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# ProductInformation

**GlycoProfile™ II**
**Enzymatic In-Solution N-Deglycosylation Kit**

Product Code **PP0201**
Storage Temperature 2–8 °C

## TECHNICAL BULLETIN

### Product Description

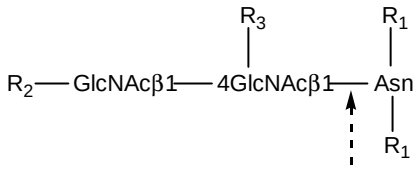
Glycosylation is one of the most common post-translational modifications of proteins in eukaryotic cells. These glycoproteins are involved in a wide range of biological functions such as receptor binding, cell signaling, immune recognition, inflammation, and pathogenicity. Mammalian glycoproteins contain three major types of oligosaccharides (glycans): N-linked, O-linked, and glycosylphophatidylinositol (GPI) lipid anchors. N-Linked glycans are linked to the protein backbone via an amide bond to asparagine residues in an Asn-Xaa-Ser/Thr motif, where Xaa can be any amino acid, except proline. O-Linked glycans are attached to the hydroxyl group of serine or threonine. However, variations in the glycan structures and different degrees of saturation of available glycosylation sites often result in heterogeneity in the mass and charge of glycoproteins.

To study the structure and function of a glycoprotein, it is often desirable to remove all or just a select class of glycans. This approach allows the assignment of specific biological functions to particular components of the glycoprotein. For example, removal of N-linked glycans from glycoproteins is very useful in the elimination or reduction of heterogeneity for MALDI-TOF mass spectrometric analysis. Also, the removal of glycans may enhance or reduce blood clearance rates and/or the potency of therapeutic glycoproteins.

Various chemical and enzymatic methods are possible for the removal of glycans from glycoproteins. Chemical methods are often harsh and may damage or destroy the target protein. Enzymatic methods are relatively mild and provide complete removal of a selected class of glycans without protein or glycan degradation.

Peptide N-glycosidase F (PNGase F) is one of the most widely used enzymes for the removal of the N-linked glycans from glycoproteins. The enzyme requires a minimal substrate consisting of a tripeptide with the glycan-linked asparagine as the central residue (see Figure 1). The resultant protein is left intact with the only modification being the deamination of the asparagine residue to aspartic acid at the site of attachment. The released N-linked glycans, when separated from the protein, are then suitable for further compositional, structural, or other analyses. PNGase F will not remove glycans containing an $\alpha(1\rightarrow3)$ linked fucose core structure, such as plant glycoproteins.

**Figure 1.**
PNGase F Cleavage site




$R_1$ = N- and C-substitution by groups other than H
$R_2$ = H or the rest of an oligosaccharide
$R_3$ = H or α1,6 fucose

GlycoProfile™ II has been optimized to provide a convenient and reproducible method to remove N-linked glycans from glycoproteins and is compatible with subsequent MALDI-TOF mass spectrometric analysis without interference from any of the reaction components. Reaction conditions may require optimization of denaturation time, reaction time, and PNGase F concentration for deglycosylation of different glycoproteins.

**Reagents**
GlycoProfile II contains sufficient enzyme, glycoprotein standard, and reagents for a minimum of 20 reactions when the sample size is between one to two mg of a typical glycoprotein. The kit contains 5 reagents:

Proteomics Grade PNGase F – The enzyme is supplied in a vial containing 50 units (50 IUB milliunits) of PNGase F (Product Code P 7367)

Ribonuclease B (RNase B) – A glycoprotein standard, supplied in a vial containing 0.5 mg of protein (Product Code R 7884)

10X Reaction Buffer – 158 mg ammonium bicarbonate (Product Code I 1283)

Octyl β-D-glucopyranoside – 100 mg (Product Code O 9882)

2-Mercaptoethanol – 0.90 ml (Product Code M 3148)

**Equipment and Reagents Required But Not Provided**
- Ultrapure water
- Eppendorf® tubes (Product Code T 4691 or equivalent)
- 37 °C and 100 °C heating block or water bath
- Bench-top centrifuge (microcentrifuge)

**Precautions and Disclaimer**
This product is for laboratory use only, not for drug, household, or other uses. Consult the Material Safety Data Sheet for information regarding hazards and safe handling practices. It is recommended to read the entire technical bulletin prior to starting the procedure.

