Source: Cloned from *Streptococcus pneumoniae* and expressed in *E. coli* (1).

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.

**Specific Activity:** -160,000 units/mg.

**Molecular Weight:** 74,000 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:** 80 units of α2-3 Neuraminidase S were incubated with 0.1 mM of flourously-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) for digestion of substrate with the following substrates:

- β-N-Acetylglucosaminidase: GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC ND
- β-N-Acetylglucosaminidase: GalNAcβ1-4Galβ1-4Glc-AMC ND
- α-N-Acetylglucosaminidase: GalNAcα1-3(Fucα1-2)Galβ1-4Glc-AMC ND

**Specificity:**

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**Specific Activity:** -160,000 units/mg.
Endo F1, F2, H: Dansylated invertase high mannose. ND
Endo F2, F3: Dansylated fibrinogen biantennary. ND

Protease Assay: After incubation of 400 units of \(\alpha\)2-3 Neuraminidase S with 0.2 nmol of a standard mixture of proteins in a 20 µl reaction, for 20 hours at 37°C, no proteolytic activity could be detected by SDS–PAGE.

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

Heat Inactivation: 75°C for 10 minutes.

Reaction Conditions: Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate. Typical reaction conditions are as follows:
1. Combine 1 µg of glycoprotein or 100 nM of oligosaccharide and H2O (if necessary) to make a 9 µl total reaction volume.
2. Add 1 µl of 10X GlycoBuffer 1 to make a 10 µl total reaction volume.
3. Add 1 µl of \(\alpha\)2-3 Neuraminidase S.
4. Incubate at 37°C for 1 hour.

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.

Heat Inactivation: 75°C for 10 minutes.

References: