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 glycosidases, 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 sialylation, 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 glycosides as well as some complex O-linked glycosides. 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 glycosids)

Deglycosylation Enzyme Mix supplied in: 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C) and 0.1 mM Na₂EDTA.

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 deaminites the asparagine 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.

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 β-1-4 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.

β(1-4) Galactosidase, is a recombinant enzyme cloned from Bacteroides fragilis (5). It is a highly specific exoglycosidase that catalyzes the hydrolysis of β(1-4) 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 H₂O.
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 H₂O.

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 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 H₂O.
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

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