

## Product Information

### **$\alpha(2\rightarrow3,6,8,9)$ Neuraminidase, Proteomics Grade from *Arthrobacter ureafaciens* Complete with 5x Reaction Buffer**

Product Code **N 3786**

Storage Temperature 2 – 8 °C

## TECHNICAL BULLETIN

### **Product Description**

One of the distinguishing features of the proteome in eukaryotic cells is that most proteins are subject to post-translational modification, of which glycosylation is the most common form. It is estimated that more than half of all proteins are glycoproteins. Two major classes of oligosaccharides (glycans) may be attached to proteins. N-linked glycans may be attached to the amide side chain of Asn residues, which form part of the amino acid triplet AsnXaaSer/Thr, where Xaa is any amino acid except Pro. O-linked glycans may be added to the hydroxyl side chain of Ser or Thr residues. The terminal residues on these carbohydrate chains are commonly N-acetylneuraminic acids (sialic acids).

Neuraminidase can be used directly on intact glycoproteins as a gentle means of removing sialic acid groups for quantification, while leaving the glycan chain attached to the protein. More detailed structural analysis of these glycan chains can be performed by a number of methods.<sup>1,2</sup> After release of the whole glycan from the protein, by either chemical or enzymatic means, an essential first step is often the removal of all sialic acid residues. This is followed by treatment with highly specific endo and/or exoglycosidases to determine sequence and structure.

Proteomics Grade Neuraminidase is a highly purified enzyme from *Arthrobacter ureafaciens* that releases  $\alpha(2\rightarrow3)$ ,  $\alpha(2\rightarrow6)$ ,  $\alpha(2\rightarrow8)$ , and  $\alpha(2\rightarrow9)$  linked sialic acids. The relative rates of cleavage are reported to be  $\alpha(2\rightarrow6) > \alpha(2\rightarrow3) > \alpha(2\rightarrow8)$  and  $\alpha(2\rightarrow9)$ ;<sup>3</sup> however, these rates make little practical difference as sufficient enzyme is used to ensure cleavage of all sialic acid residues. This wide spectrum of activity makes it ideal for complete non-specific removal of sialic acid groups prior to analysis.

The enzyme is lyophilized from 10 mM sodium/potassium phosphate buffer without any added stabilizers. The low levels of buffer salts make it compatible with subsequent analysis by MALDI-TOF MS or HPLC.

The enzyme has the following properties:<sup>3,4</sup>

- The enzyme consists of three active species with molecular weights of approximately 52, 66, and 88 kDa.
- Optimal pH range is 4.5 – 5.5.
- The enzyme is stable in the pH range of 4.5 – 9.5.
- Unlike some other sialidases the *Arthrobacter* enzyme does not require calcium for activity and is not inhibited by EDTA or thiol inhibitors such as iodoacetate or p-chloromercuribenzoate.

The enzyme is tested and the absence of contaminating exo and endoglycosidase activities is confirmed. Protease activity is not detected after incubation of 5 Sigma units of the enzyme with denatured BSA at 37 °C for 24 hours.

### **Components**

This product (Product Code N 3786) includes lyophilized, Proteomics Grade neuraminidase and a reaction buffer that may be used for the convenient desialylation of glycoproteins or glycans. There is sufficient enzyme and buffer to desialylate a minimum of 10 glycoprotein samples (approximately 200  $\mu\text{g}$  each), following the described procedure using fetuin as a model glycoprotein.

5x Reaction Buffer (Product Code N 3536) – Buffer to provide suitable pH for the neuraminidase desialylation reaction. One vial of 5x concentrate (1.5 ml) that reconstitutes to a final volume of 7.5 ml.

$\alpha(2\rightarrow3,6,8,9)$  Neuraminidase, Proteomics Grade (Product Code N 3286) – The lyophilized enzyme is supplied in a vial containing 25 Sigma units of neuraminidase.

**Unit Definition:** One Sigma unit is the amount of activity that will release 1 nmole of 4-methylumbelliferone from 2-(4-methylumbelliferyl)  $\alpha$ -D-N-acetylneuraminic acid per minute at pH 5.5 at 37° C. One Sigma unit is equivalent to a standard International milliunit (mIU).

