Selectin Biosciences Inc.

α(2-3) Neuraminidase

α(2-3) Neuraminidase (N-acetylneuraminate glycohydrolase EC 3.2.1.18) cleaves exclusively the non-reducing terminal α(2-3) unbranched sialic acid residues from complex carbohydrates and glycoproteins. There is no detectable activity on α(2-6) or α(2-8) linkages or on branched α(2-3) linkages (see Figure 1). To cleave all non-reducing terminal sialic acid residues including branched sialic acids (linked to an internal residue) from complex carbohydrates and glycoproteins, use α(2-3,6,8,9) Neuraminidase.

α(2-3) Neuraminidase is isolated from a clone of *Streptococcus pneumonia* (formerly *Diplococcus pneumonia*). The enzyme has been extensively characterized using oligosaccharide standards.

α(2-3) Neuraminidase is useful for:

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

Product Code: GE 20

Specifications

Activity: ≥150 U/mg, >5 U/mL

Storage: Store at 4°C. Do not freeze.

Formulation: The enzyme is provided as a sterile-filtered solution in 50 mM Sodium Phosphate pH 7.5.

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

Product Description

Molecular weight: ~75,000 Daltons

Specificity: Only non-reducing terminal unbranched α(2-3) sialic acids (see Figure 1).

Purity: Each lot of α(2-3,6) Neuraminidase is tested for contaminating protease as follows: 10 µg of denatured BSA is incubated for 24 hours with 2 µL of enzyme. SDS-PAGE analysis of the treated BSAshows no evidence of degradation.

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

pH Range: 50 mM sodium phosphate (pH 6.0) provides the optimal buffer for enzyme activity with 3'-siayllactose, 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 α(2-3) Neuraminidase is defined as the amount of enzyme required to produce 1 µmole of methylumbelliferone in 1 minute at 37°C, pH 5.0 from MU-NANA [2'-(4-methylumbelliferyl)-α-D-N-acetylneuraminic acid].

Reagents

- 5X reaction buffer 6.0 – 250 mM sodium phosphate, pH 6.0

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Procedure for De-sialylation

1. Add up to 100 µg of glycoprotein or 1 nmol of oligosaccharide to tube.
2. Add water to a total volume of 14 µL
3. Add 4 µL 5X Reaction Buffer 5.0.
4. Add 2 µL of α(2-3) Neuraminidase.
5. Incubate at 37°C for 1 hour.

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

References

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3. Kobata, A. Use of endo- and exoglycosidases for structural studies of glycoconjugates. **Anal Biochem 100:**1-14 (1979).
4. Glasgow, L R., J. C. Paulson and R. L. Hill. Systematic purification of five glycosidases from Streptococcus pneumonia. **J. Biol Chem 252:**8615-8623 (1977).
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6. Prime, S. J. Dearnley, A. M. Venton, R. B. Parekh and C. J. Edge. Oligosaccharide sequencing based on exo- and endoglycosidase digestion and liquid chromatographic analysis of the products. **J Chromatogr A 720:**263-274 (1996).

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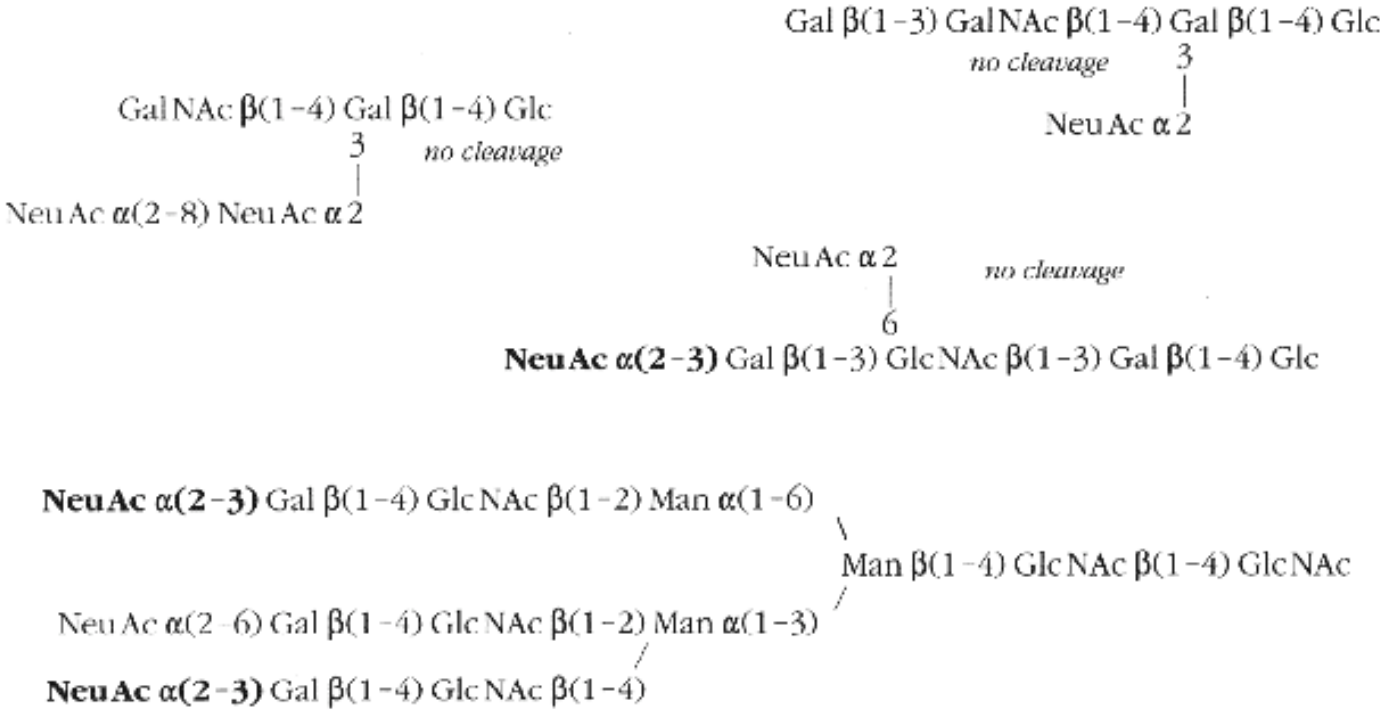
Figure 1 – Linkage specificities showing cleavable residues (in bold) for α(2-3) Neuraminidase



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