

α 2-3,6,8 Neuraminidase



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P0720S 015150117011

P0720S



2,000 units 50,000 U/ml Lot: 0151501
RECOMBINANT Store at -20°C Exp: 1/17

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

Specificity:

Neu5Ac α 2 \downarrow 3 R
 α 2 - 6 R
>> α 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 Na₂EDTA.

Reagents Supplied with Enzyme:
10X GlycoBuffer 1

Reaction Conditions:

1X GlycoBuffer 1:
50 mM Sodium Acetate (pH 5.5 @ 25°C)
and 5 mM CaCl₂. 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 Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc-7-amino-4-methyl-coumarin (AMC), in 5 minutes at 37°C in a total reaction volume of 10 μ l.

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

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

β -N-Acetyl-glucosaminidase:
GlcNAc β 1-4GlcNAc β 1-4GlcNAc-AMC ND

α -Fucosidase:
Fuc α 1-2Gal β 1-4Glc-AMC Gal β 1-4
(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc-AMC ND

β -Galactosidase:
Gal β 1-3GlcNAc β 1-4Gal β 1-4Glc-AMC ND

α -Galactosidase:
Gal α 1-3Gal β 1-4Gal α 1-3Gal-AMC ND

α -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:
Xyl β 1-4Xyl β 1-4Xyl β 1-4Xyl-AMC ND

(see other side)

CERTIFICATE OF ANALYSIS

α 2-3,6,8 Neuraminidase



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α -Fucosidase:
Fuc α 1-2Gal β 1-4Glc-AMC Gal β 1-4
(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc-AMC ND

β -Galactosidase:
Gal β 1-3GlcNAc β 1-4Gal β 1-4Glc-AMC ND

α -Galactosidase:
Gal α 1-3Gal β 1-4Gal α 1-3Gal-AMC ND

α -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:
Xyl β 1-4Xyl β 1-4Xyl β 1-4Xyl-AMC ND

(see other side)

CERTIFICATE OF ANALYSIS

β -Mannosidase:
Man β 1-4Man β 1-4Man-AMC ND

Endo F₁, F₂, H:
Dansylated invertase high mannose. ND

Endo F₂, F₃:
Dansylated fibrinogen biantennary. ND

PNGase F:
Fluoresceinated fetuin triantennary. ND

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

Note: This enzyme shows a preference for α 2,3 and α 2,6 linkages over α 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.



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