



CERTIFICATE OF ANALYSIS

PRODUCT NAME:	AdvanceBio InstantAB Human IgG N-glycan library
PRODUCT CODE:	GKIB-005
LOT NUMBER:	DP16K0801Z
PACK SIZE:	70 pmol (qualitative standard for glycan identification)
FORM:	Dry solid
STORAGE:	Store at -20 °C in the dark before and after reconstitution
EXPIRATION:	September 2026
STRUCTURE:	The Human IgG N-Linked Glycan Library consists of complex biantennary oligosaccharides consistent with N-glycans on normal human IgGs ^{1,2,3,4} (see Table 1). The reducing termini are derivatized with the fluorescent dye, InstantAB.

Quality Control:

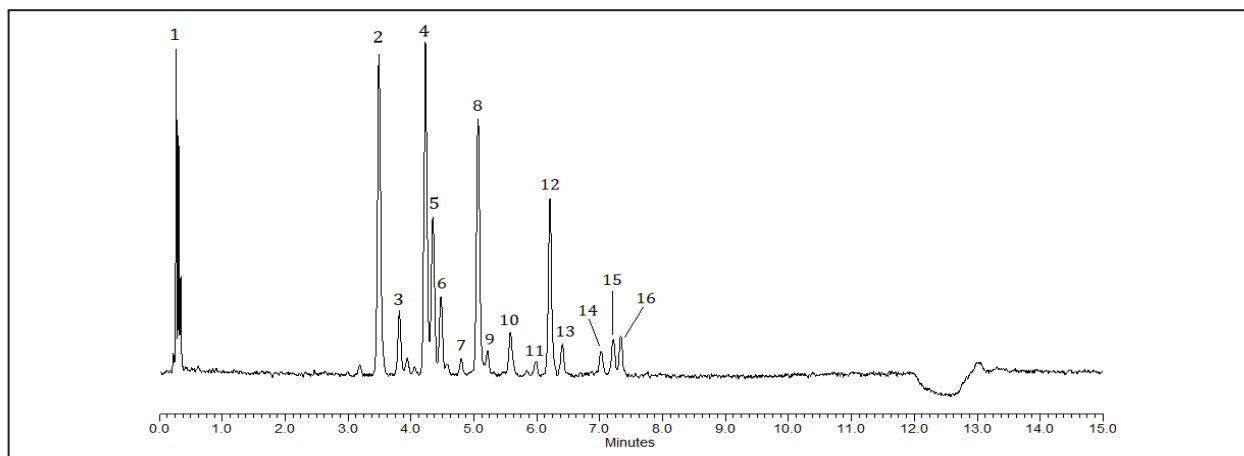


Figure 1 - UHPLC Results: 2–4 pmol (1 μ L, aqueous) of the InstantAB-labeled library was injected on a UHPLC System (Waters ACQUITY H Class) utilizing a 15-minute method under the conditions below:

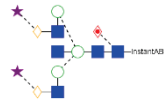
Time (min)	Flow (mL/min)	% ACN	% Buffer
0.0	1.0	75.0	25.0
12.0	1.0	52.5	47.5
12.1	0.5	40.0	60.0
12.5	0.5	40.0	60.0
12.6	0.5	75.0	25.0
12.7	1.0	75.0	25.0
15.0	1.0	75.0	25.0

Column: Waters ACQUITY UPLC BEH Glycan Column (1.7 μ m, 2.1 x 100 mm)
 ACN: Acetonitrile
 Buffer: 100 mM ammonium formate, pH 4.4
 Flow rate: As stated in table, in mL/min
 Temperature: 60 $^{\circ}$ C
 Max Pressure: 15,000 psi
 Fluorescence Detection: λ_{ex} = 278 nm, λ_{em} = 344 nm

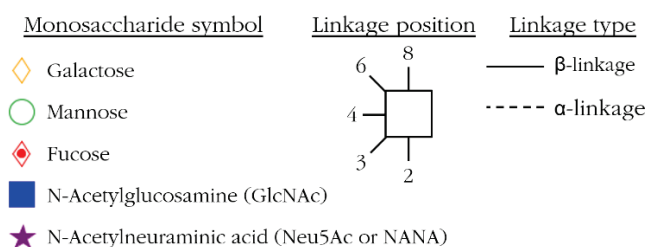
Table 1 - Peak Identification of InstantAB Labeled Human IgG N-Linked Glycan Library

Peak Number	Glycan Identification	Agilent	Common	Oxford (New)	Structure ^{5,6}
1	Free Dye (InstantAB)				
2	Asialo-, agalacto- biantennary with core fucose	NGA2F	G0F	F(6)A2	
3	Asialo-, agalacto- biantennary with core fucose and with bisecting N-Acetylglucosamine	NGA2FB	G0FB	F(6)A2B	

