CERTIFICATE OF ANALYSIS

PRODUCT NAME:	GLYKO® INSTANTAB™ α(2-3) SIALYLATED BIANTENNARY LIBRARY
PRODUCT CODE:	GKIB-232
LOT NUMBER:	DP16G2001a
PACK SIZE:	70 pmol (qualitative standard for glycan identification)
FORM:	Dry solid
STORAGE:	Store at -20°C in the dark before and after reconstitution
EXPIRATION:	April 2022, may be used for 1 year after reconstitution
STRUCTURE:	The InstantAB α(2-3) Sialylated Biantennary Library contains α(2-3) sialylated N-glycans whose reducing termini are derivatized with the fluorescent dye, InstantAB. The α(2-3) sialic acid linkage is the type found on glycoproteins produced in Chinese hamster ovary (CHO) cells[1]. In contrast, human intravenous immunoglobulin (IVIG) IgG Fc N-glycans are predominantly α(2-6)-sialylated[2]. Depending on the separation method, it may be possible to resolve α(2-3) and α(2-6) sialic acid linkage isomers. For example, α(2-3)-sialylated N-glycans are known to have a shorter HILIC retention time than isomeric N-glycans with α(2-6) sialic acid linkages[3]. Sialic acid linkage position may also be determined by exoglycosidase digests with Sialidase S (GK80021), which releases non-reducing terminal α(2-3)-linked sialic acid, and Sialidase A™ (GK80040) which releases α(2-3,6,8,9)-linked sialic acid.

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Quality Control:

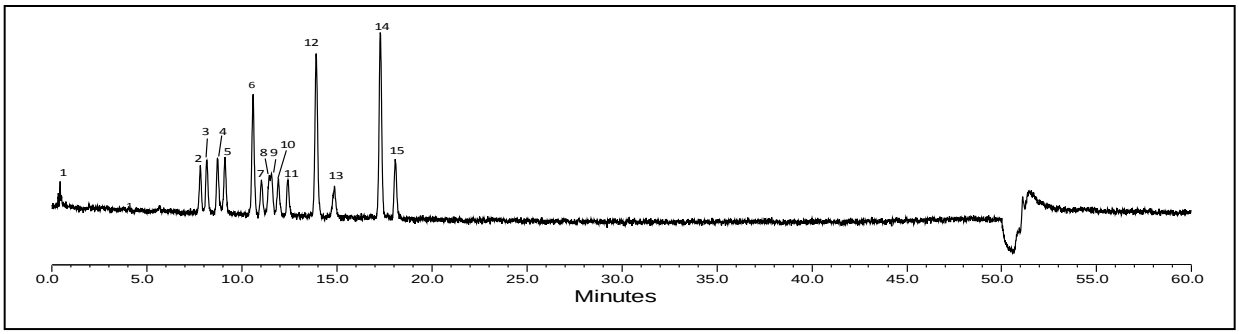
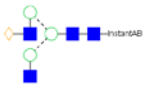
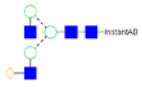
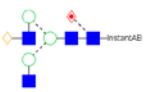


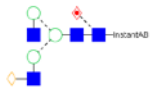
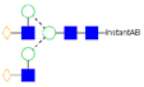
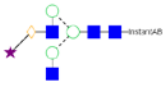
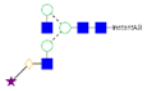
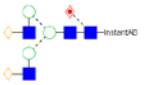
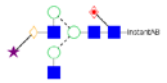
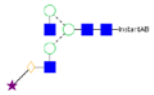
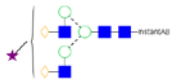
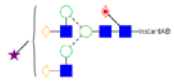
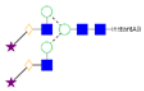
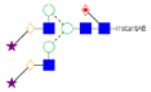
Figure 1 - **UPLC® Results**: 3 -6 pmol (1 µl, aqueous) of the InstantAB-labeled glycan library was injected on a Waters ACQUITY UPLC® H Class System utilizing a 60-minute method under the conditions below (see Table 1 for peak ID; the number of peaks observed depends on the running conditions employed):

