CERTIFICATE OF ANALYSIS

PRODUCT NAME:	GLYKO® HUMAN α1-ACID GLYCOPROTEIN N-LINKED GLYCAN LIBRARY
PRODUCT CODE:	GKLB-001
LOT NUMBER:	DP06C0901
PACK SIZE:	20 µg (qualitative standard for glycan identification)
PACKED WITH:	0.5 mg Human α_1-Acid Glycoprotein (WS0173, lot W060030), source for the glycan library
FORM:	Glycan Library: dry solid Human α_1-acid glycoprotein: dry solid
STORAGE:	Store at -20°C in the dark before and after reconstitution
EXPIRATION:	August 2021, may be used for 1 year after reconstitution (extended from prior exp. date based on re-assay)
RE-ASSAY DATE:	August 2016
STRUCTURE :	Human α_1-acid glycoprotein is heavily glycosylated (~45% carbohydrates)[1] and contains five N-glycosylation sites. The Human α_1-acid Glycoprotein N-linked Glycan Library represents a total pool of N-linked glycans released from human α_1-acid glycoprotein; constituting a heterogenous mixture of core non-fucosylated bi-, tri- and tetraantennary glycans with various degree of sialylation (NeuAc) and some with outer arm fucose residues and lactosamine repeats, consistent with N-glycans previously reported for human α_1-acid glycoprotein.[2,3] The biantennary glycans can have one or two sialic acid residues. The triantennary and tetraantennary glycans can have from one to four sialic acid residues and also may be substituted with fucose which results in formation of sialyl Lewis X-like structure: {NeuAc (α2-3) Gal (β1-4) [Fuc (α1-3)] GlcNAc-} and/or lactosamine repeat(s): Gal (β1-4) GlcNAc. The sialic acid residues are found both in α(2-3) and α(2-6) linkages.[4] The composition of the library is approximately as follows: mono-sialylated glycans ~1%, di-sialylated ~20%, tri-sialylated ~51% and tetra-sialylated ~27%.

3832 Bay Center Place
Hayward, CA 94545-3619

TOLL FREE (800) 457-9444
PHONE (510) 638-6900
FAX (510) 638-6919

info@prozyme.com
www.prozyme.com

Quality Control:

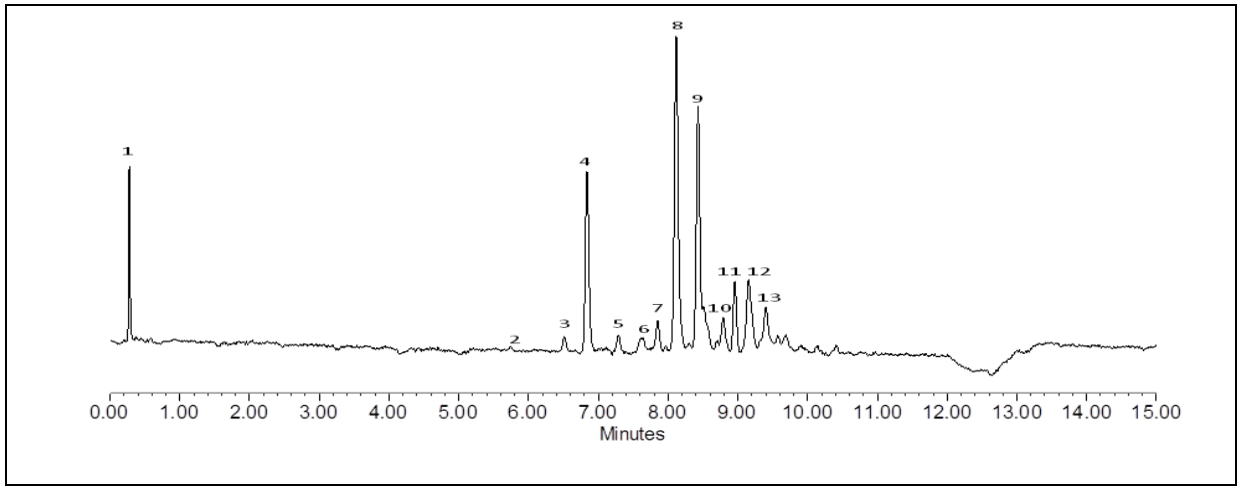
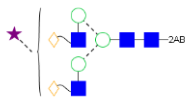
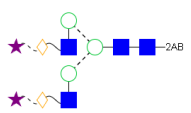


