Enzymatic Deglycosylation Kit

Instruction Manual

Catalog Number
170-6500

BIO-RAD

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Section 1
Introduction

The Enzymatic Deglycosylation kit enzymatically cleaves all N and most O-linked oligosaccharides from glycoproteins or glycopeptides. PNGase F removes all Asn-linked oligosaccharides while the combination of NANase II and O-Glycosidase DS removes all Ser/Thr linked Gal(β1,3)GalNAc(α1) and all sialic acid substituted Gal(β1,3)LGalNAc(α1). Modifications of the Ser/Thr linked Gal(β1,3)GalNAc core other than by neuraminic acid (such as galactose, GlcNAc or fucose substitutions) will inhibit O-Glycosidase cleavage. To insure complete deglycosylation of such modified cores, the use of additional enzymes may be required.

Section 2
Kit Components and Specifications

Component	Specificity	Concentration	Volume	Storage
NANase II	Releases α2-3 and α2-6 linked N-acetylneuraminic acid from complex oligosaccharides	10 U/ml (in 20 mM Tris-HCl pH 7.5, 25 mM NaCl)	40 µl	4 °C
O-Glycosidase DS	Releases unsubstituted Gal(β1,3)GalNAc(α1) disaccharide attached to serine or threonine.	1 U/ml (in 20 mM Tris-HCl pH 7.5, 25 mM NaCl)	40 µl	4 °C
PNGase F	Releases all Asn-linked oligosaccharides from glycoproteins.	2.5 U/ml (in 20 mM Tris-HCl pH 7.5, 50 mM NaCl, 1 mM EDTA)	40 µl	4 °C
Bovine Fetuin Control	N/A	500 µg	N/A	4 °C
5x Reaction Buffer	N/A	250 mM sodium phosphate, pH 6.0	200 µl	RT
pH Adjustment Buffer	N/A	0.5 M sodium phosphate dibasic	200 µl	RT
Denaturing Solution	N/A	2% SDS, 1 M β-Mercaptoethanol	200 µl	RT
NP-40	N/A	>99 %	100 µl	RT

RT = Room Temperature

Section 3
Protocols

Two deglycosylation protocols are provided: one with and one without a denaturing step. The choice of protocols depends on the glycoprotein. Some proteins require denaturation prior to PNGase F digestion. Initially, carry out both protocols with your protein. If denaturation releases more oligosaccharides then without, use the denaturing protocol for all subsequent studies.

3.1 Sample Preparation

Isolate glycoprotein according to your usual procedures.

Solid Sample: Dissolve up to 100 µg glycoprotein in 12 µl of distilled water. Add 4 µl of 5x Reaction Buffer.

Liquid Sample: Add 4 µl of 5x Reaction Buffer to 12 µl of glycoprotein solution (up to 100 µg glycoprotein).

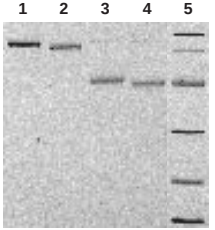
3.2 Non-Denaturing Protocol

1. Add 2 µl of NANase II to the reaction vial.
2. Add 2 µl of O-Glycosidase to the reaction vial.
3. Incubate at 37 °C for 1 hour.
4. Add 10 µl of distilled water and 10 µl of pH Adjustment Buffer.
5. Add 2.0 µl of PNGase F to the reaction vial.
6. Incubate at 37 °C for 24 hours.
7. Check the efficiency of the deglycosylation reaction by running samples (before and after deglycosylation) on a SDS-PAGE gel. Stain with Coomassie Blue or Silver Stain to observe the shift in mobility of your sample. Alternatively, use the Immun-Blot® Kit for Glycoprotein Detection to determine the deglycosylation efficiency.

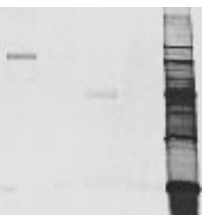
3.3 Denaturing Protocol

1. Add 2 µl of NANase II to the reaction vial.
2. Add 2 µl of O-Glycosidase to the reaction vial.
3. Incubate at 37 °C for 1 hour.

4. Add 10 µl of deionized water and 10 µl of pH Adjustment Buffer.
5. Add 2.5 µl of Denaturing Solution and heat for 5 minutes at 100 °C.
6. Cool on ice for 5 minutes.
7. Add 2.5 µl of NP-40 to the reaction vial and mix.
8. Add 2.0 µl of PNGase F to the reaction vial.
9. Incubate at 37 °C for 3 hours.
10. Check the efficiency of the deglycosylation reaction by running samples (before and after deglycosylation) on a SDS-PAGE gel. Stain with Coomassie Blue or Silver Stain to observe the shift in mobility of your sample. Alternatively, use the Immun-Blot Kit for Glycoprotein Detection to determine the deglycosylation efficiency.



A. 12 % Ready Gel stained with Coomassie® blue. Mobility shift indicates proteins were deglycosylated.



B. A parallel gel blotted onto nitrocellulose. Glycosylation determined with the Immun-Blot Kit for Glycoprotein Detection. Deglycosylated proteins do not react and are not detected.

Fig. 1. Human transferrin and Ovalbumin were deglycosylated with the Enzymatic Deglycosylation Kit and detected with the Immun-Blot Kit for Glycoprotein Detection. Lane 1: Human transferrin; Lane 2: Deglycosylated Human transferrin; Lane 3: Ovalbumin; Lane 4: Deglycosylated Ovalbumin; Lane 5: Biotinylated SDS-PAGE Standards, low range.

3.4 Experimental Control

Bovine Fetuin is included in the kit for use as a positive control. Use this control to verify that the deglycosylation reaction was carried out correctly. Dilute the Bovine Fetuin Control (500 µg) in 50 µl of distilled water to yield a 10 mg/ml working solution and perform the deglycosylation reaction protocol. Store reconstituted control at -20 °C.

Section 4
Product Information

Catalog Number	Product Description
170-6500	**Enzymatic Deglycosylation Kit**
170-6490	**Immun-Blot Kit for Glycoprotein Detection**
170-6501	**N-Linked Oligosaccharide Profiling Kit**
170-6510	**N-Linked Oligosaccharide Sequencing Kit**

Section 5
Technical Support

If you require additional technical assistance contact your local Bio-Rad representative or in the U.S. dial 1-800-4BIORAD and press 2 for the technical service department.