Time (min)	Flow (ml/min)	%ACN	%Buffer
0.0	1.0	75.0	25.0
50.0	1.0	52.5	47.5
50.1	0.5	40.0	60.0
50.5	0.5	40.0	60.0
50.6	0.5	75.0	25.0
50.7	1.0	75.0	25.0
60.0	1.0	75.0	25.0

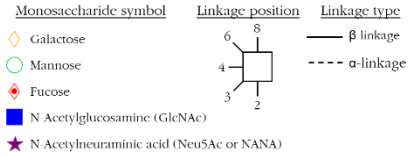
Column: Waters ACQUITY UPLC BEH Glycan Column (1.7 µm, 2.1 x 150 mm)
ACN: Acetonitrile
Buffer: 100 m*M* ammonium formate, pH 4.4
Flow rate: As stated in table, in ml/min
Temperature: 60° C
Max Pressure: 15,000 psi
Fluorescence Detection: λ_{ex} = 278 nm, λ_{em} = 344 nm

Table 1 - Peak Identification of InstantAB α(2-3) Sialylated Biantennary Library

Peak Number	Glycan Identification	ProZyme	Common	Oxford (New)	Structure[4,5]
1	Free Dye (InstantAB)				
2	Asialo, mono-galactosylated biantennary	NA2G1	G1[6]	A2[6]G(4)1	
3	Asialo, mono-galactosylated biantennary	NA2G1	G1[3]	A2[3]G(4)1	
4	Asialo, mono-galactosylated biantennary, core substituted with fucose	NA2G1F	G1F[6]	F(6)A2[6]G(4)1	

Peak Number	Glycan Identification	ProZyme	Common	Oxford (New)	Structure[4,5]
5	Asialo, mono-galactosylated biantennary, core substituted with fucose	NA2G1F	G1F[3]	F(6)A2[3]G(4)1	
6	Asialo, galactosylated biantennary	NA2	G2	A2G(4)2	
7	Mono-α(2-3)-sialylated, mono-galactosylated biantennary	NA2G1S1	G1[6]S1	A2[6]G(4)1S(3)1	
8	Mono-α(2-3)-sialylated, mono-galactosylated biantennary	NA2G1S1	G1[3]S1	A2[3]G(4)1S(3)1	
9	Asialo, galactosylated biantennary core substituted with fucose	NA2F	G2F	F(6)A2G(4)2	
10	Mono-α(2-3)-sialylated, mono-galactosylated biantennary, core substituted with fucose	NA2G1FS1	G1F[6]S1	F(6)A2[6]G(4)1S(3)1	
11	Mono-α(2-3)-sialylated, mono-galactosylated biantennary, core substituted with fucose	NA2G1FS1	G1F[3]S1	F(6)A2[3]G(4)1S(3)1	
12	Mono-α(2-3)-sialylated, galactosylated biantennary	A1	G2S1	A2G(4)2S(3)1	
13	Mono-α(2-3)-sialylated, galactosylated biantennary, core substituted with fucose	A1F	G2FS1	F(6)A2G(4)2S(3)1	
14	Di-α(2-3)-sialylated, galactosylated biantennary	A2	G2S2	A2G(4)2S(3)2	
15	Di-α(2-3)-sialylated, galactosylated biantennary, core substituted with fucose	A2F	G2FS2	F(6)A2G(4)2S(3)2	

Structure Key[4,5]:



Structural Analysis: The purity and structural integrity of the glycan library was assessed by UPLC[6] (as described above) and MALDI-TOF[7,8], ESI-MS or LC-MS. Agreement was found between the results from mass spectrometry and UPLC.

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 InstantAB-labeled library standard injected on a UPLC column is typically 3 -6 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. For suggested methods see Rapid UPLC Methods for Screening Labeled N-Glycans at:

www.prozyme.com/protocols/

REFERENCES

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