CERTIFICATE OF ANALYSIS

PRODUCT NAME:	GLYKO® 2AB-VERIFICATION STANDARD
PRODUCT CODE:	GKSB-965
LOT NUMBER:	DP15I2201a
PACK SIZE:	1 each (2-AB glycans from 50 µg of Human IgG)
FORM:	Dry solid
STORAGE:	Store at -20°C in the dark before and after reconstitution
EXPIRATION:	January 2022, may be used for 1 year after reconstitution
PACKAGED WITH:	2 x 0.5 mg vial WS0162 Human IgG Glycoprotein lot W160269b, Expiration: Nov 2021
STRUCTURE:	The 2-AB-Verification Standard consists of a library of Human IgG N-linked glycans whose reducing termini are derivatized with the fluorescent dye, 2-AB (2-aminobenzamide). The labeled glycans were released using GlykoPrep® from the same lot of material that is packaged in the Human IgG Glycoprotein control vials (WS0162) included in the packaging. The library is intended for use as a verification standard for the GlykoPrep platform.

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Quality Control:

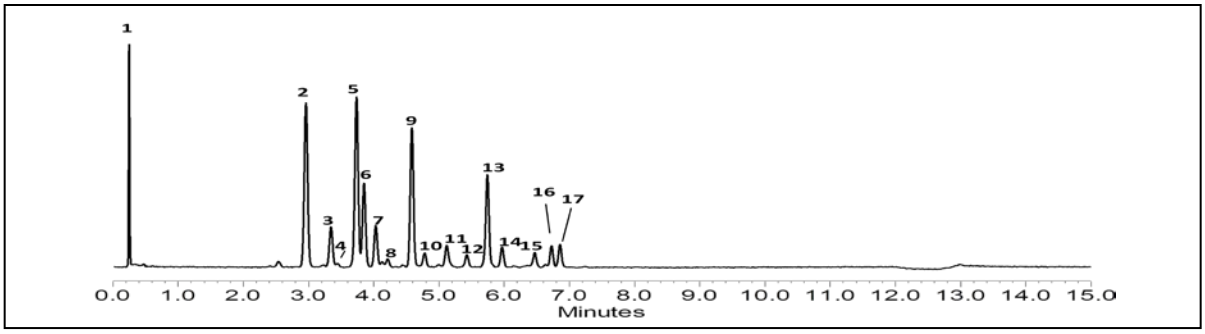
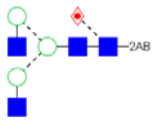
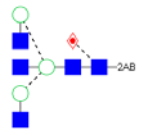
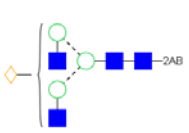


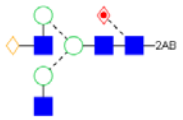
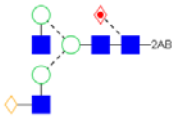
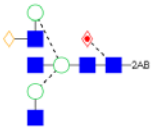
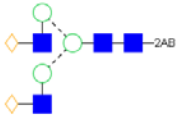
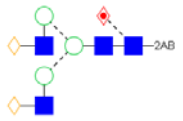
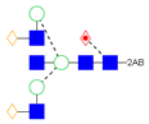
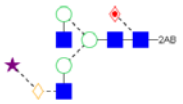
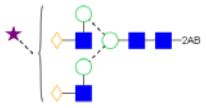
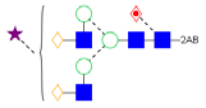
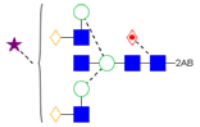
Figure 1 - **UPLC® Results**: 10 - 20 pmol (1 µl, aqueous) of the 2-AB-labeled library was injected on a Waters ACQUITY UPLC® H Class System utilizing a 15-minute method under the conditions below:

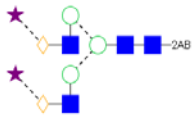
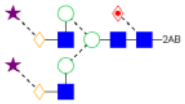
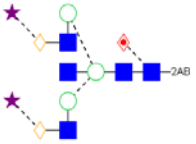
Time (min)	Flow (ml/min)	%ACN	%Buffer
00.0	1.0	75.0	25.0
12.0	1.0	52.5	47.5
12.1	0.5	40.0	60.0
12.5	0.5	40.0	60.0
12.6	0.5	75.0	25.0
12.7	1.0	75.0	25.0
15.0	1.0	75.0	25.0

