4Glc-AMC Gal β 1-4
(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc-AMC ND

β -Galactosidase:
Gal β 1-3GlcNAc β 1-4Gal β 1-4Glc-AMC ND

α -Galactosidase:
Gal α 1-3Gal β 1-4Gal α 1-3Gal-AMC ND

α -Neuraminidase:
Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β
1-4Glc-AMC ND

α -Mannosidase:
Man α 1-3Man β 1-4GlcNAc-AMC
Man α 1-6Man α 1-6(Man α 1-3)Man-AMC ND

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

(See other side)

CERTIFICATE OF ANALYSIS

β -Xylosidase:
Xyl β 1-4Xyl β 1-4Xyl β 1-4Xyl-AMC ND

β -Mannosidase:
Man β 1-4Man β 1-4Man-AMC ND

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

Endo F₂, F₃:
Dansylated fibrinogen biantennary. ND

PNGase F:
Fluoresceinated fetuin triantennary. ND

Protease Assay: After incubation of 50 units of β -N-Acetyl-hexosaminidase, with 0.2 nmol of a standard mixture of proteins in a 20 μ l reaction, for 20 hours at 37°C, no proteolytic activity could be detected by SDS-PAGE.

***Note:** Non-branched oligosaccharides only.

Page 2 (P0721)

β -Xylosidase:
Xyl β 1-4Xyl β 1-4Xyl β 1-4Xyl-AMC ND

β -Mannosidase:
Man β 1-4Man β 1-4Man-AMC ND

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

Endo F₂, F₃:
Dansylated fibrinogen biantennary. ND

PNGase F:
Fluoresceinated fetuin triantennary. ND

Protease Assay: After incubation of 50 units of β -N-Acetyl-hexosaminidase, with 0.2 nmol of a standard mixture of proteins in a 20 μ l reaction, for 20 hours at 37°C, no proteolytic activity could be detected by SDS-PAGE.

***Note:** Non-branched oligosaccharides only.

Page 2 (P0721)

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.

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.