Data Sheet

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Agilent AdvanceBio α(1-2)-Mannosidase, Recombinant

Specifications

Specification	Value
Part Number	GK80075
Activity	≥25 mU/mL (2 mU vial, 80 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 10 mM sodium acetate, 250 µg/mL BSA (pH 5.0)

Introduction

Agilent AdvanceBio α(1-2)-Mannosidase (α-d-mannoside mannohydrolase, EC 3.2.1.24) achieves specific cleavage of nonreducing terminal α(1-2)-linked mannose residues from complex carbohydrates and glycoproteins.[1]
To release terminal mannose with broader linkage specificity, use Agilent α(1-2,3,6)-Mannosidase (part number GKX-5010).

AdvanceBio α(1-2)-Mannosidase is purified from a strain of *Pichia pastoris* expressing a cloned gene from *Aspergillus phoenicis*. The activity of the enzyme has been extensively characterized using oligosaccharide and glycoprotein standards.

AdvanceBio α(1-2)-Mannosidase is useful for the following applications:

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

Product description

Supplied reagents (research pack only)

5x Reaction Buffer D (500 mM sodium acetate, pH 5.0) (part number WS0397).

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 releasing nonreducing terminal α(1-2)-linked mannose (Figure 1). It is useful in identifying this type of linkage and presence of high mannose-type N-glycans that are digested to the Man-5 structure. The number of antennae does not affect cleavage-rate.

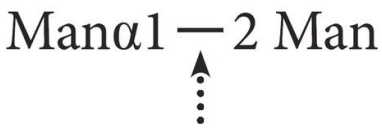

Figure 1. Specificity of Agilent AdvanceBio α(1-2)-Mannosidase to terminal α(1-2)-linked mannose (Man).

Molecular weight

~70 kDa

pH range

Optimum: pH 5.0

Range: pH 5.0 to 6.0

Sodium acetate 100 mM (pH 5.0) 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(1-2)-Mannosidase is defined as the amount of enzyme required to catalyze the release of 1 µmol of mannose from methyl-2-O-α-D-mannopyranosyl-α-D-mannopyranoside per minute at pH 5.0 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 demannosylate isolated glycan, the optimum substrate concentration is 15 µM in 100 mM sodium acetate, (pH 5.0) with an enzyme concentration of 1 to 2 mU/mL at 37 °C for 16 to 24 hours.

Procedure for demannosylation

1. Add up to 100 µg of glycoprotein 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 D.
4. Add 2 µL of AdvanceBio α(1-2)-Mannosidase.
5. Incubate at 37 °C for 16 to 24 hours.

References

1. Ichishima, E. *et al.* Purification of an Acidic α-d-Mannosidase from Aspergillus Saitoi and Specific Cleavage of 1,2-α-d-Mannosidic Linkage in Yeast Mannan. *Biochim. Biophys. Acta - Enzymol.* **1981**, *658(1)*, 45–53. https://doi.org/10.1016/0005-2744(81)90248-5.
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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