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Product Information

α(2→3,6,8,9) Neuraminidase, Proteomics Grade from *Arthrobacter ureafaciens*

Catalog Number **N3786**
Storage Temperature 2–8 °C

CAS RN 9001-67-6
EC 3.2.1.18
Synonyms: Acyl-neuraminyl Hydrolase, Sialidase, N-Acetylneuraminidase, N-Acetylneuraminate glycohydrolase

Product Description

One distinguishing feature of the proteome in eukaryotic cells is that most proteins are subject to post-translational modification (PTM): Glycosylation is the most common PTM. 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-acylneuraminic 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.[1,2] 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 of the remaining glycan chains 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 α(2→3)-, α(2→6)-, α(2→8)-, and α(2→9)-linked sialic acids. The relative rates of cleavage have been reported as follows:[3]

α(2→6) > α(2→3) > α(2→8) and α(2→9)

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:[3,4]

- It consists of three active species with molecular masses 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, nor by thiol inhibitors such as iodoacetate or *p*-chloromercuribenzoate.

This enzyme product is tested for the presence of other enzyme activities. Contaminating exo and endoglycosidase activities are not detected. 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 (Catalog Number N3786) 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 (~200 µg each), following the described procedure using fetuin as a model glycoprotein.

5× Reaction Buffer (Catalog Number N3536): Buffer to provide suitable pH for the neuraminidase desialylation reaction. One vial of 5× concentrate (1.5 ml) reconstitutes to a final volume of 7.5 ml.

α(2→3,6,8,9) Neuraminidase, Proteomics Grade (Catalog Number N3286): 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)-α-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 (Catalog Number F3004)
- Ultrapure water
- Clear-view™ Snap-Cap microtubes (Catalog Number T4691)
- 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 (Catalog Numbers M3913, S8445)
- 2× Laemmli Sample Buffer Concentrate (Catalog Number S3401)
- Brilliant Blue R Concentrate (Catalog Number B8647)
- SDS-PAGE gel destain solution

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 µl 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.
- 1× 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.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

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.

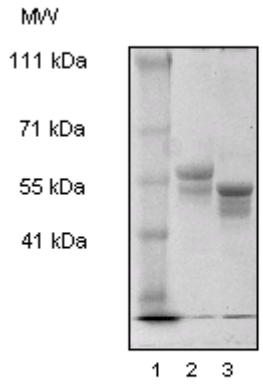
Procedure

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 1× Reaction Buffer.
2. Add 100 µl of the fetuin control solution to a microtube marked “Control”. Add 100 µl of the test sample to a microtube marked “Test”.
3. Add 4 µl (2 Sigma units) of prepared Neuraminidase Enzyme Solution to the Test sample and 4 µl of the prepared 1× 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 2× Laemmli Sample Buffer Concentrate and heat for 5 minutes at 100 °C.

Results
Analyze the sample by SDS-PAGE. 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" (Lane 2) and desialylated "Test" (Lane 3) sample on 7% Tris-Acetate SDS PAGE:



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

Related Products	Catalog Number
ProteoPrep™ Reduction and Alkylation Kit	PROTRA
ProteoSilver™ Plus Silver Staining Kit	PROTSIL2
ProteoMass™ MALDI-MS Calibration Kits	MSCAL1, MSCAL2, MSCAL3
ProteoProfile™ Trypsin In-Gel Digest Kit	PP0100
ProteoProfile Enzymatic In-Gel Deglycosylation Kit	PP0200
PNGase F, Proteomics Grade	P7367
EZBlue™ Gel Staining Reagent	G1041
2′-(4-methylumbelliferyl)-α-D-N-acetyl-neuraminic acid (sodium salt)	M8639

References
1. Rudd, P.M., *et al.*, *Nature*, **388(6638)**, 205-207 (1997).
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(5)**, 1573-1585 (1979).
4. Ohta, Y., *et al.*, *J. Biochem.*, **106**, 1086-1089 (1989).

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