I want to deglycosylate my antibody. Should I follow the denaturation protocol suggested in the Technical Data Sheet for N-Glycanase® (PNGase F)?

Antibodies are glycoproteins that bind to antigens and to specialized cells of the immune system. Different structural regions of antibody molecules have been identified, which have different functional properties. Figure 1 shows immunoglobulin G (IgG), the most abundant immunoglobulin in serum. IgG molecules have 2 Fab domains, which contains the antigen-binding sites. A third region, the Fc domain, is important in certain aspects of the immune response.

Almost all IgG's are N-glycosylated in the Fc portion of the heavy chains (see Figure 2). Some IgG's may also contain N-linked glycans in the Fab region.

Each IgG contains four polypeptides—two heavy chains (~55 kda) and two light chains (~25 kda). These chains are held together by covalent disulfide bonds and noncovalent interactions. Upon denaturation and reduction of the disulfide bonds, a single IgG molecule gives rise to two heavy chain and two light chain polypeptides which are easily resolved into two distinct molecular weight species by SDS-PAGE.

IgG's in their native conformation are water soluble, but some interior regions of the molecule are highly hydrophobic. Upon denaturation the heavy chain of IgG's becomes insoluble. Hence, complete deglycosylation of glycans present in the Fc region of the heavy chain is difficult under denaturing conditions. However, it is possible to achieve nearly complete deglycosylation of IgG samples by treating with N-Glycanase-PLUS without denaturation.

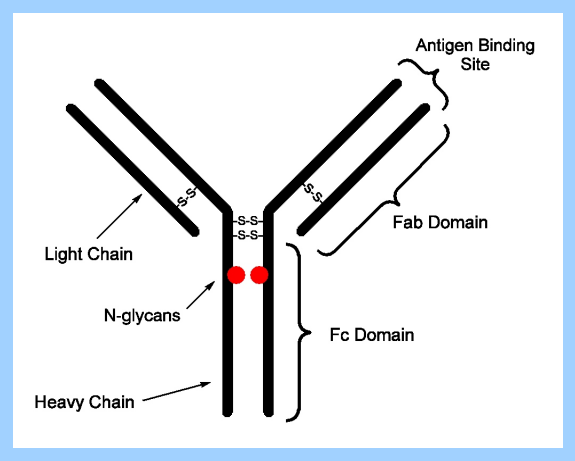

Figure 1 - Structure of Immunoglobulin G (IgG)

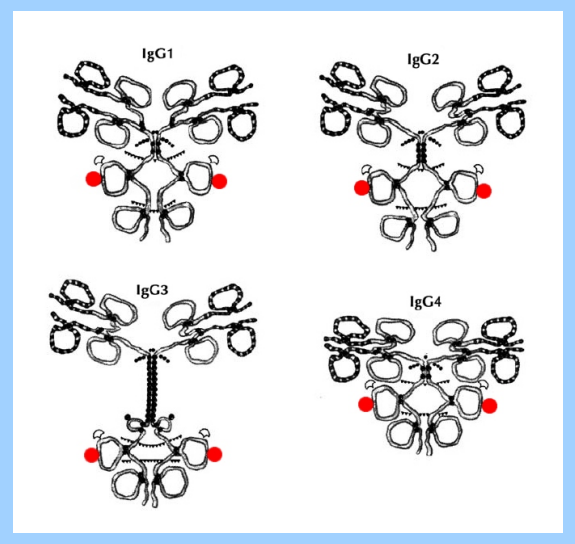

Figure 2 - Schematic Representation of N-linked Glycans on IgG Isotypes (red dots on the Fc region show the sites of N-linked substitution).

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The protocol presented here is a method to specifically remove N-linked glycans from IgG molecules while leaving the antibody intact for further study. This procedure may not cleave N-glycans in the Fab region.

PROCEDURE

Reagents

GKE-5010 N-Glycanase-PLUS (10 U/ml)

Antibody samples: ~1 mg/ml solution of IgG in 20 m*M* Tris-HCl buffer pH 8.2

NOTE: The retail package of N-Glycanase-PLUS ships with 5x Reaction Buffer (phosphate buffer) and 5x Tris Reaction Buffer. Phosphate buffers should be avoided if mass spectrometry is used in downstream analysis.

In addition, Denaturation Solution and Detergent Solution are included ONLY for use in the Denaturing Protocol. These solutions should not be used in the protocol described here.

Protocol

1. Add 1-2 µl of N-Glycanase-PLUS for every 100 µg of IgG sample.

2. Incubate at 37°C for 6 - 24 hours.

NOTE: To protect the biological activity of IgG and avoid degradation of the oligosaccharides, do not incubate for more than 24 hours.

3. For glycan analysis: precipitate the deglycosylated IgG from the enzyme digest either by heat denaturation or ethanol precipitation.

   Glycans remain in solution after these treatments and can be recovered from the supernatant. Further glycan analysis can be carried out using FACE® gel electrophoresis, HPLC chromatography with 2-AB labeling or mass spectrometry.

   For analysis of the deglycosylated antibody: separate the protein from the enzyme digest on an immobilized Protein A column. The antibody is bound to the column while the released glycans are removed by washing the column prior to eluting the antibody. Save the flow-through and wash fractions for recovery of the released glycans.

NOTE: Do not use dialysis to separate the antibody from the enzyme digest because deglycosylated antibody tends to bind to the membrane or precipitate during dialysis.

4. Deglycosylation may be verified by MALDI-TOF-MS of the treated protein. The N-linked glycans on the Fc portion of the heavy chains contribute only ~2% of the molecular weight of IgG's, so deglycosylation cannot be monitored using the gel shift on SDS-PAGE.

   As a consequence of enzyme action, the asparagine on the antibody to which the glycan was attached is converted to aspartic acid, but otherwise the protein chain remains intact. The conversion of each asparagine adds a negative charge to the protein. For small proteins and large charge differences, the progress of deglycosylation may be monitored on an IEF gel where deglycosylated product is resolved from the original material. For antibodies however, IEF results may be equivocal.