Deglycosylation Enhancement Kit

Instruction Manual

Catalog Number 170-6508

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4006087 Rev A

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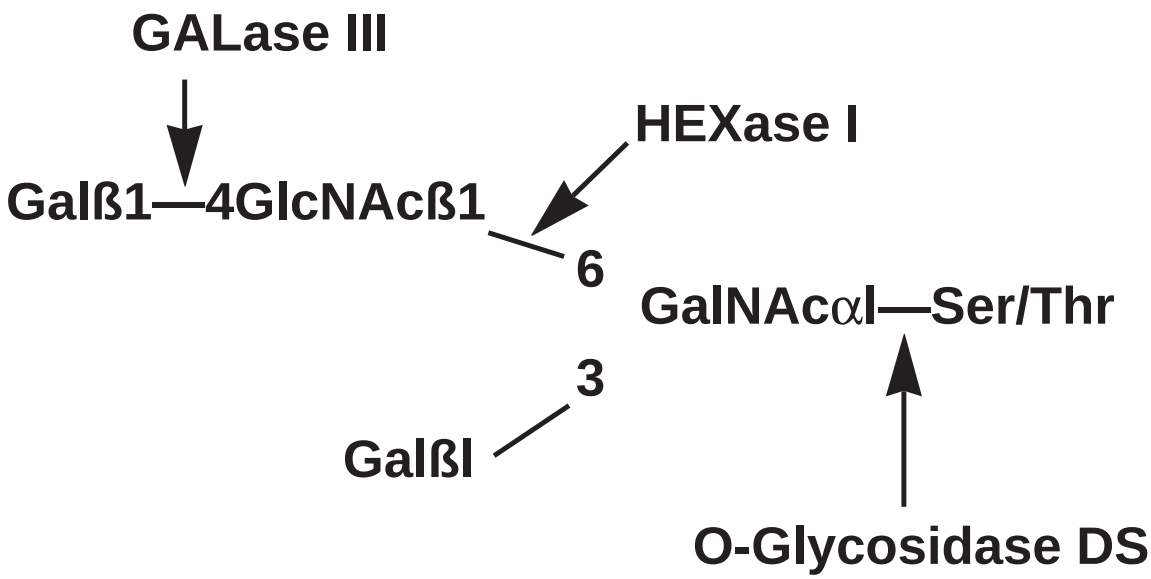
Section 1
Introduction

The Deglycosylation Enhancement Kit (catalog number 170-6508) enhances deglycosylation by the additional release of ß-galactose and ß-GlcNAc modified cores. The kit includes HEXase I which releases non-reducing terminal ß-linked N-acetyl-glucosamine and GALase III which releases non-reducing terminal (ß1,4) linked galactose. These enzymes together with the enzymes provided in the Enzymatic Deglycosylation Kit (170-6500) will release Gal(ß1,3)GalNAc(α1) with ß-galactose or ß-GlcNAc substitutions. For example:



Additional modifications such as α-fucose or α-galactose to these structures require additional enzymes for complete O-linked deglycosylation. To insure complete deglycosylation of such modified cores, the use of additional enzymes may be required.

Note: The Deglycosylation Enhancement Kit protocol differs from the one contained in the Enzymatic Deglycosylation Kit. To use the Deglycosylation Enhancement Kit effectively, follow the deglycosylation protocol contained in this instruction manual.

Section 2
Kit Components and Specifications

	HEXase I	GALase III	2x Reaction Buffer pH 7.5
Specificity	Non-reducing terminal ß-linked N-acetyl-glucosamine	Non-reducing terminal (ß1,4) linked galactose	N/A
Concentration	8 U/ml	1.5 U/ml	100 mM sodium phosphate, pH 7.5
Volume	40 µl	40 µl	
Storage	4 °C	4 °C	4 °C

Section 3
Protocols

The HEXase I and GALase III supplied in this kit will degrade N-linked oligosaccharides present in your sample. To minimize N-linked oligosaccharide degradation, release N-linked oligosaccharides with PNGase F prior to O-linked oligosaccharides release.

Two deglycosylation protocols are provided. One protocol includes a denaturing step and the other omits the denaturing step. Some glycoproteins require denaturation prior to PNGase F digestion. Denaturation of these glycoproteins with SDS/ß-mercaptoethanol will increase the efficiency of N-linked oligosaccharide release 10 fold. The choice of protocols depends on the glycoprotein. Carry out both protocols with your protein. If denaturation releases more oligosaccharides than without, use the denaturing protocol for all subsequent studies.

3.1 Sample Preparation

Isolate glycoprotein according to usual procedures.

Solid Sample:	Dissolve up to 100 µg of glycoprotein in 20 µl of distilled water. Add 25 µl of 2x Reaction Buffer, pH 7.5.
Liquid Sample:	Dilute 20 µl of glycoprotein solution (containing up to 100 µg) with 25 µl of 2x Reaction Buffer, pH 7.5.

3.2 Non-Denaturing Protocol

1. Aliquot 45 µl of prepared sample (Section 3.1).
2. Add 2 µl of PNGase F and incubate for 24 hours at 37 °C.
3. Add 3 volumes of cold ethanol. Chill on ice for 5 minutes.
4. Centrifuge the sample to pellet the glycoprotein and decant the supernatant.
5. Wash the pellet with cold 70% ethanol and decant the supernatant.

6. Dry the pellet in a centrifugal vacuum evaporator.
7. Add 30 µl of distilled water to re-suspend the glycoprotein pellet.
8. Add 10 µl of 5x Reaction Buffer 6.
9. Add 2 µl of each:

   NANase II

   GALase III

   HEXase I

   O-Glycosidase DS
10. Incubate for 2 hours at 37 °C.
11. 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. Alternatively use the Immun-Blot® Kit for glycoprotein detection (catalog number 170-6490) to determine the efficiency of the deglycosylation reaction.

3.3 Denaturing Protocol

1. Aliquot 45 µl of prepared sample (Section 3.1).

2. Add 2.5 µl of Denaturing Solution to sample. Mix gently and heat at 100 °C for 5 minutes.
3. Chill on ice and add 2.5 µl of NP-40 solution.
4. Add 2 µl of PNGase F and incubate for 3 hours at 37 °C.
5. Add 3 volumes of cold ethanol. Chill on ice for 5 minutes.
6. Centrifuge the sample to pellet the glycoprotein and decant the supernatant.
7. Wash the pellet with cold 70% ethanol and decant supernatant.
8. Dry the pellet in a centrifugal vacuum evaporator..
9. Add 30 µl of distilled water to re-suspend the glycoprotein pellet.
10. Add 10 µl of 5x Reaction Buffer.
11. Add 2 µl of each:

    NANase II

    GALase III

    HEXase I

    O-Glycosidase DS
12. Incubate for 2 hours at 37 °C.

13. 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. Alternatively use the Immun-Blot® kit for glycoprotein detection (catalog number 170-6490) to determine if deglycosylation is complete.

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

Catalog Number	Product Description
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**
170-6503	**Oligosaccharide Electrophoresis Buffer Refill**
170-6811	**Monosaccharide Compostional 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.