Neuraminidase A

**Detailed Specificity:**

α2-3,6,8,9 Neuraminidase A will cleave branched sialic acid residues that are linked to an internal residue. This oligosaccharide from fetuin is an example of a side-branch sialic acid residue that can efficiently be cleaved (1).

Source: Cloned from *Arthrobacter ureafaciens* and expressed in *E. coli* (2).

Supplied in: 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C) and 1 mM EDTA.

Reagents Supplied with Enzyme:

10X GlycoBuffer 1
(0.5 M Sodium Acetate, pH 5.5 @ 25°C and 50 mM CaCl₂)

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α-Neu5Ac from 1 nmol Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Unit Definition Assay: Two fold dilutions of α2-3,6,8,9 Neuraminidase A are incubated with 1 nmol AMC-labeled substrate and 1X GlycoBuffer 1 in a 10 µl reaction. The reaction mix is incubated at 37°C for 1 hour. Separation of reaction products are visualized via thin layer chromatography (3).

Specific Activity: -316,000 units/mg.

Molecular Weight: 100,000 daltons.

Quality Assurance: No contaminating exoglycosidase or endoglycosidase F1, F2 or F3 activity could be detected. No contaminating proteolytic activity could be detected.

**Quality Controls**

Glycosidase Assays: 100 units of α2-3,6,8,9 Neuraminidase A are incubated with 0.1 mM of fluorescently-labeled oligosaccharides and glycopeptides, in a 10 µl reaction for 20 hours at 37°C. The reaction products were analyzed by TLC for digestion of substrate.

No other glycosidase activities were detected (ND) with the following substrates:

- **β-N-Acetyld glucosaminidase**: GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC ND
- **β-N-Acetylgalactosaminidase**: GalNAcβ1-4Galβ1-4Glc-AMC ND

(see other side)
β-Arrestin II
Molecular Mass: 100 kDa

Notes on Use:
- Reactions may be scaled-up linearly to accommodate larger reaction volumes.
- The amount of exoglycosidase enzyme required varies when different substrates are used. Start with 1–2 µl for 1 µg of glycoprotein or 100 nM of oligosaccharide for one hour in a 10–25 µl reaction. If there is still undigested material, let the reaction go overnight.
- Higher concentrations of enzyme as well as longer incubation times may be necessary for cleavage of branched structures.

References: