Neuraminidase

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.

Specificity:
Neu5Ac α 2 – 3 R
>> α 2 – 6 R
>> α 2 – 8 R

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Quality Controls
Glycosidase Assays: 500 units of α-2-3 Neuraminidase were incubated with 0.1 mM of fluorescently-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:
GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC ND

α-Fucosidase:
Fucx1-2Galβ1-4Glc-AMCGalβ1-4 (Fucx1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND

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

α-Galactosidase:
Galα1-3Galβ1-4Galα1-3Gal-AMC ND

(See other side)
α-Mannosidase:
Man\(\alpha\)-1-3Manβ1-4GlcNAc-AMC
Man\(\alpha\)-1-6Man\(\alpha\)-1-6(Man\(\alpha\)-1-3)Man-AMC  ND

β-Glucosidase:
Glcβ1-4Glcβ1-4Glc-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 F\(_3\), F\(_3\):
Dansylated fibrinogen biantennary. ND

PNGase F:
Fluoresceinated fetuin triantennary. ND

Protease Assay: After incubation of 500 units of α2-3 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: Store at 4°C or in small aliquots at −20°C. Avoid repeated freeze/thaw cycles.

References: