

# $\alpha$ 2-3 Neuraminidase S



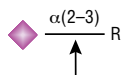
P0743S



400 units 8,000 U/ml Lot: 0011501  
RECOMBINANT Store at -20°C Exp: 1/17

**Description:** Neuraminidase is the common name for Acetyl-neuraminyl hydrolase (Sialidase).  $\alpha$ 2-3 Neuraminidase S is a highly specific exoglycosidase that catalyzes the hydrolysis of  $\alpha$ 2-3 linked N-acetyl-neuraminic acid residues from glycoproteins and oligosaccharides.

### Specificity:



NeuAc  $\blacklozenge$  R = any sugar

**Source:** Cloned from *Streptococcus pneumoniae* and expressed in *E. coli* (1).

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

### Reagents Supplied with Enzyme:

10X GlycoBuffer 1  
(0.5 M Sodium Acetate, pH 5.5 @ 25°C and 50 mM CaCl<sub>2</sub>)

**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-AMC, in 1 hour at 37°C in a total reaction volume of 10  $\mu$ l.

**Unit Definition Assay:** Two fold dilutions of  $\alpha$ 2-3 Neuraminidase S 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 1 hour. Separation of reaction products are visualized via thin layer chromatography (2).

**Specific Activity:** ~160,000 units/mg.

**Molecular Weight:** 74,000 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:** 80 units of  $\alpha$ 2-3 Neuraminidase S 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.

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

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

**$\beta$ -N-Acetylgalactosaminidase:**  
GalNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC ND

**$\alpha$ -N-Acetylgalactosaminidase:**  
GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4Glc-AMC ND

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

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

**$\alpha$ -Galactosidase:**  
Gal $\alpha$ 1-3Gal $\beta$ 1-4Gal-AMC ND  
Gal $\alpha$ 1-6Gal $\alpha$ 1-6Glc $\alpha$ 1-2Fru-AMC ND

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

**$\alpha$ -Glucosidase:**  
Glc $\alpha$ 1-6Glc $\alpha$ 1-4Glc-AMC ND

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

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

(see other side)

CERTIFICATE OF ANALYSIS

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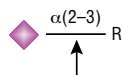
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GalNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC ND

**$\alpha$ -N-Acetylgalactosaminidase:**  
GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4Glc-AMC ND

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

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

**$\alpha$ -Galactosidase:**  
Gal $\alpha$ 1-3Gal $\beta$ 1-4Gal-AMC ND  
Gal $\alpha$ 1-6Gal $\alpha$ 1-6Glc $\alpha$ 1-2Fru-AMC ND

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

**$\alpha$ -Glucosidase:**  
Glc $\alpha$ 1-6Glc $\alpha$ 1-4Glc-AMC ND

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

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

(see other side)

CERTIFICATE OF ANALYSIS

**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

**Protease Assay:** After incubation of 400 units of  $\alpha$ 2-3 Neuraminidase S 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.

**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  $\mu$ g of glycoprotein or 100 nM of oligosaccharide and H<sub>2</sub>O (if necessary) to make a 9  $\mu$ l total reaction volume.
2. Add 1  $\mu$ l of 10X GlycoBuffer 1 to make a 10  $\mu$ l total reaction volume.
3. Add 1  $\mu$ l of  $\alpha$  2-3 Neuraminidase S.
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  $\mu$ l for 1  $\mu$ g of glycoprotein or 100 nM of oligosaccharide for one hour in a 10–25  $\mu$ l reaction. If there is still undigested material, let the reaction go overnight.

#### References:

1. Chen, M. New England Biolabs, Inc., unpublished results.
2. Wong-Madden, S. T. and Landry, D. (1995) *Glycobiology* 5, 19–28.



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