

# $\alpha$ 2-3,6,8 Neuraminidase



1-800-632-7799  
info@neb.com  
www.neb.com



P0720S 017141216121

## P0720S



2,000 units 50,000 U/ml Lot: 0171412

RECOMBINANT Store at -20°C Exp: 12/16

**Description:** Neuraminidase is the common name for Acetyl-neuraminyl hydrolase (Sialidase). This Neuraminidase catalyzes the hydrolysis of  $\alpha$ 2-3,  $\alpha$ 2-6 and  $\alpha$ 2-8 linked N-acetyl-neuraminic acid residues from glycoproteins and oligosaccharides.

### Specificity:

Neu5Ac  $\alpha$  2  $\downarrow$  3 R  
 $\alpha$  2 - 6 R  
>>  $\alpha$  2 - 8 R

New Reaction Buffer

**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<sub>2</sub>EDTA.

### Reagents Supplied with Enzyme:

10X GlycoBuffer 1

### Reaction Conditions:

1X GlycoBuffer 1:  
50 mM Sodium Acetate (pH 5.5 @ 25°C)  
and 5 mM CaCl<sub>2</sub>. 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  $\alpha$ -Neu5Ac from 1 nmol Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-7-amino-4-methyl-coumarin (AMC), in 5 minutes at 37°C in a total reaction volume of 10  $\mu$ l.

**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<sub>2</sub>EDTA.

### Reagents Supplied with Enzyme:

10X GlycoBuffer 1

### Reaction Conditions:

1X GlycoBuffer 1:  
50 mM Sodium Acetate (pH 5.5 @ 25°C)  
and 5 mM CaCl<sub>2</sub>. 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  $\alpha$ -Neu5Ac from 1 nmol Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-7-amino-4-methyl-coumarin (AMC), in 5 minutes at 37°C in a total reaction volume of 10  $\mu$ l.

**Unit Definition Assay:** Two fold dilutions of  $\alpha$ 2-3,6,8 Neuraminidase are incubated with 1 nmol AMC-labeled substrate and 1X GlycoBuffer 1 in a 10  $\mu$ l 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  $\alpha$ 2-3,6,8 Neuraminidase were incubated with 0.1 mM of fluorescently-labeled oligosaccharides and glycopeptides, in a 10  $\mu$ l 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.

**Unit Definition Assay:** Two fold dilutions of  $\alpha$ 2-3,6,8 Neuraminidase are incubated with 1 nmol AMC-labeled substrate and 1X GlycoBuffer 1 in a 10  $\mu$ l 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  $\alpha$ 2-3,6,8 Neuraminidase were incubated with 0.1 mM of fluorescently-labeled oligosaccharides and glycopeptides, in a 10  $\mu$ l 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:

**$\beta$ -N-Acetyl-glucosaminidase:**  
GlcNAc $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-AMC ND

**$\alpha$ -Fucosidase:**  
Fuc $\alpha$ 1-2Gal $\beta$ 1-4Glc-AMC Gal $\beta$ 1-4  
(Fuc $\alpha$ 1-3)GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC ND

**$\beta$ -Galactosidase:**  
Gal $\beta$ 1-3GlcNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC ND

**$\alpha$ -Galactosidase:**  
Gal $\alpha$ 1-3Gal $\beta$ 1-4Gal $\alpha$ 1-3Gal-AMC ND

**$\alpha$ -Mannosidase:**  
Man $\alpha$ 1-3Man $\beta$ 1-4GlcNAc-AMC  
Man $\alpha$ 1-6Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man-AMC ND

**$\beta$ -Glucosidase:**  
Glc $\beta$ 1-4Glc $\beta$ 1-4Glc-AMC ND

**$\beta$ -Xylosidase:**  
Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl-AMC ND

(see other side)

CERTIFICATE OF ANALYSIS

# $\alpha$ 2-3,6,8 Neuraminidase



1-800-632-7799  
info@neb.com  
www.neb.com



P0720S 017141216121

## P0720S



2,000 units 50,000 U/ml Lot: 0171412

RECOMBINANT Store at -20°C Exp: 12/16

**Description:** Neuraminidase is the common name for Acetyl-neuraminyl hydrolase (Sialidase). This Neuraminidase catalyzes the hydrolysis of  $\alpha$ 2-3,  $\alpha$ 2-6 and  $\alpha$ 2-8 linked N-acetyl-neuraminic acid residues from glycoproteins and oligosaccharides.

### Specificity:

Neu5Ac  $\alpha$  2  $\downarrow$  3 R  
 $\alpha$  2 - 6 R  
>>  $\alpha$  2 - 8 R

New Reaction Buffer

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

**$\beta$ -N-Acetyl-glucosaminidase:**  
GlcNAc $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-AMC ND

**$\alpha$ -Fucosidase:**  
Fuc $\alpha$ 1-2Gal $\beta$ 1-4Glc-AMC Gal $\beta$ 1-4  
(Fuc $\alpha$ 1-3)GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC ND

**$\beta$ -Galactosidase:**  
Gal $\beta$ 1-3GlcNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC ND

**$\alpha$ -Galactosidase:**  
Gal $\alpha$ 1-3Gal $\beta$ 1-4Gal $\alpha$ 1-3Gal-AMC ND

**$\alpha$ -Mannosidase:**  
Man $\alpha$ 1-3Man $\beta$ 1-4GlcNAc-AMC  
Man $\alpha$ 1-6Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man-AMC ND

**$\beta$ -Glucosidase:**  
Glc $\beta$ 1-4Glc $\beta$ 1-4Glc-AMC ND

**$\beta$ -Xylosidase:**  
Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl-AMC ND

(see other side)

CERTIFICATE OF ANALYSIS

**$\beta$ -Mannosidase:**  
Man $\beta$ 1-4Man $\beta$ 1-4Man-AMC ND

**Endo F<sub>1</sub>, F<sub>2</sub>, H:**  
Dansylated invertase high mannose. ND

**Endo F<sub>2</sub>, F<sub>3</sub>:**  
Dansylated fibrinogen biantennary. ND

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

**Protease Assay:** After incubation of 500 units of  $\alpha$ 2-3,6,8 Neuraminidase with 0.2 nmol of a standard mixture of proteins in a 20  $\mu$ l reaction, for 20 hours at 37°C, no proteolytic activity could be detected by SDS-PAGE.

**Note:** This enzyme shows a preference for  $\alpha$ 2,3 and  $\alpha$ 2,6 linkages over  $\alpha$ 2,8 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.



NEW ENGLAND BIOLABS® is a registered trademark of New England Biolabs, Inc.

**$\beta$ -Mannosidase:**  
Man $\beta$ 1-4Man $\beta$ 1-4Man-AMC ND

**Endo F<sub>1</sub>, F<sub>2</sub>, H:**  
Dansylated invertase high mannose. ND

**Endo F<sub>2</sub>, F<sub>3</sub>:**  
Dansylated fibrinogen biantennary. ND

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

**Protease Assay:** After incubation of 500 units of  $\alpha$ 2-3,6,8 Neuraminidase with 0.2 nmol of a standard mixture of proteins in a 20  $\mu$ l reaction, for 20 hours at 37°C, no proteolytic activity could be detected by SDS-PAGE.

**Note:** This enzyme shows a preference for  $\alpha$ 2,3 and  $\alpha$ 2,6 linkages over  $\alpha$ 2,8 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.



NEW ENGLAND BIOLABS® is a registered trademark of New England Biolabs, Inc.