**Neuraminidase**

**Source:** Cloned from *Clostridium perfringens* (1) and overexpressed in *E. coli* at NEB (2).

Supplied in: 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C) and 5 mM Na₂EDTA.

Reagents Supplied with Enzyme: 10X G1 Reaction Buffer

Reaction Conditions: 1X G1 Reaction Buffer: 50 mM Sodium Citrate (pH 6.0 @ 25°C). Incubate at 37°C.

Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate.

**Unit Definition:** One unit is defined as the amount of enzyme required to cleave > 95% of the terminal Neu5Ac from 1 nmol Neu5Aco2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ-7-amino-4-methyl-coumarin (AMC), in 5 minutes at 37°C in a total reaction volume of 10 µl.

**Unit Definition Assay:** Two fold dilutions of Neuraminidase are incubated with 1 nmol AMC-labeled substrate and 1X G1 Reaction Buffer in a 10 µl reaction. The reaction mix is incubated at 37°C for 5 minutes. Separation of reaction products are visualized via thin layer chromatography (3).

**Specific Activity:** ~225,000 units/mg.

**Molecular Weight:** 43,000 daltons.

**Quality Assurance:** No contaminating exoglycosidase or proteolytic activity could be detected.

**Quality Controls**

**Glycosidase Assays:** 500 units of Neuraminidase were 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.

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

**Molecular Weight:** 43,000 daltons.

**Quality Controls**

**Glycosidase Assays:** 500 units of Neuraminidase were 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.

**Physical Purity:** Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.
β-Mannosidase:
Manβ1-4Manβ1-4Man-AMC ND
Endo F1, F2, H:
Dansylated invertase high mannose. ND
Endo F2, F3:
Dansylated fibrinogen bi-antennary. ND
PNGase F:
Fluoresceinated fetuin tri-antennary. ND

Protease Assay: After incubation of 500 units of Neuraminidase 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.

Note: This enzyme shows a preference for α2,3 and α2,6 linkages over α2,8 linkages (4).

References: