



NANase II

Instruction Manual

Catalog Number
170-6882

BIO-RAD

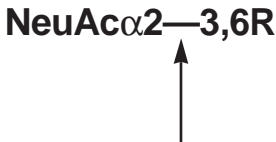
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Section 1

Introduction

NANase II is an *E. coli* expressed recombinant β 2-3,6 neuraminidase. It will remove all terminal β 2-3,6 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	α 2-3,6 linked N-acetylneuraminic acid from complex oligosaccharides	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 μ l	200 μ l
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 μ mole 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 μ l of distilled water. Add 4 μ l of 5x Reaction Buffer, pH 6.
Liquid Sample: Dilute 14 μ l of oligosaccharide solution (containing up to 1 nanomole) with 4 μ l of 5x Reaction Buffer, pH 6.
2. Add 2 μ l NANase II to the reaction vial. Total reaction volume is 20 μ l.
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
<i>Carbohydrate Analysis Kits</i>	
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
<i>Carbohydrate Analysis Instruments</i>	
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.