CERTIFICATE OF ANALYSIS

PRODUCT NAME:	AdvanceBio 2-AB Biantennary and High Mannose Partitioned Library
PRODUCT CODE:	GKSB-520
LOT NUMBER:	DP22E5033
PACK SIZE:	200 pmol (qualitative standard for glycan identification) 1 each WS0311 2-AB-(Fucosyl Biantennary Library) 1 each WS0312 2-AB-(Afucosyl Biantennary Library) 1 each WS0313 2-AB-(High Mannose Library)
FORM:	Dry solid
STORAGE:	Store at –20 °C in the dark before and after reconstitution
EXPIRATION:	August 2029, may be used for 1 year after reconstitution
STRUCTURE:	The 2-AB-(Biantennary & High Mannose Partitioned Library) consists of 3 blended libraries of N-linked glycans whose reducing termini are derivatized with the fluorescent dye, 2-AB (2-aminobenzamide). The libraries were partitioned to minimize overlap of peaks to facilitate glycan peak identification.

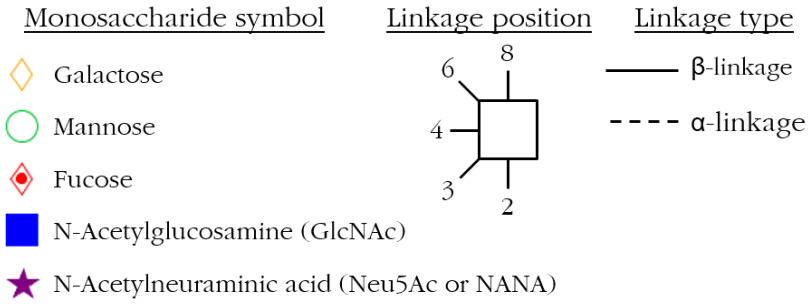
Quality Control:

UHPLC Running Conditions: 6–9 pmol (1 µL) of each 2-AB labeled glycan 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
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 mM 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

Structure Key [1,2]:



WS0311 2-AB Fucosyl Biantennary Library QC Results

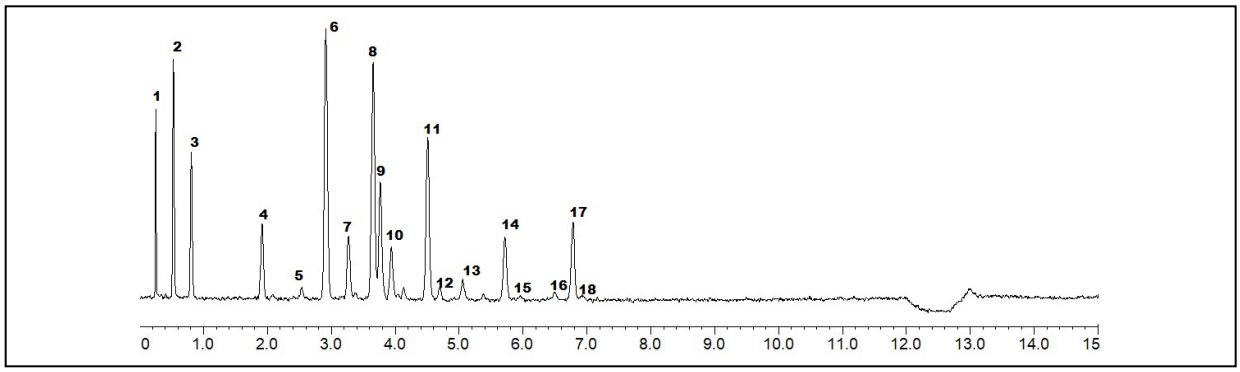
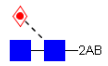
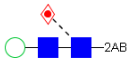
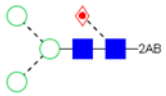
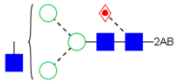
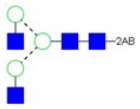
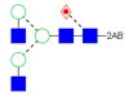
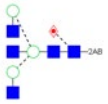
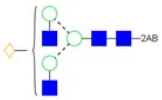
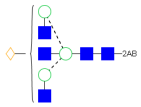
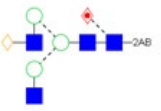
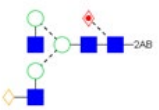
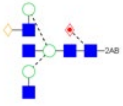
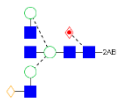
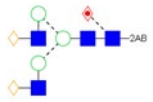
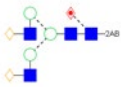
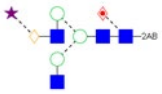
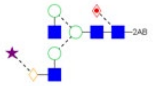


Figure 1 - UHPLC Results for WS0311 - See Table 1 for peak ID.

