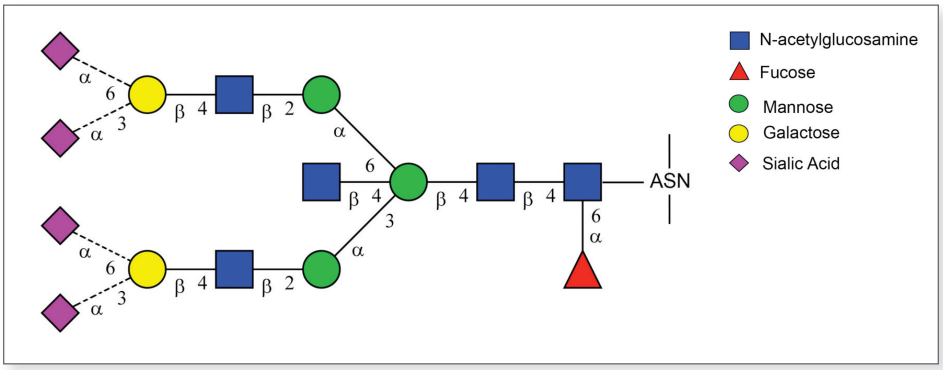
β-*N*-Acetylglucosaminidase Application Note

Removal of terminal *N*-acetylglucosamine from the biantennary *N*-linked sugars of IgG.

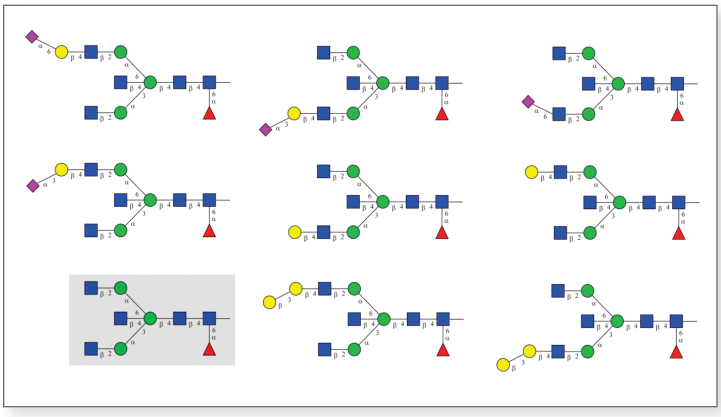
IgG contains one asparagine-linked biantennary carbohydrate in the Fc region of the heavy chain (1). Even though the heterogeneous biantennary structure of different molecules can be characterized by the variability of the presence of terminal galactose, the overall oligosaccharide pattern is constant for healthy individuals (2).



Mature biantennary carbohydrate structure for IgG.

Changes in the extent of galactosylation in the *N*-glycan of IgG are related to several diseases such as cancer (3), arthritis (4), Castleman's disease (5), periodontitis (6), and coeliac disease (7). Other emerging developments are in the use of recombinantly produced glycoproteins for therapeutic applications. Functional consequences can result from improper glycosylation. In the productionof monoclonal antibodies it has been shown that varying dissolved oxygen concentrations in murine hybridoma cell lines during growth can influence the extent of galactosylation (8).

Specific glycosidases can be used to characterize the diversity of the carbohydrate structures in IgG. This application note describes the use of a β-*N*-Acetylglucosaminidase (recombinant expressed in *E. coli*) to remove terminal *N*-acetylglucosamine from the biantennary *N*-glycoprotein IgG. The amount of *N*-acetylglucosamine that is released is a direct result of the extent of galactosylation.



