PROzyme®

**NOTICE:** ProZyme was purchased by Agilent in July 2018. Documents for products and product lots manufactured before August 2019 will contain references to ProZyme. For more information about these products and support, go to: **www.agilent.com/en/contact-us.**

Agilent

# SIALIDASE C™

## SPECIFICATIONS

**Product Code:** GK80030
**Specific Activity:** ≥40 U/mg
**Activity:** ≥10 U/ml
Shipped with cold pack for next day delivery.
Store at 2-8°C. **DO NOT FREEZE.**
**Formulation:** A sterile-filtered solution in 20 m*M* Tris-HCl, 25 m*M* NaCl (pH 7.5).

Glyko® Sialidase C™/NANase II (N-acetyl-neuraminate glycohydrolase, EC 3.2.1.18) is a sequencing-grade enzyme that cleaves all non-reducing terminal non-branched α(2-3) and α(2-6) sialic acid residues from complex carbohydrates and glycoproteins. Relative cleavage rates for different linkages are:

α(2-3) > α(2-6)

Sialidase C™ will not cleave branched sialic acids (linked to an internal residue). Use Glyko® Sialidase A™/NANase III (GK80040) for α(2-8) or branched sialic acids. To cleave only non-reducing terminal α(2-3) unbranched sialic acid residues, use Glyko® Sialidase S™/NANase III (GK80020).

Sialidase C™ is isolated from a strain of *E. coli* expressing a cloned gene from *Clostridium perfringens*. The enzyme has been extensively characterized using oligosaccharide standards.

Sialidase C™ is useful for:

- Structural analysis of oligosaccharides
- Determining sialic acid linkage
- Glycoprotein deglycosylation
- Removing heterogeneity from glycoproteins

## PRODUCT DESCRIPTION

**Supplied Reagents** (research pack only)

- WS0049 5x Reaction Buffer B (250 m*M* sodium phosphate, pH 6.0)

**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 according to the method described by Twining (1984).

**Specificity:** all non-reducing terminal α(2-3) and α(2-6) unbranched sialic acids (see Figure 1)

**Molecular Weight:** ~41,000 daltons

**pH Optimum:** 50 m*M* sodium phosphate (pH 6.0) provides the optimal buffer for enzyme activity with 3'-sialyllactose, a standard substrate. 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 Glyko® Sialidase C™ is defined as the amount of enzyme required to produce 1 µmole of p-nitrophenol from pNP-α-D-N-acetylneuraminic acid per minute at pH 5.5 and 37°C.

**Additional Reagents** (not supplied)

- 250 µ*M* 2-O-(*p*-nitrophenyl)-α-D-N-acetylneuraminic acid (Toronto Research Chemicals #N502500) in 100 m*M* 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 1*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 tube.
2. Add water to a total of 14 µl.
3. Add 4 µl 5x Reaction Buffer B.
4. Add 2 µl Sialidase C™.
5. Incubate at 37°C for 1 hour.

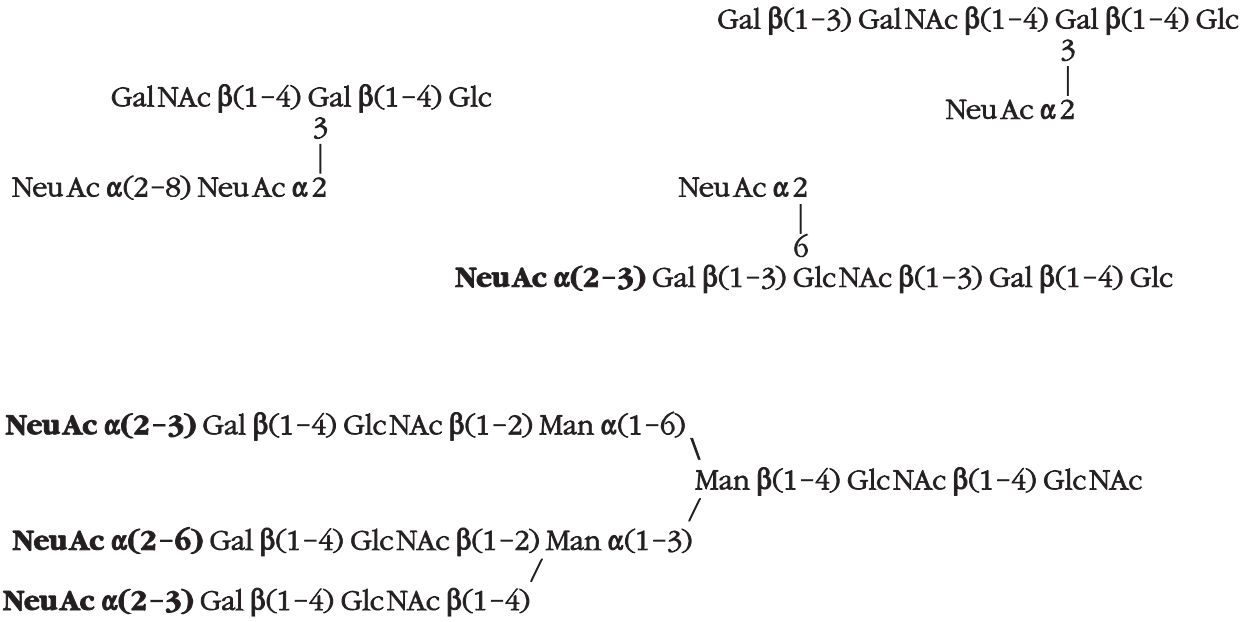
De-sialylation may be monitored by SDS-PAGE if the size differential between native and de-sialylated protein is sufficient for detection.

## REFERENCES


1. Corfield AP, Higa H, Paulson JC, Schauer R. The specificity of viral and bacterial sialidases for alpha(2-3) and alpha(2-6)-linked sialic acids in glycoproteins. Biochim Biophys Acta. 1983;744:121-26.
2. Dwek RA , Edge CJ, Harvey DJ, Wormald MR, Parekh RB. Analysis of glycoprotein-associated oligosaccharides. Ann Rev Biochem. 1993;62:65-100.
3. Kobata A. Use of endo- and exoglycosidases for structural studies of glycoconjugates. Anal Biochem. 1979;100:1-14.
4. Prime SJ Dearnley AM, Venton AM, Parekh RB, Edge CJ. Oligosaccharide sequencing based on exo- and endoglycosidase digestion and liquid chromatographic analysis of the products. J Chromatogr A. 1996;720:263-74.
5. Roggentin P, Rothe B, Lottspeich F, Schauer R. Cloning and sequencing of a *Clostridium perfringens* sialidase gene. FEBS Lett. 1988;238:31-34.
6. Roggentin P, Kleineidam RG, Schauer R. Diversity in the properties of two sialidase isoenzymes produced by *Clostridium perfringens* spp. Biol Chem Hoppe-Seyler. 1995;376:569-75.
7. Twining SS. Fluorescein Isothiocyanate-labeled Casein Assay for Proteolytic Enzymes. Anal Biochem. 1984;143:30-4.

**Figure 1 - Linkage specificities showing cleavable residues (in bold) for Sialidase C**

Gal - galactose; Glc - glucose; Man - mannose; GalNAc - N-acetylgalactosamine; GlcNAc - N-acetylglucosamine; NeuAc - N-acetylneuraminic acid (sialic acid)





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