

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



1-800-632-7799  
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P0721S 001120414041

## P0721S

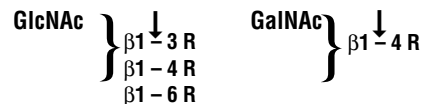


**500 units    5,000 U/ml    Lot: 0011204**  
**RECOMBINANT    Store at -20°C    Exp: 4/14**

**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.

**\*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 Na<sub>2</sub>EDTA.

**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  $\beta$ -D-N-acetyl-galactosamine from

1 nmol of GalNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-7-amino-4-methyl-coumarin (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 G2 Reaction Buffer 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

**$\alpha$ -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

(See other side)

CERTIFICATE OF ANALYSIS

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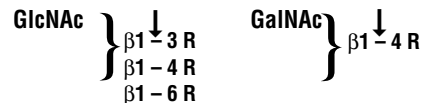


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(Fuc $\alpha$ 1-3)GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC    ND

**$\beta$ -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

**$\alpha$ -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

(See other side)

CERTIFICATE OF ANALYSIS

**$\beta$ -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

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

**Protease Assay:** After incubation of 50 units of  $\beta$ -N-Acetyl-hexosaminidase, 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.

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**$\beta$ -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

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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.

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