

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



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



P0721S 001150117011

## P0721S



500 units 5,000 U/ml Lot: 0011501

RECOMBINANT Store at -20°C Exp: 1/17

**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 001150117011

## P0721S



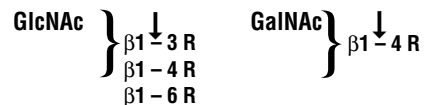
500 units 5,000 U/ml Lot: 0011501

RECOMBINANT Store at -20°C Exp: 1/17

**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.

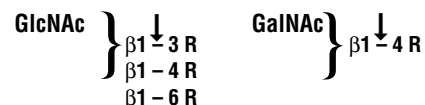
**Reagents Supplied with Enzyme:**  
10X GlycoBuffer 1

### 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 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.

**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.