

α 1-3, 6 Galactosidase



1-800-632-7799
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www.neb.com



P0731S 004151116111

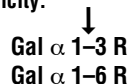
P0731S



100 units 4,000 U/ml Lot: 0041511
RECOMBINANT Store at 4°C Exp: 11/16

Description: α 1-3, 6 Galactosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of α 1-3, 6 linked D-galactopyranosyl residues from oligosaccharides.

Specificity:



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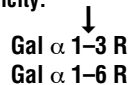
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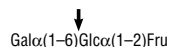
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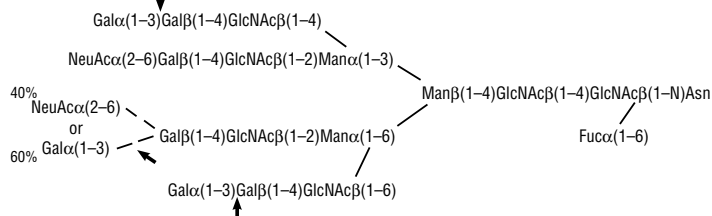


Detailed Specificity: Specificity can vary depending on incubation time and branching structure.

A) 0.1 nm/ μ l substrate, 1 hour incubation



B) 0.1 nm/ μ l substrate, 18 hour incubation



C) 0.1 nm/ μ l substrate, 1 hour incubation, not cleaved

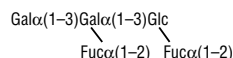
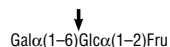


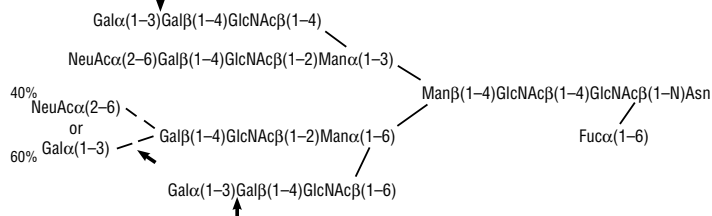
Figure 1: Detailed specificity of α 1-3, 6 Galactosidase. Reactions (A) and (C) contained 4 units of α 1-3, 6 Galactosidase, 1X GlycoBuffer 1 and 1X BSA in a total reaction volume of 10 μ l. Reaction (C) shows that branched fucose inhibits cleavage. Reaction (B) contained 24 units of α 1-3, 6 Galactosidase and 100 units of Neuraminidase, followed by a heat kill at 65°C for 10 minutes and a 2 hour digestion with 16 units of β 1-4 Galactosidase. The reaction in (B) contained 1X GlycoBuffer 1 and 1X BSA in a total reaction volume of 20 μ l. The reactions were incubated at 37°C. Complete digestion of the α 1-3, 6 Galactosidase was determined by an observation of complete transformation of the substrate in (B) to the non-reducing terminal N-acetylglucosamine tetra antennary oligosaccharide.

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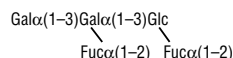


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Source: Cloned from *Xanthomonas manihotis* and expressed in *E. coli* (1).

Supplied in: 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C) and 1 mM Na₂EDTA.

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-galactose from 1 nmol Gal α 1-3Gal β 1-4Gal-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 μ l.

(See other side)

CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS

Unit Definition Assay: Two fold serial dilutions of α 1-3, 6 Galactosidase are incubated with 1 nmol AMC-labeled substrate in 1X GlycoBuffer 1 and 1X 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: 137,000 units/mg

Molecular Weight: 70,000 daltons.

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

Quality Controls

Glycosidase Assays:

12 units of α 1-3, 6 Galactosidase were incubated with 0.1 mM of fluorescently-labeled oligosaccharides and glycopeptides, in a 10 μ l reaction for 20 hours at 37°C. The reaction products were analyzed by TLC for digestion of substrate.

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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:

β -N-Acetylglucosaminidase:
GlcNAc β 1-4GlcNAc β 1-4GlcNAc-AMC ND

α -N-Acetylgalactosaminidase:
GalNAc α 1-3(Fuc α 1-2)Gal β 1-4Glc-AMC ND

α -Fucosidase:
Fuc α 1-2Gal β 1-4Glc-AMC ND

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

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

α -Neuraminidase:
Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc-AMC ND

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β -N-Acetylglucosaminidase:
GlcNAc β 1-4GlcNAc β 1-4GlcNAc-AMC ND

α -N-Acetylgalactosaminidase:
GalNAc α 1-3(Fuc α 1-2)Gal β 1-4Glc-AMC ND

α -Fucosidase:
Fuc α 1-2Gal β 1-4Glc-AMC ND

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

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

α -Neuraminidase:
Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc-AMC ND

α -Mannosidase:
Man α 1-3Man β 1-4GlcNAc-AMC ND

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

α -Glucosidase:
Glc α 1-6Glc α 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

Endo F₂, F₃:
Dansylated fibrinogen biantennary. ND

PNGase F:
Fluoresceinated fetuin triantennary. ND

α -Mannosidase:
Man α 1-3Man β 1-4GlcNAc-AMC ND

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

α -Glucosidase:
Glc α 1-6Glc α 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

Endo F₂, F₃:
Dansylated fibrinogen biantennary. ND

PNGase F:
Fluoresceinated fetuin triantennary. ND

Protease Assay: After incubation of 28 units of α 1-3, 6 Galactosidase with 0.2 nmol of a standard mixture of proteins in a 20 μ l reaction, for 20 hours at 37°C, no proteolytic activity could be detected by SDS-PAGE.

Note: Recommended storage temperature is 4°C. Avoid repeated freeze/thaw cycles

Heat Inactivation: 65°C for 10 minutes.

References:

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



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