

β 1-4 Galactosidase



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P0730S 002150417041

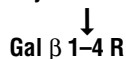
P0730S



400 units 8,000 U/ml Lot: 0021504
RECOMBINANT Store at -20°C Exp: 4/17

Description: β 1-4 Galactosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of β 1-4 linked D-galactopyranosyl residues from oligosaccharides.

Specificity:



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

New Reaction Buffer

β 1-4 Galactosidase



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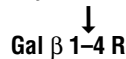
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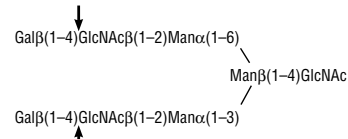
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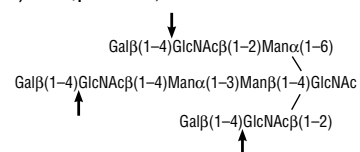
Detailed Specificity: Specificity can vary depending on incubation time and branching structure.

New Reaction Buffer

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



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



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

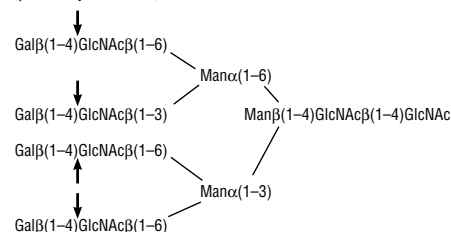
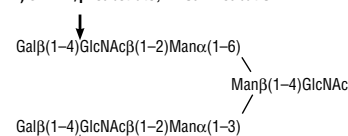
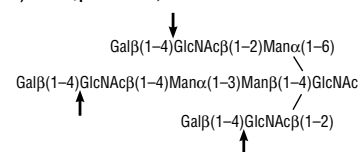


Figure 1: Detailed specificity of β 1-4 Galactosidase. Reactions (A), (B) and (C) contained 2 units, 4 units and 8 units of β 1-4 Galactosidase, respectively, and 1X GlycoBuffer 1 total reaction volume of 10 μ l. Reactions were incubated at 37°C.

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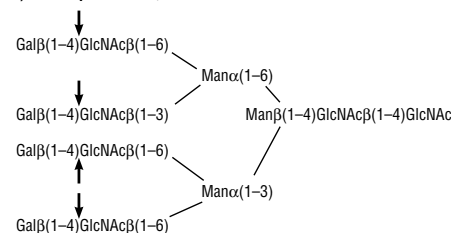


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Source: Cloned from *Bacteroides fragilis* and expressed in *E. coli* (1).

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

Reaction Conditions:

1X GlycoBuffer 1:
50 mM Sodium Acetate (pH 5.5 @ 25°C)
and 5 mM CaCl₂. 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, β -D-galactose from 1 nmol Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc-7-amino-4-methylcoumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 μ l.

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Unit Definition Assay: Two fold serial dilutions of β 1-4 Galactosidase are incubated with 1 nmol AMC-labeled substrate in 1X GlycoBuffer 1, 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: 50,000 units/mg

Molecular Weight: 94,000 daltons.

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

Quality Controls

Glycosidase Assays: 32 units of β 1-4 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.

(See other side)

CERTIFICATE OF ANALYSIS

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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

α -Galactosidase:

Gal α 1-3Gal β 1-4Gal-AMC ND

Gal α 1-6Gal α 1-6Glc α 1-2Fru-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

PNase F:

Fluoresceinated fetuin triantennary. ND

Protease Assay: After incubation of 112 units of β 1-4 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 -20°C.

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.



U.S. Patent No. 6,358,724

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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

α -Galactosidase:

Gal α 1-3Gal β 1-4Gal-AMC ND

Gal α 1-6Gal α 1-6Glc α 1-2Fru-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

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Man β 1-4Man β 1-4Man-AMC ND

Endo F₁, F₂, H:

Dansylated invertase high mannose. ND

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