CERTIFICATE OF ANALYSIS

PRODUCT NAME:	InstantPC™-(Human IgG N-Linked Glycan Library)
PRODUCT CODE:	GKPC-005
LOT NUMBER:	DP18C2701a
PACK SIZE:	~25 injections (qualitative standard for glycan identification)
FORM:	Dry solid
STORAGE:	Store at -20°C in the dark before and after reconstitution
EXPIRATION:	October 2019 May be used for 6 months after reconstitution in 100 m*M* ammonium formate, pH 4.4 – 5.0, or for 1 month after reconstitution in water.
STRUCTURE[1,2,3]:	The Human IgG N-Linked Glycan Library consists of complex biantennary oligosaccharides consistent with N-glycans on normal human IgGs [1,2,3]. The glycosylamine form of the glycan is labeled with the fluorescent dye, InstantPC.

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Quality Control:

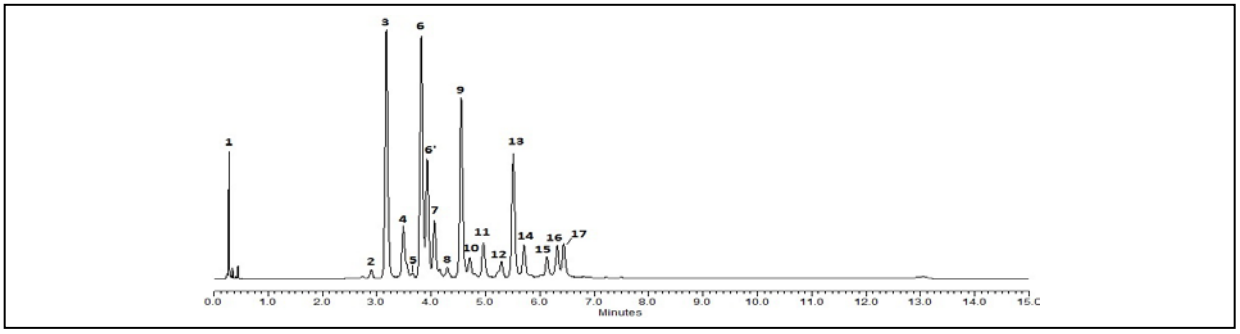
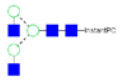
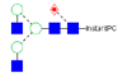
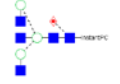
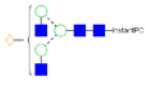


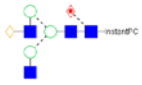
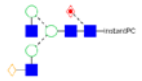
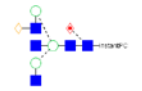
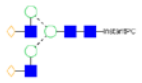
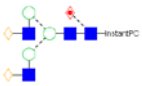
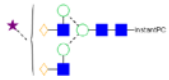
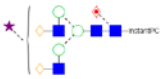
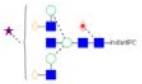
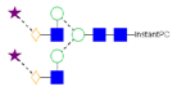
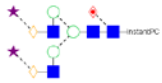
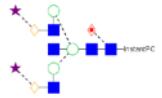
Figure 1 - **UPLC® Results**: 1 µl, aqueous, of the InstantPC-labeled glycan was injected on a Waters ACQUITY UPLC® H Class System utilizing a 15-minute method under the conditions below (see Table 1 for peak ID; the number of peaks observed depends on the running conditions employed):

Time (min)	Flow	%ACN	%Buffer
00.0	1.0	75.0	25.0
12.0	1.0	50.0	50.0
12.1	0.5	40.0	60.0
12.5	0.5	40.0	60.0
12.6	0.5	75.0	25.0
13.0	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} = 285 nm, λ_{em} = 345 nm

Table 1 – UPLC Peak Identification of InstantPC-(Human IgG N-Llinked Library). Although no sialic acid linkage analysis was performed on this material, linkages are shown as α(2-6) as these are the predominant sialic acid linkage present on human IgG Fc N-glycans[4].

Peak Number	Glycan Identification	ProZyme	Common	Oxford (New)	Structure [5,6]
1	Free Dye (InstantPC)				
2	Asialo-, agalacto-biantennary	NGA2	G0	A2	
3	Asialo-, agalacto-biantennary with core fucose	NGA2F	G0F	F(6)A2	
4	Asialo-, agalacto-biantennary, with core fucose and bisecting GlcNAc	NGA2FB	G0FB	F(6)A2B	
5	Asialo-, monogalactosylated biantennary	NA2G1	G1	A2G(4)1	

