**α2-3 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 α2-3 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

**Quality Controls**

**Glycosidase Assays:** 500 units of α2-3 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:**
  - Fucx1-2Gaβ1-4Glc-AMCGalβ1-4 (Fucx1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND

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

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

(See other side)

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

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

(See other side)
α-Mannosidase:
Manα1→3Manβ1→4GlcNAc-AMC
Manα1→6Manα1→6(Manα1→3)Men-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 F1, F2, F3, H:
Dansylated invertase high mannose. ND

Endo F4, F5:
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: