



CERTIFICATE OF ANALYSIS

PRODUCT NAME: AdvanceBio InstantAB Biantennary and High Mannose Partitioned Library

PRODUCT CODE: GKIB-520

LOT NUMBER: P16C0601

PACK SIZE: 70 pmol of each library (qualitative chromatographic standard for N-glycan identification)

1 each WS0330 InstantAB -(Fucosyl Library)
1 each WS0331 InstantAB -(Afucosyl Library)
1 each WS0332 InstantAB -(High Mannose Library)

FORM: Dry solid

STORAGE: Store at -20 °C in the dark before and after reconstitution

EXPIRATION: October 2025, may be used for 1 year after reconstitution (extended from prior exp. date based on re-assay)

RE-ASSAY DATE: October 2020

STRUCTURE: The InstantAB Biantennary and High Mannose Partitioned Library consists of three blended libraries of N-linked glycans whose reducing termini are derivatized with the fluorescent dye, InstantAB. The libraries were partitioned to minimize overlap of peaks to facilitate glycan peak identification.

LABORATORY REAGENT

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Quality Control:

UPLC Running Conditions: 2–5 pmol (1 μ L, aqueous) of each of the InstantAB-labeled libraries was injected on a Waters ACQUITY UPLC H Class System 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 μ m, 2.1 x 100 mm)

ACN: Acetonitrile

Buffer: 100 mM ammonium formate, pH 4.4

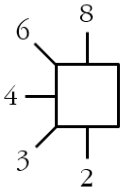
Flow rate: As stated in table, in mL/min

Temperature: 60 $^{\circ}$ C

Max Pressure: 15,000 psi

Fluorescence Detection: λ_{ex} = 278 nm, λ_{em} = 344 nm

Structure Key^{1,2}:

<u>Monosaccharide symbol</u>	<u>Linkage position</u>	<u>Linkage type</u>
 Galactose		 β -linkage
 Mannose		 α -linkage
 Fucose		
 N-Acetylglucosamine (GlcNAc)		
 N-Acetylneuraminic acid (Neu5Ac or NANA)		

WS0330 InstantAB Fucosyl Biantennary Library QC Results

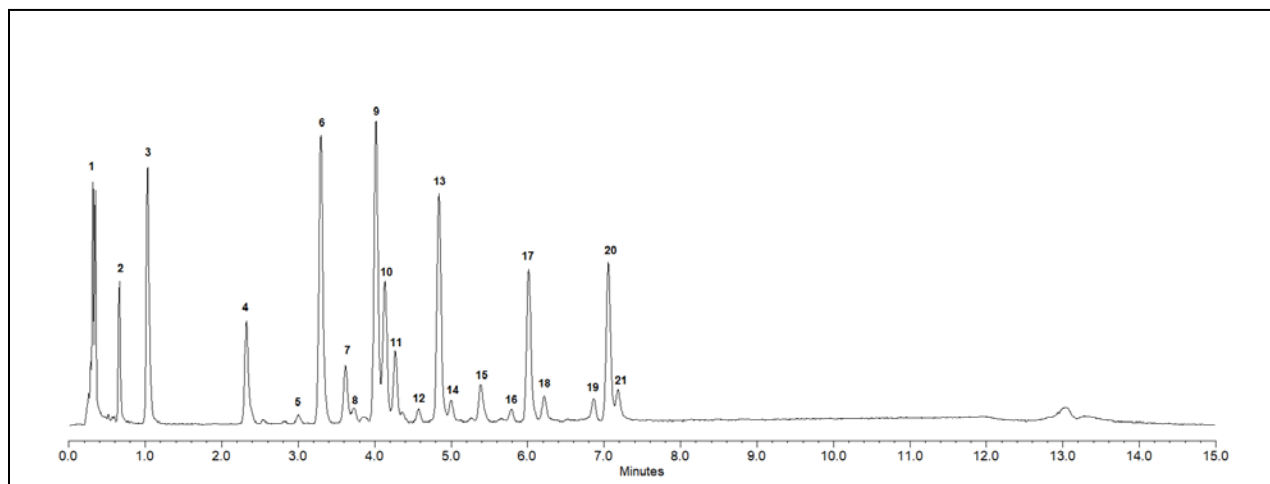
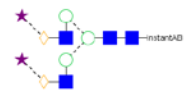
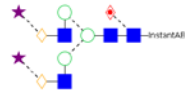
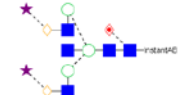


Figure 1 - ACQUITY UPLC Results for WS0330 - See Table 1 for peak ID.

