

# $\beta$ -N-Acetyl-hexosaminidase<sub>f</sub>



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



P0721S 001150817081

## P0721S



500 units 5,000 U/ml Lot: 0011508

RECOMBINANT Store at -20°C Exp: 8/17

**Description:**  $\beta$ -N-Acetyl-hexosaminidase<sub>f</sub> is a recombinant protein fusion of  $\beta$ -N-Acetyl-hexosaminidase (1) and maltose binding protein. It has identical activity to  $\beta$ -N-Acetyl-hexosaminidase.  $\beta$ -N-Acetyl-hexosaminidase<sub>f</sub> catalyzes the hydrolysis of terminal  $\beta$ -D-N-acetyl-galactosamine and glucosamine residues from oligosaccharides.

New Reaction Buffer

# $\beta$ -N-Acetyl-hexosaminidase<sub>f</sub>



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



P0721S 001150817081

## P0721S



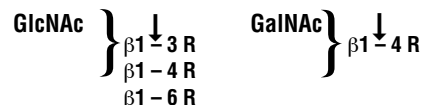
500 units 5,000 U/ml Lot: 0011508

RECOMBINANT Store at -20°C Exp: 8/17

**Description:**  $\beta$ -N-Acetyl-hexosaminidase<sub>f</sub> is a recombinant protein fusion of  $\beta$ -N-Acetyl-hexosaminidase (1) and maltose binding protein. It has identical activity to  $\beta$ -N-Acetyl-hexosaminidase.  $\beta$ -N-Acetyl-hexosaminidase<sub>f</sub> catalyzes the hydrolysis of terminal  $\beta$ -D-N-acetyl-galactosamine and glucosamine residues from oligosaccharides.

New Reaction Buffer

### Specificity:



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

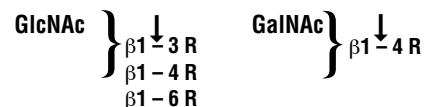
Supplied in: 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C) and 5 mM Na<sub>2</sub>EDTA.

**Reagents Supplied with Enzyme:**  
10X GlycoBuffer 1

**Reaction Conditions:**  
1X GlycoBuffer 1:  
50 mM Sodium Acetate (pH 5.5 @ 25°C)  
and 5 mM CaCl<sub>2</sub>. Incubate at 37°C.

Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate.

### 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 Na<sub>2</sub>EDTA.

**Reagents Supplied with Enzyme:**  
10X GlycoBuffer 1

**Reaction Conditions:**  
1X GlycoBuffer 1:  
50 mM Sodium Acetate (pH 5.5 @ 25°C)  
and 5 mM CaCl<sub>2</sub>. 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  $\beta$ -D-N-acetyl-galactosamine from 1 nmol of GalNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-7-amino-4-methylcoumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10  $\mu$ l.

**Unit Definition Assay:** Two fold dilutions of  $\beta$ -N-Acetyl-hexosaminidase, are incubated with 1 nmol AMC-labeled substrate in 1X GlycoBuffer 1 in a 10  $\mu$ 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.

**Unit Definition:** One unit is defined as the amount of enzyme required to cleave > 95% of the terminal  $\beta$ -D-N-acetyl-galactosamine from 1 nmol of GalNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-7-amino-4-methylcoumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10  $\mu$ l.

**Unit Definition Assay:** Two fold dilutions of  $\beta$ -N-Acetyl-hexosaminidase, are incubated with 1 nmol AMC-labeled substrate in 1X GlycoBuffer 1 in a 10  $\mu$ 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  $\beta$ -N-Acetyl-hexosaminidase<sub>f</sub> were incubated with 0.1 mM of fluorescently-labeled oligosaccharides and glycopeptides, in a 10  $\mu$ 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:

**$\alpha$ -Fucosidase:**  
Fuc $\alpha$ 1-2Gal $\beta$ 1-4Glc-AMC Gal $\beta$ 1-4  
(Fuc $\alpha$ 1-3)GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC ND

**$\beta$ -Galactosidase:**  
Gal $\beta$ 1-3GlcNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC ND

**$\alpha$ -Galactosidase:**  
Gal $\alpha$ 1-3Gal $\beta$ 1-4Gal $\alpha$ 1-3Gal-AMC ND

(See other side)

CERTIFICATE OF ANALYSIS

### Quality Controls

**Glycosidase Assays:** 50 units of  $\beta$ -N-Acetyl-hexosaminidase<sub>f</sub> were incubated with 0.1 mM of fluorescently-labeled oligosaccharides and glycopeptides, in a 10  $\mu$ 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:

**$\alpha$ -Fucosidase:**  
Fuc $\alpha$ 1-2Gal $\beta$ 1-4Glc-AMC Gal $\beta$ 1-4  
(Fuc $\alpha$ 1-3)GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC ND

**$\beta$ -Galactosidase:**  
Gal $\beta$ 1-3GlcNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC ND

**$\alpha$ -Galactosidase:**  
Gal $\alpha$ 1-3Gal $\beta$ 1-4Gal $\alpha$ 1-3Gal-AMC ND

(See other side)

CERTIFICATE OF ANALYSIS

**$\alpha$ -Neuraminidase:**  
Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$   
1-4Glc-AMC ND

**$\alpha$ -Mannosidase:**  
Man $\alpha$ 1-3Man $\beta$ 1-4GlcNAc-AMC  
Man $\alpha$ 1-6Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man-AMC ND

**$\beta$ -Glucosidase:**  
Glc $\beta$ 1-4Glc $\beta$ 1-4Glc-AMC ND

**$\beta$ -Xylosidase:**  
Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl-AMC ND

**$\beta$ -Mannosidase:**  
Man $\beta$ 1-4Man $\beta$ 1-4Man-AMC ND

**Endo F<sub>1</sub>, F<sub>2</sub>, H:**  
Dansylated invertase high mannose. ND

**Endo F<sub>2</sub>, F<sub>3</sub>:**  
Dansylated fibrinogen biantennary. ND

Page 2 (P0721)

**$\alpha$ -Neuraminidase:**  
Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$   
1-4Glc-AMC ND

**$\alpha$ -Mannosidase:**  
Man $\alpha$ 1-3Man $\beta$ 1-4GlcNAc-AMC  
Man $\alpha$ 1-6Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man-AMC ND

**$\beta$ -Glucosidase:**  
Glc $\beta$ 1-4Glc $\beta$ 1-4Glc-AMC ND

**$\beta$ -Xylosidase:**  
Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl-AMC ND

**$\beta$ -Mannosidase:**  
Man $\beta$ 1-4Man $\beta$ 1-4Man-AMC ND

**Endo F<sub>1</sub>, F<sub>2</sub>, H:**  
Dansylated invertase high mannose. ND

**Endo F<sub>2</sub>, F<sub>3</sub>:**  
Dansylated fibrinogen biantennary. ND

Page 2 (P0721)

**PNGase F:**  
Fluoresceinated fetuin triantennary. ND

**Protease Assay:** After incubation of 50 units of  $\beta$ -N-Acetyl-hexosaminidase<sub>i</sub> with 0.2 nmol of a standard mixture of proteins in a 20  $\mu$ l 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.



NEW ENGLAND BIOLABS® is a registered trademark of New England Biolabs, Inc.

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

**PNGase F:**  
Fluoresceinated fetuin triantennary. ND

**Protease Assay:** After incubation of 50 units of  $\beta$ -N-Acetyl-hexosaminidase<sub>i</sub> with 0.2 nmol of a standard mixture of proteins in a 20  $\mu$ l 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.



NEW ENGLAND BIOLABS® is a registered trademark of New England Biolabs, Inc.

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.