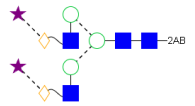
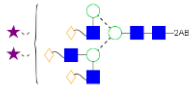
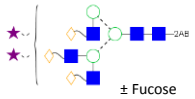
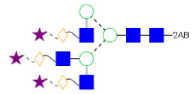
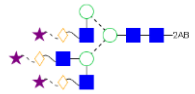
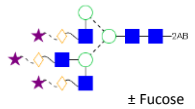
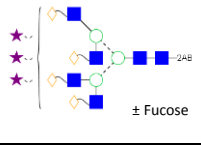
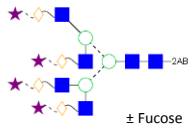
Figure 1 - **UPLC® Results**: 6 - 9 pmol (1 µl, aqueous) of the 2-AB-labeled glycan library was injected on a Waters ACQUITY UPLC® H Class System utilizing a 15-minute method under the conditions below:

Time (min)	Flow	%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

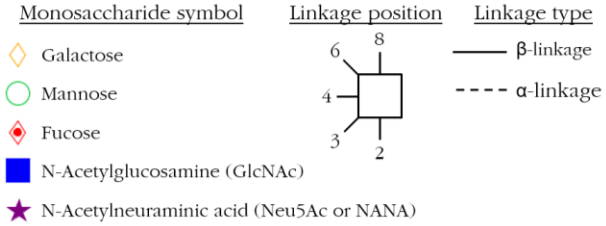
Column: Waters ACQUITY UPLC BEH Glycan Column (1.7 µm, 2.1 x 100 mm)
ACN: Acetonitrile
Buffer: 100 m*M* ammonium formate, pH 4.4
Flow rate: As stated in table, in ml/min
Temperature: 60° C
Max Pressure: 15,000 psi
Fluorescence Detection: λ_{ex} = 330 nm, λ_{em} = 420 nm

Table 1 - Peak Identification of 2-AB Labeled Human α1-Acid Glycoprotein N-Linked Glycan Library.

Peak Number	Glycan Identification	ProZyme	Common	Oxford (New)	Structure[5,6]
1	Free Dye (2-AB)				
2	Mono-α(2-6)-sialylated, galactosylated biantennary	A1	G2S1	A2G2S(6)1	
3	Di-α(2-3, 2-6)-sialylated, galactosylated biantennary	A2	G2S2	A2G2S(3,6)2	

Peak Number	Glycan Identification	ProZyme	Common	Oxford (New)	Structure[5,6]
4	Di-α(2-6)-sialylated, galactosylated biantennary	A2	G2S2	A2G2S(6)2	
5	Di-α-sialylated, galactosylated triantennary	NA3S2	A3G3S2	A3G3S2	
6	Di-α-sialylated, galactosylated triantennary and di-α-sialylated, galactosylated triantennary with fucose	NA3S2 ± Fuc	A3G3S2 ± Fuc	A3G3S2 ± Fuc	
7	Tri-α(2-3, 2-3, 2-6)-sialylated, galactosylated triantennary	A3	A3G3S3	A3G3S(3,3,6)3	
8	Tri-α(2-3, 2-6, 2-6)-sialylated, galactosylated triantennary	A3	A3G3S3	A3G3S(3,6,6)3	
9	Tri-α(2-6)-sialylated, galactosylated triantennary and tri-α-sialylated, galactosylated triantennary with fucose	A3 ± Fuc	A3G3S3 ± Fuc	A3G3S3 ± Fuc	
10 + 11 + 12 + 13	Tri-α-sialylated, galactosylated tetrantennary, tri-α-sialylated, galactosylated tetrantennary with fucose, tetra-α-sialylated, galactosylated tetrantennary and tetra-α-sialylated, galactosylated tetrantennary with fucose	NA4S3 + A4 ± Fuc	A4G4S3 + A4G4S4 ± Fuc	A4G4S3 + A4G4S4 ± Fuc	 

Structure Key[5,6]:



Preparation: Human α_1-acid glycoprotein was digested with N-Glycanase® Plus (ProZyme product code GKE-5010). Released N-linked oligosaccharides were then labeled with 2-AB by reductive amination and purified from excess labeling reagents.[7,8]

Structural Analysis: The purity and structural integrity of the glycan library was assessed by UPLC[9,10] (GU values) and MALDI-TOF mass spectrometry[11,12] or LC-MS. Good agreement was found between the results from mass spectrometry and UPLC.

Application: Confirmation of identification of specific N-glycans by UPLC analysis for samples prepared on the GlykoPrep® Sample Preparation Platform.

Handling & Reconstitution:
The oligosaccharide 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 UPLC 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 your mobile phase. For suggested methods see Rapid UPLC Methods for Screening Labeled N-Glycans at:

www.prozyme.com/protocols/

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