Column: Waters ACQUITY UPLC BEH Glycan Column (1.7 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} = 330 nm, λ_{em} = 420 nm

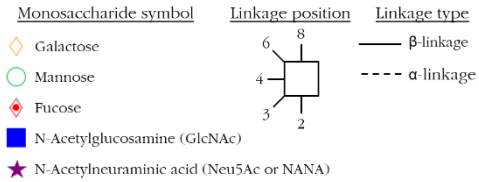
Table 1 - Peak Identification of 2-AB Verification Standard

Peak Number	Glycan Identification	ProZyme	Common	Oxford (New)	Structure [1,2]
1	Free Dye (2-AB)				
2	Asialo, agalactosylated biantennary, core substituted with fucose	NGA2F	G0F	F(6)A2	
3	Asialo, agalactosylated biantennary, core substituted with fucose and bisecting N-Acetylglucosamine	NGA2FB	G0FB	F(6)A2B	
4	Asialo, mono-galactosylated biantennary	NA2G1	G1	A2G(4)1	

Peak Number	Glycan Identification	ProZyme	Common	Oxford (New)	Structure [1,2]
5	Asialo, mono-galactosylated biantennary, core substituted with fucose	NA2G1F	G1F[6]	F(6)A2[6]G(4)1	
6	Asialo, mono-galactosylated biantennary, core substituted with fucose	NA2G1F	G1F[3]	F(6)A2[3]G(4)1	
7	Asialo, mono-galactosylated biantennary, core substituted with fucose and bisecting N-Acetylglucosamine	NA2G1FB	G1FB	F(6)A2BG1	
8	Asialo, galactosylated biantennary	NA2	G2	A2G(4)2	
9	Asialo, galactosylated biantennary core substituted with fucose	NA2F	G2F	F(6)A2G(4)2	
10	Asialo, galactosylated biantennary core substituted with fucose and bisecting N-Acetylglucosamine	NA2FB	G2FB	F(6)A2BG(4)2	
11	Mono-sialylated, mono-galactosylated biantennary, core substituted with fucose	NA2G1FS1	G1FS1	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, core substituted with fucose	A1F	G2FS1	F(6)A2G(4)2S(6)1	
14	Mono-sialylated, galactosylated biantennary, core substituted with fucose and bisecting N-Acetylglucosamine	A1FB	G2FBS1	F(6)A2BG2S(6)1	

Peak Number	Glycan Identification	ProZyme	Common	Oxford (New)	Structure [1,2]
15	Di-sialylated, galactosylated biantennary	A2	G2S2	A2G(4)2S(6)2	
16	Di-sialylated, galactosylated biantennary, core substituted with fucose	A2F	G2FS2	F(6)A2G(4)2S(6)2	
17	Di-sialylated, galactosylated biantennary, core substituted with fucose and bisecting N-Acetylglucosamine	A2FB	G2FBS2	F(6)A2BG(4)2S(6)2	

Structure Key[1,2]:



Preparation: Utilizing the GlykoPrep platform, Human IgG was digested and the released N-linked oligosaccharides labeled with 2-AB and cleaned up to remove excess labeling reagent. Each vial contains the 2-AB glycans released from 50 µg of Human IgG which is approximately 300 - 600 pmol of labeled glycans.

The WS0162 Human IgG control vials included in the package were dispensed and dried from the same source lot of Human IgG as the labeled glycan library.

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

Application: Verification of the GlykoPrep Sample Preparation Platform.

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, GKSB-965 2-AB-Labeled Glycan Library:

Dissolve the GKSB-965 vial in the same volume eluted from one CU cartridge when processing the WS0162 human IgG with GlykoPrep/2-AB (typically ~25 - 50 µl). The amount of 2-AB-labeled library standard injected on a UPLC column is typically 6 - 25 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 (TNGP101) available at:

www.prozyme.com/tech_notes.html

Directions For Use, WS0162 Human IgG: To obtain a 1 mg/ml solution of human IgG, redissolve a vial of WS0162 in 0.5 ml of water (or aqueous buffer). Transfer 50 µg (50 µl) of the solution to a clean vial and add 50 µl of WS0226 Denaturation Reagent. Incubate for 5 minutes and proceed with digestion step as described in the GlykoPrep protocol.

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. James DC and 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 (1997).

4. Papac DI, Wong A and 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.

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