

Agilent AdvanceBio Sialidase V, Recombinant

Specifications

Specification	Value
Part Number	GK80060
Activity	≥5 U/mL (1 U vial, 200 μL)
Storage	Caution: do not freeze 2 to 8 °C
Shipping	Shipped on cold pack for next day delivery
Formulation	A sterile-filtered solution in 50 mM sodium acetate, 150 mM NaCl, pH 5.5

Introduction

Agilent AdvanceBio $\alpha(2-3,6,8)$ -Sialidase V (N-acetyl-neuraminidase glycohydrolase, EC 3.2.1.18) cleaves all nonreducing terminal $\alpha(2-3,6,8)$ -linked-sialic acid from oligosaccharides, glycoproteins, and complex carbohydrates. These linkages are found in a wide range of oligosaccharides, polysaccharides, and glycoconjugates, such as glycoproteins and glycolipids. The rate of hydrolysis of $\alpha(2-3)$ over $\alpha(2-6)$ and/or $\alpha(2-8)$ linkages is approximately three-fold.^{1,2} For $\alpha(2-8)$ linkages, GK80040 Sialidase A is recommended.

Sialidases are also known as neuraminidases.

AdvanceBio $\alpha(2-3,6,8)$ -Sialidase V is isolated from a strain of *Vibrio cholerae* and recombinantly expressed in *E. coli*. Removal of AdvanceBio Sialidase V by IMAC purification after desialylation is possible, as the recombinant enzyme is fused with a His tag. Note that denaturing condition may be required for this removal. The sialidase activity has been characterized extensively using oligosaccharide and glycoprotein standards.

AdvanceBio Sialidase V is useful for the following applications:

- Structural analysis of oligosaccharides
- Distinguishing different sialic acid linkages
- Removing heterogeneity from glycoproteins
- Probing cell surface molecules (such as receptors)

Product description

Supplied reagents (research pack only)

5x Reaction Buffer C (250 mM sodium acetate, 20 mM calcium chloride, 0.5 mg/mL BSA, pH 5.5) (part number WS0389).

Purity

The absence of exoglycosidase contaminants was confirmed by extended incubations with the corresponding pNP-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.³

Specificity

This enzyme is highly specific for N-acetyl (Neu5Ac) or N-glycolyl (Neu5Gc) neuraminic acid at the nonreducing terminus in α 2-3, 6, or 8 linkages to various monosaccharides (e.g. galactose; neuraminic acid, NeuAc-NeuAc, N-acetylglucosamine, or N-acetylgalactosamine), as shown in Figure 1.

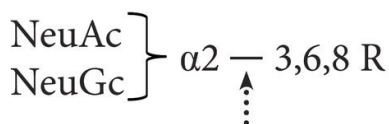


Figure 1. Specificity of Agilent AdvanceBio Sialidase V to N-acetylneuraminic acid (NeuAc) or N-glycolylneuraminic acid (NeuGc) at the nonreducing terminus in α 2-3,6, or 8 linkages.

Molecular weight

~83 kDa

pH range

Optimum: pH 5.5

Range: pH 5.0 to 6.0

Sodium acetate 250 mM (pH 5.5) 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.

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Assay

One unit of AdvanceBio Sialidase V is defined as the amount of enzyme required to catalyze the release of 1 μ mol of methylumbelliferone from MU-NANA [2'-(4-methylumbelliferyl)- α -d-N-acetylneuraminic acid] per minute at pH 5.5 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 and sample type. For example, to desialylate isolated glycans, the optimum substrate concentration is 5 to 30 μ M in 50 mM sodium acetate, 4 mM CaCl₂, 100 μ g/mL BSA (pH5.5) with an enzyme concentration of 1 to 2 U/mL at 37 °C for 18 hours.

Procedure for desialylation

1. Add up to 100 μ g of glycoprotein or 1 nmol of oligosaccharide to a tube.
2. Add deionized water to a total of 14 μ L.
3. Add 4 μ L of 5x Reaction Buffer C.
4. Add 2 μ L of AdvanceBio Sialidase V.
5. Incubate at 37 °C for 18 hours.

References

1. Kobata, A. Use of Endo- and Exoglycosidases for Structural Studies of Glycoconjugates. *Anal. Biochem.* **1979**, *100*(1), 1–14. [https://doi.org/10.1016/0003-2697\(79\)90102-7](https://doi.org/10.1016/0003-2697(79)90102-7).
2. Ada, G. L.; French, E. L.; Lind, P. E. Purification and Properties of Neuraminidase from *Vibrio Cholerae*. *Microbiology* **1961**, *24*(3), 409–421. <https://doi.org/10.1099/00221287-24-3-409>.
3. 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>.