


**β1-3 Galactosidase**




P0726S 006131015101


**P0726S**

500 units 10,000 U/ml Lot: 0061310 Exp: 10/15

RECOMBINANT Store at -20°C (see note)



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

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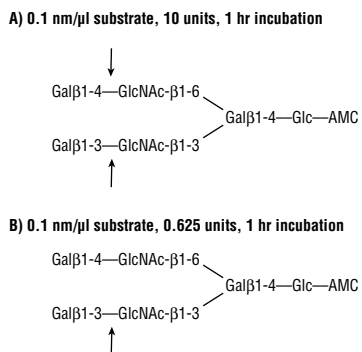
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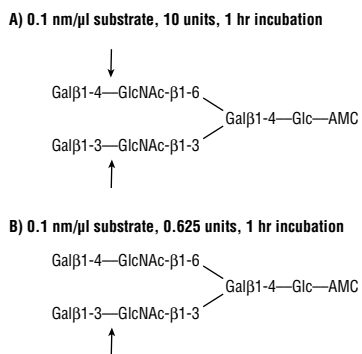
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**Detailed Specificity:** The GlcNAc(β1-6) residue is the only anomeric configuration that can effect the specificity of the enzyme enabling cleavage of the non-reducing β1-4Galactose (Fig. 1).



**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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**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 0.1 mM Na<sub>2</sub>EDTA.

**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.

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**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 fluorescently-labeled oligosaccharides and glycopeptides, in a 10 μl reaction for 20 hours at 37°C. The reaction products were analyzed by TLC of digestion of substrate.

(See other side)

CERTIFICATE OF ANALYSIS

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(See other side)

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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:

**$\beta$ -N-Acetyl-glucosaminidase:**  
GlcNAc $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-AMC ND

**$\alpha$ -Fucosidase:**  
Fuc $\alpha$ 1-2Gal $\beta$ 1-4Glc-AMCGal $\beta$ 1-4  
(Fuc $\alpha$ 1-3)GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC ND

**$\alpha$ -Galactosidase:**  
Gal $\alpha$ 1-3Gal $\beta$ 1-4Gal-AMC ND

**$\alpha$ -Neuraminidase:**  
Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$   
1-4Glc-AMC ND

**$\alpha$ -Mannosidase:**  
Man $\alpha$ 1-3Man $\beta$ 1-4GlcNAc-AMC  
Man $\alpha$ 1-6Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man-AMC ND

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Fuc $\alpha$ 1-2Gal $\beta$ 1-4Glc-AMCGal $\beta$ 1-4  
(Fuc $\alpha$ 1-3)GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC ND

**$\alpha$ -Galactosidase:**  
Gal $\alpha$ 1-3Gal $\beta$ 1-4Gal-AMC ND

**$\alpha$ -Neuraminidase:**  
Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$   
1-4Glc-AMC ND

**$\alpha$ -Mannosidase:**  
Man $\alpha$ 1-3Man $\beta$ 1-4GlcNAc-AMC  
Man $\alpha$ 1-6Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man-AMC ND

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**$\beta$ -Glucosidase:**  
Glc $\beta$ 1-4Glc $\beta$ 1-4Glc-AMC ND

**$\beta$ -Xylosidase:**  
Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl-AMC ND

**$\beta$ -Mannosidase:**  
Man $\beta$ 1-4Man $\beta$ 1-4Man-AMC ND

**Endo F<sub>1</sub>, F<sub>2</sub>, H:**  
Dansylated invertase high mannose. ND

**Endo F<sub>2</sub>, F<sub>3</sub>:**  
Dansylated fibrinogen biantennary. ND

**PNGase F:**  
Fluoresceinated fetuin triantennary. ND

**Protease Assay:** After incubation of 100 units of  $\beta$ 1-3 Galactosidase with 0.2 nmol of a standard mixture of proteins in a 20  $\mu$ l 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.

**$\beta$ -Glucosidase:**  
Glc $\beta$ 1-4Glc $\beta$ 1-4Glc-AMC ND

**$\beta$ -Xylosidase:**  
Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl-AMC ND

**$\beta$ -Mannosidase:**  
Man $\beta$ 1-4Man $\beta$ 1-4Man-AMC ND

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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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