Table 1 - Peak Identification of InstantAB Fucosyl Biantennary Library (WS0330)

Peak Number	Glycan Identification	ProZyme	Common	Oxford (New)	Structure ^{1,2}
1	Free Dye (InstantAB)				
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 with core fucose	M3N2F	N/A	F(6)M3	
5	Asialo-, agalacto-biantennary	NGA2	G0	A2	
6	Asialo-, agalacto-biantennary with core fucose	NGA2F	G0F	F(6)A2	
7	Asialo-, agalacto-biantennary with core fucose and with bisecting N-Acetylglucosamine	NGA2FB	G0FB	F(6)A2B	

Peak Number	Glycan Identification	ProZyme	Common	Oxford (New)	Structure ^{1,2}
19	Di-sialylated, galactosylated biantennary	A2	G2S2	A2G(4)2S(6)2	
20	Di-sialylated, galactosylated biantennary with core fucose	A2F	G2FS2	F(6)A2G(4)2S(6)2	
21	Di-sialylated, galactosylated biantennary with core fucose and bisecting N-Acetylglucosamine	A2FB	G2FBS2	F(6)A2BG(4)2S(6)2	

WS0331 InstantAB Afucosyl Biantennary Glycan Library QC Results

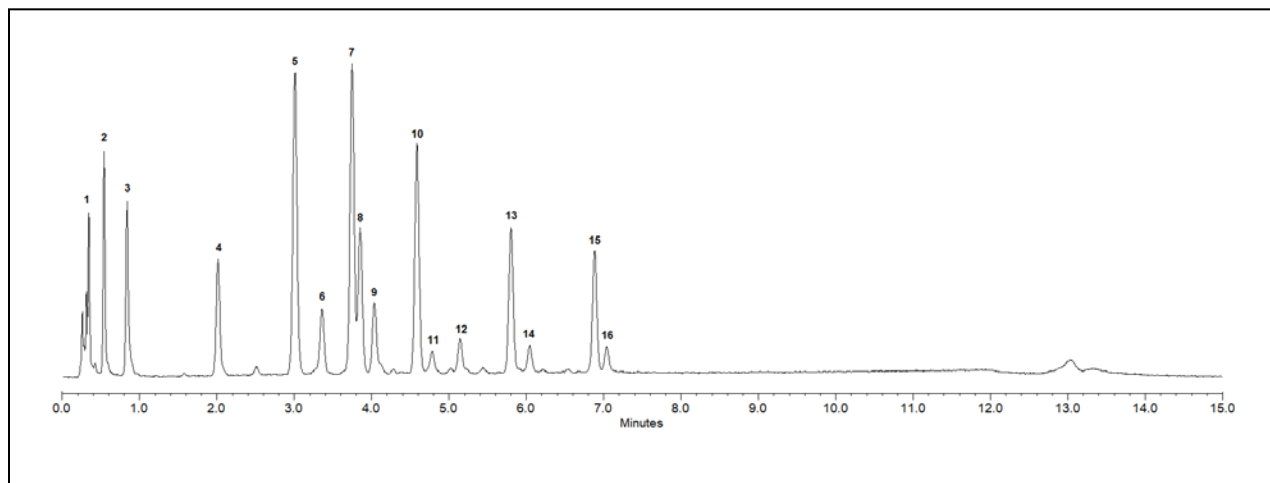
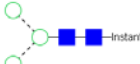
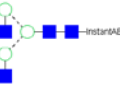
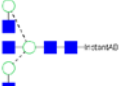
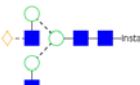
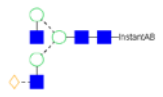
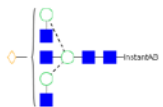
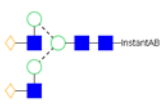
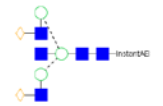
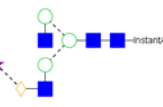
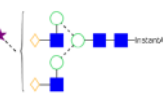
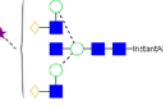
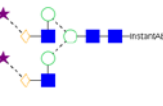
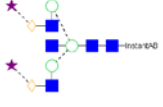


Figure 2 - ACQUITY UPLC Results for WS0331 - See Table 2 for peak ID.

