CERTIFICATE OF ANALYSIS

PRODUCT NAME:	GLYKO® 2-AB-(HUMAN IgG N-LINKED GLYCAN LIBRARY)
PRODUCT CODE:	GKSB-005
LOT NUMBER:	DP10K0802
PACK SIZE:	200 pmol (qualitative standard for glycan identification)
FORM:	Dry solid
STORAGE:	Store in the dark at -20°C before and after reconstitution
EXPIRATION:	April 2016, may be used for 1 year after reconstitution
STRUCTURE:	The Human IgG N-Linked Glycan Library (ProZyme product code GKLB-005) consists of complex biantennary oligosaccharides consistent with N-glycans on normal human IgGs[1,2,3] (see Table 1). The reducing termini are derivatized with the fluorescent dye, 2-AB (2-aminobenzamide).

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Quality Control:

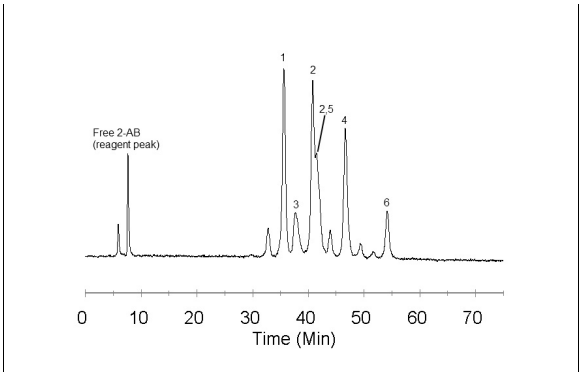

Figure 1 - HPLC results: Human IgG N-linked glycan library labeled according to the Signal™ 2-AB Labeling Kit (GKK-404), analyzed on a GlycoSep™ N column (GKI-4728) in ammonium formate/acetonitrile. Peak numbers refer to glycans identified by MALDI-TOF of the parent lot (Table 1).

Preparation: Human polyclonal IgG (containing >99% IgGs; IgG1 being at least 70% of the total IgG content, balance is IgG2 and IgG3)[1,5] was digested with N-Glycanase® Plus (ProZyme product code GKE-5010). Released N-linked oligosaccharides were purified using size-exclusion chromatography. Glycans were quantified using the phenol-sulfuric acid method.[6] The library was then labeled with 2-amino-benzamide (2-AB) by reductive amination[3] and purified from excess labeling reagents.

Structural Analysis: The purity and structural integrity of the glycan library was assessed by one or more of the following techniques: normal-phase HPLC on a GlycoSep™ N column[7], MALDI-TOF mass spectrometry[8,9] and FACE™[10] (detailed procedures and results are available upon request). Good agreement was found between the results from MALDI-MS and HPLC.

Applications: For calibration and validation of Glyko® GlycoSep HPLC columns.[4] These include the GlycoSep N column (Product Code GKI-4728) and GlycoSep C column (Product Code GKI-4721).

Handling: The labeled oligosaccharide is shipped as a dried solid. 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 water or buffer, re-cap and mix thoroughly to redissolve all the oligosaccharide. For maximal recovery, ensure that the cap lining is also rinsed and 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; high temperatures or low pH will cause desialylation.

Reconstitution: Use HPLC-grade water or an aqueous buffer to dissolve the glycan library. Store the reconstituted glycan library at -20°C in working aliquots. Avoid multiple freeze/thaw cycles.

Directions For Use: Chromatography running conditions for GlycoSep HPLC columns are given in the protocol manuals shipped with the columns. The amount of 2-AB-labeled standard injected on a GlycoSep column is typically around 20 pmol (*e.g.*, reconstitute the contents of the vial in 50 µl of water, then take 5 µl and adjust to the desired column buffer and load volume).

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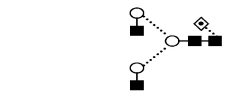
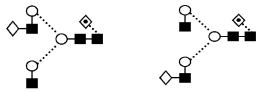
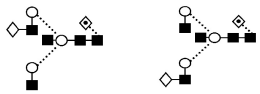
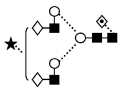
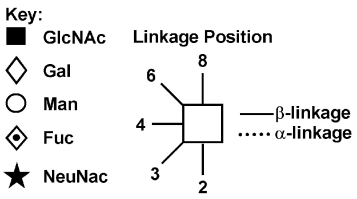
Table 1 - Human IgG N-Linked Glycan Library (Positive mode MALDI-TOF was run on parent lot to confirm masses of glycans corresponding to peaks with intensity of at least 5%).

Peak	Detected Mass*	Calc Mass*	Assigned glycan structure†	
1	1485.8	1485.5	G0F (NGA2F)	
2	1647.8	1647.6	G1F (NA2G1F)	
3	1688.8	1688.6	G0FB (NGA2FB)	
4	1809.8	1809.6	G2F (NA2F)	
5	1850.8	1850.7	G1FB (NA2G1FB)	
6	N/A**	2100.7	G2FS1 (A1F)	

* Monoisotopic mass $[M + Na]^+$ of the glycan

† Schematic representation of glycans from Rudd *et al.*[5]

** Sialylated glycans are not detected in positive mode MALDI-TOF



LICENSE TO USE

Purchase of the Signal 2-AB Labeling Kit or 2-AB-labeled standards from ProZyme or its authorized distributors grants a Use Sublicense under Glyko's® 2-AB patents. By accepting delivery of the 2-AB Kit or labeled standards [Material(s)] and by subsequently using them in glycan analysis, Recipient agrees to be bound by the following terms and restrictions:

1. A Use Sublicense is granted Recipient for in-house use of Material(s) only.

2. The Material(s) will not be made available by Recipient to any outside parties in any form, separately or in combinations, for any monetary or other consideration or at no charge, except that the Materials may be made available to outside parties who agree to be bound by all the terms and restrictions of this Agreement for purposes of evaluation only.

3. Recipient will not make commercial use of the Material(s) unless it first secures a license agreement from ProZyme, Inc. for such commercial use.

4. Recipient is solely responsible for qualification of the products for the Recipient's specific use.

5. The Material(s) will not be used *in vivo* in humans.

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