



Endo-Beta-Galactosidase

Endo- β -Galactosidase

Source

recombinant gene from *Bacteroides fragilis* in *E. Coli*

EC 3.2.1.97

Catalog Number

E-XBG01	60 μ l
E-XBG01-20	20 μ l
E-XBG01-200	200 μ l

Recommended Reagents

included with E-XBG01:

1 vial: Reaction buffer - 400 μ L
250mM Sodium phosphate, pH 5.8

Activity \geq 14 U/ml

Specific Activity \geq 140 U/mg

pH Optimum 5.8

Molecular Weight 32,000 daltons

Formulation

The enzyme is provided as a sterile-filtered solution in 20 mM Tris-HCl, pH 7.5

Storage

Store enzyme at 4°C. Do not freeze.

Stability

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

Applications

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

Specificity

Internal β (1-4) galactose linkages in unbranched, repeating poly-N-acetyllactosamine [GlcNAc β (1-3)Gal β (1-4)]_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 β (1-3)GlcNAc β (1-3)Gal β (1-4)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).

Endo- β -Glycosidase

Specifications - Protocol

Specific Activity

One unit of endo- β -Galactosidase is defined as the amount that will liberate one μ mole of reducing sugar per minute at 37°C and pH 5.8 from bovine corneal keratan sulfate.

Purity

Endo- β -Galactosidase is tested for contaminating protease as follows: 10 μ g of denatured BSA is incubated for 24 hours at 37°C with 2 μ l 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.

Directions for use

For glycoproteins:

1. Add up to 100 μ g of glycoprotein to a tube.
2. Add 4 μ l 5X buffer and water to 19 μ l.
3. Add 1 μ l 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, P.I., 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 synthesis of N-acetylglucosaminerepeating 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 Vliegthart, J.F. (1995) Structural analysis of the N- and O-linked carbohydrate chains of recombinant human erythropoietin expressed in Chinese hamster ovary cells. Sialylation patterns and branch location of dimeric N-acetylglucosamine units. Eur. J. Biochem. 228, 981-1008.

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