

β 1-4 Galactosidase



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



P0730S 003130515051

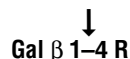
P0730S



400 units 8,000 U/ml Lot: 0021305
RECOMBINANT Store at -20°C Exp: 5/15

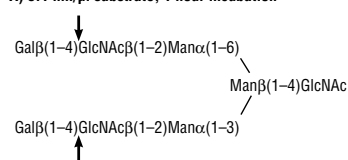
Description: β 1-4 Galactosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of β 1-4 linked D-galactopyranosyl residues from oligosaccharides.

Specificity:

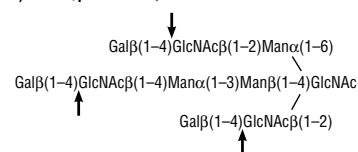


Detailed Specificity: Specificity can vary depending on incubation time and branching structure.

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

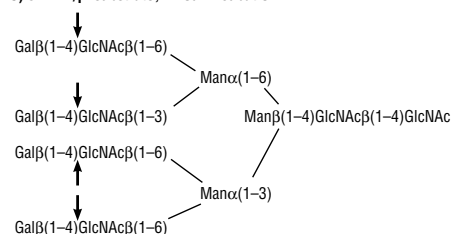


Figure 1: Detailed specificity of β 1-4 Galactosidase. Reactions (A), (B) and (C) contained 2 units, 4 units and 8 units of β 1-4 Galactosidase, respectively, and either 1X G4 or 1X G6 Reaction Buffer in a total reaction volume of 10 μ l. Reactions were incubated at 37°C.

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 G4 Reaction Buffer

Reaction Conditions:
1X G4 Reaction Buffer
50 mM Sodium Citrate (pH 6.0 @ 25°C) and 100 mM NaCl. 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.

Unit Definition Assay: Two fold serial dilutions of β 1-4 Galactosidase are incubated with 1 nmol AMC-labeled substrate in 1X G4 Reaction Buffer, 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.

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

(See other side)

CERTIFICATE OF ANALYSIS

β 1-4 Galactosidase



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



P0730S 003130515051

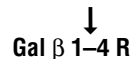
P0730S



400 units 8,000 U/ml Lot: 0021305
RECOMBINANT Store at -20°C Exp: 5/15

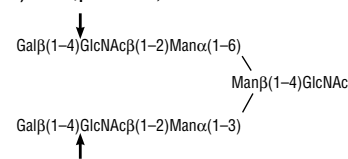
Description: β 1-4 Galactosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of β 1-4 linked D-galactopyranosyl residues from oligosaccharides.

Specificity:

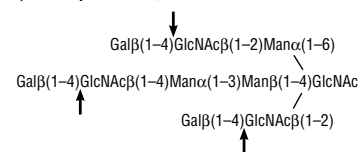


Detailed Specificity: Specificity can vary depending on incubation time and branching structure.

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

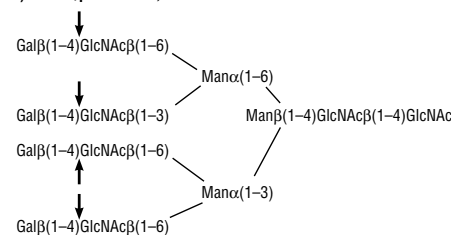


Figure 1: Detailed specificity of β 1-4 Galactosidase. Reactions (A), (B) and (C) contained 2 units, 4 units and 8 units of β 1-4 Galactosidase, respectively, and either 1X G4 or 1X G6 Reaction Buffer in a total reaction volume of 10 μ l. Reactions were incubated at 37°C.

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 G4 Reaction Buffer

Reaction Conditions:
1X G4 Reaction Buffer
50 mM Sodium Citrate (pH 6.0 @ 25°C) and 100 mM NaCl. 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.

Unit Definition Assay: Two fold serial dilutions of β 1-4 Galactosidase are incubated with 1 nmol AMC-labeled substrate in 1X G4 Reaction Buffer, 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.

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

(See other side)

CERTIFICATE OF ANALYSIS

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:	

Page 2 (P0730)

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:	

Page 2 (P0730)

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

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

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