β1-4 Galactosidase

Description: β1-4 Galactosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of β1-4 linked D-galactopyranosyl residues from oligosaccharides.

Specificity:

Gal β 1–4 R

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

A) 0.1 nmol/µl substrate, 1 hour incubation

Galβ(1–4)GlcNAcβ(1–2)Manuβ(1–6)β

Galβ(1–4)GlcNAcβ(1–2)Manuβ(1–3)β

B) 0.1 nmol/µl substrate, 1 hour incubation

Galβ(1–4)GlcNAcβ(1–2)Manuβ(1–6)β

Galβ(1–4)GlcNAcβ(1–2)Manuβ(1–3)β

C) 0.1 nmol/µl substrate, 1 hour incubation

Galβ(1–4)GlcNAcβ(1–6)β

Galβ(1–4)GlcNAcβ(1–3)β

Galβ(1–4)GlcNAcβ(1–2)β

Galβ(1–4)GlcNAcβ(1–6)β

Galβ(1–4)GlcNAcβ(1–3)β

Galβ(1–4)GlcNAcβ(1–2)β

Figure 1: Detailed specificity of β1-4 Galactosidase. Reactions (A), (B) and (C) contained 2 units, 4 units and 8 units of β1-4 Galactosidase, respectively, and either 1X G4 or 1X G6 Reaction Buffer in a total reaction volume of 10 µl. 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 Na₂EDTA.

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, β-D-galactose from 1 nmol Galβ(1–4)GlcNAcβ(1–3)Galβ(1–4)Glc-7-amino-4-methylcoumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

Unit Definition Assay: Two fold serial dilutions of β1-4 Galactosidase are incubated with 1 nmol AMC-labeled substrate in 1X G4 Reaction Buffer, 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 (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 β1-4 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 for digestion of substrate.

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

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

**β-N-Acetylglucosaminidase:**
GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC

**α-N-Acetylgalactosaminidase:**
GalNAcα1-3(Fucα1-2)Galβ1-4Glc-AMC

**α-Fucosidase:**
Fucα1-2Galβ1-4Glc-AMC

**α-Galactosidase:**
Galα1-3Galβ1-4Glc-AMC

**α-Mannosidase:**

**α-Neuraminidase:**
Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC

**α-Mannosidase:**
Manα1-3Manβ1-4GlcNAc-AMC
Manα1-6Manα1-6(Manα1-3)Man-AMC

**β-Glucosidase:**
Glcβ1-4Glcβ1-4Glc-AMC

**α-Glucosidase:**
Glcα1-6Glcα1-4Glc-AMC

**β-Xylosidase:**
Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC

**β-Mannosidase:**
Manβ1-4Manβ1-4Man-AMC

**Endo Fα, Fβ, H:**
Dansylated invertase high mannose.

**ENDO Fα, Fβ:**
Dansylated fibrinogen biotantenary.

**PNGase F:**
Fluoresceinated fetuin triantennary.

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**Protease Assay:** After incubation of 112 units of β-1-4 Galactosidase with 0.2 nmol of a standard mixture of proteins in a 20 µl 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:**

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