Table 2- Peak Identification of InstantAB Afucosyl Biantennary Library (WS0331)

Peak Number	Glycan Identification	ProZyme	Common	Oxford (New)	Structure ^{1,2}
1	Free Dye (InstantAB)				
2	Chitobiose	N2	N/A	N/A	
3	4-β-mannosyl chitobiose	MNN	N/A	N/A	
4	Conserved trimannosyl core	M3N2	N/A	M3	
5	Asialo-, agalacto-biantennary	NGA2	G0	A2	
6	Asialo-, agalacto-biantennary with bisecting N-Acetylglucosamine	NGA2B	G0B	A2B	
7	Asialo-, mono-galacto-biantennary	NA2G1	G1[6]	A2[6]G(4)1	

Peak Number	Glycan Identification	ProZyme	Common	Oxford (New)	Structure ^{1,2}
8	Asialo-, mono-galacto-biantennary	NA2G1	G1[3]	A2[3]G(4)1	
9	Asialo-, mono-galactosylated biantennary with bisecting N-Acetylglucosamine	NA2G1B	G1B	A2BG(4)1	
10	Asialo-, galactosylated biantennary	NA2	G2	A2G(4)2	
11	Asialo-, galactosylated biantennary with bisecting N-Acetylglucosamine	NA2B	G2B	A2BG2	
12	Mono-sialylated, mono-galactosylated, biantennary	NA2G1S1	G1S1	A2[3]G(4)1S(6)1	
13	Mono-sialylated, galactosylated biantennary	A1	G2S1	A2G(4)2S(6)1	
14	Mono-sialylated, galactosylated biantennary with bisecting N-Acetylglucosamine	A1B	G2S1B	A2BG(4)2S(6)1	
15	Di-sialylated, galactosylated biantennary	A2	G2S2	A2G(4)2S(6)2	
16	Di-sialylated, galactosylated biantennary with bisecting N-Acetylglucosamine	A2B	G2S2B	A2BG(4)2S(6)2	

WS0332 InstantAB High Mannose Biantennary Library QC Results

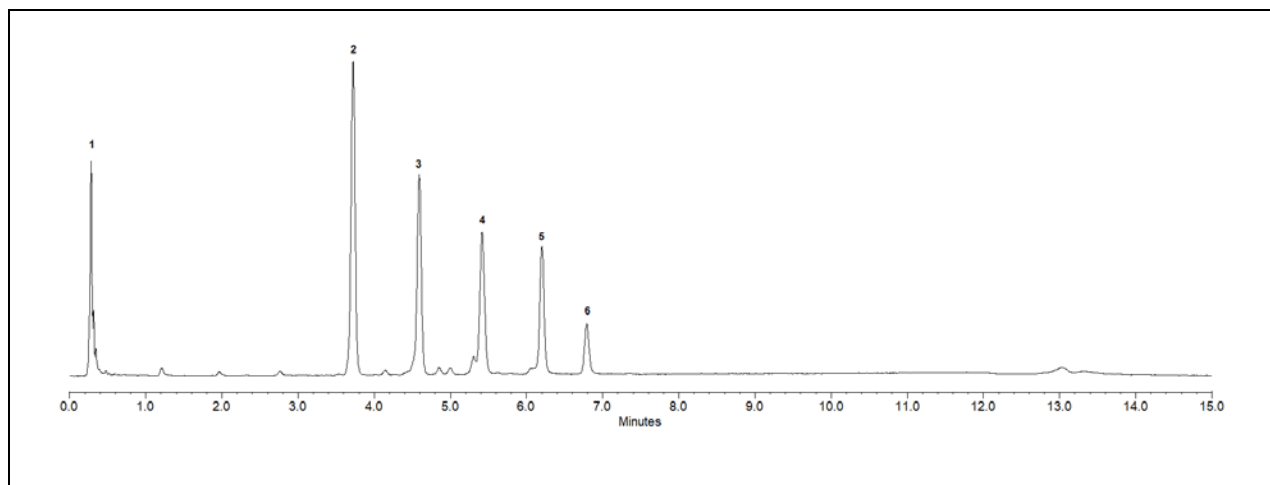


Figure 3 - ACQUITY UPLC Results of WS0332 - See Table 3 for Peak ID.

Table 3- Peak Identification of InstantAB High Mannose Biantennary Library (WS0332)

Peak Number	Glycan Identification	ProZyme	Common	Oxford (New)	Structure ^{1,2}
1	Free Dye (InstantAB)				
2	Oligomannose 5	Man5	Man5	M5	
3	Oligomannose 6	Man6	Man6	M6	
4	Oligomannose 7	Man7	Man7	M7	
5	Oligomannose 8	Man8	Man8	M8	
6	Oligomannose 9	Man9	Man9	M9	

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

Application: As a peak reference 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 InstantAB-labeled library standard injected on the ACQUITY UPLC BEH Glycan Column is typically 2–5 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.

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.

Authorized Signature