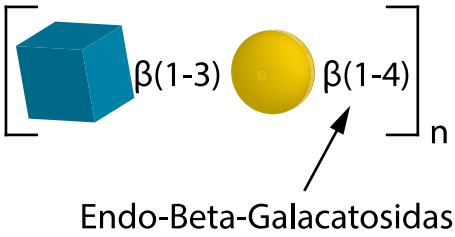

Endo-β-Galactosidase

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

EC 3.2.1.97

Catalog Number

E-XBG01	60 µl
E-XBG01-20	20 µl
E-XBG01-200	200 µl

Recommended Reagents
included with E-XBG01:
1 vial: Reaction buffer - 400 µL
250mM Sodium phosphate, pH 5.8

Activity ≥ 15 U/ml
Specific Activity ≥150 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 24 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 [GlcNAcβ(1-3) Galβ(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. Oligosaccharidesof the neo-lacto group are cleaved at greatly educed rates depending on the deviation from the preferred substrate.

For example, Galβ(1-3)GlcNAcβ(1-3)Galβ(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

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Specific Activity
One unit of endo-β-Galactosidase 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.

Purity
Endo-β-Galactosidase is tested for contaminating protease as follows: 10 ug 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.

Directions for use
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.

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