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

**Detailed Specificity:** The GlcNAc(β-1-6) 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

\[ \beta 1-3 \rightarrow \beta 1-4 \]

\[ \beta 1-3 \rightarrow \beta 1-4 \]

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

\[ \beta 1-3 \rightarrow \beta 1-4 \]

\[ \beta 1-3 \rightarrow \beta 1-4 \]

**Figure 1:** Selliing concentration of the enzyme will cut the β1-4Galactose linkage as shown in (A) due to the adjacent GlcNAcβ1-6 anomer. This cleavage will not occur if the selling concentration of the enzyme is diluted 16-fold, as shown in (B).

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

**Unit Definition:** Two fold dilutions of β1-3 Galactosidase are incubated with 1 nmol AMC-labeled substrate in 1X G6 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).

**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 flourescently-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.

**Supplement with 100 µg/ml BSA. Incubate at 37°C. Separation of reaction products are visualized via thin layer chromatography (1).**

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

**Unit Definition:** Two fold dilutions of β1-3 Galactosidase are incubated with 1 nmol AMC-labeled substrate in 1X G6 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).

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**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:**
  - Fucα1-2Galβ1-4Glc-AMCGalβ1-4
t  - Fucα1-3GlcNAcβ1-3Galβ1-4Glc-AMC ND

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

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

- **α-Mannosidase:**
  - Manα1-3Manα1-4GlcNAc-AMC ND
  - 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 F₁, F₂, H:**
  - Dansylated invertase high mannose. ND

- **Endo F₁, F₂, F₃:**
  - Dansylated fibrinogen biantennary. ND

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

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

- **Endo F₁, F₂, H:**
  - Dansylated invertase high mannose. ND

- **Endo F₁, F₂, F₃:**
  - 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:**