Beta(1-4) Galactosidase

β(1-4) Galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23] releases only β(1-4)-linked, non-reducing terminal galactose from complex carbohydrates and glycoproteins. β(1-4) galactose is by far the most common linkage found in N-linked oligosaccharides. The enzyme is active on tetra-antennary oligosaccharides, as on disaccharides containing β(1-4) linked galactose. Fucose linked to the penultimate N-acetylglucosamine will block cleavage of the galactose. Up to 100 ug of asialofetuin can be completely β(1-4) Galactosidase-degalactosylated in less than 1 hour using 3 mU of enzyme.

β(1-4) Galactosidase is isolated from a clone of Streptococcus pneumonia expressed in *E. coli*.

β(1-4) Galactosidase is useful for:

- Structural analysis of oligosaccharides
- Distinguishing different galactose linkages
- Removing heterogeneity from glycoproteins

Specifications

Activity

≥ 6 U/mg, ~ 3 U/mL

Storage

Store at 4°C. Do not freeze.

Formulation

The enzyme is provided as a sterile solution in 20 *mM* Tris-HCl pH 7.5, 25 *mM* NaCl.

Stability

Stable at least 12 months when stored properly. Several days exposure to ambient temperatures will not reduce activity.

Product Description

Molecular Weight

250,000 Daltons

Purity

Each lot of β(1-4) Galactosidase is tested for contaminating protease as follows: 10 µg of denatured BSA is incubated for 24 hours at 37°C with 2 µL of enzyme. SDS-PAGE analysis of the treated BSA shows no evidence of degradation.

The production host strain has been extensively tested and does not produce any detectable glycosidases.

Specificity

Non-reducing terminal β(1-4) galactose. The number of antennae does not affect the cleavage rate. Fucose linked to the penultimate N-acetylglucosamine will block cleavage of the galactose.

Assay

One unit of β(1-4) Galactosidase is defined as the amount of enzyme required to produce 1 µmole of *p*-nitrophenol (*p*NP) in 1 minute at 37°C, pH 5.0 from *p*-nitrophenyl-β-D-galactopyranoside.

Reagents

- 5X Reaction buffer 6.0 - 250 *mM* $NaHPO_4$, pH 6.0

Suggestions for Use

Procedure for Degalactocosylation

1. Add up to 100 µg of asialglycoprotein or 1 µmole of oligosaccharide to a tube.
2. Add water to a total of 13 µL.
3. Add 4 µL 5X Reaction Buffer.
4. Add 2 µL of β(1-4) Galactosidase.
5. Incubate at 37°C for 1 hour.

For glycoproteins, cleavage may be monitored by SDS-PAGE if the size differential between native an de-galactosylated protein is sufficient for detection.

Note: The optimum pH for cleavage of oligosaccharides is ~pH6.0.

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References

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2. Kobata, A. Use of endo- and exoglycosidases for structural studies of glycoconjugates. Anal. Biochem. 100:1-14 (1979).

3. Prime, S., J. Dearnley, A.M. Venton, R.B. Parekh and C.J. Edge. Oligosaccharide sequencing based on exo- adn endoglycosidase digestion and liquid chromatographic analysis of the products. J. Chromatogr. A. 720:263-274 (1996).

4. Dwek, R.A., C.J. Edge, D.J. Harvey, M. R. Wormald and R.B. Parekh. Analysis of glycoprotein-associated oligosaccharides. Ann. Rev. Biochem. 62:65-100.

Order Information

Catalog No.	Product Description	Package Size	Temp. °C
100577-1	**β(1-4) Galactosidase** *(Streptococcus pneumonia* recombinant*)*	60 µL	+4

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