**Neuraminidase A**

**α2-3,6,8,9 Neuraminidase A**

**P0722S**

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**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:**

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**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).

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

**Molecular Weight:** 100,000 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:** 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:

**β-N-Acetylgalactosaminidase:**

GalNAcβ1-4GlcNAcβ1-4GlcNAc-AMC ND

**β-N-Acetylgalactosaminidase:**

GalNAcβ1-4Galβ1-4Glc-AMC ND

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**β-N-Acetylgalactosaminidase:**

GalNAcβ1-4Galβ1-4Glc-AMC ND

(see other side)
**β-N-Acetylgalactosaminidase:**
GalNAcc1-3(Fucocc1-2)Galβ1-4Glc-AMC ND

**β-Fucosidase:**
Galβ1-1-4(Fucocc1-3)GlcNAcc1-3Galβ1-4Glc-AMC ND
Fucocc1-2Galβ1-4Glc-AMC ND

**β-Galactosidase:**
Galβ1-3GlcNAcc1-4Galβ1-4Glc-AMC ND
Galβ1-4GlcNAcc1-3Galβ1-4Glc-AMC ND

**α-Galactosidase:**
Galβ1-3(Fucocc1-2)Galβ1-4Xyl-AMC ND

**α-Fucosidase:**
Manβ1-4Fucocc1-2GlcNAc-AMC ND

**α-Glucosidase:**
Glcc1-6Glcocc1-4Glc-AMC ND

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

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

**Endo F1, F3:**
Dansylated invertase high mannosyl. ND

**Endo F2, F3:**
Dansylated fibrinogen biantennary. 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 analysis.

**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 α2,3,6,8,9 Neuraminidase A.
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.
- Higher concentrations of enzyme as well as longer incubation times may be necessary for cleavage of branched structures.