Alpha (2-3,6,8,9) Neuraminidase

α(2-3,6,8,9) Neuraminidase (EC 3.2.1.18) cleaves all non-reducing terminal sialic acid residues from complex carbohydrates and glycoproteins. The relative cleavage rates for different linkages are: α(2-6) > α(2-3) > α(2-8), α(2-9)

In addition, the enzyme will cleave branched sialic acids (linked to an internal residue). This property makes it unique among sialidases (Figure 1). 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 α(2-3) neuraminidase. α(2-3,6,8,9) neuraminidase is isolated from a clone of *Arthrobacter ureafaciens*. The enzyme has been extensively characterized using oligosaccharide standards.

α(2-3,6,8,9) neuraminidase is useful for:

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

Specifications

Activity
≥ 135 U/mg, ≥ 5 U/mL

Storage
Store at 4°C. Do not freeze.

Formulation
The enzyme is provided as a sterile solution in 20 *mM* Tris HCl pH 7.5, 25 *mM* Sodium Chloride.

Stability
Stable at least 12 months when stored properly. Several days exposure to ambient temperatures will not reduce activity.

Product Description

Molecular Weight
70,000 Daltons

Purity
α(2-3,6,8,9) neuraminidase is tested for contaminating protease as follows; 10 µg of denatured BSA is incubated for 24 hours at 37°C with 2 µL of enzyme. SDS-PAGE analysis of the treated BSA shows no evidence of degradation.

The production host strain has been extensively tested and does not produce any detectable glycosidases.

Specificity:
All non-reducing terminal branched and unbranched sialic acids (see Figure 1).

pH Range:
Optimum: pH 6
Range: pH 4.5 - 7

The recommended buffer concentrate provides the optimal pH for enzyme activity with the 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 α(2-3,6,8,9) neuraminidase activity is defined as the amount of enzyme required to produce 1 µmole methylumbelliferone in 1 minute at 37°C, pH 5 from 2'-(4-methylumbelliferyl)-α-D-N acetylneuraminic.

Reagents

- 5X Reaction buffer 6 - 250 *mM* sodium phosphate pH 6.0

Suggestions for Use

Procedure for Desialylation
Desialylation may be monitored by SDS-PAGE if the size differential between native and desialylated protein is sufficient for detection.

1. Add up to 100 µg of glycoprotein or 1 nmol of oligosaccharide to tube.

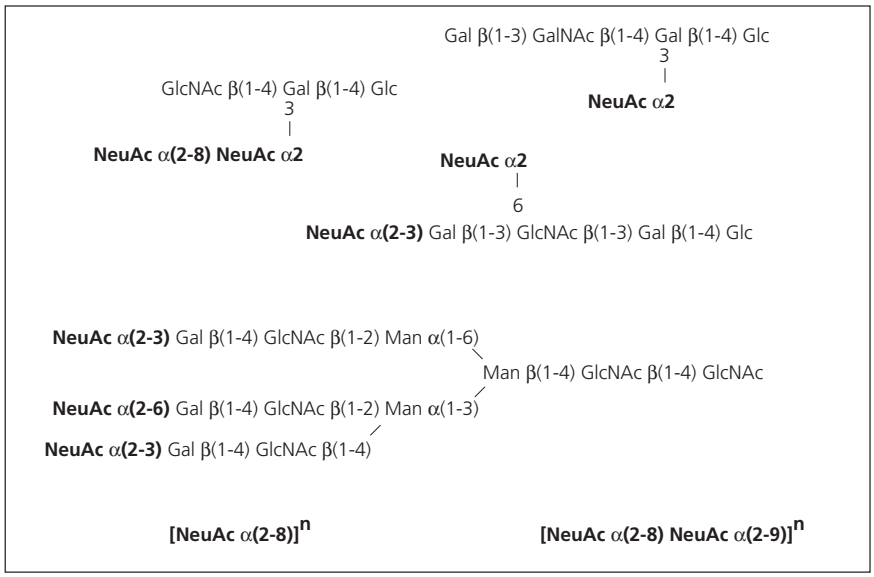
2. Add water to 13 µL and 4 µL 5X Reaction Buffer
3. Add 2 µL α(2-3, 6, 8, 9) neuraminidase
4. Incubate at 37°C for 1 hour.

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

References

1. Corfield, A. P., H. Higa, J. C. Paulson and R. Schauer. The specificity of viral and bacterial sialidases for alpha(2-3) and alpha(2-6)-linked sialic acids in glycoproteins. Biochim Biophys Acta 744:121-126 (1983).
2. Dwek, R. A. , C. J. Edge, D. J. Harvey, M. R. Wormald and R. B. Parekh. Analysis of glycoprotein-associated oligosaccharides. Ann Rev Biochem 62:65-100 (1993).
3. Kobata, A. Use of endo- and exoglycosidases for structural studies of glycoconjugates. Anal Biochem 100:1-14 (1979).
4. Ohta, Y., Y. Tsukada and T. Sugimori. Purification and properties of neuraminidase isoenzymes in Arthrobacter ureafaciens mutant. J Biochem (Tokyo) 106:1086-1089 (1989).
5. Prime, S. J. Dearnley, A. M. Venton, R. B. Parekh and C. J. Edge. Oligosaccharide sequencing based on exoand endoglycosidase digestion and liquid chromatographic analysis of the products. J Chromatogr A 720:263-274 (1996).
6. Uchida, Y., Y. Tsukada and T. Sugimori. Enzymatic properties of neuraminidases from Arthrobacter ureafaciens. J Biochem (Tokyo) 86:573-585 (1979).

Figure 1 - Linkage specificities showing cleavable residues (in bold) for α(2-3,6,8,9) neuraminidase



Gal = Galactose; Glc = Glucose; Man = Mannose; GalNAc = N-acetylgalactosamine; GlcNAc = N-acetylglucosamine; NeuAc = N-acetylneuraminic Acid (Sialic Acid)

Order Information

Catalog No.	Product Description	Package Size	Temp. °C
120057-1	**Alpha (2-3,6,8,9) Neuraminidase** *(Arthrobacter ureafaciens recombinant)*	60 µL	+4

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