α2-3 Neuraminidase S

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

P0743S 00115091709 1

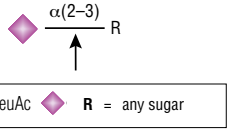
P0743S

400 units **8,000 U/ml** **Lot: 0011509**

RECOMBINANT **Store at –20°C** **Exp: 9/17**

Description: Neuraminidase is the common name for Acetyl-neuraminyl hydrolase (Sialidase). α2-3 Neuraminidase S is a highly specific exoglycosidase that catalyzes the hydrolysis of α2-3 linked N-acetyl-neuraminic acid residues from glycoproteins and oligosaccharides.

Specificity:



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 $CaCl_2$)

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-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Unit Definition Assay: Two fold dilutions of α2-3 Neuraminidase S 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 (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 α2-3 Neuraminidase S 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.

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

β-*N*-Acetylglucosaminidase:
GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC ND

β-*N*-Acetylgalactosaminidase:
GalNAcβ1-4Galβ1-4Glc-AMC ND

α-N-Acetylgalactosaminidase:
GalNAcα1-3(Fucα1-2)Galβ1-4Glc-AMC ND

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

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

α-Galactosidase:
Galα1-3Galβ1-4Gal-AMC ND
Galα1-6Galα1-6Glcα1-2Fru-AMC ND

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

α-Glucosidase:
Glcα1-6Glcα1-4Glc-AMC ND

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

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

(see other side)

CERTIFICATE OF ANALYSIS

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Specificity:

α(2–3) R
NeuAc R = any sugar

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

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GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC ND

β-*N*-Acetylgalactosaminidase:
GalNAcβ1-4Galβ1-4Glc-AMC ND

α-N-Acetylgalactosaminidase:
GalNAcα1-3(Fucα1-2)Galβ1-4Glc-AMC ND

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

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

α-Galactosidase:
Galα1-3Galβ1-4Gal-AMC ND
Galα1-6Galα1-6Glcα1-2Fru-AMC ND

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

α-Glucosidase:
Glcα1-6Glcα1-4Glc-AMC ND

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

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

(see other side)

CERTIFICATE OF ANALYSIS

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

Endo F_2, F_3:
Dansylated fibrinogen biantennary. ND

Protease Assay: After incubation of 400 units of α2-3 Neuraminidase S 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.

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 H_2O (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 α 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 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.

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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Dansylated invertase high mannose. ND

Endo F_2, F_3:
Dansylated fibrinogen biantennary. ND

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