β1-3 Galactosidase

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

P0726S 006140216021

P0726S

500 units 10,000 U/ml Lot: 0061402 Exp: 2/16

RECOMBINANT Store at –20°C (see note)

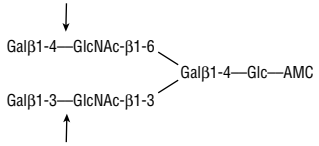
Description: β1-3 Galactosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of β1-3 and, at a much lower rate, β1-6 linked D-galactopyranosyl residues from oligosaccharides. The approximate kinetic data show > 100-fold preference for β1-3 over β1-6 linkages (1,2) and > 500-fold preference from β1-3 over β1-4 linkages (3).

Specificity:

Gal β 1 – 3 R
> β 1 – 6 R
>> β 1 – 4 R

Detailed Specificity: The GlcNAc(β1-6) residue is the only anomeric configuration that can effect the specificity of the enzyme enabling cleavage of the non-reducing β1-4Galactose (Fig. 1).

A) 0.1 nm/µl substrate, 10 units, 1 hr incubation



B) 0.1 nm/µl substrate, 0.625 units, 1 hr incubation

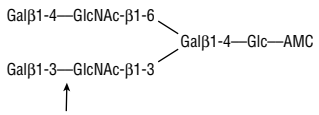


Figure 1: *Selling concentration of the enzyme will cut the β1-4Galactose linkage as shown in (A) due to the adjacent GlcNAcβ1-6 anomer. This cleavage will not occur if the selling concentration of the enzyme is diluted 16-fold, as shown in (B).*

Source: Cloned from *Xanthomonis manihotis* and expressed in *E. coli* (4).

Supplied in: 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C) and 0.1 mM Na_2EDTA.

Reagents Supplied with Enzyme:
10X G2 Reaction Buffer
100X BSA

Reaction Conditions:
1X G2 Reaction Buffer:
50 mM Sodium Citrate (pH 4.5 @ 25°C)

Supplement with 100 µg/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 β-D-galactose from 1 nmol of Galβ1-3GlcNAcβ1-3Galβ1-4Glc-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 dilutions of β1-3 Galactosidase are incubated with 1 nmol AMC-labeled substrate in 1X G2 Reaction Buffer, supplemented with 100 µg/ml BSA, 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 (1).

Specific Activity: 17,000 units/mg

Molecular Weight: 66,000 daltons.

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

Quality Control Assays

Glycosidase Assay: 100 units of β1-3 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

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> β 1 – 6 R
>> β 1 – 4 R
Detailed Specificity: The GlcNAc(β1-6) residue is the only anomeric configuration that can effect the specificity of the enzyme enabling cleavage of the non-reducing β1-4Galactose (Fig. 1).
A) 0.1 nm/µl substrate, 10 units, 1 hr incubation
Galβ1-4—GlcNAc-β1-6
Galβ1-4—Glc—AMC
Galβ1-3—GlcNAc-β1-3
B) 0.1 nm/µl substrate, 0.625 units, 1 hr incubation
Galβ1-4—GlcNAc-β1-6
Galβ1-4—Glc—AMC
Galβ1-3—GlcNAc-β1-3
Figure 1: Selling concentration of the enzyme will cut the β1-4Galactose linkage as shown in (A) due to the adjacent GlcNAcβ1-6 anomer. This cleavage will not occur if the selling concentration of the enzyme is diluted 16-fold, as shown in (B).
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Molecular Weight: 66,000 daltons.
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(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-Acetyl-glucosaminidase:
GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC ND

α-Fucosidase:
Fucα1-2Galβ1-4Glc-AMCGalβ1-4
(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND

α-Galactosidase:
Galα1-3Galβ1-4Gal-AMC ND

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

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

β-Glucosidase:
Glcβ1-4Glcβ1-4Glc-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 100 units of β1-3 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 has changed to –20°C.

Avoid repeated freeze/thaw cycles.

References:

1. Wong-Madden, S.T. and Landry, D. (1995) *Glycobiology* 5, 19–28.
2. Guthrie, E.P. and Taron C. New England Biolabs, Inc., unpublished results.
3. Monks, B., New England Biolabs, Inc., unpublished results.
4. Taron, C.H. et al. (1995) *Glycobiology* 5, 603–610.

U.S. Patent No. 7,094,563

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-Acetyl-glucosaminidase:
GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC ND

α-Fucosidase:
Fucα1-2Galβ1-4Glc-AMCGalβ1-4
(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND

α-Galactosidase:
Galα1-3Galβ1-4Gal-AMC ND

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

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

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β-Glucosidase:
Glcβ1-4Glcβ1-4Glc-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

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