

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



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P0734S 006141116111

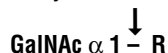
# P0734S



3,000 units    20,000 U/ml    Lot: 0061411  
RECOMBINANT    Store at -20°C (see note)    Exp: 11/16

**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:



New Reaction Buffer

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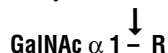
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**Note:** *p*-nitrophenyl- $\alpha$ -D-N-acetyl-galactosaminide a substrate for this enzyme, however, the *p*-nitrophenyl- $\alpha$ -D-N-acetyl-glucosaminide is NOT a substrate for this enzyme.

**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 1 mM EDTA.

### Reagents Supplied with Enzyme:

10X GlycoBuffer 1  
100X BSA

### Reaction Conditions:

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

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**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.

**Unit Definition Assay:** Two fold dilutions of  $\alpha$ -N-Acetyl-galactosaminidase are incubated with 1 nmol AMC-labeled substrate in 1X GlycoBuffer 1, 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.

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### 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.

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

(See other side)

CERTIFICATE OF ANALYSIS

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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

(See other side)

CERTIFICATE OF ANALYSIS

<b><math>\alpha</math>-Galactosidase:</b> Gal $\alpha$ 1-3Gal $\beta$ 1-4Gal-AMC	ND
<b><math>\alpha</math>-Neuraminidase:</b> Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC	ND
<b><math>\alpha</math>-Mannosidase:</b> Man $\alpha$ 1-6Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man-AMC	ND
<b><math>\beta</math>-Glucosidase:</b> Glc $\beta$ 1-4Glc $\beta$ 1-4Glc-AMC	ND
<b><math>\beta</math>-Xylosidase:</b> Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl-AMC	ND
<b><math>\beta</math>-Mannosidase:</b> Man $\beta$ 1-4Man $\beta$ 1-4Man-AMC	ND
<b>Endo F<sub>1</sub>, F<sub>2</sub>, H:</b> Dansylated invertase high mannose.	ND

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<b><math>\alpha</math>-Galactosidase:</b> Gal $\alpha$ 1-3Gal $\beta$ 1-4Gal-AMC	ND
<b><math>\alpha</math>-Neuraminidase:</b> Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC	ND
<b><math>\alpha</math>-Mannosidase:</b> Man $\alpha$ 1-6Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man-AMC	ND
<b><math>\beta</math>-Glucosidase:</b> Glc $\beta$ 1-4Glc $\beta$ 1-4Glc-AMC	ND
<b><math>\beta</math>-Xylosidase:</b> Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl-AMC	ND
<b><math>\beta</math>-Mannosidase:</b> Man $\beta$ 1-4Man $\beta$ 1-4Man-AMC	ND
<b>Endo F<sub>1</sub>, F<sub>2</sub>, H:</b> Dansylated invertase high mannose.	ND

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

#### References:

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



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

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#### References:

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