PNGase F is the most effective enzymatic method for removing almost all O-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 α(1-3)-linked to the glycan core are, however, resistant to PNGase F which can occur on some plant and insect glycoproteins.

**Deglycosylation Enzyme Mix:**

- **PNGase F Glycerol Free:** 500,000 units/ml
- **10 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-β-N-Acetylgalactosaminidase, is a recombinant enzyme cloned from *Enterooccus faecalis* (1). It catalyzes the removal of the core 1 and core 3 α-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 amidas 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.

**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 PNGase F 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 PNGase F Reaction Buffer, 5 µl 10% NP-40, 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.