Table 1 - Peak Identification of 2-AB Fucosyl Biantennary Library (WS0311)

Peak Number	Glycan Identification	Prozyme	Common	Oxford (New)	Structure[1,2]
1	Free Dye (2-AB)				
2	6-α-fucosyl chitobiose	N2F	N/A	N/A	
3	4'-β-mannosyl chitobiose with core fucose	MNNF	N/A	N/A	
4	Conserved trimannosyl core, substituted with fucose	M3N2F	N/A	F(6)M3	
5	Asialo-, agalacto-, biantennary complex N-Glycan with core fucose, -1 N-Acetylglucosamine	NGA2F-N	G0F-N	F(6)A1	
6	Asialo-, agalacto-biantennary	NGA2	G0	A2	
7	Asialo-, agalacto-biantennary with core fucose	NGA2F	G0F	F(6)A2	
8	Asialo-, agalacto-biantennary with core fucose and with bisecting N-Acetylglucosamine	NGA2FB	G0FB	F(6)A2B	
9	Asialo-, mono-galacto-biantennary	NA2G1	G1	A2G(4)1	
10	Asialo, mono-galactosylated biantennary with bisecting N-Acetylglucosamine	NA2G1B	G1B	A2G(4)1B	
11 + 12	Asialo-, mono-galacto-biantennary with core fucose	NA2G1F	G1F[6] + G1F[3]	F(6)A2[6]G(4)1 + F(6)A2[3]G(4)1	 
13 + 14	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	

Peak Number	Glycan Identification	Prozyme	Common	Oxford (New)	Structure[1,2]
					
15	Asialo-, galactosylated biantennary with core fucose	NA2F	G2F	F(6)A2G(4)2	
16	Asialo-, galactosylated biantennary with core fucose and bisecting N-Acetylglucosamine	NA2FB	G2FB	F(6)A2BG(4)2	
17 + 18	Mono-α(2-6)-sialylated, mono-galactosylated, biantennary with core fucose	NA2G1FS1	G1FS1[6] + G1FS1[3]	F(6)A2[6]G(4)1S(6)1 + F(6)A2[3]G(4)1S(6)1	 

WS0312 2-AB Afucosyl Biantennary Library QC Results

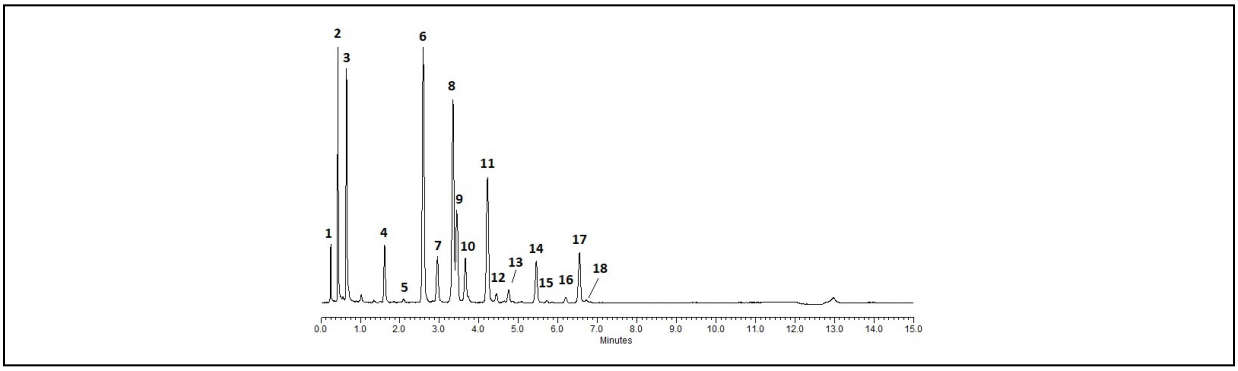
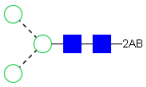
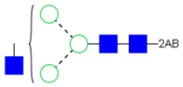
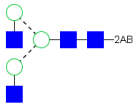
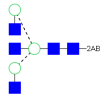
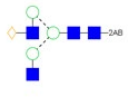
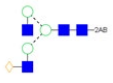
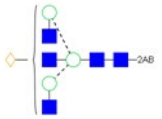
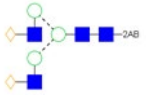
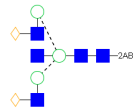
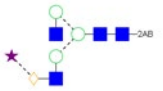
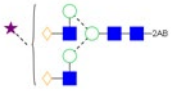
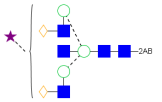
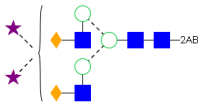
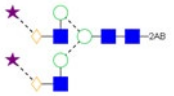
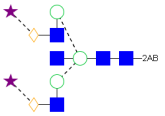


Figure 2 - UHPLC Results for WS0312 - See Table 2 for peak ID.

