α2-3,6,8 Neuraminidase

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

P0720S 015140816081

P0720S

2,000 units **50,000 U/ml** **Lot: 0151408**
RECOMBINANT **Store at –20°C** **Exp: 8/16**

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

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 α2-3,6,8 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: ~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 α2-3,6,8 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

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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 α2-3,6,8 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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