**Galactosidase**

1-800-632-7799
info@neb.com
www.neb.com

**1-4 Galactosidase**

**P0730S**

400 units 8,000 U/ml Lot: 0031405
RECOMBINANT Store at -20°C Exp: 5/16

**Description:** 1-4 Galactosidase is a highly specific endo-β-1-4-galactosidase that catalyzes the hydrolysis of β-1-4 linked D-galactopyranosyl residues from oligosaccharides.

**Specificity:**

Gal β 1-4 R

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

**Unit Definition Assay:** Two fold serial dilutions of 1-4 Galactosidase are incubated with 1 nmol of a particular substrate. The amount of enzyme required to cleave > 95% of the terminal, β-D-galactose from 1 nmol of the terminal, β-D-galactose from 1 nmol of a particular substrate.

**Reaction Conditions:**

Reagents Supplied with Enzyme:

- 10X G4 Reaction Buffer
- 100 mM NaCl
- 50 mM Sodium Citrate (pH 6.0 @ 25°C) and 1 mM Na2EDTA.

**Quality Controls**

- Glycosidase Assays: 32 units of β-1-4 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.

**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
- 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 of a particular substrate.

**Unit Definition Assay:** Two fold serial dilutions of 1-4 Galactosidase are incubated with 1 nmol of a particular 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 β-1-4 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.

**Figure 1:** Detailed specificity of β-1-4 Galactosidase. Reactions (A), (B) and (C) contained 2 units, 4 units and 8 units of β-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.
No other glycosidase activities were detected (ND) with the following substrates:

**β-N-Acetylgalactosaminidase:**
GalNAcβ1-3(Fucx1-2)Galβ1-4Glc-AMC

**α-N-Acetylgalactosaminidase:**
GalNAcα1-3(Fucx1-2)Galβ1-4Glc-AMC

**α-Fucosidase:**
Fucx1-2Galβ1-4Glc-AMC

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

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

**α-Mannosidase:**
Manα1-3Manβ1-4GlcNAc-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 biantennary.

**PNGase F:**
Fluoresceinated fetuin triantennary.

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:

U.S. Patent No. 6,358,724