

# $\beta$ -N-Acetylglucosaminidase S



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P0744S 001150416041

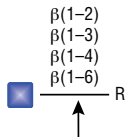
## P0744S



**100 units**    **4,000 U/ml**    **Lot: 0011504**  
**RECOMBINANT**    **Store at 4°C**    **Exp: 4/16**

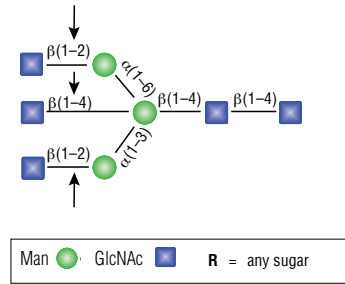
**Description:**  $\beta$ -N-Acetylglucosaminidase S is a highly specific exoglycosidase that catalyzes the hydrolysis of terminal, non-reducing  $\beta$ -N-Acetylglucosamine residues from oligosaccharides.

### Specificity:



### Detailed Specificity:

$\beta$ -N-Acetylglucosaminidase S is able to efficiently cleave bisecting  $\beta$ -N-Acetylglucosaminidase residues.



**Source:** Cloned from *Streptococcus pneumoniae* and expressed in *E. coli* (1).

Supplied in: 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C) and 1 mM EDTA.

### Reagents Supplied with Enzyme:

10X GlycoBuffer 1  
(0.5 M Sodium Acetate, pH 5.5 @ 25°C and 50 mM CaCl<sub>2</sub>)

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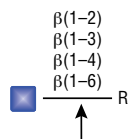
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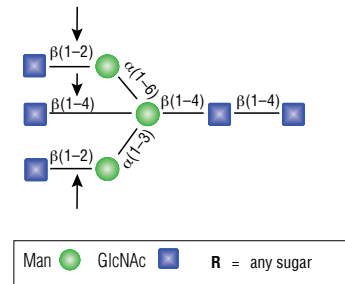
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**Unit Definition:** One unit is defined as the amount of enzyme required to cleave > 95% of the terminal, non-reducing  $\beta$ -N-Acetylglucosamine from 1 nmol GlcNAc $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-7-amino-4-methylcoumarin (AMC), in 1 hour at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

**Unit Definition Assay:** Two fold dilutions of  $\beta$ -N-Acetylglucosaminidase S are incubated with 1 nmol AMC-labeled substrate and 1X GlycoBuffer 1 in a 10  $\mu$ l reaction. The reaction mix is incubated at 37°C for 1 hour. Separation of reaction products are visualized via thin layer chromatography (2).

**Specific Activity:** ~2,700 units/mg.

**Molecular Weight:** 125,000 daltons.

**Quality Assurance:** No contaminating exoglycosidase or endoglycosidase F1, F2 or F3 activity could be detected. No contaminating proteolytic activity could be detected.

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### Quality Controls

**Glycosidase Assays:** 16 units of  $\beta$ -N-Acetylglucosaminidase S 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:

**$\beta$ -N-Acetylgalactosaminidase:**  
GalNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC    ND

**$\alpha$ -N-Acetylgalactosaminidase:**  
GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4Glc-AMC    ND

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

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

(see other side)

CERTIFICATE OF ANALYSIS

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

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

(see other side)

CERTIFICATE OF ANALYSIS

**$\alpha$ -Galactosidase:**  
Gal $\alpha$ 1-3Gal $\beta$ 1-4Gal-AMC ND  
Gal $\alpha$ 1-6Gal $\alpha$ 1-6Glc $\alpha$ 1-2Fru-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 ND  
Man $\alpha$ 1-6Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man-AMC ND

**$\alpha$ -Glucosidase:**  
Glc $\alpha$ 1-6Glc $\alpha$ 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

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**$\alpha$ -Galactosidase:**  
Gal $\alpha$ 1-3Gal $\beta$ 1-4Gal-AMC ND  
Gal $\alpha$ 1-6Gal $\alpha$ 1-6Glc $\alpha$ 1-2Fru-AMC ND

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**$\alpha$ -Glucosidase:**  
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**Endo F<sub>2</sub>, F<sub>3</sub>:**  
Dansylated fibrinogen biantennary. ND

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**Protease Assay:** After incubation of 24 units of  $\beta$ -*N*-Acetylglucosaminidase S 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.

**Physical Purity:** Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

**Reaction Conditions:** Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate. Typical reaction conditions are as follows:

1. Combine 1  $\mu$ g of glycoprotein or 100 nM of oligosaccharide and H<sub>2</sub>O (if necessary) to make a 9  $\mu$ l total reaction volume.
2. Add 1  $\mu$ l of 10X GlycoBuffer 1 to make a 10  $\mu$ l total reaction volume.
3. Add 1  $\mu$ l of  $\beta$ -*N*-Acetylglucosaminidase S.
4. Incubate at 37°C for 1 hour.

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2. Add 1  $\mu$ l of 10X GlycoBuffer 1 to make a 10  $\mu$ l total reaction volume.
3. Add 1  $\mu$ l of  $\beta$ -*N*-Acetylglucosaminidase S.
4. Incubate at 37°C for 1 hour.

#### Notes on Use:

- Reactions may be scaled-up linearly to accommodate larger reaction volumes.
- The amount of exoglycosidase enzyme required varies when different substrates are used. Start with 1–2  $\mu$ l for 1  $\mu$ g of glycoprotein or 100 nM of oligosaccharide for one hour in a 10–25  $\mu$ l reaction. If there is still undigested material, let the reaction go overnight.
- $\beta$ -*N*-Acetylglucosaminidase S cannot be heat inactivated.

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#### References:

1. Chen, M. New England Biolabs, Inc., unpublished results.
2. Wong-Madden, S. T. and Landry, D. (1995) *Glycobiology* 5, 19–28.



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#### References:

1. Chen, M. New England Biolabs, Inc., unpublished results.
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