α-N-Acetyl-galactosaminidase

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P0734S 006121014101

P0734S

3,000 units **20,000 U/ml** **Lot: 0061210**
RECOMBINANT **Store at –20°C (see note)** **Exp: 10/14**

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:

GalNAc α 1 ↓ – R

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 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 µg/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 (GalNAcα1-3)(Fucα1-2)Galβ1-4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

Unit Definition Assay: Two fold dilutions of α-N-Acetyl-galactosamininidase are incubated with 1 nmol AMC-labeled substrate in 1X G1 Reaction Buffer, supplemented with 100 µg/ml BSA, in a 10 µl 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-galactosamininidase were incubated with 0.1 mM of flourescently-labeled oligosaccharides and glycopeptides, in a 10 µl 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:
GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC ND

α-Fucosidase:
Fucα1-2Galβ1-4Glc-AMC Galβ1-4 (Fucα1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND

β-Galactosidase:
Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC ND

α-Galactosidase:
Galα1-3Galβ1-4Gal-AMC ND

α-Neuraminidase:
Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ 1-4Glc-AMC ND

α-Mannosidase:
Manα1-6Manα1-6(Manα1-3)Man-AMC ND

β-Glucosidase:
Glcβ1-4Glcβ1-4Glc-AMC ND

(See other side)

CERTIFICATE OF ANALYSIS

α-N-Acetyl-galactosaminidase

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GalNAc α 1 ↓ – R

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β-N-Acetyl-glucosaminidase:
GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC ND

α-Fucosidase:
Fucα1-2Galβ1-4Glc-AMC Galβ1-4 (Fucα1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND

β-Galactosidase:
Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC ND

α-Galactosidase:
Galα1-3Galβ1-4Gal-AMC ND

α-Neuraminidase:
Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ 1-4Glc-AMC ND

α-Mannosidase:
Manα1-6Manα1-6(Manα1-3)Man-AMC ND

β-Glucosidase:
Glcβ1-4Glcβ1-4Glc-AMC ND

(See other side)

CERTIFICATE OF ANALYSIS

β-Xylosidase:
Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC ND

β-Mannosidase:
Manβ1-4Manβ1-4Man-AMC ND

Endo F_1, F_2, H:
Dansylated invertase high mannose. ND

Endo F_2, F_3:
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:

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