Peak Number	Glycan Identification	ProZyme	Common	Oxford (New)	Structure [5,6]
6	Asialo-, monogalactosylated biantennary, with core fucose	NA2G1F	G1F[6]	F(6)A2[6]G(4)1	
6'	Asialo-, monogalactosylated biantennary, with core fucose	NA2G1F	G1F[3]	F(6)A2[3]G(4)1	
7	Asialo-, monogalactosylated biantennary, with core fucose and bisecting GlcNAc	NA2G1FB	G1FB[6]	F(6)A2[6]BG(4)1	
8	Asialo-, galactosylated biantennary	NA2	G2	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 GlcNAc	NA2FB	G2FB	F(6)A2BG(4)2	
11	Mono-sialylated, monogalactosylated biantennary, with core fucose	NA2G1FS1	G1FS1[3]	F(6)A2[3]G(4)1S(6)1	
12	Mono-sialylated, galactosylated biantennary	A1	G2S1	A2G(4)2S(6)1	
13	Mono-sialylated, galactosylated biantennary, with core fucose	A1F	G2FS1	F(6)A2G(4)2S(6)1	
14	Mono-sialylated, galactosylated biantennary, with core fucose and bisecting GlcNAc	A1FB	G2FS1B	F(6)A2BG(4)2S(6)1	
15	Di-sialylated, galactosylated biantennary	A2	G2S2	A2G(4)2S(6)2	
16	Di-sialylated, galactosylated biantennary, with core fucose	A2F	G2FS2	F(6)A2G(4)2S(6)2	
17	Di-sialylated, galactosylated biantennary, with core fucose and bisecting GlcNAc	A2FB	G2FS2B	F(6)A2BG(4)2S(6)2	

Structure Key[5,6]:

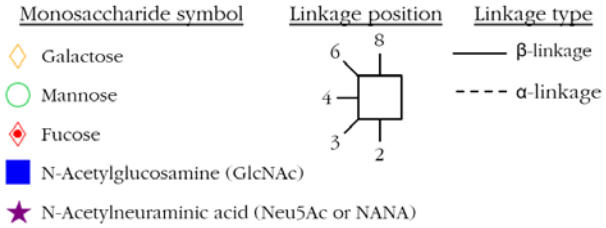


Table 2 – Glycan Masses of InstantPC-(Human IgG N-Linked Library)

Glycan ID	InstantPC-(Glycan) Monoisotopic Mass[7]	$[M+2H]^{+2}$	$[M+3H]^{+3}$
NGA2	1577.6343	789.8244	526.8854
NGA2F	1723.6922	862.8534	575.5713
NGA2FB	1926.7716	964.3931	643.2645
NA2G1	1739.6871	870.8508	580.9030
NA2G1F	1885.7450	943.8798	629.5889
NA2G1FB	2088.8244	1045.4195	697.2821
NA2	1901.7399	951.8772	634.9206
NA2F	2047.7978	1024.9062	683.6065
NA2FB	2250.8772	1126.4459	751.2997
NA2G1FS1	2176.8404	1089.4275	726.6207
A1	2192.8353	1097.4249	731.9524
A1F	2338.8932	1170.4539	780.6383
A1FB	2541.9726	1271.9936	848.3315
A2	2483.9307	1242.9726	828.9842
A2F	2629.9886	1316.0016	877.6701
A2FB	2833.0680	1417.5413	945.3633

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

InstantPC-labeled glycan masses are shown in Table 2.

Application: Qualitative reference standard for the separation and identification of N-glycan structures labeled with InstantPC by LC and LC-MS.

Handling & Reconstitution: The labeled oligosaccharide library 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 library at -20° C. Allow the vial to equilibrate to ambient temperature before use.

Directions For Use: The amount of InstantPC-labeled glycan standard injected on a UPLC column is typically 1 µl. For our Quality Control testing, one vial was dissolved in 30 µl of water and 1 µl injected on the ACQUITY BEH Glycan column.

We suggest reconstituting with 100 m*M* ammonium formate, pH 4.4 – 5.0 for storage at -20° C for up to 6 months. This buffer is often used as a HILIC mobile phase. Water may also be used for reconstitution, but the recommended storage period is shorter, -20° C for up to 1 month.

For larger injection volumes of InstantPC-labeled glycans (> 1 µl), do not use ACN alone to dilute the glycan to match the high organic % at the start of HILIC methods, as this may cause sialylated InstantPC glycans to precipitate. Use 1 part glycan in ammonium formate or water to 3 parts 1:1 [v/v] ACN:DMF, for a final concentration of 25% aqueous buffer, 37.5 % DMF, 37.5% ACN. Dilute only as much as is needed, and freeze the main stock at -20° C. For example, for a 10 µl injection, dilute 5 µl of glycan stock in ammonium formate or water with 15 µl 1:1 [v/v] ACN:DMF.

For further information on LC and LC-MS methods for InstantPC-labeled glycans, please contact ProZyme:

info@prozyme.com

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. Anthony RM, Nimmerjahn F, Ashline DJ, Reinhold VN, Paulson JC, Ravetch JV. A recombinant IgG Fc that recapitulates the anti-inflammatory activitiy of IVIG. Science 2008 Apr;320(5874):373-376

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. Calculating the Mass of Glycans Labeled with InstantPC.

   Mass added to glycan with a free reducing end:
   $Mass_{Glycan\ (free\ reducing\ end)} + C_{14}N_3O_2H_{19} = Mass_{InstantPC\text{-}Labeled\ Glycan}$

   Mass added by $C_{14}N_3O_2H_{19}$ (Da):
   Monoisotopic: 261.14773
   Average: 261.3

   Mass added to glycosylamine:
   $Mass_{Glycan\ (glycosylamine)} + C_{14}N_2O_3H_{18} = Mass_{InstantPC\text{-}Labeled\ Glycan}$

   Mass added by $C_{14}N_2O_3H_{18}$ (Da):
   Monoisotopic: 262.13174
   Average: 262.3

8. 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.

9. 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.

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