

# $\beta$ 1-4 Galactosidase



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P0730S 002120814081

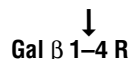
## P0730S



400 units 8,000 U/ml Lot: 0021208  
RECOMBINANT Store at -20°C Exp: 8/14

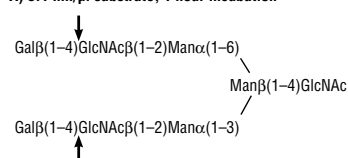
**Description:**  $\beta$ 1-4 Galactosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of  $\beta$ 1-4 linked D-galactopyranosyl residues from oligosaccharides.

### Specificity:

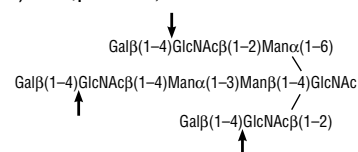


**Detailed Specificity:** Specificity can vary depending on incubation time and branching structure.

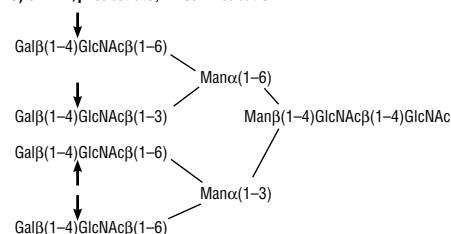
### A) 0.1 nm/ $\mu$ l substrate, 1 hour incubation



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



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



**Figure 1:** Detailed specificity of  $\beta$ 1-4 Galactosidase. Reactions (A), (B) and (C) contained 2 units, 4 units and 8 units of  $\beta$ 1-4 Galactosidase, respectively, and either 1X G4 or 1X G6 Reaction Buffer in a total reaction volume of 10  $\mu$ l. 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<sub>2</sub>EDTA.

**Reagents Supplied with Enzyme:**  
10X G4 Reaction Buffer

### Reaction Conditions:

1X G4 Reaction Buffer  
50 mM Sodium Citrate (pH 6.0 @ 25°C) and 100 mM NaCl. 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,  $\beta$ -D-galactose from 1 nmol Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-7-amino-4-methylcoumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10  $\mu$ l.

**Unit Definition Assay:** Two fold serial dilutions of  $\beta$ 1-4 Galactosidase are incubated with 1 nmol AMC-labeled substrate in 1X G4 Reaction Buffer, 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:** 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  $\beta$ 1-4 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.

**Physical Purity:** Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

(See other side)

CERTIFICATE OF ANALYSIS

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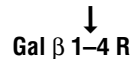
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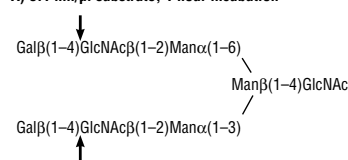
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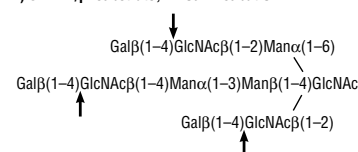


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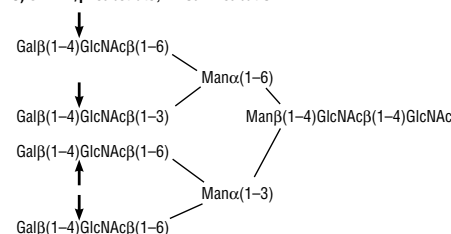
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**Physical Purity:** Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

(See other side)

CERTIFICATE OF ANALYSIS

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

**$\alpha$ -Galactosidase:**  
Gal $\alpha$ 1-3Gal $\beta$ 1-4Gal-AMC ND  
Gal $\alpha$ 1-6Gal $\alpha$ 1-6Glc $\alpha$ 1-2Fru-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

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

**Protease Assay:** After incubation of 112 units of  $\beta$ 1-4 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 -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

Page 2 (P0730)

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

**$\alpha$ -Galactosidase:**  
Gal $\alpha$ 1-3Gal $\beta$ 1-4Gal-AMC ND  
Gal $\alpha$ 1-6Gal $\alpha$ 1-6Glc $\alpha$ 1-2Fru-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

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Page 2 (P0730)