CERTIFICATE OF ANALYSIS

PRODUCT NAME:	AdvanceBio InstantAB huIgG and RNase B N-Glycan Library
PRODUCT CODE:	GKIB-025
LOT NUMBER:	DP14G3001A
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:	January 2027, may be used for 1 year after reconstitution (extended from prior exp. date based on re-assay)
RE-ASSAY DATE:	January 2022
STRUCTURE[1,2,3]:	The InstantAB-Human IgG + RNase B N-Linked Glycan Library consists of 2 blended libraries of N-linked glycans whose reducing termini are derivatized with the fluorescent dye, InstantAB. The libraries were blended to provide a comprehensive profile of biantennary, high mannose and sialylated oligosaccharides.

Agilent

Quality Control:

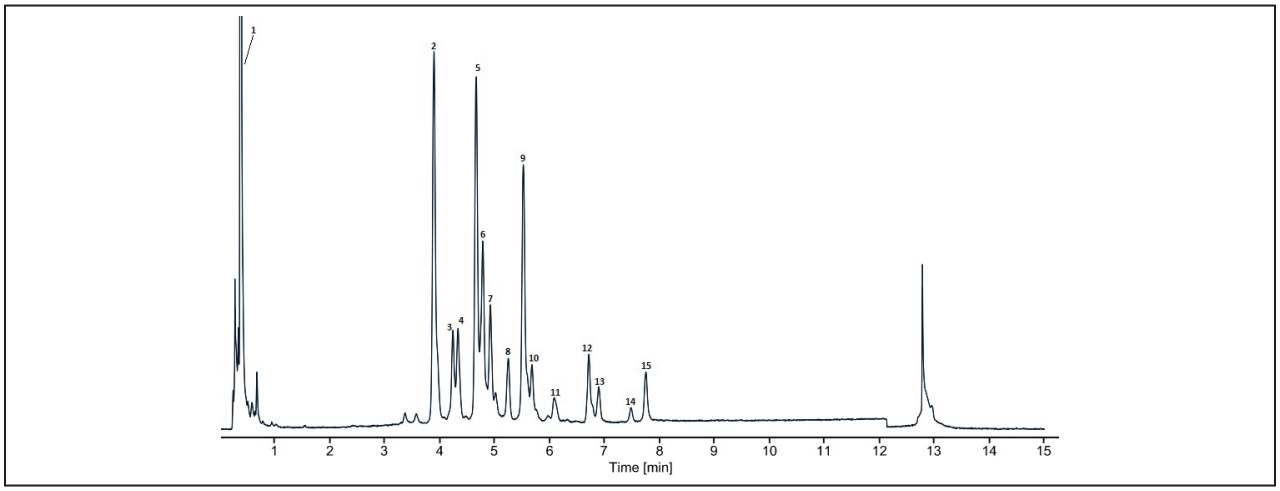
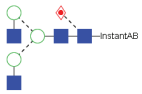
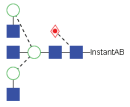


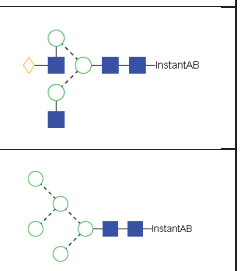
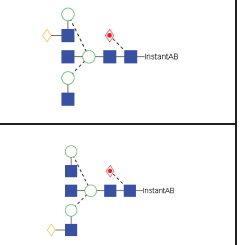
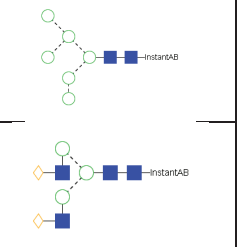
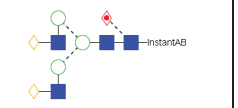
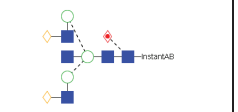
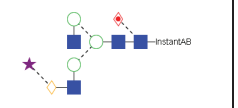
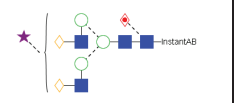
Figure 1 - UHPLC Results: 2 - 4 pmol (1 µl, aqueous) of the InstantAB-labeled library was injected on a UHPLC System (Agilent Infinity II) utilizing a 15-minute method under the conditions below:

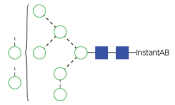
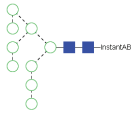
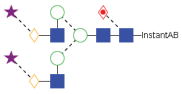
Time (min)	Flow (ml/min)	%ACN	%Buffer
00.0	1.0	75.0	25.0
12.0	1.0	52.5	47.5
12.1	0.5	40.0	60.0
12.5	0.5	40.0	60.0
12.6	0.5	75.0	25.0
12.7	1.0	75.0	25.0
15.0	1.0	75.0	25.0