#### Reagents and Equipment Required But Not Provided

- Fetuin from calf serum (Product Code F 3004)
- Ultrapure water
- Siliconized Eppendorf® tubes (Product Code T 4691)
- 37 °C heating block or heating bath
- 100 °C heating block
- Bench-top centrifuge (microcentrifuge)
- Micropipettes
- SDS-PAGE apparatus
- 7% or 10% homogenous SDS-PAGE gels
- SDS-PAGE Molecular Weight Markers (Product Code M 3788)
- 2x SDS Sample Buffer (Product Code S 3401)
- Coomassie® Protein Stain (Product Code B 8647)
- SDS-PAGE gel destain solution

#### Precautions and Disclaimer

This product is for laboratory use only, not for drug, household, or other uses. Consult the MSDS for information regarding hazards and safe handling practices. It is recommended to read the entire technical bulletin prior to starting the procedure.

#### Preparation Instructions

Before opening either the enzyme or the buffer vial, centrifuge each briefly to ensure the contents are at the base of the tube.

- Neuraminidase Enzyme Solution – Reconstitute the enzyme in an appropriate volume of ultrapure water, depending on the application. In general it is suggested to prepare a 500 Sigma unit/ml solution. To do this, add 50  $\mu$ l of water to the 25 Sigma unit vial, agitate the contents gently, and briefly centrifuge the vial. Place the vial on ice for 5 minutes, mix the contents once more, and finally centrifuge briefly once again. For a solution with higher enzyme activity dissolve the contents of the vial in a smaller volume of water.

- 1x Neuraminidase Reaction Buffer – Dilute aliquots from the tube 5-fold with ultrapure water to give a solution of 50 mM sodium phosphate, pH 6.0, as required.

#### Storage/Stability

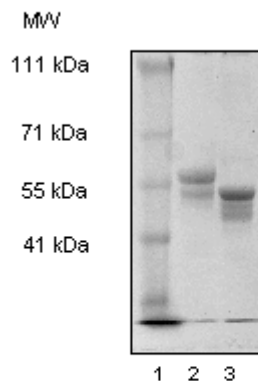
Both the enzyme and buffer are stable for 1 year if stored unopened at 2 – 8 °C. Once reconstituted the enzyme solution should be stored at 2 – 8 °C and used within 7 days. The diluted buffer should be stored at 2 – 8 °C and used within 7 days.

#### Procedure

This procedure efficiently desialylates a standard glycoprotein, calf serum fetuin. The desialylation is monitored by increased mobility of the fetuin on SDS-PAGE.

1. Prepare a fetuin control solution and a test sample (1.7 mg/ml) in 1x Reaction Buffer.
2. Add 100  $\mu$ l of the fetuin control solution to an Eppendorf tube marked control and add 100  $\mu$ l of the test sample to an Eppendorf tube marked test.
3. Add 4  $\mu$ l (2 Sigma units) of prepared Neuraminidase Enzyme Solution to the test sample and 4  $\mu$ l of the prepared 1x Neuraminidase Reaction Buffer to the control tube.
4. Cap the tubes and incubate each sample at 37 °C for 3 hours.
5. Stop each reaction by heating for 5 minutes at 100 °C.
6. Allow each sample to cool and centrifuge briefly to ensure all liquid is at the base of the tube.
7. Remove a suitable aliquot from each tube. To this aliquot, add an equal volume of 2x SDS Sample Buffer and heat for 5 minutes at 100 °C.
8. Analyze the sample by SDS-PAGE and determine the mobility of the fetuin bands against a suitable set of molecular weight markers (Figure 1). The desialylated fetuin sample in Lane 3 migrates further down the gel than the untreated control sample in Lane 2.

**Figure 1.**  
Analysis of the fetuin control and desialylated test sample on 7% Tris-Acetate SDS PAGE



Lane 1 MW Standards  
Lane 2 Fetuin Control  
Lane 3 Fetuin treated with neuraminidase

Related Products	Product Code
ProteoPrep™ Reduction and Alkylation Kit	PROT-RA
ProteoSilver™ Plus Silver Staining Kit	PROT-SIL2
ProteoMass™ MALDI-MS Calibration Kits	MS-CAL
ProteoProfile™ Trypsin In-Gel Digest Kit	PP0100
ProteoProfile Enzymatic In-Gel Deglycosylation Kit	PP0200
PNGase F, Proteomics Grade	P 7367
EZBlue™ Gel Staining Reagent	G 1041
2-(4-methylumbelliferyl) $\alpha$ -D-N-acetylneuraminic acid	M 8639

#### References

1. Rudd, P.M. et al., *Nature*, **388**, 205-207 (1977).
2. Fukuda, M., and Kobata, A., eds., *Glycobiology: A Practical Approach*, IRL Press (Oxford, UK: 1993), pp. 165-242.
3. Uchida, Y. et al., *J. Biochem.*, **86**, 1573-1585 (1979).
4. Ohta, Y. et al., *J. Biochem.*, **106**, 1086-1089 (1989).

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