



O-Glycosidase DS

Instruction Manual

Catalog Number
170-6881

BIO-RAD

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Section 1

Introduction

O-Glycosidase DS is a recombinant endoglycosidase that cleaves the Ser/Thr linkage of the disaccharide Gal (β 1-3)GalNAc(α 1) from glycoproteins and glycopeptides. It will remove all non-sialylated Ser/Thr-linked Gal (β 1-3)GalNAc(α 1) disaccharides from glycoproteins and glycopeptides. Sialylated Ser/Thr-linked Gal (β 1-3)GalNAc(α 1) disaccharides can also be removed when O-Glycosidase DS is used in combination with a neuraminidase. Reaction buffer and two protocols (with and without neuraminidase treatment) are provided.



Section 2 Kit Components and Specifications

Component	O-Glycosidase DS (MW=200 kD)	5x Reaction Buffer pH 5
Specificity	Ser/Thr-linked Gal (β 1-3)GalNAc(α 1)	N/A
Concentration	1 U*/ml (in 20 mM Tris pH 7.5, 25 mM NaCl)	250 mM sodium phosphate, pH 5.0
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 Gal β -3GalNAc α 1-p-nitrophenol in 01 minute at 37 °C, pH 5.0.

Section 3 Protocol

3.1 Protocol with Neuraminidase

1. Isolate the glycoprotein and dilute it as follows.
Dried Sample: Resuspend up to 100 μ g of glycoprotein in 13 μ l of distilled water. Add 4 μ l of 5x Reaction Buffer, pH 5.
Liquid Sample: Dilute 13 μ l of oligosaccharide solution (containing up to 100 μ g) with 4 μ l of 5x Reaction Buffer, pH 5.
2. Add Neuraminidase (1 μ l of NANase II, catalog number 170-6882).
3. Incubate at 37 °C for 1 hour.
4. Add 2 μ l of O-Glycosidase DS to the reaction vial. Total reaction volume is 20 μ l.
5. Incubate at 37 °C for 3 hours.

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

3.2 Protocol without Neuraminidase

- Isolate the glycoprotein and dilute it as follows:

Dried Sample: Resuspend up to 100 μg of glycoprotein in 14 μl of distilled water. Add 4 μl of 5x Reaction Buffer, pH 5.

Liquid Sample: Dilute 14 μl of oligosaccharide solution (containing up to 100 μg) with 4 μl of 5x Reaction Buffer, pH 5.
- Add 2 μl of O-Glycosidase DS to the reaction vial. Total reaction volume is 20 μl .
- Incubate at 37 °C for three hours.
- Run the treated and untreated glycoprotein in a SDS-PAGE gel. Deglycosylated proteins will exhibit an increase in mobility due to the reduction in molecular weight.

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.