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## Product Information

### NEURAMINIDASE

from *Clostridium perfringens*  
Sigma Prod. Nos. N2876, N2133, N2904,  
N3001, and N5631

**CAS NUMBER:** 9001-67-6

**E.C. NUMBER:** 3.2.1.18

### SYNONYMS:

Sialidase; receptor destroying enzyme; acylneuraminydase; Exo- $\alpha$ -sialidase

### KINETIC PARAMETERS:

Neuraminidase from *C. perfringens* is most active at pH 5.0-5.5; it is about 30-50% as active at pH 7.0 in phosphate buffer.<sup>1</sup>

The proteolytic activity in many neuraminidases was not inhibited by serum protease inhibitors, soybean trypsin inhibitor or DIFP using fibrinogen as a substrate. EDTA at 0.1 M completely inhibited gelatinase activity, but also inhibited 90% of neuraminidase activity on mucin.<sup>2</sup> However, a purified neuraminidase did not require calcium or other divalent metal ions for activity, and was not inhibited by EDTA.<sup>1</sup>

### PHYSICAL PROPERTIES / STRUCTURE:

The molecular weight has been determined a variety of ways, from SDS and acid gel PAGE, to gel filtration under denaturing conditions. Reported values ranged from 50,000 to 100,000, most often as 64,000-66,000 as a monomer.<sup>2,3,4</sup> Although the monomer mass appears to be about 50-60 kDa, neuraminidases tend to polymerize under certain conditions. This species seems to give an active dimer under acid conditions, but after brief exposure to pH 9.0, two active forms of MW 50 and 100 kDa were separated by gel filtration at pH 7.3.<sup>2</sup>

One reference reported the existence of 5 isoforms with pI values of 4.7, 4.9, 5.1, 5.4 and 5.6.<sup>3</sup> Another claimed only one isoform with pI 5.1.<sup>5</sup> Sigma has not tested the preparations.

### STORAGE / STABILITY AS SUPPLIED:

The freeze-dried solid is stable for at least three years stored at -20°C. The highly purified N2133 should be stable at least one year.

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**SOLUBILITY / SOLUTION STABILITY:**

These products dissolve in water containing 0.2% BSA (albumin) at 2 mg/mL, giving a clear tan or brown solution; solutions of N2133 are usually colorless. Neuraminidase is a comparatively stable enzyme in solution. When dissolved at 120 µg/mL in 10 mM phosphate buffer, pH 6, containing 25 to 150 mM KCl, the enzyme not only retained activity at 0-4°C for over 30 months but, actually increased in activity in storage. However, for an enzyme concentration of 2 µg/mL under the same conditions, activity was lost unless albumin (BSA) was added at 0.03%.<sup>1</sup> A sample at 0.01 unit/mL in 0.2 M phosphate buffer (pH 6.0) held at 57-58°C steadily lost activity, approximately 70% over three hours.<sup>2</sup>

**USAGE / GENERAL REMARKS:**

Neuraminidases are used to cleave N-acetyl neuraminic acid (sialic acid) from a variety of glycoproteins. The enzyme from *Clostridium perfringens* cleaves terminal sialic acid residues which are  $\alpha$ -2,3-  $\alpha$ -2,6- or  $\alpha$ -2,8-linked to Gal, GlcNac, GalNAc, AcNeu, GlcNeu, oligosaccharides, glycolipids or glycoproteins. The relative rate of cleavage decreases in the order:  $\alpha$ -2-3 >  $\alpha$ -2-6 .  $\alpha$ -2-8. Neuraminidase from *C. perfringens* cleaves  $\alpha$ -2-3 linked sialic acid residues most efficiently, compared to *A. ureafaciens*, (Sigma N3642) which preferentially cleaves  $\alpha$ -2-6 linked residues.<sup>6,7</sup>

A number of references have discussed the action of neuraminidases on gangliosides and sialoglycolipids.<sup>8,9</sup> Neuraminidase cleavage of sialic acid groups has been used to study recognition by antibodies of glycoprotein structures.<sup>10,11</sup> The use of neuraminidase in the estimation of N-acetylneuraminic acid was compared favorably to two other methods.<sup>12</sup>

The use of neuraminidase to remove sialic acid residues from glycoproteins on cell surfaces has been frequently reported. Generally, procedures have indicated using neuraminidase in PBS at 37°C for 30 minutes, followed by several washings with PBS.<sup>13-16</sup> Treatment of tissue sections with neuraminidase at much lower concentrations require longer incubation: for 1-4 U/mL in 0.1 M acetate buffer pH 4.2-5, from 2 to 20 hours at 37°C.<sup>17a</sup>

Some lectins (for example, *Arachis hypogaea*) do not agglutinate native red cells unless they are enzymatically treated with neuraminidase.<sup>17b</sup>

T lymphocytes will bind to and form rosettes with ovine erythrocytes; the rosette formation occurs best with cells which have been treated with neuraminidase. The rosettes can then be separated from B cells by density gradient centrifugation.<sup>17c</sup>

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**USAGE / GENERAL REMARKS:** (continued)

A chromogenic substrate, BCI- $\alpha$ -D-N-acetylneuraminic acid (Sigma product number B4666), was developed to facilitate the screening of bacterial colonies or plaques for detecting either natural or mutant neuraminidase. It had a  $K_m = 8.9 \times 10^{-4}$  M and was stable under assay conditions.<sup>18</sup>

Neuraminidase from *Clostridium perfringens* has different properties from the enzyme from other species. For example, it does not require divalent metals for activation, in contrast to the enzyme from *Vibrio cholerae*.<sup>1</sup> Cleavage specificity does differ and pH optima may also differ.

**METHOD OF PREPARATION:**

The five products from *Clostridium perfringens* differ in specific activity due to different purification methods.

N2876 - prepared by ammonium sulfate fractionation, dialyzed against water, nearly salt-free. May contain protease and NAN-aldose activities. Activity: 0.5-6.0 units/mg solid

N2904 - the same product as N2876, but sterilized by gamma-irradiation. (No longer available)

N3001 - chromatographically purified from N2876, dialyzed against water. May contain protease and NAN-aldose. Protein >95% (Biuret). Activity: 6-10 units/mg solid.

N5631 - similar to N3001, but protein approx. 90% (Biuret). May contain protease and NAN-aldose. Activity: 10-20 units/mg solid.

N2133 - purified by affinity chromatography using p-aminophenyl oxamic acid agarose (Sigma product A3765).<sup>6,19</sup> Protein content approximately 85% (Biuret). Activity: 150-400 units/mg protein. Lot-specific values for protease (using casein as substrate) and N-acetylneuraminic acid aldolase activity are reported.<sup>1</sup>

An ion-exchange method for removing protease activity from neuraminidase has been described.<sup>2</sup>

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**UNIT DEFINITION:**

One unit will liberate 1.0  $\mu$ mole of N-acetylneuraminic acid per minute at pH 5.0 at 37°C using NAN-lactose or bovine submaxillary mucin. The products are packaged in terms of NAN-lactose units.<sup>20</sup> The assay procedures are available on request from Technical Service and are posted on Sigma's Internet website.

**REFERENCES:**

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