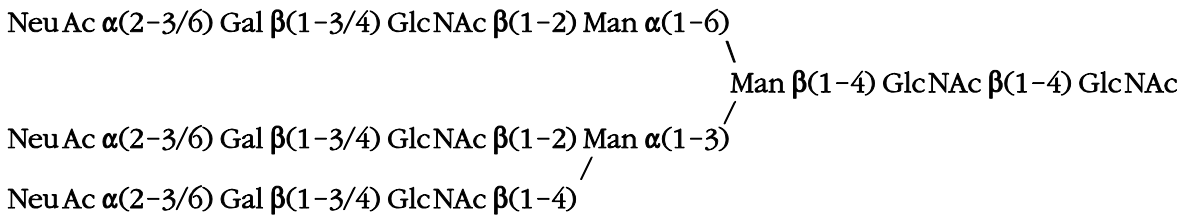
CERTIFICATE OF ANALYSIS

PRODUCT NAME:	GLYKO® TRI-SIALYLATED, GALACTOSYLATED, TRIANTENNARY COMPLEX N-GLYCAN (A3)
PRODUCT CODE:	GKC-335300
LOT NUMBER:	DP12K2701a
PACK SIZE:	10 µg (qualitative standard for glycan identification)
PURITY:	≥90% of glycan by HPLC
FORM:	Dry solid
STORAGE:	Store at -20°C before and after reconstitution
EXPIRATION:	August 2018, may be used for 1 year after reconstitution

STRUCTURE:



MOLECULAR WEIGHT: 2880.6 (average)[1]

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Quality Control:

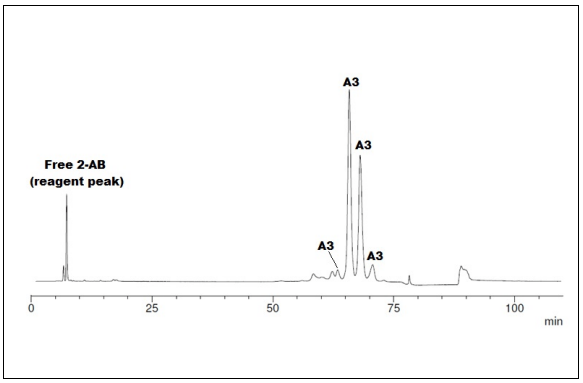

Figure 1 - **HPLC results**:
A3 labeled according to the Signal™ 2-AB Labeling Kit (GKK-404) and analyzed on a GlycoSep™ N column (GKI-4728) in ammonium formate/acetonitrile.

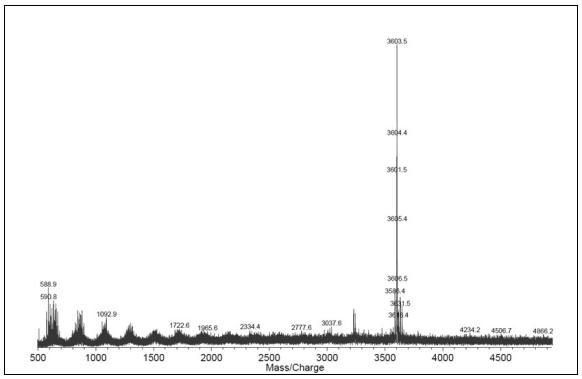

Figure 2 - MALDI-TOF of A3 $[M + Na]^+$

Example of UPLC Results:

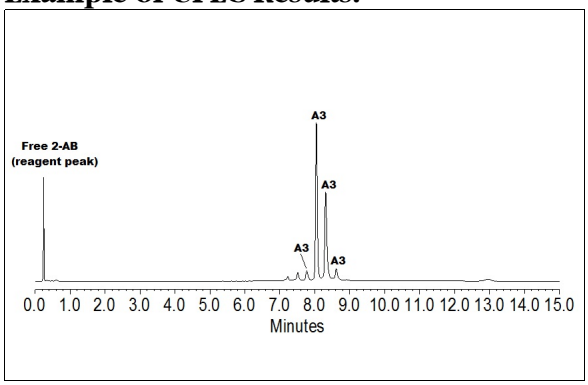

Figure 3 - A3 labeled with 2-AB and analyzed on a Waters ACQUITY® UPLC® BEH Glycan column (1.7 μm, 2.1 x 100 mm) in ammonium formate/acetonitrile.

Isolation: A3 complex-type N-linked oligosaccharide is typically released from a glycoprotein using N-Glycanase® or anhydrous hydrazine[8], separated from peptide material by adsorption chromatography, then purified further using a combination of glycosidase digestion and chromatographic techniques.

Structural Analysis: The purity and structural integrity of the glycan is assessed by one or more of the following techniques: HPLC[2], UPLC, mass spectrometry[3,4], FACE™[5], ^{1}H-NMR[6] and HPAEC-PAD[7].

The product supplied is a mixture of isomers differing in the type of sialic acid linkages [NeuAcα(2-3) or NeuAcα(2-6)] and the Gal residue linkage of one of the arms [Galβ(1-4) or Galβ(1-3)]. The composite structure on the front page indicates mixed Gal linkages on all three arms because it has not been determined which arm has the given linkage. Note that the isomeric mixture of the sialic acid linkages results in 2 main peaks when run on a GlycoSep™ N HPLC column, which differ due to the presence of either one or two NeuAcα(2-3/6) linkages.

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

Handling: The 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 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

1. Average molecular weight was calculated using the ExPASy GlycanMass calculator: http://us.expasy.org/tools/glycomod/glycanmass.html

2. Guile GR, Rudd PM, Wing DR, Prime SB and Dwek RA. A rapid and high-resolution high-performance liquid chromatographic method for separating glycan mixtures and analyzing oligosaccharide profiles**.** Anal Biochem 1996 Sep 5;240(2):210-226.

3. James DC and Jenkins N. Analysis of N-glycans by matrix-assisted laser desorption/ionization mass spectrometry. In: Jackson P, Gallagher JT, editors. A laboratory guide to glycoconjugate analysis, BioMethods Vol. 9. Basel: Birkhäuser; 1997. p. 91-112.

4. Papac DI, Wong A and 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.

5. Starr CM, Masada RI, Hague C, Skop E and Klock JC. Fluorophore-assisted carbohydrate electrophoresis in the separation, analysis, and sequencing of carbohydrates. J Chromatogr A 1996 Jan 12;720(1-2):295-321.

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7. Townsend RR, Hardy MR, Hindsgaul O and Lee YC. High-performance anion-exchange chromatography of oligosaccharides using pellicular resins and pulsed amperometric detection. Anal Biochem 1988 Nov 1;174(2):459-70.

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Authorized Signature