β1-3 Galactosidase

**Description:** β1-3 Galactosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of β1-3- and, at a much lower rate, β1-6-linked α-galactopyranosyl residues from oligosaccharides. The approximate kinetic data show >100-fold preference for β1-3 over β1-6 linkages (1,2) and >500-fold preference from β1-3 over β1-4 linkages (3).

**Specificity:**
- Gal β1-3 R
- > Gal β1-6 R
- >> Gal β1-4 R

**Detailed Specificity:** The GlcNAc(β1-6) residue is the only anomeric configuration that can effect the specificity of the enzyme enabling cleavage of the non-reducing β1-4Galactose (Fig. 1).

A) 0.1 nm/µl substrate, 10 units, 1 hr incubation

![Reaction Products](Fig1A)

B) 0.1 nm/µl substrate, 0.625 units, 1 hr incubation

![Reaction Products](Fig1B)

**Source:** Cloned from *Xanthomonas manihotis* and expressed in *E. coli* (4).

**Unit Definition Assay:** Two fold dilutions of β1-3 Galactosidase are incubated with 1 nmol AMC-labeled substrate in 1X G2 Reaction Buffer, supplemented with 100 µg/ml BSA, 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 (1).

**Unit Definition:** One unit is defined as the amount of enzyme required to cleave >95% of the terminal β-α-galactose from 1 nmol of Galβ1-3GlcNAcβ1-3Galβ1-4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

**Specific Activity:** 17,000 units/mg

**Molecular Weight:** 66,000 daltons.

**Quality Assurance:** No contaminating exoglycosidase or proteolytic activity could be detected.

**Quality Control Assays**

**Glycosidase Assay:** 100 units of β1-3 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.

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

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

- **β-N-Acetyl-glucosaminidase**: GlcNAcβ1-4GlcNAcβ1-4GlcNAC-AMC ND

- **α-Fucosidase**: Fucx1-2Galβ1-4Glc-AMCα1-4 (Fucx1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND

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

- **α-Neuraminidase**: Neu5Acc2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC ND

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

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

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

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

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

- **Endo Fα, Fβ**: Dansylated fibrinogen biantennary. ND

- **PNGase F**: Fluoresceinated fetuin triantennary. ND

| Protease Assay | After incubation of 100 units of β1-3 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 has changed to −20°C.

Avoid repeated freeze/thaw cycles.

References:


U.S. Patent No. 7,094,563