CERTIFICATE OF ANALYSIS

PRODUCT NAME:	GLYKO® HUMAN IgG N-LINKED GLYCAN LIBRARY
PRODUCT CODE:	GKLB-005
LOT NUMBER:	DP14A1608d
PACK SIZE:	20 µg Glycan Library (qualitative chromatographic standard for N-glycan identification)
PACKED WITH:	0.5 mg Human IgG Glycoprotein (WS0162, lot W150167a), source for the Glycan Library
PURITY:	Suitable for mass spectrometry
FORM:	Glycan Library: Dry solid Human IgG Glycoprotein: Dry solid
STORAGE:	Store at -20°C in the dark before and after reconstitution
EXPIRATION:	February 2022, may be used for 1 year after reconstitution
STRUCTURE:	The Human IgG N-Linked Glycan Library consists of complex biantennary oligosaccharides consistent with N-glycans on normal human IgGs[1,2,3] (see Table 1). Human IgG Glycoprotein: Predominantly IgG1, IgG2, and IgG3[4]

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Quality Control:

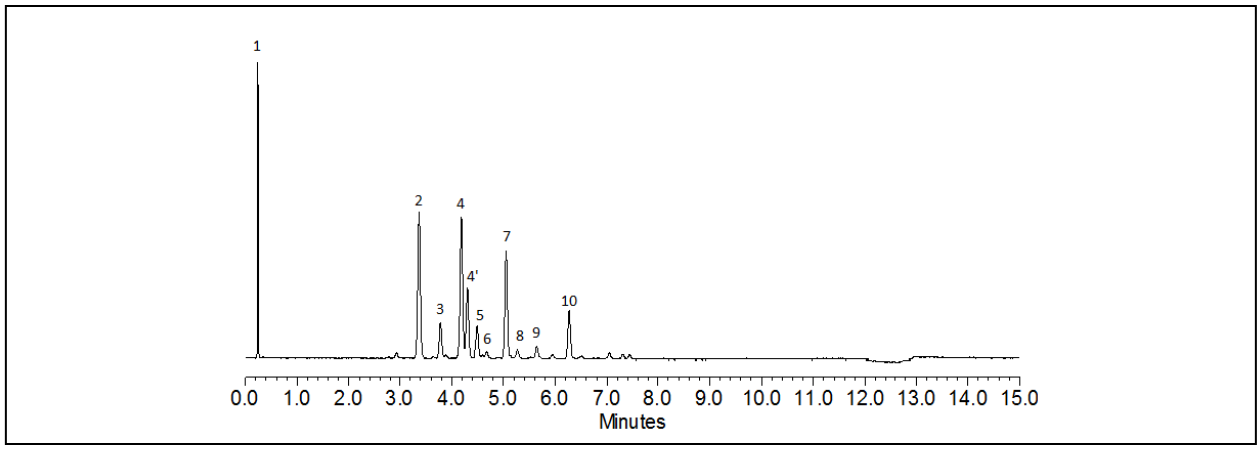
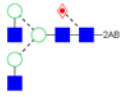
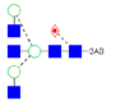
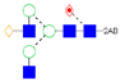


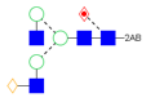
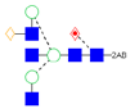
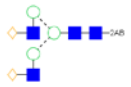
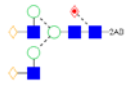
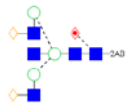
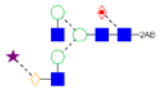
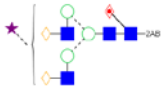
Figure 1 - UPLC® Results: 5 pmol (1 µl, aqueous) of the 2-AB-labeled library was injected on a Waters ACQUITY UPLC® H Class System utilizing a 15-minute method under the conditions below:

Time (min)	Flow	%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

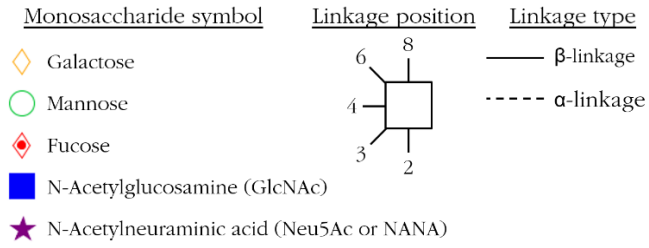
Column: Waters ACQUITY UPLC BEH Glycan Column (1.7 µm, 2.1 x 100 mm)
ACN: Acetonitrile
Buffer: 100 m*M* ammonium formate, pH 4.4
Flow rate: As stated in table, in ml/min
Temperature: 60° C
Max Pressure: 15,000 psi
Fluorescence Detection: λ_{ex} = 330 nm, λ_{em} = 420 nm

Table 1 - Peak Identification of 2-AB Labeled Human IgG N-Linked Glycan Library.

Peak Number	Glycan Identification	ProZyme	Common	Oxford (New)	Structure[4,5]
1	Free Dye (2-AB)				
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	
4	Asialo-, monogalactosylated biantennary with core fucose	NA2G1F	G1F[6]	F(6)A2[6]G(4)1	

Peak Number	Glycan Identification	ProZyme	Common	Oxford (New)	Structure[4,5]
4'	Asialo-, mono-galactosylated biantennary with core fucose	NA2G1F	G1F[3]	F(6)A2[3]G(4)1	
5	Asialo, mono-galactosylated biantennary, core substituted with fucose and bisecting N-Acetylglucosamine	NA2G1FB	G1FB	F(6)A2[6]BG(4)1	
6	Asialo, galactosylated biantennary	NA2	G2	A2G(4)2	
7	Asialo, galactosylated biantennary with core fucose	NA2F	G2F	F(6)A2G(4)2	
8	Asialo, galactosylated biantennary with core fucose and bisecting N-Acetylglucosamine	NA2FB	G2FB	F(6)A2BG(4)2	
9	Mono-α(2-6)-sialylated, mono-galactosylated biantennary with core fucose	NA2G1FS1	G1FS1	F(6)A2[3]G(4)1S(6)1	
10	Mono-α(2-6)-sialylated, galactosylated biantennary with core fucose	A1F	G2FS1	F(6)A2G(4)2S(6)1	

Structure Key[5,6]:



Preparation: Human polyclonal IgG (containing >99% IgGs; IgG1 being at least 70% of the total IgG content, balance is IgG2 and IgG3)[1,4] was digested with N-Glycanase® PLUS (ProZyme product code GKE-5010). Released N-linked oligosaccharides were then labeled with 2-AB and purified from excess labeling reagents.

Structural Analysis: The purity and structural integrity of the glycan library was assessed by UPLC[7,8] (GU values) and MALDI-TOF mass spectrometry[9,10] or LC-MS. Good agreement was found between the results from mass spectrometry and UPLC.

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

Handling & Reconstitution: The labeled oligosaccharide 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 2-AB-labeled library standard injected on a UPLC column is typically 6-9 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. For suggested methods see Rapid UPLC Methods for Screening Labeled N-Glycans at:

www.prozyme.com/protocols/

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. Herrra 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. 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.
8. Royle L, Campbell MP, Radcliffe CM, White DM, Harvey DJ, Abrahams JL, Kim YG, Henry GW, Shadick NA, Weinblatt ME, Lee DM, Rudd PM and Dwek RA. HPLC-based analysis of serum N-glycans on a 96-well plate platform with dedicated database software. Anal Biochem. 2008 May 1;376(1):1-12.
9. 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.
10. 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.

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