Data Sheet

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Agilent Sialidase A

Specifications	
Product Code	GK80040
Specific Activity	40 U/mg
Activity	≥5 U/mL
Shipping	Shipped on ice pack for next day delivery.
Storage	2 to 8 °C DO NOT FREEZE
Formulation	A sterile-filtered solution in 20 mM Tris-HCl, 25 mM NaCl (pH 7.5)

Introduction

Agilent (formerly ProZyme) sequencing-grade Sialidase A (N-acetyl-neuraminate glycohydrolase, EC 3.2.1.18) cleaves all nonreducing terminal sialic acid residues from complex carbohydrates and glycoproteins (Figure 1). The relative cleavage rates for different linkages are:

α(2-6) > α(2-3) > α(2-8),α(2-9)

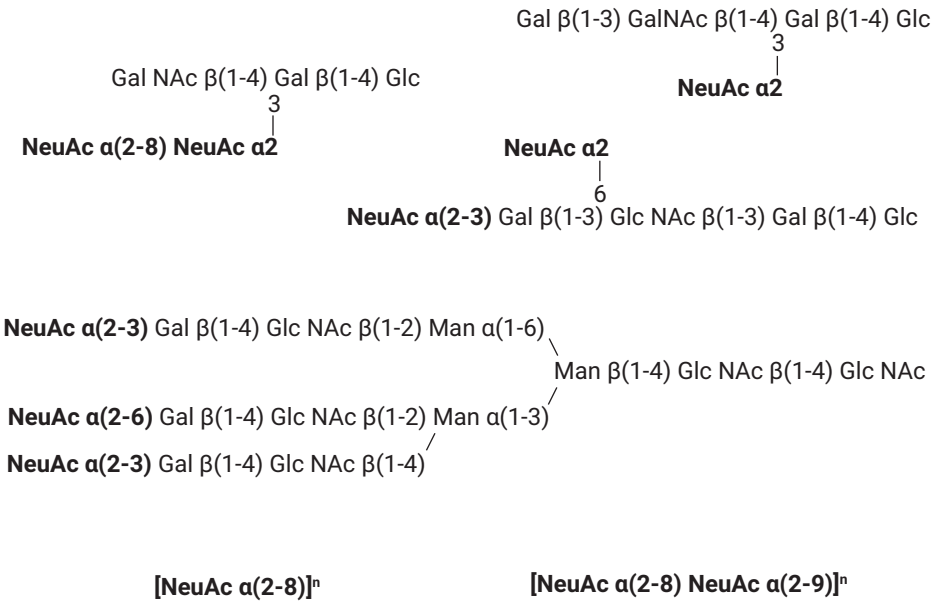


Figure 1. Linkage specificities showing cleavable residues (in bold) for Sialidase A. Gal: galactose; Glc: glucose; Man: mannose; GalNAc: N-acetylgalactosamine; GlcNAc: N-acetylglucosamine; NeuAc: N-acetylneuraminic acid (sialic acid)

In addition, Sialidase A will cleave branched sialic acids (linked to an internal residue). This property makes it unique among sialidases. High concentrations of enzymes and prolonged incubation times may be required for cleaving branched residues. To cleave only non-reducing terminal α(2-3) unbranched sialic acid residues, use Agilent Glyko Sialidase S (formerly ProZyme).

Glyko Sialidase A is isolated from a strain of *E. coli* expressing a cloned gene from *Arthrobacter ureafaciens*. The enzyme has been extensively characterized using oligosaccharide standards.

Sialidase A, because of its purity and broad linkage specificity, has been extensively used in the analysis of both glycoproteins and glycolipids.[1,2] It can be used in conjunction with the GKK-407 Signal DMB Sialic Acid Labeling Kit and GKI-4727 GlycoSep R HPLC Column (available from Agilent) for the release and chromatographic analysis of sialic acid species.

Glyko Sialidase A is useful for:

- Structural analysis of oligosaccharides
- Determining sialic acid linkage (in conjunction with other sialidases having different specificities, such as Glyko GK80030 Sialidase C and GK80021 Sialidase S; these and other Glyko sialidases are available from Agilent)
- Glycoprotein deglycosylation
- Removing heterogeneity from glycoproteins

Product description

Supplied reagents (research pack only)

5x Reaction Buffer (250 mM sodium phosphate, pH 6.0)

Molecular weight

~88 kDa

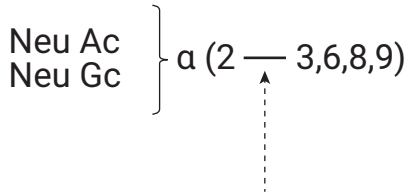
Purity

The absence of exoglycosidase contaminants was confirmed by extended incubations with the corresponding pNP-glycosides. See certificate of analysis for specific assays performed.

No protease activity was detectable after incubation of the enzyme with 0.2 mg resorufin-labeled casein for ~18 hours at 37 °C.[3]

Specificity

Sialidase A cleaves all nonreducing terminal branched and unbranched sialic acids (see Figure 1). The enzyme releases α(2-3)-, α(2-6)-, α(2-8)- and α(2-9)-linked N-acetylneuraminic acid from complex carbohydrates. The initial rate of hydrolysis of α(2-6) linkages is reported to be approximately twice that of α(2-3)-linked sialic acid however, in practice, this kinetic selectivity is of little consequence during extended incubations.[4] Effective digestion of glycolipid substrates is facilitated by the addition of a detergent, such as sodium taurodeoxycholate to the incubation.[5]



pH range

Optimum: pH 6.0

Range: pH 4.5–8.0

50 mM sodium phosphate (pH 6.0) provides the optimal buffer for enzyme activity with sialyllactose, a standard substrate. With NAN-lactose the optimum pH is 5.0 to 5.5; with colominic acid (poly NeuAc) the optimum pH is 4.3 to 4.5. If glycosidase treatment is performed at a suboptimal pH because of glycoprotein solubility or activity requirements, expect some diminution in enzyme activity.

Assay

One unit of Glyko Sialidase A is defined as the amount of enzyme required to catalyze the release of 1 µmole of p-nitrophenol from pNP-α-D-N-acetylneuraminic per minute at pH 5.5 and 37 °C.

Additional reagents (not supplied)

250 µM 2-*o*-(*p*-nitrophenyl)-α-D-N-acetylneuraminic acid (Toronto Research Chemicals #N502501) in 100 mM sodium phosphate (pH 5.5)

0.5 M sodium carbonate

Procedure

1. Adjust spectrophotometer to read 405 nm.
2. Add 395 µL of substrate solution to two tubes and warm to 37 °C.
3. Add 5 µL of enzyme to one tube and mix.
4. After 30 seconds, add 0.6 mL 0.5 M sodium carbonate to both tubes.
5. Blank spectrophotometer to control tube (without enzyme).
6. Read the absorbance at 405 nm.

Suggestions for use

Procedure for De-sialylation

1. Add up to 100 µg of glycoprotein or 1 nmole of oligosaccharide to a tube.
   Note: To cleave more than one nmole of substrate, increase reaction volume and enzyme proportionally.
2. Add de-ionized water to a total of 14 µL.
3. Add 4 µL 5x Reaction Buffer.
4. Add 2 µL Sialidase A.
5. Incubate at 37 °C for one hour.

Note: longer incubation times are necessary if branched sialic acids are present.

De-sialylation may be monitored by SDS-PAGE if the size differential between native and de-sialylated protein is sufficient for detection.

References

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