Table 2- Peak Identification of 2-AB Afucosyl Biantennary Library (WS0312)

Peak Number	Glycan Identification	Prozyme	Common	Oxford (New)	Structure[1,2]
1	Free Dye (2-AB)				
2	Chitobiose	N2	N/A	N/A	
3	4'-β-mannosyl chitobiose	MNN	N/A	N/A	
4	Conserved trimannosyl core	M3N2	Man3	M3	
5	Asialo-, agalacto-, biantennary complex N-Glycan, -1 N-Acetylglucosamine	NGA2-N	G0-N	A1	
6	Asialo-, agalacto-biantennary	NGA2	G0	A2	
7	Asialo-, agalacto-biantennary with bisecting N-Acetylglusosamine	NGA2B	G0B	A2B	
8 + 9	Asialo-, mono-galactosylated biantennary	NA2G1	G1[6] + G1[3]	A2[6]G(4)1 + A2[3]G(4)1	 

Peak Number	Glycan Identification	Prozyme	Common	Oxford (New)	Structure[1,2]
10	Asialo-, mono-galactosylated biantennary with bisecting N-Acetylglusosamine	NA2G1B	G1B	A2BG(4)1	
11	Asialo-, galactosylated biantennary	NA2	G2	A2G(4)2	
12	Asialo-, galactosylated biantennary with bisecting N-Acetylglucosamine	NA2B	G2B	A2BG2	
13	Mono-α(2-6)-sialylated, mono-galactosylated, biantennary	NA2G1S1	G1S1	A2[3]G(4)1S(6)1	
14	Mono-α(2-6)-sialylated, galactosylated biantennary	A1	G2S1	A2G(4)2S(6)1	
15	Mono-α(2-6)-sialylated, galactosylated biantennary with bisecting N-Acetylglucosamine	A1B	G2S1B	A2BG(4)2S(6)1	
16	Di-α(2,3/2,6)-sialylated, galactosylated biantennary	A2	G2S2	A2G2S(3,6)2	
17	Di-α(2-6)-sialylated, galactosylated biantennary	A2	G2S2	A2G(4)2S(6)2	
18	Di-α(2-6)-sialylated, galactosylated biantennary with bisecting N-Acetylglucosamine	A2B	G2S2B	A2BG(4)2S(6)2	

WS0313 2-AB High Mannose Library QC Results

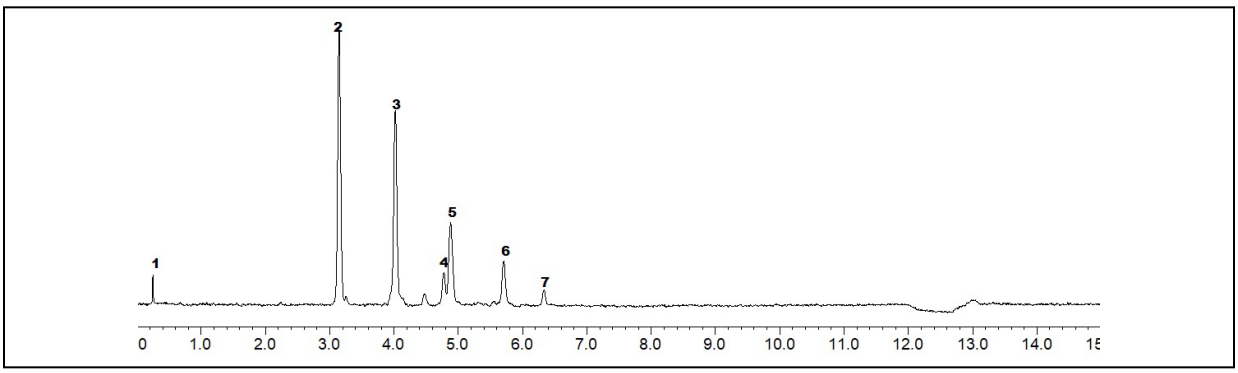
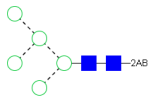
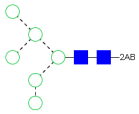
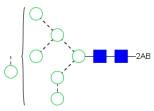
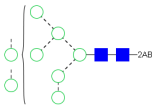
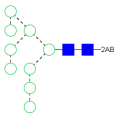


Figure 3 - UHPLC Results for WS0313 - See Table 3 for peak ID.

Table 3- Peak Identification of 2-AB High Mannose Library (WS0313)

Peak Number	Glycan Identification	Prozyme	Common	Oxford (New)	Structure[1,2]
1	Free Dye (2-AB)				
2	Oligomannose 5	Man5	Man5	M5	
3	Oligomannose 6	Man6	Man6	M6	
4 + 5	Oligomannose 7	Man7	Man7	M7	
6	Oligomannose 8	Man8	Man8	M8	
7	Oligomannose 9	Man9	Man9	M9	

Structural Analysis: The purity and structural integrity of the glycan libraries were assessed by a combination of methods including UHPLC[3,4] (GU values) and LC/MS.

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 2-AB-labeled library standard injected on a UHPLC 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 the mobile phase.

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. 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.

4. 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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