CERTIFICATE OF ANALYSIS

PRODUCT NAME:	GLYKO® HUMAN α_1-ACID GLYCOPROTEIN N-LINKED GLYCAN LIBRARY
PRODUCT CODE:	GKLB-001
LOT NUMBER:	DP06C0901
PACK SIZE:	20 µg Glycan Library (based on hexose content, not a quantitative standard)
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 before and after reconstitution
EXPIRATION:	September 2016, may be used for 1 year after reconstitution (extended from prior exp. date based on re-assay)
RE-ASSAY DATE:	September 2011
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 as shown with an asterisk (*) in Figure 1 on the following page. 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%. Triantennary glycans are a major component and constitute ~44% of total glycans present in the Human α_1-acid Glycoprotein N-linked Glycan Library.

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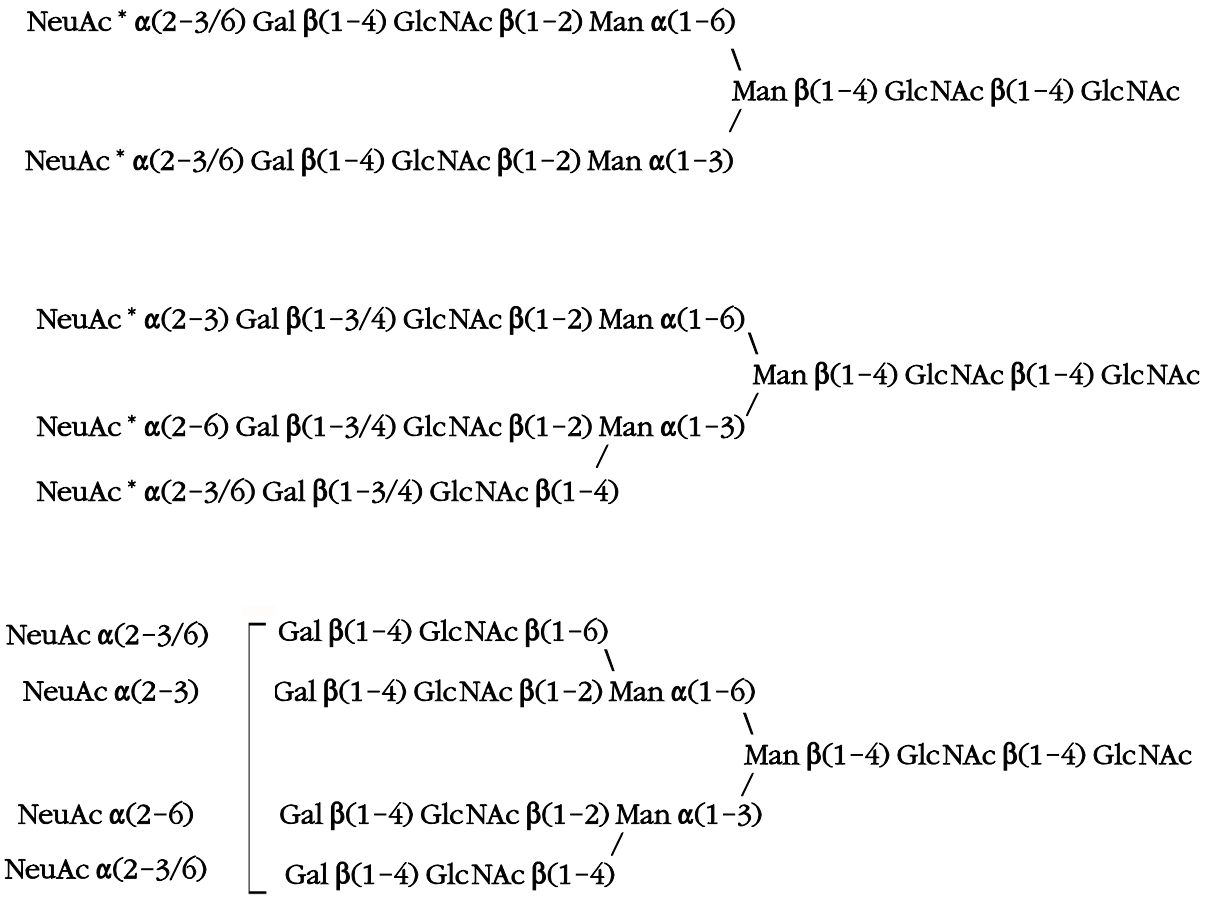
Figure 1



QUALITY CONTROL:

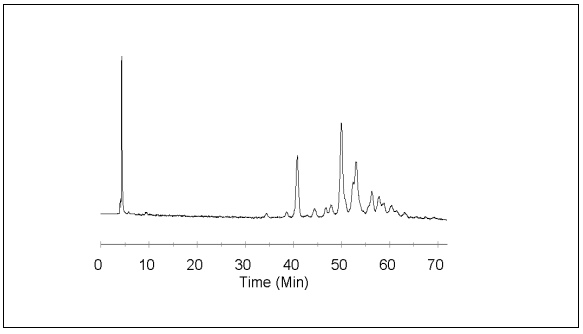


Figure 2 - Normal phase HPLC of 2-AB-labeled Human α_1-acid Glycoprotein N-linked Glycan Library.

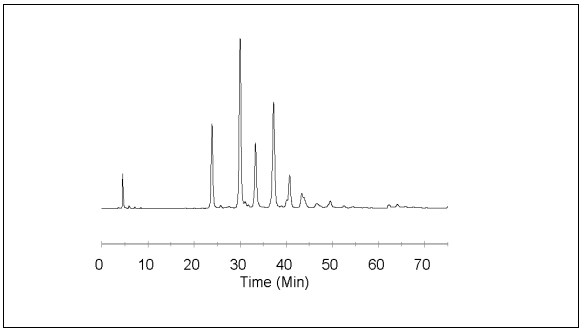


Figure 3 - Normal phase HPLC of 2-AB-labeled desialylated Human α_1-acid Glycoprotein N-linked Glycan Library.

Isolation: Human α_1-acid glycoprotein was digested with N-Glycanase® Plus (ProZyme product code GKE-5010) under denaturing conditions. Purified N-glycans were quantified using the resorcinol-sulfuric acid method.[5]

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[6,7], MALDI-TOF mass spectrometry[8] and FACE[9] (detailed procedures and results are available upon request). Good agreement was found between the results from MALDI-MS and HPLC.

The glycan library was also subjected to enzymatic desialylation by Sialidase A™ (ProZyme product code GK80040) and analyzed by normal-phase HPLC on a GlycoSep™ N Column[4] and MALDI-TOF mass spectrometry[5] to confirm the identity of the underlying neutral glycans.

Applications:

- qualitative standard for various analytical procedures
- radio-labeling, fluorescent-labeling or formation of a variety of oligosaccharide derivatives
- substrate for glycosidase and glycosyl transferase assays

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

Handling: The oligosaccharide library 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 which come into contact with the glycan are free of glycosidases and carbohydrate contaminants.

Minimize exposure to elevated temperatures or extremes of pH; high temperatures or low pH will cause desialylation. High pH will cause epimerization of the reducing terminal GlcNAc.

REFERENCES

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