Selectin Biosciences Inc.

Endo-β-Galactosidase

Recombinant from *Bacteroides fragilis*

Endo-β-Galactoctosidase(EC 3.2.1.103) cleaves internal β(1-4) galactose linkages in unbranched, repeating poly-N-acetyllactosamine structures. Sulfated structures such as keratan sulfate are also cleaved. Branching and/or fucosylation of the substrate may decrease or eliminate cleavage.

Endo-β-Galactoctosidase is useful for identifying and removing poly-N-acetyllactosamine structures on many biologically important glycoconjugates.

Product Code: GE 38

Specifications

Activity: 150 U/mg 15 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.

Stability: Stable at least 12 months when stored properly. Several days exposure to ambient temperature will not reduce activity. Active for at least 5 days under reaction conditions.

Product Description

Molecular Weight

32,000 Daltons

Purity

Endo-β-Galactoctosidase 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 strain of E. coli has been extensively tested and does not produce any detectable glycosidases.

Specificity: Internal β(1-4) galactose linkages in unbranched, repeating poly-N-acetyllactosamine $[GlcNAc\beta(1\text{-}3)Gal\beta(1\text{-}4)]_n$ structures are the preferred substrate. Sulfated structures such as keratan sulfate are also cleaved. Branching and/or fucosylation of the substrate may decrease or eliminate cleavage. Sulfation of C-6 on galactose will block cleavage. Oligosaccharides of the neo-lacto group are cleaved at greatly reduced rates depending on the deviation from the preferred substrate. For example, $Gal\beta(1\text{-}3)GlcNAc\beta(1\text{-}3)Gal\beta(1\text{-}4)Glc$ is cleaved at $5X10^{-5}$ the rate of keratan sulfate(see ref.4). Specificity is similar to the *Escherichia freundii* enzyme except that it is limited to cleaving N-acetyllactosamine extensions on tetraantennary structures of erythropoietin (see ref 5).

pH Range: Optimum: pH 5.8

Assay: One unit of Endo-β-Galactoctosidase is defined as the amount that will liberate one μmole of reducing sugar per minute at 37°C and pH 5.8 from bovine corneal keratan sulfate.

Reagents

- 5X Reaction buffer is 250 mM sodium phosphate pH 5.8

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Suggestions for Use:

Procedure for glycoproteins:

1. Add up to 100 μg of glycoprotein to a tube.
2. Add 4 ul 5X buffer and water to 19 μl.
3. Add 1 μl enzyme.
4. Incubate at 37°C for 2 hrs.

Procedure for oligosaccharides: Same as above except incubate from several hours to several days depending on the substrate. Add bovine serum albumen to 2 mg/ml to stabilize the protein during extended incubations.

References:

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2. Scudder,P., Hanfland, Pl, Uemura, K. & Feizi, T. (1984) Endo-β-galactosidases of Bacteroides fragilis and Escherichia freundii hydrolyze linear but not branched oligosaccharide domains of glycolipids of the neolacto series. **J. Biol. Chem . 259**, 6586-6592.

3. Scudder, P. Tang, P.W., Hounsell, E.F., Lawson, A.M., Mehmet, H. & Feizi, T. (1986) Isolation and characterization of sulfated oligosaccharides released from bovine corneal keratan sulphate by the action of endo-β-galactosidase. Eur. **J. Biochem. 157**, 365-373.

4. Murata, T., Hattori, T. Amarume, S. Koicki, A. & Usui, T. (2003) Kinetic studies on endo-β-galactosidase by a novel colorimetric assay and sythesis of N-acetyllactosamine-repeating oligosaccharide β-glycosides using its transglycosylation activity. Eur. **J. Biochem 270**, 3709-3719.

5. Hokke, C.H., Bergwerff, A.A., Van Dedem, D.W., Kamerling, J.P, and Vliegenthart, J.F. (1995) Structural analysis of the N- and O-linked carbohydrate chains of recombinant human erythropoietin expressed in Chinese hamster overay cells. Sialylation patterns and branch location of dimeric N-acetyllactosamine units. Eur. **J. Biochem. 228**, 981-1008.

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