CERTIFICATE OF ANALYSIS

PRODUCT NAME:	GLYKO® INSTANTPC™ α(2-6) SIALYLATED TRIANTENNARY LIBRARY
PRODUCT CODE:	GKPC-263
LOT NUMBER:	DP17I0804a
PACK SIZE:	~25 injections (qualitative standard for glycan identification)
FORM:	Dry solid
STORAGE:	Store at -20°C in the dark before and after reconstitution
EXPIRATION:	May 2019 May be used for 6 months after reconstitution in 100 m*M* ammonium formate, pH 4.4 – 5.0, or for 1 month after reconstitution in water.
STRUCTURE:	The InstantPC α(2-6) Sialylated Triantennary Library contains α(2-6) sialylated N-glycans whose reducing termini are derivatized with the fluorescent dye, InstantPC. The α(2-6) sialic acid linkage is the predominant linkage type on human intravenous immunoglobulin (IVIG) IgG Fc N-glycans[1]. This differs from glycoproteins produced in Chinese hamster ovary (CHO) cells, where N-glycans are α(2-3)-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 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.

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 :

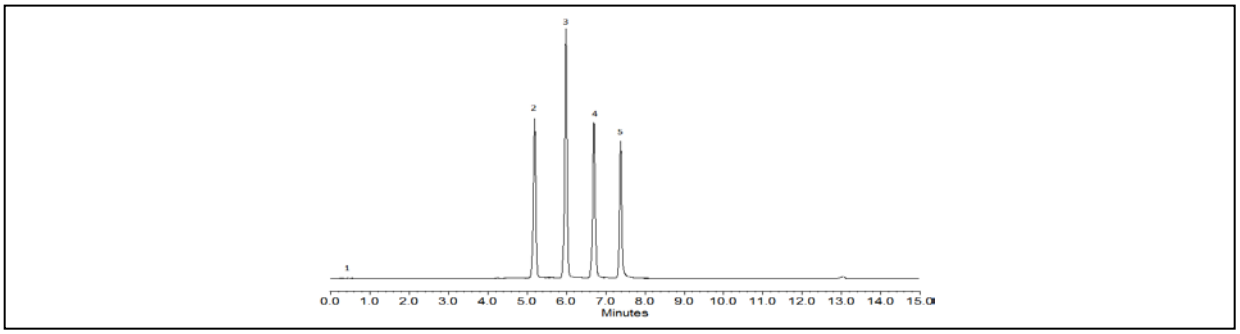
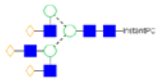
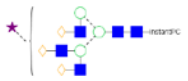
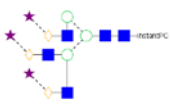


Figure 1 - **UPLC® Results**: 1 µl (aqueous) of the InstantPC-labeled glycan library was injected on a Waters ACQUITY UPLC® H Class System utilizing a 15-minute method under the conditions below (see Table 1 for peak ID; the number of peaks observed depends on the running conditions employed):

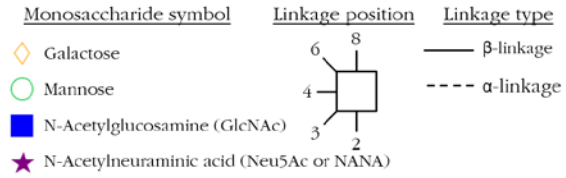
Time (min)	Flow (ml/min)	%ACN	%Buffer
0.0	1.0	75.0	25.0
12.0	1.0	50.0	50.0
12.1	0.5	40.0	60.0
12.5	0.5	40.0	60.0
12.6	0.5	75.0	25.0
13.0	1.0	75.0	25.0
15.0	1.0	75.0	25.0

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} = 285 nm, λ_{em} = 345 nm

Table 1 - Peak Identification of InstantPC α(2-6) Sialylated Triantennary Library

Peak Number	Glycan Identification	ProZyme	Oxford (New)	Structure[4,5]
1	Free Dye (InstantPC)			
2	Asialo, galactosylated triantennary	NA3	A3G(4)3	
3	Mono-α(2-6)-sialylated, galactosylated triantennary	NA3S1	A3G(4)3S(6)1	
4	Di-α(2-6)-sialylated, galactosylated triantennary	NA3S2	A3G(4)3S(6)2	
5	Tri-α(2-6)-sialylated, galactosylated triantennary	A3	A3G(4)3S(6)3	

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 InstantPC-labeled glycan standard injected on a UPLC column is typically 1 µl. For our Quality Control testing, one vial was dissolved in 30 µl of water and 1 µl injected on the ACQUITY BEH Glycan column.

We suggest reconstituting with 100 m*M* ammonium formate, pH 4.4 – 5.0 for storage at -20° C for up to 6 months. This buffer is often used as a HILIC mobile phase. Water may also be used for reconstitution, but the recommended storage period is shorter, -20° C for up to 1 month.

For larger injection volumes of InstantPC-labeled glycans (> 1 µl), do not use ACN alone to dilute the glycan to match the high organic % at the start of HILIC methods, as this may cause sialylated InstantPC glycans to precipitate. use 1 part glycan in ammonium formate or water to 3 parts 1:1 [v/v] ACN:DMF, for a final concentration of 25% aqueous buffer, 37.5 % DMF, 37.5% ACN. Dilute only as much as is needed, and freeze the main stock at -20° C. For example, for a 10 µl injection, dilute 5 µl of glycan stock in ammonium formate or water with 15 µl 1:1 [v/v] ACN:DMF.

For further information on LC and LC-MS methods for InstantPC-labeled glycans, please contact ProZyme:

info@prozyme.com

REFERENCES

1. Anthony RM, Nimmerjahn F, Ashline DJ, Reinhold VN, Paulson JC, Ravetch JV. A recombinant IgG Fc that recapitulates the anti-inflammatory activity of IGIV. Science 2008 Apr 18; 320(5874): 373–376.

2. Lee EU, Roth J, Paulson JC. Alteration of terminal glycosylation sequences on N-Linked oligosaccharides of Chinese hamster ovary cells by expression of β-Galactoside α2,6-Sialyltransferase. J Biol Chem. 1989 August 15; 264(23): 13848-13855.

3. Raymond C, Robotham A, Spearman M, Butler M, Kelly J, Durocher Y. Production of α2,6-sialylated IgG1 in CHO cells. mAbs 2015 May/June; 7(3): 571-583.4.

4. 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.

5. 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.

6. Ahn J, Bones J, Yu YQ, Rudd PM, Gilar M. Separation of 2-aminobenzamide labeled glycans using hydrophilic interaction chromatography columns packed with 1.7 microm sorbent. J Chromatogr B Analyt Technol Biomed Life Sci. 2010 Feb 1; 878(3-4): 403-8

7. James DC, 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.

8. Papac DI, Wong A, 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.

Digitally signed by Shiva Pourkaveh
Date: 2018.03.12 13:25:42 -07'00'

Authorized Signature