**Beta-1,4 Galactosidase**

**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:**

Gal 1→4 R

**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 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, beta-o-galactoside from 1 nmol Galβ-4GlcNAcβ-3Galβ-4Glc-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 beta-1,4 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 beta-1,4 Galactosidase were incubated with 0.1 mM of fluorescently-labeled oligosaccharides and glycopeptides, in a 10 µl reaction 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 ND

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

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

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

**α-Galactosidase:**
Galα1-6Galα1-6Glcα1-2Fru-AMC ND

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

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

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

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

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

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

Endo F₆, F₇, H:
Dansylated invertase high mannose. ND

Endo F₆, F₇:
Dansylated fibrinogen biantennary. ND

PNGase F:
Fluoresceinated fetuin triantennary. ND

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**Protease Assay:** After incubation of 112 units of β1-4 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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