

α -N-Acetyl-galactosaminidase



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P0734S 006150817081

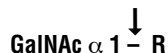
P0734S



3,000 units 20,000 U/ml Lot: 0061508
RECOMBINANT Store at -20°C (see note) Exp: 8/17

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

Specificity:



New Reaction Buffer

Note: *p*-nitrophenyl- α -D-N-acetyl-galactosaminide a substrate for this enzyme, however, the *p*-nitrophenyl- α -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 CaCl₂. Supplement with 100 μ 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 α -D-N-acetyl-galactosamine from 1 nmol (GalNAc α 1-3)(Fuc α 1-2)Gal β 1-4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 μ l.

Unit Definition Assay: Two fold dilutions of α -N-Acetyl-galactosaminidase are incubated with 1 nmol AMC-labeled substrate in 1X GlycoBuffer 1, supplemented with 100 μ g/ml BSA, in a 10 μ 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 α -N-Acetyl-galactosaminidase were incubated with 0.1 mM of fluorescently-labeled oligosaccharides and glycopeptides, in a 10 μ 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:

β -N-Acetyl-glucosaminidase:
GlcNAc β 1-4GlcNAc β 1-4GlcNAc-AMC ND

α -Fucosidase:
Fuc α 1-2Gal β 1-4Glc-AMC Gal β 1-4
(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc-AMC ND

β -Galactosidase:
Gal β 1-3GlcNAc β 1-4Gal β 1-4Glc-AMC ND

(See other side)

CERTIFICATE OF ANALYSIS

α -N-Acetyl-galactosaminidase



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Molecular Weight: 47,000 daltons.

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

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Glycosidase Assays: 20 units of α -N-Acetyl-galactosaminidase were incubated with 0.1 mM of fluorescently-labeled oligosaccharides and glycopeptides, in a 10 μ 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:

β -N-Acetyl-glucosaminidase:
GlcNAc β 1-4GlcNAc β 1-4GlcNAc-AMC ND

α -Fucosidase:
Fuc α 1-2Gal β 1-4Glc-AMC Gal β 1-4
(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc-AMC ND

β -Galactosidase:
Gal β 1-3GlcNAc β 1-4Gal β 1-4Glc-AMC ND

(See other side)

CERTIFICATE OF ANALYSIS

α-Galactosidase: Gal α 1-3Gal β 1-4Gal-AMC	ND
α-Neuraminidase: Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc-AMC	ND
α-Mannosidase: Man α 1-6Man α 1-6(Man α 1-3)Man-AMC	ND
β-Glucosidase: Glc β 1-4Glc β 1-4Glc-AMC	ND
β-Xylosidase: Xyl β 1-4Xyl β 1-4Xyl β 1-4Xyl-AMC	ND
β-Mannosidase: Man β 1-4Man β 1-4Man-AMC	ND
Endo F₁, F₂, H: Dansylated invertase high mannose.	ND

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α-Galactosidase: Gal α 1-3Gal β 1-4Gal-AMC	ND
α-Neuraminidase: Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc-AMC	ND
α-Mannosidase: Man α 1-6Man α 1-6(Man α 1-3)Man-AMC	ND
β-Glucosidase: Glc β 1-4Glc β 1-4Glc-AMC	ND
β-Xylosidase: Xyl β 1-4Xyl β 1-4Xyl β 1-4Xyl-AMC	ND
β-Mannosidase: Man β 1-4Man β 1-4Man-AMC	ND
Endo F₁, F₂, H: Dansylated invertase high mannose.	ND

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Endo F₂, F₃: Dansylated fibrinogen biantennary.	ND
PNGase F: Fluoresceinated fetuin triantennary.	ND

Protease Assay: After incubation of 20 units of α -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.



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References:

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