# Neuraminidase

NEW ENGLAND BioLabs Inc.
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P0720S 014120614061

# P0720S

RR

**2,000 units** **50,000 U/ml** **Lot: 0141206**

**RECOMBINANT** **Store at –20°C** **Exp: 6/14**

**Description:** Neuraminidase is the common name for Acetyl-neuraminyl hydrolase (Sialidase). This Neuraminidase catalyzes the hydrolysis of α2-3, α2-6 and α2-8 linked N-acetyl-neuraminic acid residues from glycoproteins and oligosaccharides.

**Specificity:**

**Neu5Ac α 2 – 3 R**
**α 2 – 6 R**
>> α 2 – 8 R

**New Unit Definition**

**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 $Na_2EDTA$.

**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 α-Neu5Ac from 1 nmol Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-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 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 flourescently-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.

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

**β-N-Acetyl-glucosaminidase:**
GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC ND

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

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

**α-Galactosidase:**
Galα1-3Galβ1-4Galα1-3Gal-AMC ND

**α-Mannosidase:**
Manα1-3Manβ1-4GlcNAc-AMC
Manα1-6Manα1-6(Manα1-3)Man-AMC ND

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

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

*(see other side)*

CERTIFICATE OF ANALYSIS

Neuraminidase

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α-Fucosidase:
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β-Galactosidase:
Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC ND

α-Galactosidase:
Galα1-3Galβ1-4Galα1-3Gal-AMC ND

α-Mannosidase:
Manα1-3Manβ1-4GlcNAc-AMC
Manα1-6Manα1-6(Manα1-3)Man-AMC ND

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

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

*(see other side)*

CERTIFICATE OF ANALYSIS

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

**Endo $F_1$, $F_2$, H:**
Dansylated invertase high mannose. ND

**Endo $F_2$, $F_3$:**
Dansylated fibrinogen biantennary. ND

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

1. Roggentin, P. et al. (1988) *FEBS* 238 (1), 31–34.
2. Guan, C., New England Biolabs, Inc., unpublished results.
3. Wong-Madden, S. T. and Landry, D. (1995) *Glycobiology* 5, 19–28.
4. Monks, B., New England Biolabs, Inc., unpublished results.

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