Data Sheet

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Agilent AdvanceBio β(1-4)-Galactosidase, Recombinant

Specifications

Specification	Value
Part Number	GK80080
Activity	≥2 U/mL (200 mU vial, 100 µL)
Storage	Caution: do not freeze 2 to 8 °C
Shipping	Shipped on ice pack for next day delivery
Formulation	A sterile-filtered solution in 20 mM Tris HCl, 25 mM NaCl, pH 7.5

Introduction

Agilent AdvanceBio β(1-4)-Galactosidase (β-d-galactoside galactohydrolase, EC 3.2.1.23) catalyzes the hydrolysis of nonreducing terminal β(1-4)-linked galactose residues from complex carbohydrates and glycoproteins.[1] β(1-4)-galactose is by far the most common linkage found in N-linked oligosaccharides. The enzyme is as active on both tetra- and bi-antennary oligosaccharides containing β(1-4)-linked galactose. Fucose linked to the penultimate N-acetylglucosamine will block cleavage of the galactose. This activity, specifically towards β(1-4)-linked galactose, contrasts with that of our other β-galactosidases (e.g. from bovine testis, part number GKX-5013; or jack bean, part number GKX-5012), which exhibit a broader cleavage of different linkages. Used in conjunction, these enzymes provide a powerful means to determine linkage positions of nonreducing β-galactose residues.

AdvanceBio β(1-4)-Galactosidase is purified from *E. coli* expressing a cloned gene from a strain of *Streptococcus pneumoniae*. Removal of AdvanceBio β(1-4)-Galactosidase by IMAC purification after degalactosylation is possible, as the recombinant enzyme is fused with a His tag. The enzyme activity has been extensively characterized using oligosaccharide and glycoprotein standards.

AdvanceBio β(1-4)-Galactosidase is useful for the following applications:

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

Product description

Supplied reagents (research pack only)

5x Reaction Buffer B (250 mM sodium phosphate, pH 6.0) (part number WS0049).

Purity

The absence of exoglycosidase contaminants was confirmed by extended incubations with the corresponding *p*NP-glycosides. See the certificate of analysis for specific assays performed. The absence of protease contamination was verified by incubating the enzyme with 0.2 mg of resorufin-labeled casein for ~18 hours at 37 °C.[2]

Specificity

This enzyme is highly specific for nonreducing terminal Galβ(1-4)GlcNAc or Galβ(1-4)GalNAc linkages (Figure 1). This bond specificity slightly compromises at enzyme concentrations above 100 mU/mL as a small amount of hydrolysis of Galβ(1-3) linkages occurs. The number of antennae does not affect cleavage rate. Fucose linked to the penultimate N-acetylglucosamine will block cleavage of the galactose.

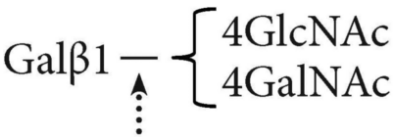

Figure 1. Specificity of Agilent AdvanceBio β(1-4)-Galactosidase to β(1-4)-Galactose linkages with galactose (Gal) and N-acetylglucosamine (GlcNAc).

Molecular weight

~240 kDa

pH range

Optimum: pH 6.0

Range: pH 5.0 to 7.0

Sodium phosphate 100 mM (pH 6.0) provides the optimal pH for enzyme activity with substrates, such as the oligosaccharide shown in Figure 1. If glycosidase treatment is performed at suboptimal pH because of glycoprotein solubility or activity requirements, expect some diminution in enzyme activity.

Assay

One unit of AdvanceBio β(1-4)-Galactosidase is defined as the amount of enzyme required to catalyze the release of 1 µmol of *o*-nitrophenyl (*o*NP) from *o*-nitrophenyl-β-galactopyranoside per minute, at pH 6.0 and 37 °C.

Suggestions for use

Before use, mix gently and briefly centrifuge the vial to ensure that all material is at the base of the vial.

Ensure that reagents, substrates, and laboratory ware are free from contaminants and proteases.

Conditions for use vary depending on the application and sample type. For example, to remove nonreducing terminal β(1-4) galactose residues from isolated glycans, the oligosaccharide concentration is typically 10 to 20 µM in 50 mM sodium phosphate (pH 6.0), with an enzyme concentration of 80 mU/mL.

Procedure for degalactosylation

1. Add up to 100 µg of asialoglycoprotein or 1 nmol of oligosaccharide to a tube.
2. Add deionized water to a total volume of 14 µL.
3. Add 4 µL of 5x Reaction Buffer B.
4. Add 2 µL of β(1-4)-Galactosidase*.
5. Incubate at 37 °C for up to 18 hours.

In rare cases, cleavage of galactose from glycoproteins may be monitored by SDS-PAGE if the size differential between native and degalactosylated protein is sufficient for detection. Usually, detection of degalactosylation can only be made by removing the glycans from the protein after enzyme digestion, followed by labeling with a fluorescent tag and analyzing with HPLC.

* Adjusting the enzyme amount when distinguishing Galβ(1-4) from Galβ(1-3)-linkages is important for the application.

References

1. Paulson, J. C. *et al.* Sialyl- and Fucosyltransferase in the Biosynthesis of Asparaginyl-Linked Oligosaccharides in Glycoproteins. Mutually Exclusive Glylcosylation by β-Galactoside A2 → 6 Sialyltransferase and N-Acetylglucosaminide A1 → 3 Fucosyltransferase. *J. Biol. Chem.* **1978**, *253(16)*, 5617–5624. https://doi.org/10.1016/S0021-9258(17)30311-3.
2. Schickaneder, E. *et al.* Casein-Resorufon, a New Substrate for a Highly Sensitive Protease Assay. *Fresenius' Zeitschrift für Anal. Chemie* **1988**, *330(4)*, 360. https://doi.org/10.1007/BF00469282.

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