

β 1-4 Galactosidase S



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P0720S 015140716071

P0745S



400 units **8,000 U/ml** **Lot: 0011407**

RECOMBINANT **Store at -20°C** **Exp: 7/16**

Description: β 1-4 Galactosidase S is a highly specific exoglycosidase that catalyzes the hydrolysis of β 1-4 linked galactose residues from oligosaccharides.

Specificity:



Gal R = any sugar

Source: Cloned from *Streptococcus pneumoniae* and expressed in *E. coli* (1).

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
(0.5 M Sodium Acetate, pH 5.5 @ 25°C and 50 mM CaCl₂)

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal, β -D-galactose from 1 nmol Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 μ l.

Unit Definition Assay: Two fold dilutions of β 1-4 Galactosidase S are incubated with 1 nmol AMC-labeled substrate and 1X GlycoBuffer 1 in a 10 μ l reaction. The reaction mix is incubated at 37°C for 1 hour. Separation of reaction products are visualized via thin layer chromatography (2).

Specific Activity: ~123,000 units/mg.

Molecular Weight: 231,000 daltons.

Quality Assurance: No contaminating exoglycosidase or endoglycosidase F1, F2 or F3 activity could be detected. No contaminating proteolytic activity could be detected.

Quality Controls

Glycosidase Assays: 80 units of β 1-4 Galactosidase S were incubated with 0.1 mM of fluorescently-labeled oligosaccharides and glycopeptides, in a 10 μ l reaction for 20 hours at 37°C. The reaction products were analyzed by TLC for digestion of substrate.

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

β -N-Acetylglucosaminidase: ND
GlcNAc β 1-4GlcNAc β 1-4GlcNAc-AMC

β -N-Acetylgalactosaminidase: ND
GalNAc β 1-4Gal β 1-4Glc-AMC

α -N-Acetylgalactosaminidase: ND
GalNAc α 1-3(Fuc α 1-2)Gal β 1-4Glc-AMC

α -Fucosidase: ND
Fuc α 1-2Gal β 1-4Glc-AMC

β -Galactosidase: ND
Gal β 1-3GlcNAc β 1-4Gal β 1-4Glc-AMC

α -Galactosidase: ND
Gal α 1-3Gal β 1-4Gal-AMC
Gal α 1-6Gal α 1-6Glc α 1-2Fru-AMC

α -Neuraminidase: ND
Neu5Aca2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc-AMC

α -Mannosidase: ND
Man α 1-3Man β 1-4GlcNAc-AMC
Man α 1-6Man α 1-6(Man α 1-3)Man-AMC

α -Glucosidase: ND
Glc α 1-6Glc α 1-4Glc-AMC

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

β -Mannosidase: ND
Man β 1-4Man β 1-4Man-AMC

(see other side)

CERTIFICATE OF ANALYSIS



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Endo F₁, F₂, H:
Dansylated invertase high mannose. ND

Endo F₂, F₃:
Dansylated fibrinogen biantennary. ND

Protease Assay: After incubation of 400 units of β 1-4 Galactosidase S 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.

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

Heat Inactivation: 75°C for 10 minutes.

Reaction Conditions: Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate. Typical reaction conditions are as follows:

1. Combine 1 μ g of glycoprotein or 100 nM of oligosaccharide and H₂O (if necessary) to make a 9 μ l total reaction volume.
2. Add 1 μ l of 10X GlycoBuffer 1 to make a 10 μ l total reaction volume.
3. Add 1 μ l of β 1-4 Galactosidase S.
4. Incubate at 37°C for 1 hour.

Notes on Use:

- Reactions may be scaled-up linearly to accommodate larger reaction volumes.
- The amount of exoglycosidase enzyme required varies when different substrates are used. Start with 1–2 μ l for 1 μ g of glycoprotein or 100 nM of oligosaccharide for one hour in a 10–25 μ l reaction. If there is still undigested material, let the reaction go overnight.

References:

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



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