**α-2,3,6,8 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 GlycoBuffer 1

**Reaction Conditions:**
1X GlycoBuffer 1:
50 mM Sodium Acetate (pH 5.5 @ 25°C) and 5 mM CaCl₂. 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 α-Neu5Ac from 1 nmol Neu5Aco2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-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 α-2,3,6,8 Neuraminidase 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 5 minutes. Separation of reaction products are visualized via thin layer chromatography (3).

**Specific Activity:** ~200,000 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 α-2,3,6,8 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 Cooamassie Blue detection.

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

**Quality Assays:**

- **α-1-4 Xylosidase:** (ND)
- **α-1-3 Galactosidase:** (ND)
- **α-1-4 Mannosidase:** (ND)
- **α-1-6 Mannosidase:** (ND)
- **α-1-3 Fucosidase:** (ND)
- **β-1-4 N-Acetyl-glucosaminidase:** (ND)
- **β-1-4 Glucosidase:** (ND)
- **β-1-4 Galactosidase:** (ND)
- **β-1-3 Xylosidase:** (ND)

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

- **β-1-2 N-Acetyl-glucosaminidase:** (ND)
- **β-1-4 Fucosidase:** (ND)
- **β-1-3 Galactosidase:** (ND)
- **β-1-3 Mannosidase:** (ND)
- **β-1-4 Glucosidase:** (ND)
- **β-1-3 Xylosidase:** (ND)

(ND = Not Determined)

(Certificate of Analysis)

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**α-2,3,6,8 Neuraminidase**

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

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**Glycosidase Assays:** 500 units of α-2,3,6,8 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 Cooamassie Blue detection.

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

**Quality Assays:**

- **α-1-4 Xylosidase:** (ND)
- **α-1-3 Galactosidase:** (ND)
- **α-1-4 Mannosidase:** (ND)
- **α-1-6 Mannosidase:** (ND)
- **α-1-3 Fucosidase:** (ND)
- **β-1-4 N-Acetyl-glucosaminidase:** (ND)
- **β-1-4 Glucosidase:** (ND)
- **β-1-3 Xylosidase:** (ND)

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

- **β-1-2 N-Acetyl-glucosaminidase:** (ND)
- **β-1-4 Fucosidase:** (ND)
- **β-1-3 Galactosidase:** (ND)
- **β-1-3 Mannosidase:** (ND)
- **β-1-4 Glucosidase:** (ND)
- **β-1-3 Xylosidase:** (ND)

(ND = Not Determined)

(Certificate of Analysis)
**β-Mannosidase:**
Manβ1-4Manβ1-4Man-AMC  ND

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

**Endo F₁, F₂:**
Dansylated fibrinogen biantennary.  ND

**PNGase F:**
Fluoresceinated fetuin triantennary.  ND

**Protease Assay:** After incubation of 500 units of α2-3,6,8 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:**

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