

β1-3 Galactosidase



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P0726S 006150117011

P0726S



500 units **10,000 U/ml** **Lot: 0061501**
RECOMBINANT **Store at -20°C** **Exp: 1/17**

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).

New Reaction Buffer

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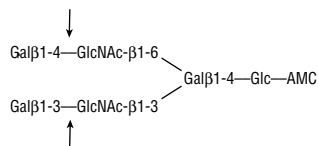
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Specificity:

↓
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

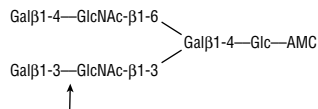


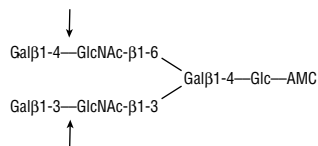
Figure 1: Selling concentration of the enzyme will cut the β1-4Galactose linkage as shown in (A) due to the adjacent GlcNAcβ1-6 anomer. This cleavage will not occur if the selling concentration of the enzyme is diluted 16-fold, as shown in (B).

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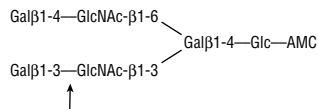


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Source: Cloned from *Xanthomonas manihotis* and expressed in *E. coli* (4).

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

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 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.

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Unit Definition Assay: Two fold dilutions of β1-3 Galactosidase are incubated with 1 nmol AMC-labeled substrate in 1X GlycoBuffer 1, 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 fluorescently-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.

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-Acetyl-glucosaminidase:
GlcNAc β 1-4GlcNAc β 1-4GlcNAc-AMC ND

α -Fucosidase:
Fuc α 1-2Gal β 1-4Glc-AMCGal 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

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
Man α 1-6Man α 1-6(Man α 1-3)Man-AMC ND

Page 2 (P0726)

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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
Man α 1-6Man α 1-6(Man α 1-3)Man-AMC ND

Page 2 (P0726)

β -Glucosidase:
Glc β 1-4Glc β 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 100 units of β 1-3 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.

β -Glucosidase:
Glc β 1-4Glc β 1-4Glc-AMC ND

β -Xylosidase:
Xyl β 1-4Xyl β 1-4Xyl β 1-4Xyl-AMC ND

β -Mannosidase:
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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

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