**Preparation Instructions**
It is recommended to use ultrapure water when reconstituting the reagents.
- PNGase F Enzyme Solution – Spin the vial briefly to collect the solid at the bottom. Add 100 µl of water, agitate gently, spin, and store on ice. The enzyme concentration is 500 units/ml. This solution may be diluted accordingly, before use, to obtain different concentrations of enzyme.
- 1X Reaction Buffer - Add 10 ml of water to the bottle. Stir to dissolve the solid completely. The bottle now contains 10X Reaction Buffer (200 mM ammonium bicarbonate solution). Before use, dilute one part of the 10X Reaction Buffer with nine parts of water to produce a 1X Reaction Buffer.
- RNase B Standard - Spin vial briefly to collect the solid at the bottom. Add 450 µl of 1X Reaction Buffer, agitate, and spin briefly to obtain a 1.1 mg/ml solution.
- Denaturant Solution – Reconstitute the octyl β-D-glucopyranoside with 4 ml of water. Mix to dissolve and then add 35 µl of 2-mercaptoethanol followed by 965 µl of water to obtain the Denaturant Solution containing 2% octyl β-D-glucopyranoside with 100 mM 2-mercaptoethanol.

**Storage/Stability**
It is recommended to store GlycoProfile II at 2-8 °C. The kit as supplied is stable for at least 1 year when stored properly.

A reconstituted solution of the PNGase F enzyme (500 units/ml) can be stored for two weeks at 2-8 °C without significant loss of activity. The ammonium bicarbonate reaction buffer should be used within two weeks after reconstitution when stored at 2-8 °C.

**Procedure**

This procedure has been developed to achieve optimal deglycosylation with minimal interference in subsequent analysis either by SDS-PAGE or mass spectrometry of the intact protein or tryptic peptide fragments. The quantity of PNGase F enzyme recommended in the procedure is sufficient to deglycosylate 50 µg of the RNase B standard in one hour with incubation at 37 °C. The method presented here for the in-solution deglycosylation of RNase B may be used as a general guideline for the treatment of other glycoproteins.

1. Add 90 µl of the RNase B Standard (1.1 mg/ml) into an Eppendorf tube.

2. Add 5 µl of the Denaturant Solution containing 2% octyl β-D-glucopyranoside with 100 mM 2-mercaptoethanol. Mix and spin briefly.

3. Incubate at 100 °C for 10 minutes, making sure the cap is firmly closed to prevent evaporation.

   Note: This step may be omitted with some glycoproteins, for example IgG, if it is found that the sample precipitates on heating. Other glycoproteins, for example $\alpha_1$-antitrypsin, may require longer incubation times for denaturation prior to deglycosylation.

4. Allow the mixture to cool to room temperature and spin briefly.

5. Add an additional 5 µl of 1X Reaction Buffer, mix, and spin. The glycoprotein concentration is now 1 mg/ml.

6. Transfer 50 µl aliquots of the above solution to two separate tubes, labeling one tube as “control” and the other as “test”.

7. To the test sample, add 5 µl of PNGase F Enzyme Solution (500 units/ml), which is equivalent to 2.5 units of enzyme. To the control sample, add 5 µl of water.

   Note: The amount of PNGase F Enzyme Solution added can be varied depending upon the nature of the glycoprotein.

8. Mix and spin briefly.

9. Incubate at 37 °C for 1 hour.

   Note: The reaction time may be extended to 24 hours depending on the glycoprotein used.

10. Stop the reaction by heating at 100 °C for 10 minutes.

    Note: Omit this step if the sample was not heated in step 3.

11. Allow the solution to cool and spin briefly.

12. Analyze an aliquot of each reaction mixture by SDS-PAGE to assess deglycosylation.

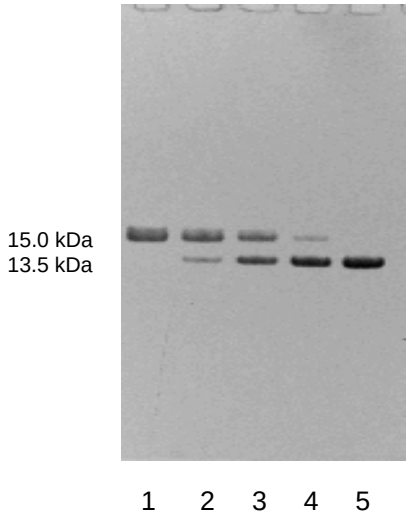
    Note: The reaction mixture may also be lyophilized for subsequent MS analysis of the deglycosylated protein or the released glycans after appropriate treatment.

