

## $\alpha$ -N-Acetyl-galactosaminidase



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P0734S 006140816081

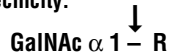
# P0734S



3,000 units 20,000 U/ml Lot: 0061408  
RECOMBINANT Store at -20°C (see note) Exp: 8/16

**Description:**  $\alpha$ -N-Acetyl-galactosaminidase is a highly specific exoglycosidase that catalyzes the hydrolysis of  $\alpha$ -linked D-N-acetyl-galactosamine residues from oligosaccharides and N-glycans attached to proteins (1).

### Specificity:



**Note:** p-nitrophenyl- $\alpha$ -D-N-acetyl-galactosaminide a substrate for this enzyme, however, the p-nitrophenyl- $\alpha$ -D-N-acetyl-glucosaminide is NOT a substrate for this enzyme.

**Source:** Cloned from *Chryseobacterium meningosepticum* and expressed in *E. coli* at NEB (1).

Supplied in: 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C) and 0.1 mM EDTA.

**Reagents Supplied with Enzyme:**  
10X G7 Reaction Buffer, 100X BSA

### Reaction Conditions:

1X G7 Reaction Buffer:  
50 mM Sodium Phosphate (pH 7.5 @ 25°C), supplement with 100  $\mu$ g/ml BSA. 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  $\alpha$ -D-N-acetyl-galactosamine from 1 nmol (GalNAc $\alpha$ 1-3)(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10  $\mu$ l.

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**Unit Definition Assay:** Two fold dilutions of  $\alpha$ -N-Acetyl-galactosaminidase are incubated with 1 nmol AMC-labeled substrate in 1X G1 Reaction Buffer, supplemented with 100  $\mu$ g/ml BSA, 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 (2).

**Specific Activity:** 20,000 units/mg

**Molecular Weight:** 47,000 daltons.

**Quality Assurance:** No contaminating exoglycosidase or proteolytic activity could be detected.

### Quality Controls

**Glycosidase Assays:** 20 units of  $\alpha$ -N-Acetyl-galactosaminidase were incubated with 0.1 mM of fluorescently-labeled oligosaccharides and glycopeptides, in a 10  $\mu$ l reaction for 20 hours at 37°C (see list below). The reaction products were analyzed by TLC for digestion of substrate (3).

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

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

No other glycosidase activities were detected (ND) with the following substrates:

**$\beta$ -N-Acetyl-glucosaminidase:**  
GlcNAc $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-AMC ND

**$\alpha$ -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-AMC ND

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

**$\alpha$ -Mannosidase:**  
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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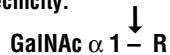
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**$\beta$ -N-Acetyl-glucosaminidase:**  
GlcNAc $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-AMC ND

**$\alpha$ -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-AMC ND

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

**$\alpha$ -Mannosidase:**  
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 20 units of  $\alpha$ -N-Acetyl-galactosaminidase with 0.2 nmol of a standard mixture of proteins, for 20 hours at 37°C, no proteolytic activity could be detected by SDS-PAGE.

**Note:** Recommended storage temperature has changed to -20°C. Avoid repeated freeze/thaw cycles.

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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

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**References:**

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

U.S. Patent No. 6,458,573 and 6,423,525

**References:**

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