**α2-3,6,8,9 Neuraminidase A**

800 units  20,000 U/ml  Lot: 0011407

**RECOMBINANT**  Store at –20°C  Exp: 7/16

**Description:** Neuraminidase is the common name for Acetyl-neuraminyl hydrolase (Sialidase). α2-3,6,8,9 Neuraminidase A catalyzes the hydrolysis of all linear and branched non-reducing terminal sialic acid residues from glycoproteins and oligosaccharides. The enzyme releases α2-3 and α2-6 linkages at a slightly higher rate than α2-8 and α2-9 linkages.

**Specificity:**

\[
\begin{align*}
\alpha(2-3) \\
\alpha(2-6) \\
>\alpha(2-8) \\
>\alpha(2-9)
\end{align*}
\]

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

**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 were incubated with 0.1 mM of fluorescein-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:

\[
\begin{align*}
\beta-N-Acetylglucosaminidase: \\
\text{GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC} & \quad \text{ND} \\
\beta-N-Acetylgalactosaminidase: \\
\text{GalNAcβ1-4Galβ1-4Glc-AMC} & \quad \text{ND}
\end{align*}
\]

**Specific Activity:** ~316,000 units/mg.

**Molecular Weight:** 100,000 daltons.

**Unit Definition:** One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α-Neu5Ac from 1 nmol Neu5Acα2-3Galβ1-3GlcNAcβ1-4Glc-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).

**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).

**Gal**  **Man**  **GlcNAc**  **NeuAc**  **R** = any sugar

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

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**α-N-Acetylgalactosaminidase:**  
GalNAcc1-3(Fucε1-3)Galβ1-4Glc-AMC ND

**α-Fucosidase:**  
Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND  
Fucε1-2Galβ1-4Glc-AMC ND

**β-Galactosidase:**  
Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC ND  
Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC ND

**β-Fucosidase:**  
Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND  
Fucα1-2Galβ1-4Glc-AMC ND

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

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

**Endo F₃:**  
Dansylated fibrinogen biantennary. ND

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

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

**Protease Assay:** After incubation of 1,000 units of α2-3,6,8,9 Neuraminidase A 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.

**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:**