

## Protein Deglycosylation Mix



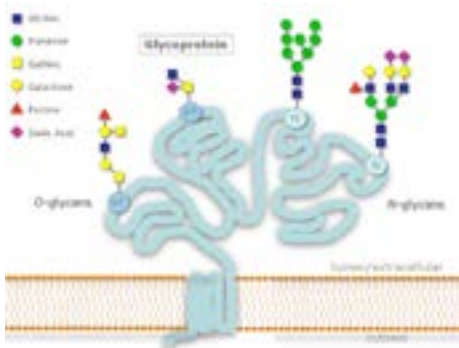
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# P6039S

20 reactions Lot: 0041504 Exp: 4/16  
Store at 4°C

**Description:** Glycosylation is one of the most common post-translational modifications of proteins, as shown in Figure 1. *N*-linked glycosylation occurs when glycans are attached to asparagine residues on the core protein. *O*-linked glycosylation occurs when glycans are attached to serine or threonine residues. Both chemical and enzymatic methods exist for removing oligosaccharides from glycoproteins. However, chemical methods such as  $\beta$ -elimination with mild alkali or mild hydrazinolysis can be harsh and may result in incomplete sugar removal and degradation of the protein; whereas, enzymatic methods are much gentler and can provide complete sugar removal with no protein degradation.



**Figure 1:** A glycoprotein modified with *O*-linked and *N*-linked glycosylation.

PNGase F is the most effective enzymatic method for removing almost all *N*-linked oligosaccharides from glycoproteins. PNGase F digestion deaminates the asparagine residue to aspartic acid, and leaves the oligosaccharide intact, keeping it suitable for further analysis. Oligosaccharides containing a fucose  $\alpha$ (1-3)-linked to the glycan core are,

however, resistant to PNGase F which can occur on some plant and insect glycoproteins. Steric hindrance slows or inhibits the action of PNGase F on certain residues of glycoproteins; therefore denaturation of the glycoprotein by heating with SDS and DTT greatly increases the rate of deglycosylation. Other commonly used endoglycosidases such as Endoglycosidase H are not suitable for general deglycosylation of *N*-linked sugars because of their limited specificities and because they leave one *N*-acetylglucosamine residue attached to the asparagine.

To remove *O*-linked glycans, monosaccharides must be removed by a series of exoglycosidases until only the Gal $\beta$ 1-3GalNAc (core 1) and/or the GlcNAc $\beta$ 1-3GalNAc (core 3) cores remain attached to the serine or threonine. The *Enterococcus faecalis* *O*-Glycosidase, also called Endo- $\alpha$ -*N*-Acetylgalactosaminidase, can then remove these core structures with no modification of the serine or threonine residues. Any modification of the core structures, including sialylation, will block the action of the *O*-Glycosidase. Sialic acid residues are easily removed by a general  $\alpha$ 2-3,6,8 Neuraminidase. In addition, exoglycosidases such as  $\beta$ (1-4) Galactosidase and  $\beta$ -*N*-Acetylglucosaminidase can be included in deglycosylation reactions to remove other complex modifications often known to be present on the core structures. This combination of enzymes will not remove all *O*-linked oligosaccharides but should remove many common oligosaccharide structures.

### Application:

This kit contains all of the enzymes, reagents, and controls needed to remove almost all *N*-linked and simple *O*-linked glycans as well as some complex *O*-linked glycans. This kit contains enzyme sufficient for 20 reactions or the cleavage of as much as 2 mg of glycoprotein.

### Kit Components:

Deglycosylation Enzyme Mix	100 $\mu$ l
10X Glycoprotein Denaturing Buffer	1 ml
10% NP-40 Buffer	1 ml
10X GlycoBuffer 2	1 ml

Substrate Control: Fetuin, 0.5 mg (Fetuin contains sialylated *N*-linked and *O*-linked glycans)

Deglycosylation Enzyme Mix supplied in: 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C) and 0.1 mM Na<sub>2</sub>EDTA.

### Deglycosylation Enzyme Mix:

20  $\mu$ l PNGase F Glycerol Free:  
500,000 units/ml

20  $\mu$ l *O*-Glycosidase:  
40,000,000 units/ml

20  $\mu$ l Neuraminidase:  
50,000 units/ml

20  $\mu$ l  $\beta$ 1-4 Galactosidase:  
8,000 units/ml

20  $\mu$ l  $\beta$ -*N*-Acetylglucosaminidase:  
4,000 units/ml

### Description of Enzymes Included in the Deglycosylation Enzyme Mix:

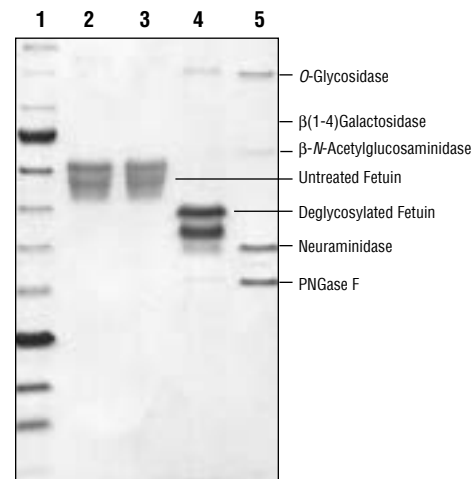
***O*-Glycosidase**, also known as Endo- $\alpha$ -*N*-Acetylgalactosaminidase, is a recombinant enzyme cloned from *Enterococcus faecalis* (1). It catalyzes the removal of core 1 and core 3 *O*-linked disaccharides from glycoproteins. The molecular weight is approximately 147 kDa.

