

Agilent AdvanceBio $\alpha(1-3,4,6)$ -Galactosidase, Recombinant

Specifications

Specification	Description
Part Number	GK80085
Activity	≥ 80 U/mL (5 U vial, 62.5 μ L)
Storage	Caution: do not freeze 2 to 8 °C
Shipping	Shipped on ice pack for next day delivery
Formulation	A sterile solution in 100 mM sodium phosphate, pH 6.5

Introduction

Agilent AdvanceBio $\alpha(1-3,4,6)$ -Galactosidase (α -D-galactoside galactohydrolase, EC 3.2.1.22) catalyzes the hydrolysis of all nonreducing terminal $\alpha(1-3,4,6)$ -linked galactose residues from oligosaccharides, complex carbohydrates, glycoproteins, or glycolipids. Terminal α -linked galactose residues have been reported to occur on several glycoproteins and are of increasing interest. $\alpha(1-3)$ -galactose is widely found in glycoconjugates from nonprimate mammals and New World monkeys, although it is absent from humans and Old World monkeys.

The enzyme is as active on $\alpha(1-3)$ -linked galactose as it is on the less abundant $\alpha(1-6)$ -linked galactose.

AdvanceBio $\alpha(1-3,4,6)$ -Galactosidase is purified from an *E. coli* strain expressing a cloned gene from green coffee bean. Removal of AdvanceBio $\alpha(1-3,4,6)$ -Galactosidase after degalactosylation by IMAC purification is possible, as the recombinant enzyme is fused with a His tag. The enzyme activity has been characterized extensively using oligosaccharide and glycoprotein standards.

AdvanceBio $\alpha(1-3,4,6)$ -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 E (500 mM sodium citrate/phosphate, pH 6.0) (part number WS0398).

Purity

The absence of exoglycosidase contaminants was confirmed by extended incubations with the corresponding *p*NP-glycosides. The absence of β -galactosidase contamination is further confirmed by extended incubation with Agilent 2-AB labeled NA2, followed by HPLC analysis. 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.¹

Specificity

This enzyme is highly specific for nonreducing terminal Gal α (1-3,4,6)Gal or Gal α (1-3,4,6)Glc linkages (Figure 1). The precise specificity of the enzyme depends on the nature of the glycoconjugate. The number of antennae does not affect cleavage rate.

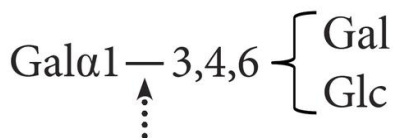


Figure 1. Specificity of Agilent AdvanceBio α (1-3,4,6)-Galactosidase to Gal α (1-3,4,6) linkages with galactose (Gal) and glucosamine (Glc).

Molecular weight

~40 kD

pH range

Optimum: pH 6.0

Range: pH 5.0 to 7.0

Sodium citrate/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 α -Galactosidase is defined as the amount of enzyme required to catalyze the release of 1 μ mol of *p*-nitrophenyl (*p*NP) from *p*-nitrophenyl- α -D-galactopyranoside per minute at pH 6 and 37 °C.

Suggestions for use

Before use, gently mix 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 or sample type. For example, to remove nonreducing terminal α -linked galactose residues from isolated glycans, the oligosaccharide concentration is typically 40 μ M in 100 mM sodium citrate/phosphate (pH 6.0), with an enzyme concentration of 5 U/mL.

Procedure for degalactosylation

1. Add up to 1 nmol of oligosaccharide to a tube.
2. Add deionized water to a total volume of 13.5 μ L.
3. Add 4 μ L of 5x Reaction Buffer E.
4. Add 2.5 μ L of α (1-3,4,6)-Galactosidase.
5. Incubate at 37 °C for 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.

Note: This enzyme remains 100% active for at least 19 hours at 37 °C, pH 6.0.

Note: This enzyme has a slow turnover. After 18 hours at 37 °C with 5 U/mL enzyme, a 40 μ M solution of asialo, biantennary, fucosylated oligosaccharide with outer arm α (1-3)-galactose is 67% de- α -galactosylated.

Note: This enzyme can be used with multiple glycosidases. An ammonium acetate buffer is recommended when running a multi-enzyme exoglycosidase digest that includes GK-80085 $\alpha(1-3)$ galactosidase. Agilent suggests a 10x reaction buffer of 500 mM ammonium acetate pH 5.5 with 0.05% azide. The reaction buffer can also be used diluted to 20x (25 mM ammonium acetate) and lower with purified glycans. This buffer works with most Agilent exoglycosidases in an overnight digestion: GK80040, GK80021, GK80080, GKX-5013, GKX-5023, GK80085, and GKX-5010. Generally, 2 μ L of each exoglycosidase is used in a 20 μ L reaction with an overnight incubation (16 hours) at 37 °C.

Reference

1. 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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