Nine of the detected thirty immature biantennary N-glycan structures have cleavable terminal N-acetylglucosamines. The only bisecting terminal N-acetylglucosamine cleaved by the N-Acetylglucosaminidase is the N-glycan framed in the highlighted box

Materials

- β-*N*-Acetylglucosaminidase (NEB #P0732)
- *N*-acetylglucosamine standard (Sigma #A8625)
- IgG from human serum (Sigma; #4506)
- 10X G1 buffer (supplied with enzyme)

(see other side)

General Protocol

1. Prepare 10 mg/ml solution of IgG in water (store in aliquots of 100 µl @ -20°C).

2. Set up the exo-glycosidase digestion reaction:

Glycoprotein Substrate 10 mg/µl	85 µl
3. G1 buffer (10x)	10 µl
4. β-*N*-Acetylglucosaminidase	5 µl (20 units)
5. Total volume	100 µl

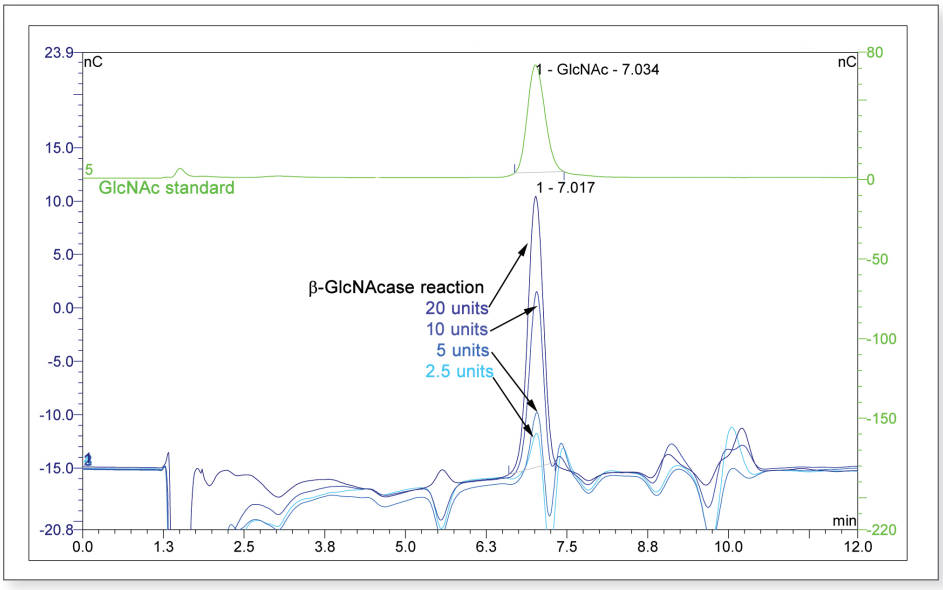
6. Incubate at 37°C for 4 hours. Add 200 µl water followed by 600 µl methanol. Chill overnight at 4°C to precipitate proteins. After the overnight precipitation, spin the sample at 14K rpm for 30 minutes, and reserve the supernatant.

7. Concentrate supernatant to dryness with a Speed Vac set at medium heat (Savant; equipped with a high vacuum pump and finger trap immersed in a Dewar containing isopropanol and dry ice). Reconstitute with 400 µl Milli-Q water.

8. De-ionize the sample from step 4 by gently rocking in 200 µl of prepared mixed bed ion exchange resin AG 501-X8 for 5 minutes (Bio-Rad; #142-6424). Collect the supernatant with a 1ml syringe using a 23 gauge needle. Note: before use, the resin must be converted to the acetate form by soaking in an equal volume of 1 M acetic acid followed washing ten times with equal volumes of water.

9. Remove the needle and load the entire sample (400 µl) from Step 5 to an activated Sep-Pak cartridge (Waters; #WAT051910). Collect the entire flow through (400 µl). Wash the Sep-Pak 2 times with 400 µl of Milli-Q water and pool the washes with the flow through. Concentrate to 70 µl using a Speed Vac. **Note:** before use, the Sep-Paks were activated by washing two times with 400 µl methanol followed by 4 times with 400 µl Milli-Q water.

10. Detect free *N*-acetylglucosamine (GlcNAc) by HPAEC-PAD Chromatography using the following conditions:
Column: CarboPac 20 with Amino Guard.
Elution: 20mM NaOH isocratic for 12 minutes, 150mM regeneration for 10 minutes, flow rate: 0.5 µl/min.
Detection: Pulse electrochemical, Au electrode, quadruple potential.
Injection sample: 30 µl, with or without internal GlcNAc standard (30 nanograms).



Superimposed chromatograms of released N-acetylglucosamine for serial decreasing amounts of β-N-Acetylglucosaminidase.

References

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