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

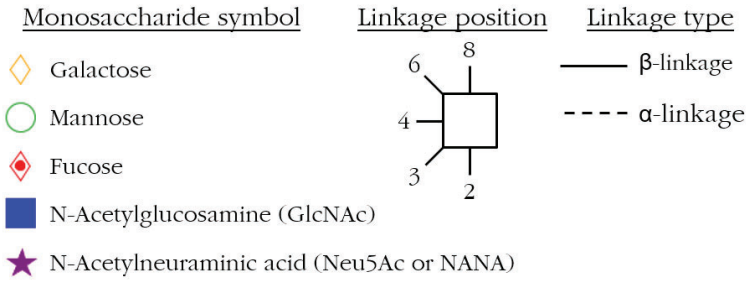
Table 1 - Peak Identification of InstantAB Labeled Human IgG + RNase B N-Linked Glycan Library.

Peak Number	Glycan Identification	Agilent	Common	Oxford (New)	Structure[2,3]
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[2,3]
4	Asialo-, mono-galacto-biantennary and Oligomannose 5	NA2G1 + Man5	G1[6] + Man5	A2[6]G(4)1 + M5	
5	Asialo-, mono-galacto-biantennary with core fucose	NA2G1F	G1F[6]	F(6)A2[6]G(4)1	
6	Asialo-, mono-galacto-biantennary with core fucose	NA2G1F	G1F[3]	F(6)A2[3]G(4)1	
7	Asialo-, mono-galactosylated biantennary with core fucose and bisecting N-Acetylglucosamine	NA2G1FB	G1FB[6] + G1FB[3]	F(6)A2B[6]G(4)1 + F(6)A2B[3]G(4)1	
8	Oligomannose 6 and Asialo-, galactosylated biantennary	Man6 + NA2	Man6 + G2	M6 + A2G(4)2	
9	Asialo-, galactosylated biantennary with core fucose	NA2F	G2F	F(6)A2G(4)2	
10	Asialo-, galactosylated biantennary with core fucose and bisecting N-Acetylglucosamine	NA2FB	G2FB	F(6)A2BG(4)2	
11	Mono-sialylated, mono-galactosylated, biantennary with core fucose	NA2G1FS1	G1FS1	F(6)A2[3]G(4)1S(6)1	
12	Mono-sialylated, galactosylated biantennary, with core fucose	A1F	G2FS1	F(6)A2G(4)2S(6)1	

Peak Number	Glycan Identification	Agilent	Common	Oxford (New)	Structure[2,3]
13	Oligomannose 8	Man8	Man8	M8	
14	Oligomannose 9	Man9	Man9	M9	
15	Di-sialylated, galactosylated biantennary with core fucose	A2F	G2FS2	F(6)A2G(4)2S(6)2	

Structure Key:



Structural Analysis: The identity of the unlabeled glycan library is confirmed by MALDI-TOF[4,5], or LC-MS. Agreement was found between the results from mass spectrometry and UHPLC[6].

Application:

- Qualitative standard for various analytical procedures
- As a migration standard for liquid chromatography

Handling & Reconstitution: The labeled oligo-saccharide is shipped as a dried solid. Use ultra-pure water or an aqueous buffer to dissolve the materials (see Directions for Use for suggested volumes).

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 ultra-pure water or aqueous buffer, re-cap and mix thoroughly to redissolve all the material.

For maximal recovery, ensure that the cap lining is also rinsed. 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 glycan 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 the mobile phase.

REFERENCES

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2. 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.

3. Harvey DJ, Merry AH, Royle L, Campbell MP, Rudd PM. Symbol nomenclature for representing glycan structures: Extension to cover different carbohydrate types. Proteomics 2011 Nov;11(22):4291-5.

4. James DC, 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.

5. Papac DI, Wong A, 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.

6. Ahn J, Bones J, Yu YQ, Rudd PM, Gilar M. Separation of 2-aminobenzamide labeled glycans using hydrophilic interaction chromatography columns packed with 1.7 microm sorbent. J Chromatogr B Analyt Technol Biomed Life Sci. 2010 Feb 1; 878(3-4): 403-8.

Christopher J. Miller Digitally signed by Christopher J. Miller Date: 2022.02.03 08:56:30 -08'00'

Authorized Signature