α2-3 Neuraminidase

**Description:** α2-3 Neuraminidase is a highly specific exoglycosidase that catalyzes the hydrolysis of α2-3 and, at a much lower rate, α2-6 linked N-Acetyl-neuraminic acid residues from oligosaccharides. This enzyme has a 260-fold preference for α2-3 sialylic linkages over α2-6 sialylic linkages and shows only trace activity against α2-8 sialylic linkages (1).

**Source:** Cloned from *Salmonella typhimurium* LT2 and overexpressed in *E. coli* (1).

**Specificity:**
- Neu5Ac α 2 – 3 R
  - >> α 2 – 6 R
  - >> α 2 – 8 R

**Reaction Conditions:**
1X GlycoBuffer 1:
- 50 mM Sodium Acetate (pH 5.5 @ 25°C)
- 5 mM CaCl₂

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

**Reagents Supplied with Enzyme:**
- 10X GlycoBuffer 1
- 100X BSA

**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 α-neu5Ac from 1 nmol of Neu5Acxx2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-7-amino-4-methyl-coumarin (AMC) in 1 hour at 37°C in a total reaction volume of 10 µl.

**Unit Definition Assay:** Two fold dilutions of α2-3 Neuraminidase are incubated with 1 nmol AMC-labeled substrate and 1X GlycoBuffer 1, supplemented with 100 µg/ml BSA, in a 10 µl reaction. The reaction mix is incubated for 1 hour at 37°C. Separation of reaction products are visualized via thin layer chromatography (3).

**Specific Activity:** ~11,300,000 units/mg.

**Molecular Weight:** 41,000 daltons.

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

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**Quality Controls**

**Glycosidase Assays:** 500 units of α2-3 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. No other glycosidase activities were detected (ND) with the following substrates:

- **β-N-Acetyl-glucosaminidase:** GlcNAcβ1-4GlcNAcβ1-4GlcNAC-AMC ND
- **α-Fucosidase:** Fucx1-2Galβ1-4Glc-AMC Galβ1-4Glc-AMC ND
- **β-Galactosidase:** Galβ1-3GlcNAcβ1-4Galβ1-4GlcNAC-AMC ND
- **α-Galactosidase:** Galα1-3Galβ1-4Galα1-3Gal-AMC ND

(See other side)
α-Mannosidase:
Manx1-3Manβ1-4GlcNAc-AMC
Manx1-6Manx1-6(Manx1-3)Man-AMC ND

β-Glucosidase:
Glcβ1-4Glcβ1-4Glc-AMC ND

β-Xylosidase:
Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC ND

β-Mannosidase:
Manβ1-4Manβ1-4Man-AMC ND

Endo F1, F2, H:
Dansylated invertase high mannose. ND

Endo F3, F4:
Dansylated fibrinogen biantennary. ND

PNGase F:
Fluoresceinated fetuin triantennary. ND

Protease Assay: After incubation of 500 units of α2-3 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: Store at 4°C or in small aliquots at –20°C. Avoid repeated freeze/thaw cycles.

References: