CERTIFICATE OF ANALYSIS

PRODUCT NAME:	AdvanceBio InstantPC CHO mAb N-Glycan Library
PRODUCT CODE:	GKPC-020
LOT NUMBER:	DP20L1501A
PACK SIZE:	1 each (contains InstantPC glycans from 20 µg purified CHO mAb) Qualitative standard for glycan identification
FORM:	Dry solid
STORAGE:	Store at –20 °C in the dark before and after reconstitution
EXPIRATION:	January 2023 (extended from prior exp. date based on re-assay) May be used for 6 months after reconstitution in 100 mM ammonium formate, pH 4.4–5.0, or for 1 month after reconstitution in water.
RE-ASSAY DATE:	April 2022
STRUCTURE:	The InstantPC CHO mAb Library contains N-glycans whose reducing termini are derivatized with the proprietary fluorescent dye, InstantPC.

Quality Control:

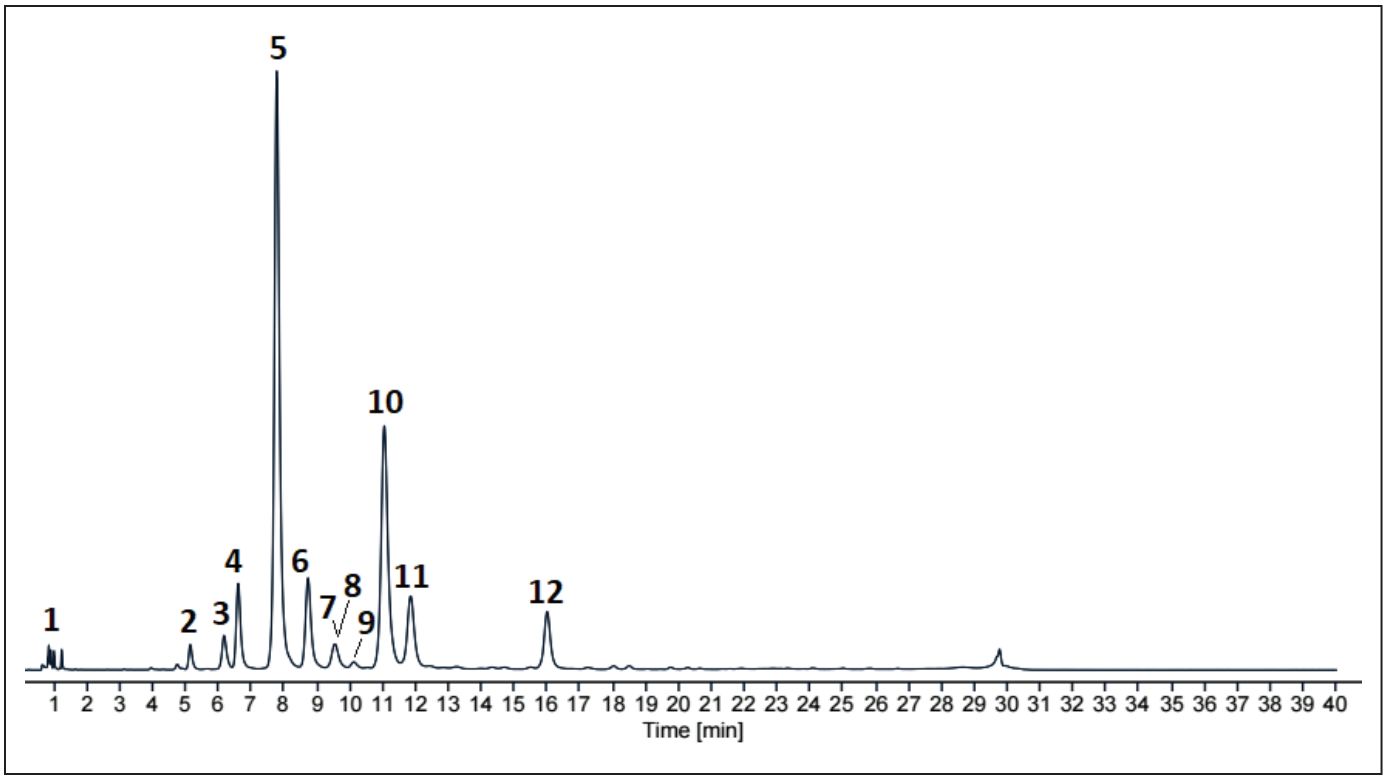
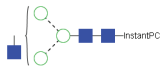
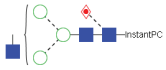
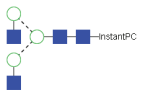
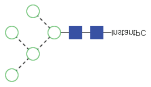
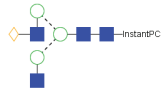
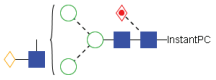
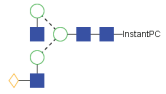
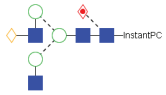
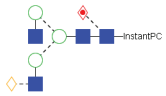
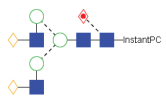


Figure 1 - UHPLC Results: 1 µL (aqueous) of the InstantPC-labeled glycan library was injected on on a UHPLC System (Agilent 1290 Infinity II) utilizing a 40-minute method under the conditions below (see Table 1 for peak ID; the number of peaks observed depends on the running conditions employed):

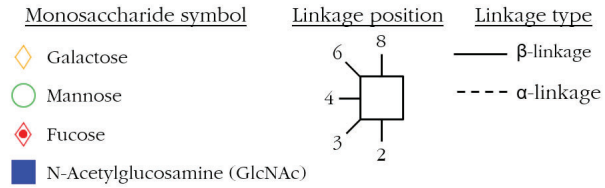
Time (min)	Flow (mL/min)	% ACN	% Buffer A
0.0	0.60	78.0	22.0
0.5	0.60	74.0	26.0
13.0	0.60	72.5	27.5
28.0	0.60	61.0	39.0
28.5	0.50	50.0	50.0
28.6	0.40	50.0	50.0
28.8	0.40	78.0	22.0
31.0	0.50	78.0	22.0
31.5	0.55	78.0	22.0
33.5	0.60	78.0	22.0
40.0	0.60	78.0	22.0

Column: Agilent AdvanceBio Glycan Mapping Column (1.8 µm, 2.1 x 150 mm)
Buffer A: 50 mM ammonium formate, pH 4.5
ACN: Acetonitrile
Flow rate: As stated in table, in mL/min
Temperature: 40 °C
Max Pressure: 15,000 psi
Fluorescence Detection: λ_{ex} = 285 nm, λ_{em} = 345 nm

Table 1 - Peak Identification of InstantPC CHO mAb Library

Peak No.	Glycan Identification	Agilent	Common	Oxford (New)	Structure[4,5]
1	Free Dye (InstantPC)				
2	Asialo-, agalacto-, biantennary, -1 N-Acetylglucosamine	NGA2-N	G0-N	A1	
3	Asialo-, agalacto-, biantennary with core fucose, -1 N-Acetylglucosamine	NGA2F-N	G0F-N	F(6)A1	
4	Asialo-, agalacto-, biantennary	NGA2	G0	A2	
5	Asialo-, agalacto-, biantennary with core fucose	NGA2F	G0F	F(6)A2	
6	Oligomannose 5	Man-5	Man5	M5	
7	Asialo-, mono-galactosylated, biantennary	NA2G1[6]	G1[6]	A2[6]G(4)1	
8	Asialo-, mono-galactosylated, biantennary with core fucose, -1 N-Acetylglucosamine	NA2G1F-N	G1F-N	F(6)A1G(4)1	
9	Asialo-, mono-galactosylated, biantennary	NA2G1[3]	G1[3]	A2[3]G(4)1	
10	Asialo-, mono-galactosylated, biantennary with core fucose	NA2G1F[6]	G1F[6]	F(6)A2[6]G(4)1	
11	Asialo-, mono-galactosylated, biantennary with core fucose	NA2G1F[3]	G1F[3]	F(6)A2[3]G(4)1	
12	Asialo-, galactosylated biantennary with core fucose	NA2F	G2F	F(6)A2G(4)2	

Structure Key[1,2]:



Structural Analysis: The purity and structural integrity of the glycan library was assessed by UHPLC[3](as described above) and MALDI-TOF[4,5] or LC-MS. Agreement was found between the results from mass spectrometry and UHPLC.

Application:

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

Handling & Reconstitution: The labeled oligo-saccharide 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 UHPLC column is typically 1 µL. The number of injections obtained from each vial depends on the reconstitution and subsequent dilution (if any) of the library.

For our Quality Control testing, one vial was dissolved in 30 µL of water and 1 µL injected on the Agilent AdvanceBio Glycan Map column. The expected number of injections from this volume would be approximately 25.

We suggest reconstituting with 100 mM ammonium formate, pH 4.4–5.0. This buffer is often used as a HILIC mobile phase and the glycan library may be stored longer term at –20 °C in this buffer. Water may also be used for reconstitution, but the shelf-life may be shorter (recommend –20 °C storage). Shelf-life of the reconstituted glycan library is not yet established.

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.

REFERENCES

1. 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.

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. 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.

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.

Christopher J. Miller

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