**PNGase F**, also known as Peptide: *N*-glycosidase F, is an enzyme purified from *Flavobacterium meningosepticum* (2). PNGase F is an amidase which cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid, and complex oligosaccharides from *N*-linked glycoproteins unless  $\alpha$ (1-3) core fucosylated. The molecular weight is approximately 36 kDa.

**Neuraminidase**, also known as Sialidase, is a recombinant enzyme cloned from *Clostridium perfringens* (3) and overexpressed in *E. coli* (4). It catalyzes the hydrolysis of  $\alpha$ 2,3,  $\alpha$ 2,6, and  $\alpha$ 2,8 linked *N*-acetylneuraminic acid residues from glycoproteins and oligosaccharides. The molecular weight is approximately 43 kDa.

**$\beta$ 1-4 Galactosidase**, is a recombinant enzyme cloned from *Bacteroides fragilis* (5). It is a highly specific exoglycosidase that catalyzes the hydrolysis of  $\beta$ 1-4 linked D-galactopyranosyl residues from oligosaccharides. The molecular weight is approximately 94 kDa.

**$\beta$ -*N*-Acetylglucosaminidase**, is a recombinant enzyme cloned from *Xanthomonas manihotis* (6). It is a highly specific exoglycosidase that catalyzes the hydrolysis of terminal, non-reducing  $\beta$ -*N*-Acetylglucosamine residues from oligosaccharides. The molecular weight is approximately 71 kDa.



**Figure 2: Enzymatic Deglycosylation of Bovine Fetuin:** 100  $\mu$ g Bovine Fetuin Control was deglycosylated using the denaturing reaction conditions. 25  $\mu$ g of the reaction was loaded onto a 10/20 SDS-PAGE gel. Lane 1: Protein Ladder (10-250 kDa) (NEB #P7703), Lane 2: 25  $\mu$ g untreated Fetuin control, Lane 3: 25  $\mu$ g denatured Fetuin control, Lane 4: 25  $\mu$ g deglycosylated denatured Fetuin, Lane 5: 5  $\mu$ l Deglycosylation Mix

### Reaction Protocols

The quantity of enzyme recommended is sufficient for the deglycosylation of 100  $\mu$ g of a glycoprotein. Reactions may be scaled-up linearly to accommodate larger amounts of glycoprotein and larger reaction volumes. Optimal incubation times may vary for particular substrates. Typical reaction conditions are as follows:

### Denaturing Reaction Conditions:

1. Dissolve 100  $\mu$ g of glycoprotein into 18  $\mu$ l H<sub>2</sub>O.
2. Add 2  $\mu$ l of 10X Glycoprotein Denaturing Buffer to make a 20  $\mu$ l total reaction volume.
3. Denature glycoprotein by heating reaction at 100°C for 10 minutes.
4. Chill denatured glycoprotein on ice and centrifuge 10 seconds.
5. To the denatured glycoprotein reaction add 5  $\mu$ l 10X GlycoBuffer 2, 5  $\mu$ l 10% NP40, and 15  $\mu$ l H<sub>2</sub>O.

**Note:** PNGase F and *O*-Glycosidase are inhibited by SDS, therefore it is essential to have NP-40 in the reaction mixture under denaturing conditions. Failure to not include NP-40 into the denaturing protocol may result in loss of activity of some enzymes.

(see other side)

6. Add 5 µl Deglycosylation Enzyme Cocktail, mix gently.
7. Incubate reaction at 37°C for 4 hours.
8. Analyze by method of choice

*Note: The simplest method of assessing the extent of deglycosylation is by mobility shifts on SDS-PAGE gels.*

**Non-Denaturing Reaction Conditions:** When deglycosylating a native glycoprotein it is recommended that an aliquot of the glycoprotein is subjected to the denaturing protocol to provide a positive control for the fully deglycosylated protein. The non-denatured reaction can then be compared to the denatured reaction to determine the extent of reaction completion.

1. Dissolve 100 µg of glycoprotein into 40 µl H<sub>2</sub>O.
2. To the native glycoprotein add 5 µl 10X GlycoBuffer 2.
3. Add 5 µl Deglycosylation Enzyme Cocktail, mix gently.
4. Incubate reaction at 37°C for 4 hours.

*Note: To deglycosylate a native glycoprotein, longer incubation time as well as more enzyme may be required.*

5. Analyze by method of choice.

*Note: The simplest method of assessing the extent of deglycosylation is by mobility shifts on SDS-PAGE gels.*

### **Storage**

It is recommended to store this kit at 4°C. All components of the kit will be stable for at least one year if stored correctly.

**Notes:** Deglycosylation Mix is not recommended for use on Mucin-like substrates.

### **References**

1. Koutsoulis, D., Landry, D. and Guthrie, E.P. (2008) *Glycobiology*, 18, 799–805.
2. Plummer, T.H. Jr. and Tarentino, A.L. (1991) *Glycobiology*, 1, 257–263.
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4. Guan, C., New England Biolabs, unpublished observations.
5. McLeod, E., New England Biolabs, Inc., unpublished observations.
6. Guthrie, E.P., Shimer, E.P., New England Biolabs, Inc., unpublished observations.

U.S. Patent No. 6,358,724 and 5,770,405.



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