

α 1-3,4,6 Galactosidase



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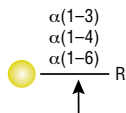
P0747S 001150216021

P0747S

200 units **8,000 U/ml** **Lot: 0011502**
Store at 4°C **Exp: 2/16**

Description: α 1-3,4,6 Galactosidase is a broad specificity exoglycosidase that catalyzes the hydrolysis of α 1-3, α 1-4, and α 1-6 linked D-galactopyranosyl residues from oligosaccharides.

Specificity:



Gal
R = any sugar

Source: Cloned from green coffee bean and expressed in *E. coli* (1).

Supplied in: 20 mM Tris-HCl (pH 7.5), 50 mM NaCl and 1 mM EDTA

Reagents Supplied with Enzyme:

10X GlycoBuffer 1
100X BSA

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

Specific Activity: 71,000 U/mg

Molecular Weight: 39,700 daltons

Unit Definition Assay: Two fold dilutions of α 1-3,4,6 Galactosidase are incubated with 1 nmol AMC-labeled substrate in 1X GlycoBuffer 1 and 1X BSA 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).

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

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

Heat Inactivation: 8 units of enzyme were inactivated by incubation at 65°C for 10 minutes.

Reaction Protocol

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 an 8 μ l total reaction volume.
2. Add 1 μ l of 10X GlycoBuffer 1 and 1 μ l of 10X BSA (diluted 1:10 from 100X concentration) to make a 10 μ l total reaction volume.
3. Add 1 μ l of α 1-3,4,6 Galactosidase.
4. Incubate at 37°C for 1 hour.

Notes: 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.

Store at 4°C, Avoid repeated freeze-thaw cycles.

(see other side)

CERTIFICATE OF ANALYSIS

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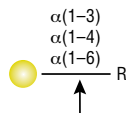
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CERTIFICATE OF ANALYSIS

Quality Controls

Glycosidase Assays: 80 units of α 1-3,4,6 Galactosidase 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:
GlcNAc β 1-4GlcNAc β 1-4GlcNAc-AMC ND

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

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

α -Fucosidase:
Gal β 1-4 (Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc-AMC ND
Fuc α 1-2Gal β 1-4Glc-AMC ND

Page 2 (P0747)

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

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

α -Fucosidase:
Gal β 1-4 (Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc-AMC ND
Fuc α 1-2Gal β 1-4Glc-AMC ND

Page 2 (P0747)

β -Galactosidase:
Gal β 1-3GlcNAc β 1-4Gal β 1-4Glc -AMC ND
Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc -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 ND
Man α 1-6Man α 1-6(Man α 1-3)Man-AMC ND

α -Glucosidase:
Glc α 1-6Glc α 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

β -Galactosidase:
Gal β 1-3GlcNAc β 1-4Gal β 1-4Glc -AMC ND
Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc -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 ND
Man α 1-6Man α 1-6(Man α 1-3)Man-AMC ND

α -Glucosidase:
Glc α 1-6Glc α 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

Protease Assay: After incubation of 200 units of α 1-3,4,6 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.

References:

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



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