Protein Deglycosylation Mix

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P6039S 004121013101

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20 reactions **Lot: 0041210** **Exp: 10/13**

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 β-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.

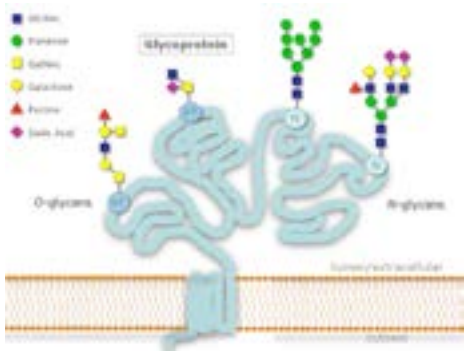

Figure 1: *A glycoprotein modified with O-linked and N-linked glycosylation.*

PNGase F is the most effective enzymatic method for removing almost all *N*-linked oligosaccharides from glycoproteins. PNGase F digestion deaminates the aspargine residue to aspartic acid, and leaves the oligosaccharide intact, keeping it suitable for further analysis. Oligosaccharides containing a fucose α(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 Galβ1-3GalNAc (core 1) and/or the GlcNAcβ1-3GalNAc (core 3) cores remain attached to the serine or threonine. The *Enterococcus faecalis O*-Glycosidase, also called Endo-α-*N*-Acetylgalactosaminidase, can then remove these core structures with no modification of the serine or threonine residues. Any modification of the core structures, including sialyation, will block the action of the *O*-Glycosidase. Sialic acid residues are easily removed by a general α2-3,6,8 Neuraminidase. In addition, exoglycosidases such as β(1-4) Galactosidase and β-*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 µl
10X Glycoprotein Denaturing Buffer	1 ml
10% NP-40 Buffer	1 ml
10X G7 Reaction Buffer	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_2EDTA.

Deglycosylation Enzyme Mix:
20 µl PNGase F Glycerol Free:
500,000 units/ml

20 µl *O*-Glycosidase:
40,000,000 units/ml

20 µl Neuraminidase:
50,000 units/ml

20 µl β1-4 Galactosidase:
8,000 units/ml

20 µl β-*N*-Acetylglucosaminidase:
4,000 units/ml

Description of Enzymes Included in the Deglycosylation Enzyme Mix:

***O*-Glycosidase**, also known as Endo-α-*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 α(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 α2,3, α2,6, and α2,8 linked *N*-acetylneuraminic acid residues from glycoproteins and oligosaccharides. The molecular weight is approximately 43 kDa.

β1-4 Galactosidase, is a recombinant enzyme cloned from *Bacteroides fragilis* (5). It is a highly specific exoglycosidase that catalyzes the hydrolysis of β1-4 linked D-galactopyranosyl residues from oligosaccharides. The molecular weight is approximately 94 kDa.

β-*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 β-*N*-Acetylglucosamine residues from oligosaccharides. The molecular weight is approximately 71 kDa.

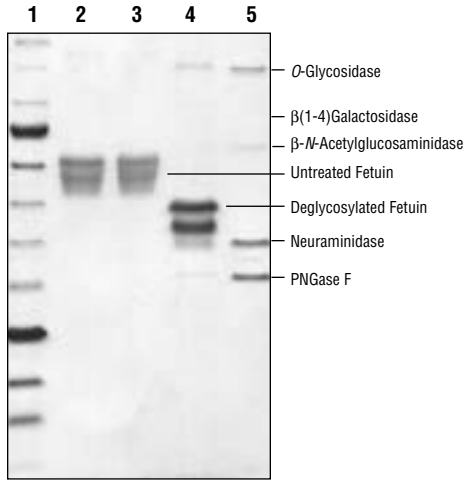

Figure 2: Enzymatic Deglycosylation of Bovine Fetuin: *100 µg Bovine Fetuin Control was deglycosylated using the denaturing reaction conditions. 25 µg of the reaction was loaded onto a 10/20 SDS-PAGE gel. Lane 1: Protein Ladder (10-250 kDa) (NEB #P7703), Lane 2: 25 µg untreated Fetuin control, Lane 3: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

Reaction Protocols

The quantity of enzyme recommended is sufficient for the deglycosylation of 100 µg 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 µg of glycoprotein into 18 µl H_2O.
2. Add 2 µl of 10X Glycoprotein Denaturing Buffer to make a 20 µl 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 µl 10X G7 Reaction Buffer, 5 µl 10% NP40, and 15 µl H_2O.

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)

CERTIFICATE OF ANALYSIS

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_2O.
2. To the native glycoprotein add 5 µl 10X G7 Reaction Buffer.
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. Koutsioulis, 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.
3. Roggentin, P. et al. (1988) *FEBS Lett.*, 238 (1), 31–34.
4. Guan, C., New England Biolabs, unpublished observations.
5. McLeod, E., New England Biolabs, Inc., unpublished observations.
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U.S. Patent No. 6,358,724 and 5,770,405.