NANase II

Instruction Manual

Catalog Number
170-6882

BIO-RAD

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4006093 Rev A

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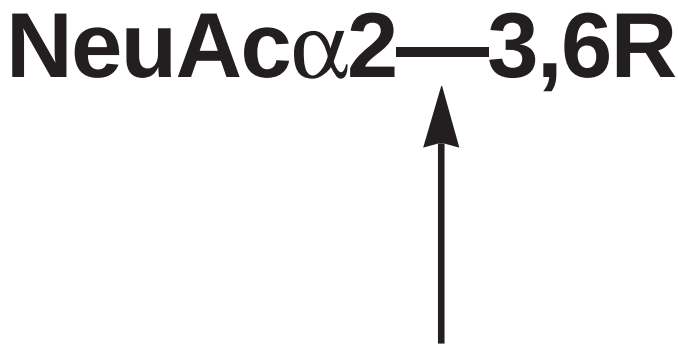
Section 1
Introduction

NANase II is an *E. coli* expressed recombinant ß2–3,6 neuraminidase. It will remove all terminal ß2-3,6 N-acetylneuraminic acid from complex carbohydrates. Reaction buffer and oligosaccharide and glycoprotein protocols are provided.



R = oligosaccharide

Section 2
Kit Components and Specifications

Component	NANase II (MW=41 kD)	5x Reaction Buffer 6
Specificity	α2–3,6 linked N-acetylneuraminic acid from complex oligosaccharicdes	N/A
Concentration	5 U*/ml (in 20 mM Tris pH 7.5, 25 mM NaCl)	250 mM sodium phosphate, pH 6.0
Volume	40 µl	200 µl
Storage	4 °C	4 °C
Shelf Life	9 months	1 year

* One unit (U) is defined as the amount of enzyme required to catalyze the release of 1 µmole of p-nitrophenol from p-nitrophenyl-N-acetyl-α-D-neuraminic acid per minute at 37 °C, pH 5.0.

Section 3
Protocol

3.1 Oligosaccharide Protocol

1. Prepare oligosaccharide sample (up to 1 nanomole).

   Dried Sample: Resuspend 1 nanomole of oligosaccharide in 14 µl of distilled water. Add 4 µl of 5x Reaction Buffer, pH 6.

   Liquid Sample: Dilute 14 µl of oligosaccharide solution (containing up to 1 nanomole) with 4 µl of 5x Reaction Buffer, pH 6.

2. Add 2 µl NANase II to the reaction vial. Total reaction volume is 20 µl.
3. Incubate at 37 °C for 1 hour.
4. To test for completion of the desialylation reaction compare the mobility of the oligosaccharide with and without NANase II digestion. Use a N- or O-Linked Oligosaccharide Profiling Kit (catalog numbers 170-6501 or 170-6815) to analyze oligosaccharide mobility shifts.

Note: To cleave more than one nanomole of substrate increase reaction volume, enzyme quantity, and incubation time proportionately.

3.2 Glycoprotein Protocol

1. Isolate glycoprotein and dilute as follows:

   Dried Sample: Resuspend up to 100 µg of glycoprotein in 15 µl of distilled water. Add 4 µl of 5x Reaction Buffer, pH 6.

   Liquid Sample: Dilute 15 µl of oligosaccharide solution (containing up to 100 µg) with 4 µl of 5x Reaction Buffer, pH 6.

2. Add 1.0 µl of NANase II.
3. Incubate at 37 °C for 1 hour.
4. Run the treated and untreated glycoprotein in separate lanes in a SDS-PAGE gel. Desialylated glycoproteins will exhibit an increase in mobility due to the reduction in molecular weight. Alternatively perform the sialic acid detection protocol with the Immun-Blot® Kit for glycoprotein detection (catalog number 170-6490) to check for completion of the desialylation reaction.

Section 4
Product Information

Catalog Number	Product Description
	Carbohydrate Analysis Kits
170-6490	**Immun-Blot Kit for Glycoprotein Detection**
170-6500	**Enzymatic Deglycosylation Kit**
170-6508	**Deglycosylation Enhancement Kit**
170-6513	**GALase III,** 1.5 U/ml, 0.04 ml
170-6880	**HEXase I,** 42 U/ml, 0.04 ml
170-6881	**O-Glycosidase DS,** 1 U/ml, 0.04 ml
170-6882	**NANase II**, 5 U/ml, 0.04 ml
170-6883	**PNGase F,** 2.5 U/ml, 0.04 ml
170-6501	**N-Linked Oligosaccharide Profiling Kit**
170-6510	**N-Linked Oligosaccharide Sequencing Kit**
170-6502	**N-Linked Oligosaccharide Gel Refill**, 6
170-6514	**N-Linked Oligosaccharide Gel and Buffer Refill**
170-6815	**O-Linked Oligosaccharide Profiling Kit**
170-6816	**O-Linked Oligosaccharide Gel and Buffer Refill Pack**
170-6817	**O-Linked Oligosaccharide Gel Refill Pack**

Catalog Number	Product Description
170-6503	**Oligosaccharide Electrophoresis Buffer Refill**
170-6811	**Monosaccharide Compositional Analysis Kit**
170-6812	**Monosaccharide Gel and Buffer Refill Pack**
170-6813	**Monosaccharide Gel Refill Pack**
170-6814	**Monosaccharide Buffer Refill Pack**
Carbohydrate Analysis Instruments	
170-6555	**Glyco Doc™ Imager**, 100/120 V
170-6557	**Glyco Doc Imaging System**, 100/120 V
170-6559	**Glyco Doc Analytical Software**

Section 5
Technical Support

If you require additional technical assistance contact your local Bio-Rad representative or in the US dial 1-800 4BIORAD.