

# $\alpha$ 1-3, 6 Galactosidase



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P0731S 002150417041

## P0731S

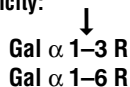


100 units 4,000 U/ml Lot: 0021504

RECOMBINANT Store at 4°C Exp: 4/16

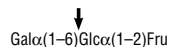
**Description:**  $\alpha$ 1-3, 6 Galactosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of  $\alpha$ 1-3, 6 linked D-galactopyranosyl residues from oligosaccharides.

### Specificity:

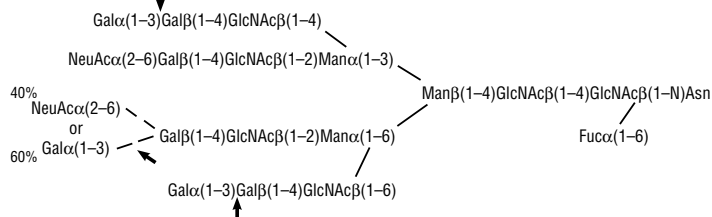


**Detailed Specificity:** Specificity can vary depending on incubation time and branching structure.

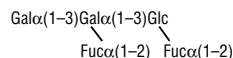
### A) 0.1 nm/ $\mu$ l substrate, 1 hour incubation



### B) 0.1 nm/ $\mu$ l substrate, 18 hour incubation



### C) 0.1 nm/ $\mu$ l substrate, 1 hour incubation, not cleaved

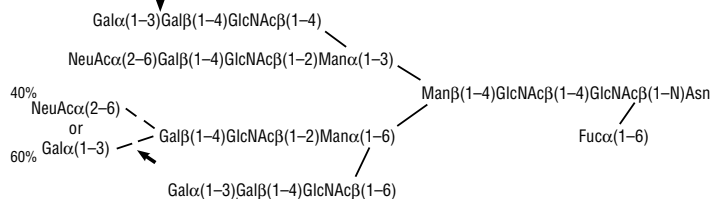


**Figure 1:** Detailed specificity of  $\alpha$ 1-3, 6 Galactosidase. Reactions (A) and (C) contained 4 units of  $\alpha$ 1-3, 6 Galactosidase, 1X G6 Reaction Buffer and 1X BSA in a total reaction volume of 10  $\mu$ l. Reaction (C) shows that branched fucose inhibits cleavage. Reaction (B) contained 24 units of  $\alpha$ 1-3, 6 Galactosidase and 100 units of Neuraminidase, followed by a heat kill at 65°C for 10 minutes and a 2 hour digestion with 16 units of  $\beta$ 1-4 Galactosidase. The reaction in (B) contained 1X G6 Reaction Buffer and 1X BSA in a total reaction volume of 20  $\mu$ l. The reactions were incubated at 37°C. Complete digestion of the  $\alpha$ 1-3, 6 Galactosidase was determined by an observation of complete transformation of the substrate in (B) to the non-reducing terminal N-acetylglucosamine tetra antennary oligosaccharide.

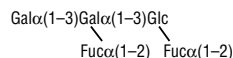
### A) 0.1 nm/ $\mu$ l substrate, 1 hour incubation



### B) 0.1 nm/ $\mu$ l substrate, 18 hour incubation



### C) 0.1 nm/ $\mu$ l substrate, 1 hour incubation, not cleaved



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**Source:** Cloned from *Xanthomonas manihotis* and expressed in *E. coli* (1).

Supplied in: 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C) and 1 mM Na<sub>2</sub>EDTA.

### Reagents Supplied with Enzyme:

10X G6 Reaction Buffer  
100X BSA

### Reaction Conditions:

1X G6 Reaction Buffer  
50 mM Sodium acetate (pH 5.5 @ 25°C) and 5 mM CaCl<sub>2</sub>. Supplement with 100  $\mu$ g/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,  $\alpha$ -D-galactose from 1 nmol Gal $\alpha$ 1-3Gal $\beta$ 1-4Gal-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10  $\mu$ l.

(See other side)

CERTIFICATE OF ANALYSIS

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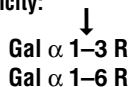


100 units 4,000 U/ml Lot: 0021504

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(See other side)

CERTIFICATE OF ANALYSIS

**Unit Definition Assay:** Two fold serial dilutions of  $\alpha$ 1-3, 6 Galactosidase are incubated with 1 nmol AMC-labeled substrate in 1X G6 Reaction Buffer and 1X BSA in a 10  $\mu$ 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:** 137,000 units/mg

**Molecular Weight:** 70,000 daltons.

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

### Quality Controls

#### Glycosidase Assays:

12 units of  $\alpha$ 1-3, 6 Galactosidase were incubated with 0.1 mM of fluorescently-labeled oligosaccharides and glycopeptides, in a 10  $\mu$ l reaction for 20 hours at 37°C. The reaction products were analyzed by TLC for digestion of substrate.

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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-Acetylglucosaminidase:**  
GlcNAc $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-AMC ND

**$\alpha$ -N-Acetylgalactosaminidase:**  
GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4Glc-AMC ND

**$\alpha$ -Fucosidase:**  
Fuc $\alpha$ 1-2Gal $\beta$ 1-4Glc-AMC ND

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

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

Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC ND

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

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No other glycosidase activities were detected (ND) with the following substrates:

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

**$\alpha$ -N-Acetylgalactosaminidase:**  
GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4Glc-AMC ND

**$\alpha$ -Fucosidase:**  
Fuc $\alpha$ 1-2Gal $\beta$ 1-4Glc-AMC ND

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

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

Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-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 ND

Man $\alpha$ 1-6Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man-AMC ND

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

**$\alpha$ -Glucosidase:**  
Glc $\alpha$ 1-6Glc $\alpha$ 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

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

Man $\alpha$ 1-6Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man-AMC ND

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

**$\alpha$ -Glucosidase:**  
Glc $\alpha$ 1-6Glc $\alpha$ 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:**  
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**Endo F<sub>2</sub>, F<sub>3</sub>:**  
Dansylated fibrinogen biantennary. ND

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

**Protease Assay:** After incubation of 28 units of  $\alpha$ 1-3, 6 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 is 4°C. Avoid repeated freeze/thaw cycles

**Heat Inactivation:** 65°C for 10 minutes.

### References:

- McLeod, E., New England Biolabs, Inc. unpublished results.
- Wong-Madden, S.T. and Landry, D. (1995) *Glycobiology* 5, 19–28.

U.S. Patent No. 5,770,405

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