**Results**

One of the simplest methods to assess the extent of deglycosylation is by mobility shift on SDS-PAGE gels. Figure 2 shows the deglycosylation of the RNase B Standard using four different amounts of PNGase F enzyme (0.05, 0.1, 0.2, and 0.4 units) incubated for 1 hour. As can be seen in Lane 5, the band at 13.5 kDa represents completely deglycosylated RNase B, compared to the band at 15 kDa for denatured RNase B without PNGase F treatment (control, Lane 1). This ability to detect mobility shifts when the N-linked glycans are removed with PNGase F will depend on the molecular weight of the native protein and the relative mass contribution by the glycans.

**Figure 2.**

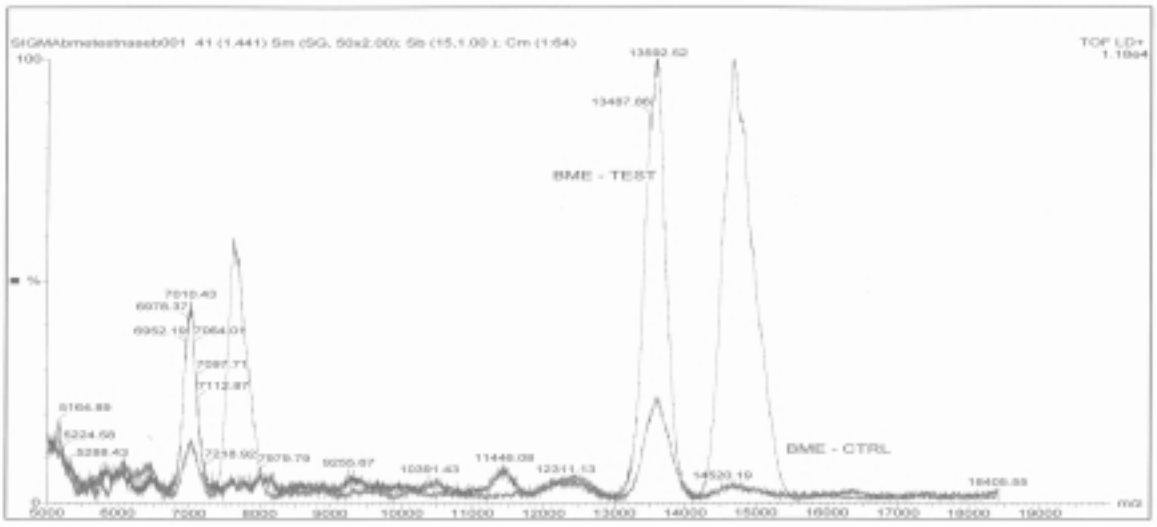
In-Solution Deglycosylation of RNase B by PNGase F Enzyme




Analysis of the deglycosylation of RNase B on 12% homogeneous SDS-PAGE gel. Lane 1 is the control (denatured RNase B), while Lanes 2, 3, 4, and 5 represent test samples with 0.05, 0.1, 0.2, and 0.4 units of PNGase F, respectively.

The deglycosylation reaction can also be monitored by MALDI-TOF MS analysis, using an appropriate matrix and operational conditions. Figure 3 shows an overlay of the MS traces of an untreated and a PNGase F-treated sample of RNase B obtained in positive ion linear mode. The result shows that upon deglycosylation, the average m/z of RNase B is reduced from 15,000 to 13,500 and the N-deglycosylated sample shows a narrower peak due to reduced heterogeneity.

**Figure 3.**
MALDI-TOF MS Analysis of Control and Deglycosylated RNase B




Overlay of two MALDI-TOF mass spectra of control and deglycosylated RNase B. Spectra were obtained with a MALDI instrument in positive ion linear mode using sinapinic acid as the matrix. The traces show the control RNase B with an average m/z of 15,000 compared to 13,500 for the deglycosylated RNase B.

**Related Products**

| Product Name | Product Code |
|---|---|
| GlycoProfile I, Enzymatic In-Gel N-Deglycosylation Kit | PP0200 |
| PNGase F, Proteomics Grade | P 7367 |
| Ribonuclease B | R 7884 |
| Trypsin Profile IGD Kit, Trypsin In-Gel Digest Kit | PP0100 |
| ProteoMass™ MALDI-MS Calibration Kits | |
| Peptide and Protein | MS-CAL1 |
| Peptide | MS-CAL2 |
| Protein | MS-CAL3 |
| Trypsin, Proteomics Grade | T 6567 |

**References**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