$\alpha 2-3$

Neuraminidase



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

P0728S



2,500 units 50,000 U/ml Lot: 0021210 RECOMBINANT Store at 4°C Exp: 10/13

Description: α 2-3 Neuraminidase is a highly specific exoglycosidase that catalyzes the hydrolysis of α 2-3 and, at a much lower rate, α2-6 linked N-Acetyl-neuraminic acid residues from oligosaccharides. This enzyme has a 260-fold preference for α 2-3 sialvl linkages over α 2-6 sialvl linkages and shows only trace activity against α 2-8 sialyl linkages (1).

Specificity:

Neu5Ac α 2 – 3 R

 $>> \alpha 2 - 6 R$ $>> \alpha 2 - 8 R$

Source: Cloned from *Salmonella typhimurium* LT2 and overexpressed in E. coli (1).

Supplied in: 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C), and 5 mM Na₂EDTA.

Reagents Supplied with Enzyme:

10X G4 Reaction Buffer 100X BSA

Reaction Conditions:

1X G4 Reaction Buffer: 50 mM Sodium Citrate (pH 6.0 @ 25°C), 100 mM NaCl. Supplement with 100 µg/ml BSA. Incubate at 37°C.

Note: To hydrolyze α 2-3 linkages selectively, an initial 10-fold dilution of this enzyme, using 1X G4 Reaction Buffer supplemented with 100 µg/ml BSA, is recommended.

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 α -Neu5Ac from 1 nmol of Neu5Ac α 2-3Gal β 1-3GlcNAcβ1-3Galβ1-4Glc-7-amino-4-methylcoumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 ul.

Unit Definition Assay: Two fold dilutions of α 2-3 Neuraminidase are incubated with 1 nmol AMC-labeled substrate and 1X G4 Reaction Buffer, supplemented with 100 µg/ml BSA, in a 10 ul reaction. The reaction mix is incubated for 1 hour at 37°C. Separation of reaction products are visualized via thin layer chromatography (3).

Specific Activity: ~11.300.000 units/mg.

Molecular Weight: 41,000 daltons.

Quality Assurance: No contaminating exoglycosidase or proteolytic activity could be detected.

Quality Controls

Glycosidase Assavs: 500 units of α 2-3 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.

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

β -N-Acetyl-glucosaminidase:

GICNACB1-4GICNACB1-4GICNAC-AMC ND

α -Fucosidase:

Fucα1-2Galβ1-4Glc-AMCGalβ1-4 (Fucα1-3)GicNAcβ1-3Galβ1-4Glc-AMC ND

β-Galactosidase:

Gal\u00e41-3GIcNAc\u00b41-4Gal\u00b41-4GIc-AMC ND α -Galactosidase:

 $Gal\alpha 1-3Gal\beta 1-4Gal\alpha 1-3Gal-AMC$

(See other side)

CERTIFICATE OF ANALYSIS

ND

$\alpha 2-3$

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α -Fucosidase:

Fucα1-2Galβ1-4Glc-AMCGalβ1-4 (Fucα1-3)GicNAcβ1-3Galβ1-4Glc-AMC ND

β-Galactosidase:

Gal\u00e41-3GIcNAc\u00b41-4Gal\u00b41-4GIc-AMC ND α -Galactosidase: ND

 $Gal\alpha 1-3Gal\beta 1-4Gal\alpha 1-3Gal-AMC$

(See other side)

CERTIFICATE OF ANALYSIS

$\alpha\text{-Mannosidase:}$ Man α 1-3Man β 1-4GlcNAc-AMC Man α 1-6Man α 1-6(Man α 1-3)Man-AMC	ND
$\beta \textbf{-Glucosidase:} \\ \text{Glc}\beta 1\text{-}4\text{Glc}\beta 1\text{-}4\text{Glc-AMC}$	ND
β -Xylosidase: Xyl $β$ 1-4Xyl $β$ 1-4X	ND
β -Mannosidase: Manβ1-4Manβ1-4Man-AMC	ND
Endo F ₁ , F ₂ , H : Dansylated invertase high mannose.	ND
Endo F₂, F₃: Dansylated fibrinogen biantennary.	ND

Fluoresceinated fetuin triantennary.

ND

ND

Protease Assay: After incubation of 500 units of α 2-3 Neuraminidase with 0.2 nmol of a standard mixture of proteins in a 20 μ I reaction, for 20 hours at 37°C, no proteolytic activity could be detected by SDS-PAGE.

Note: Store at 4°C or in small aliquots at –20°C. Avoid repeated freeze/thaw cycles.

References:

- 1. Hoyer, et al. (1991) *J. Biochem*. 110, 462–467.
- 2. Monks, B. New England Biolabs, Inc., unpublished results.
- 3. Wong-Madden, S.T. and Landry, D. (1995) *Glycobiology* 5, 19–28.

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PNGase F:

$\alpha\text{-Mannosidase:}$ Man α 1-3Man β 1-4GlcNAc-AMC Man α 1-6Man α 1-6(Man α 1-3)Man-AMC	ND
β-Glucosidase: Glc $β$ 1-4Glc $β$ 1-4Glc-AMC	ND
β -Xylosidase: Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC	ND
β -Mannosidase: Manβ1-4Manβ1-4Man-AMC	ND
Endo F ₁ , F ₂ , H : Dansylated invertase high mannose.	ND
Endo F₂, F₃: Dansylated fibrinogen biantennary.	ND

Fluoresceinated fetuin triantennary.

Protease Assay: After incubation of 500 units of α 2-3 Neuraminidase with 0.2 nmol of a standard mixture of proteins in a 20 μ I reaction, for 20 hours at 37°C, no proteolytic activity could be detected by SDS-PAGE.

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- 3. Wong-Madden, S.T. and Landry, D. (1995) Glycobiology 5, 19–28.

PNGase F: