α1-3,4,6 Galactosidase

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info@neb.com
www.neb.com

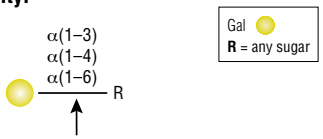
P0747S 10115111116111

P0747S

200 units	**8,000 U/ml**	**Lot: 0011511**
Store at 4°C		**Exp: 11/16**

Description: α1-3,4,6 Galactosidase is a broad specificity exoglycosidase that catalyzes the hydrolysis of α1-3, α1-4, and α1-6 linked D-galactopyranosyl residues from oligosaccharides.

Specificity:



Source: Cloned from green coffee bean and expressed in *E. coli* (1).

Supplied in: 20 mM Tris-HCl (pH 7.5), 50 mM NaCl and 1 mM EDTA

Reagents Supplied with Enzyme:
10X GlycoBuffer 1
100X BSA

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal, α-D-galactose from 1 nmol Galα1-3Galβ1-4Gal-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

Specific Activity: 71,000 U/mg

Molecular Weight: 39,700 daltons

Unit Definition Assay: Two fold dilutions of α1-3,4,6 Galactosidase are incubated with 1 nmol AMC-labeled substrate in 1X GlycoBuffer 1 and 1X BSA in a 10 µl reaction. The reaction mix is incubated at 37°C for 1 hour. Separation of reaction products are visualized via thin layer chromatography (2).

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

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

Heat Inactivation: 8 units of enzyme were inactivated by incubation at 65°C for 10 minutes.

Reaction Protocol

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

1. Combine 1 µg of glycoprotein or 100 nM of oligosaccharide and H_2O (if necessary) to make an 8 µl total reaction volume.
2. Add 1 µl of 10X GlycoBuffer 1 and 1 µl of 10X BSA (diluted 1:10 from 100X concentration) to make a 10 µl total reaction volume.
3. Add 1 µl of α1-3,4,6 Galactosidase.
4. Incubate at 37°C for 1 hour.

Notes: 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 µl for 1ug of glycoprotein or 100 nM of oligosaccharide for one hour in a 10-25 µl reaction. If there is still undigested material, let the reaction go overnight.

Store at 4°C, Avoid repeated freeze-thaw cycles.

(see other side)

CERTIFICATE OF ANALYSIS

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Specificity:

α(1–3) α(1–4) α(1–6) R — Gal; R = any sugar

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(see other side)

CERTIFICATE OF ANALYSIS

Quality Controls

Glycosidase Assays: 80 units of α1-3,4,6 Galactosidase were incubated with 0.1 mM of fluorescently-labeled oligosaccharides and glycopeptides, in a 10µl 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:

β-N-Acetylglucosaminidase:
GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC ND

β-N-Acetylgalactosaminidase:
GalNAcβ1-4Galβ1-4Glc-AMC ND

α-N-Acetylgalactosaminidase:
GalNAcα1-3(Fucα1-2)Galβ1-4Glc-AMC ND

α-Fucosidase:
Galβ1-4 (Fucα1-3)GlcNAcβ1-3Galβ 1-4Glc-AMC ND
Fucα1-2Galβ1-4Glc-AMC ND

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

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

α-Mannosidase:
Manα1-3Manβ1-4GlcNAc-AMC ND
Manα1-6Manα1-6(Manα1-3)Man-AMC ND

α-Glucosidase:
Glcα1-6Glcα1-4Glc-AMC ND

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

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

Endo F1, F2, H:
Dansylated invertase high mannose ND

Endo F2, F3:
Dansylated fibrinogen biantennary ND

Protease Assay: After incubation of 200 units of α1-3,4,6 Galactosidase with 0.2 nmol of a standard mixture of proteins in a 20 µl reaction, for 20 hours at 37°C, no proteolytic activity could be detected by SDS-PAGE.

References:

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

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α-N-Acetylgalactosaminidase:
GalNAcα1-3(Fucα1-2)Galβ1-4Glc-AMC ND

α-Fucosidase:
Galβ1-4 (Fucα1-3)GlcNAcβ1-3Galβ 1-4Glc-AMC ND
Fucα1-2Galβ1-4Glc-AMC ND

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

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

α-Mannosidase:
Manα1-3Manβ1-4GlcNAc-AMC ND
Manα1-6Manα1-6(Manα1-3)Man-AMC ND

α-Glucosidase:
Glcα1-6Glcα1-4Glc-AMC ND

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

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

Endo F1, F2, H:
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