



## CERTIFICATE OF ANALYSIS

PRODUCT NAME: GLYKO® INSTANTAB™  $\alpha(2-3)$  SIALYLATED TRIANTENNARY LIBRARY

PRODUCT CODE: GKIB-233

LOT NUMBER: DP16G2002a

PACK SIZE: 70 pmol (qualitative standard for glycan identification)

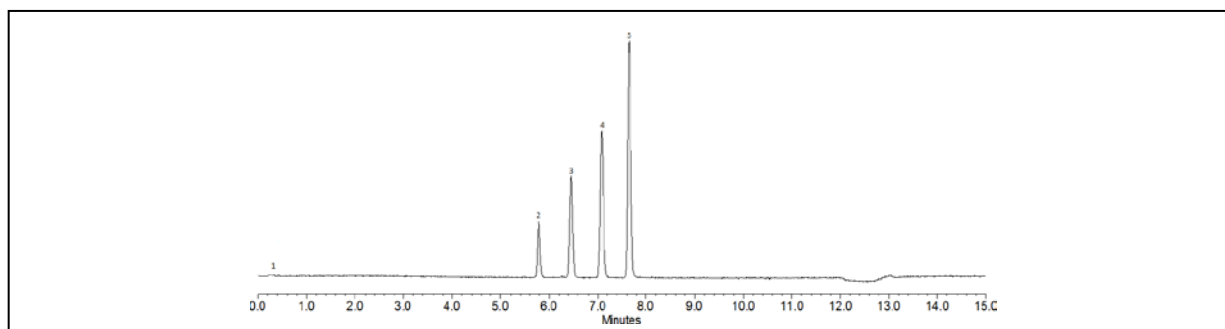
FORM: Dry solid

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

EXPIRATION: May 2022, may be used for 1 year after reconstitution

STRUCTURE: The InstantAB  $\alpha(2-3)$  Sialylated Triantennary Library contains  $\alpha(2-3)$  sialylated N-glycans whose reducing termini are derivatized with the fluorescent dye, InstantAB. The  $\alpha(2-3)$  sialic acid linkage is the type found on glycoproteins produced in Chinese hamster ovary (CHO) cells<sup>1</sup>. In contrast, human intravenous immunoglobulin (IVIG) IgG Fc N-glycans are predominantly  $\alpha(2-6)$ -sialylated<sup>2</sup>. Depending on the separation method, it may be possible to resolve  $\alpha(2-3)$  and  $\alpha(2-6)$  sialic acid linkage isomers. For example,  $\alpha(2-3)$ -sialylated N-glycans are known to have a shorter HILIC retention time than isomeric N-glycans with  $\alpha(2-6)$  sialic acid linkages<sup>3</sup>. Sialic acid linkage position may also be determined by exoglycosidase digests with Sialidase S (GK80021), which releases non-reducing terminal  $\alpha(2-3)$ -linked sialic acid, and Sialidase A™ (GK80040) which releases  $\alpha(2-3,6,8,9)$ -linked sialic acid.

## 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 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	0.5	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 mM ammonium formate, pH 4.4

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

Temperature: 60° C

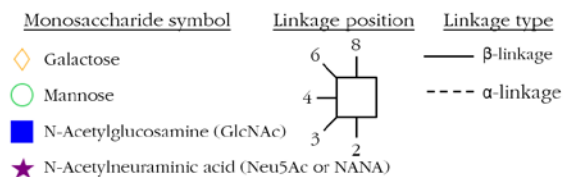
Max Pressure: 15,000 psi

Fluorescence Detection:  $\lambda_{\text{ex}} = 278 \text{ nm}$ ,  $\lambda_{\text{em}} = 344 \text{ nm}$

**Table 1 - Peak Identification of InstantAB  $\alpha$ (2-3) Sialylated Triantennary Library**

Peak Number	Glycan Identification	ProZyme	Oxford (New)	Structure <sup>4,5</sup>
1	Free Dye (InstantAB)			
2	Asialo, galactosylated triantennary	NA3	A3G(4)3	
3	Mono- $\alpha$ (2-3)-sialylated, galactosylated triantennary	NA3S1	A3G(4)3S(3)1	
4	Di- $\alpha$ (2-3)-sialylated, galactosylated triantennary	NA3S2	A3G(4)3S(3)2	
5	Tri- $\alpha$ (2-3)-sialylated, galactosylated triantennary	A3	A3G(4)3S(3)3	

## Structure Key<sup>4,5</sup>:



**Structural Analysis:** The purity and structural integrity of the glycan library was assessed by UPLC<sup>6</sup> (as described above) and MALDI-TOF<sup>7,8</sup>, 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 oligosaccharide 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  $\mu$ l of water and 1  $\mu$ l 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/](http://www.prozyme.com/protocols/)

## REFERENCES

1. Lee EU, Roth J, Paulson JC. Alteration of terminal glycosylation sequences on N-Linked oligosaccharides of Chinese hamster ovary cells by expression of  $\beta$ -Galactoside  $\alpha$ 2,6-Sialyltransferase. *J Biol Chem.* 1989 August 15; 264(23): 13848-13855.
2. 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.
3. Raymond C, Robotham A, Spearman M, Butler M, Kelly J, Durocher Y. Production of  $\alpha$ 2,6-sialylated IgG1 in CHO cells. *mAbs* 2015 May/June; 7(3): 571-583.
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.

---

Authorized Signature