β1-4 Galactosidase

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

P0730S 00315041704 1

P0730S

400 units 8,000 U/ml Lot: 0031504

RECOMBINANT Store at –20°C Exp: 4/17

Description: β1-4 Galactosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of β1-4 linked D-galactopyranosyl residues from oligosaccharides.

Specificity:

Gal β 1–4 R

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

New Reaction Buffer

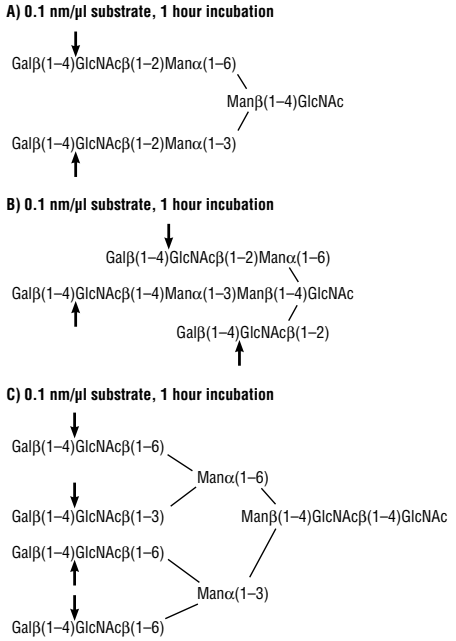

Figure 1: *Detailed specificity of β1-4 Galactosidase. Reactions (A), (B) and (C) contained 2 units, 4 units and 8 units of β1-4 Galactosidase, respectively, and 1X GlycoBuffer 1 total reaction volume of 10 µl. Reactions were incubated at 37°C.*

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 Na_2EDTA.

Reagents Supplied with Enzyme:
10X GlycoBuffer 1

Reaction Conditions:
1X GlycoBuffer 1:
50 mM Sodium Acetate (pH 5.5 @ 25°C) and 5 mM $CaCl_2$. 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 Galβ1-4GlcNAcβ1-3Galβ1-4Glc-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 serial dilutions of β1-4 Galactosidase are incubated with 1 nmol AMC-labeled substrate in 1X GlycoBuffer 1, 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: 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 β1-4 Galactosidase were incubated with 0.1 mM of flourescently-labeled oligosaccharides and glycopeptides, in a 10 µl reaction for 20 hours at 37°C. The reaction products were analyzed by TLC for digestion of substrate.

(See other side)

CERTIFICATE OF ANALYSIS

β1-4 Galactosidase

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

P0730S 00315041704 1

P0730S

400 units 8,000 U/ml Lot: 0031504

RECOMBINANT Store at –20°C Exp: 4/17

Description: β1-4 Galactosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of β1-4 linked D-galactopyranosyl residues from oligosaccharides.

Specificity:

Gal β 1–4 R

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

New Reaction Buffer

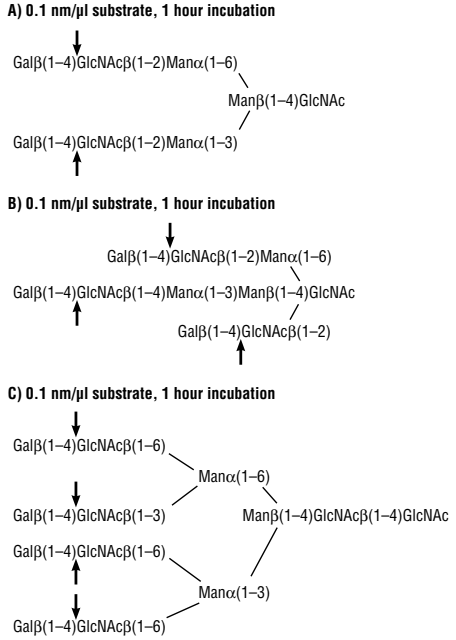

Figure 1: *Detailed specificity of β1-4 Galactosidase. Reactions (A), (B) and (C) contained 2 units, 4 units and 8 units of β1-4 Galactosidase, respectively, and 1X GlycoBuffer 1 total reaction volume of 10 µl. Reactions were incubated at 37°C.*

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 Na_2EDTA.

Reagents Supplied with Enzyme:
10X GlycoBuffer 1

Reaction Conditions:
1X GlycoBuffer 1:
50 mM Sodium Acetate (pH 5.5 @ 25°C) and 5 mM $CaCl_2$. 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 Galβ1-4GlcNAcβ1-3Galβ1-4Glc-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 serial dilutions of β1-4 Galactosidase are incubated with 1 nmol AMC-labeled substrate in 1X GlycoBuffer 1, 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: 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 β1-4 Galactosidase were incubated with 0.1 mM of flourescently-labeled oligosaccharides and glycopeptides, in a 10 µl reaction for 20 hours at 37°C. The reaction products were analyzed by TLC for digestion of substrate.

(See other side)

CERTIFICATE OF ANALYSIS

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:
GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC ND

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

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

α-Galactosidase:
Galα1-3Galβ1-4Gal-AMC ND
Galα1-6Galα1-6Glcα1-2Fru-AMC ND

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

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

β-Glucosidase:
Glcβ1-4Glcβ1-4Glc-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 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 112 units of β1-4 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.

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.

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:
GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC ND

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

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

α-Galactosidase:
Galα1-3Galβ1-4Gal-AMC ND
Galα1-6Galα1-6Glcα1-2Fru-AMC ND

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

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

β-Glucosidase:
Glcβ1-4Glcβ1-4Glc-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 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 112 units of β1-4 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.

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

NEW ENGLAND BIOLABS® is a registered trademark of New England Biolabs, Inc.

Page 2 (P0730)