Selectin Biosciences Inc.

β (1-4)-Galactosidase

Recombinant from *Streptococcus pneumonia*

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 as active on tetraantennary 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 μg of asialofetuin can be completely β(1-4)-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 *Escherichia coli*.

β(1-4) Galactosidase is useful for:

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

Product Code: GE 12

Specifications

Activity: ≥6 U/mg, ~3 U/mL

Storage: Store at 4°C.

Formulation: The enzyme is provided as a sterile solution in 20 mM Tris 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: 240,000 Daltons

Purity: Each lot of β(1-4) Galactosidase is tested for contaminating activities by incubating the enzyme 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. Number of antennae does not affect 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 (pNP) 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

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Suggestions for Use

Procedure For Degalactosylation

1. Add up to 100 µg of asialoglycoprotein or 1 nmol of oligosaccharide to tube.
2. Add water to 14 µL
3. Add 4 µL 5X Reaction Buffer
4. Add 2 µL 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 and de-galactosylated protein is sufficient for detection.

Note: The optimum for cleavage of oligosaccharides is ~ pH 6.0.

References

1. Glasgow, LR., J.C. Paulson and R.L. Hill. Systematic purification of five glycosidases from Streptococcus pneumonia. **J. Biol Chem 252:** 8615-8623(1977).
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- and 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.

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This product is intended for in vitro research only.

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