



PNGase F

Instruction Manual

Catalog Number
170-6883

BIO-RAD

Table of Contents

Section 1	Introduction	1
Section 2	Kit Components and Specifications	2
Section 3	Protocols	4
3.1	Denaturing Protocol	4
3.2	Non-Denaturing Protocol.....	5
Section 4	Product Information.....	7
Section 5	Technical Assistance.....	8

Section 1

Introduction

PNGase F is a recombinant endoglycosidase that cleaves the GlcNAc-Asn linkage of N-linked oligosaccharides in glycoproteins and glycoproteins. A reaction buffer and two protocols are provided.



R = oligosaccharide

Section 2

Kit Components and Specifications

Component	Specificity	Concentration	Volume	Storage	Shelf Life
PNGase F (MW=36 kD)	All Asn-linked oligosaccharides	2.5 U*/ml (in 20 mM Tris pH 7.5, 50 mM NaCl 1 mM EDTA)	40 µl	4 °C	9 months
2x Reaction Buffer, pH 7.5	N/A	100 mM sodium phosphate, pH 7.5	200 µl	4 °C	1 year
Denaturing Solution	N/A	2% SDS, 1 M β-Mercaptoethanol	200 µl	RT	1 year
NP-40	N/A	15%	100 µl	RT	1 year

RT = Room Temperature

* One unit (U) is defined as the amount of enzyme required to catalyze the release of N-linked oligosaccharides from 60 µmoles of denatured ribonuclease B in 1 hour at 37 °C, pH 7.5.

Section 3 Protocols

Two protocols are provided because some proteins require denaturation prior to PNGase F digestion. The choice of protocols depends on the glycoprotein. Initially, carry out both protocols with your protein. If denaturation releases more oligosaccharides than without, use the denaturing protocol for all subsequent studies.

3.1 Denaturing Protocol

Isolate the glycoprotein and dilute it as follows:

- Dried Sample: Resuspend up to 500 μg of glycoprotein in 18 μl of distilled water. Add 25 μl of 2x Reaction Buffer, pH 7.5.
- Liquid Sample: Dilute 18 μl of oligosaccharide solution (containing up to 500 μg) with 25 μl of 2x Reaction Buffer, pH 7.5.

1. Add 2.5 μl of Denaturing Solution.
2. Heat at 100 $^{\circ}\text{C}$ for 5 minutes then cool on ice.
3. Add 2.5 μl of NP-40 and mix.
4. Add 2.0 μl of PNGase F.
5. Incubate at 37 $^{\circ}\text{C}$ for 2 hours.
6. Run the treated and untreated glycoprotein in separate lanes in a SDS-PAGE gel. Deglycosylated proteins will exhibit an increase in mobility due to the reduction in molecular weight.

3.2 Non-denaturing Protocol

Isolate the glycoprotein and dilute it as follows:

- Dried Sample: Resuspend up to 500 μg of glycoprotein in 25 μl of distilled water. Add 25 μl of 2x Reaction Buffer, pH 7.5.
- Liquid Sample: Dilute 25 μl of oligosaccharide solution (containing up to 500 μg) with 25 μl of 2x Reaction Buffer, pH 7.5.
1. Add 2.0 μl of PNGase F.
 2. Incubate at 37 $^{\circ}\text{C}$ for 24 hours.

3. Run the treated and untreated glycoprotein in separate lanes in a SDS-PAGE gel. Deglycosylated proteins will exhibit an increase in mobility due to the reduction in molecular weight.

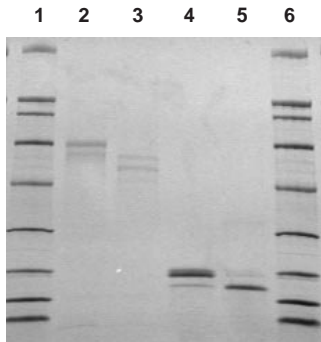


Fig. 1. Bovine fetuin and Ribonuclease B were digested with PNGase F, run in a 12 % Ready Gel and stained with Coomassie® Blue. Lanes 1 and 6: SDS-PAGE Broad Range Standards; Lane 2: Bovine fetuin; Lane 3: Bovine fetuin digested with PNGase F; Lane 4: Ribonuclease B; Lane 5: Ribonuclease B digested with PNGase F.

Section 4 Product Information

Catalog Number	Product Description
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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
<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.

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