Peak Number	Glycan Identification	Agilent	Common	Oxford (New)	Structure ^{5,6}
4	Asialo-, monogalactosylated biantennary with core fucose	NA2G1F	G1F[6]	F(6)A2[6]G(4)1	
5	Asialo-, mono-galactosylated biantennary with core fucose	NA2G1F	G1F[3]	F(6)A2[3]G(4)1	
6	Asialo, mono-galactosylated biantennary, core substituted with fucose and bisecting N-Acetylglucosamine	NA2G1FB	G1FB	F(6)A2[6]BG(4)1	
7	Asialo, galactosylated biantennary	NA2	G2	A2G(4)2	
8	Asialo, galactosylated biantennary with core fucose	NA2F	G2F	F(6)A2G(4)2	
9	Asialo, galactosylated biantennary with core fucose and bisecting N-Acetylglucosamine	NA2FB	G2FB	F(6)A2BG(4)2	
10	Mono-α(2-6)-sialylated, mono-galactosylated biantennary with core fucose	NA2G1FS1	G1FS1	F(6)A2[3]G(4)1S(6)1	
11	Mono-α(2-6)-sialylated, galactosylated biantennary	A1	G2S1	A2G(4)2S(6)1	
12	Mono-α(2-6)-sialylated, galactosylated biantennary with core fucose	A1F	G2FS1	F(6)A2G(4)2S(6)1	
13	Mono-α(2-6)-sialylated, galactosylated biantennary with core fucose and bisecting N-Acetylglucosamine	A1FB	G2FS1B	F(6)A2BG(4)2S(6)1	
14	Di-sialylated, galactosylated biantennary	A2	G2S2	A2G(4)2S(6)2	
15	Di-sialylated, galactosylated biantennary, with core fucose	A2F	G2FS2	F(6)A2G(4)2S(6)2	

Peak Number	Glycan Identification	Agilent	Common	Oxford (New)	Structure ^{5,6}
16	Di-sialylated, galactosylated biantennary, with core fucose and bisecting N-Acetylglucosamine	A2FB	G2FS2B	F(6)A2BG(4)2S(6)2	

Structure Key^{5,6}:



Structural Analysis: The identity of the unlabeled glycan library is confirmed by MALDI-TOF mass spectrometry^{7,8} or LC-MS. Agreement was found between the results from mass spectrometry and UHPLC⁹.

Application: Confirmation of identification of specific N-glycans by UHPLC analysis for samples prepared on the GlykoPrep Sample Preparation Platform.

Handling & Reconstitution:

The labeled library is shipped as a dried solid. Use ultra-pure water or an aqueous buffer to dissolve the glycan (see Directions for Use for suggested volume).

Allow the unopened vial to reach ambient temperature and tap on a solid surface to ensure that most of the material is at the bottom of the vial. Gently remove the cap, add the desired volume of water or buffer, re-cap and mix thoroughly to re-dissolve all the oligosaccharide. For maximal recovery, ensure that the cap lining is also rinsed and centrifuge the reconstituted vial briefly before use.

Make sure that any glassware, plasticware, solvents or reagents used are free of glycosidases and carbohydrate contaminants.

Minimize exposure to elevated temperatures or extremes of pH.

Store the reconstituted glycan at –20 °C. Allow the vial to equilibrate to ambient temperature before use.

Directions For Use: The amount of InstantAB-labeled library standard injected on a UHPLC column is typically 2–4 pmol of total glycan. For our Quality Control testing, one vial was dissolved in 30 µL of water and 1 µL injected on the ACQUITY column. For larger injection volumes or other LC systems we recommend further dilution as necessary for compatibility with your mobile phase.

REFERENCES

1. Raju TS, Briggs JB, Borge SM and Jones AJS. Species-specific variation in glycosylation of IgG: evidence for the species-specific sialylation and branch-specific galactosylation and importance for engineering recombinant glycoprotein therapeutics. *Glycobiology* 2000; 10(5):477-486.
2. Wormald MR, Rudd PM, Harvey DJ, Chang S-C, Scragg IG and Dwek RA. Variations in oligosaccharide-protein interactions in immunoglobulin G determine the site-specific glycosylation profiles and modulate the dynamic motion of the Fc oligosaccharides. *Biochemistry* 1997; 36:1370-1380.
3. Routier FH, Hounsell EF, Rudd PM, Takahashi N, Bond A, Hay FC, Alavi A, Axford JS and Jefferis R. Quantitation of the oligosaccharides of human serum IgG from patients with rheumatoid arthritis: a critical evaluation of different methods. *J Immunol Meth* 1998; 213:113-130.
4. Herrera AM, Saunders NB and Baker JR. Immunoglobulin composition of three commercially available intravenous immunoglobulin preparations. *J Allergy Clin Immunol* 1989; 84(4):556-561.
5. Ceroni A, Maass K, Geyer H, Geyer R, Dell A, Haslam SM. GlycoWorkbench: a tool for the computer-assisted annotation of mass spectra of glycans. *J Proteome Res.* 2008 Apr;7(4):1650-9.
6. Harvey DJ, Merry AH, Royle L, Campbell MP, Dwek RA, Rudd PM. Proposal for a standard system for drawing structural diagrams of N- and O-linked carbohydrates and related compounds. *Proteomics* 2009 Aug;9(15):3796-801.
7. James DC and Jenkins N. Analysis of N-glycans by matrix-assisted laser desorption/ionization mass spectrometry; in *A laboratory guide to glycoconjugate analysis*. BioMethods (P. Jackson and J. T. Gallagher, ed) 1997; 9:91-112 (1997).
8. Papac DI, Wong A and Jones AJS. Analysis of acidic oligosaccharides by matrix-assisted laser desorption/ionization time of flight mass spectrometry. *Anal Chem* 1996 Sep 15; 68(18):3215-3223.
9. Rudd PM, Colominas C, Royle L, Murphy N, Hart E, Merry AH, Hebestreit HF and Dwek RA. A high-performance liquid chromatography based strategy for rapid, sensitive sequencing of N-linked oligosaccharide modifications to proteins in sodium dodecyl sulphate polyacrylamide electrophoresis gel bands. *Proteomics* 2001 Feb;1(2):285-94.

Christopher J. Miller

Digitally signed by Christopher J. Miller
Date: 2021.09.30 14:54:46 -0700

Authorized Signature