



CERTIFICATE OF ANALYSIS

PRODUCT NAME: GLYKO® TRI-SIALYLATED, GALACTOSYLATED, TRIANTENNARY COMPLEX N-GLYCAN (A3)

PRODUCT CODE: GKC-335300

LOT NUMBER: DP07E1701b

PACK SIZE: 10 µg (qualitative standard for glycan identification)

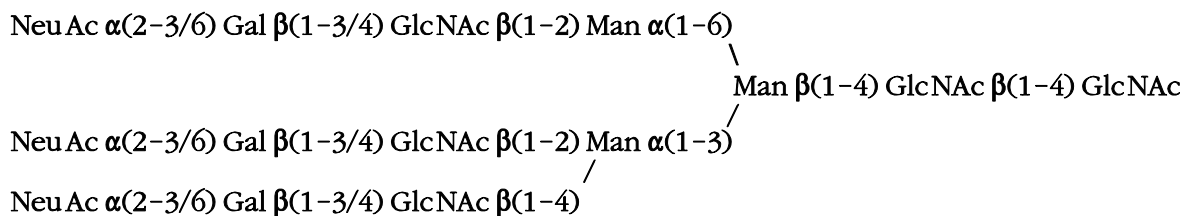
PURITY: $\geq 89\%$ of glycan by HPLC

FORM: Dry solid

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

EXPIRATION: February 2015, may be used for 1 year after reconstitution

STRUCTURE:



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Quality Control:

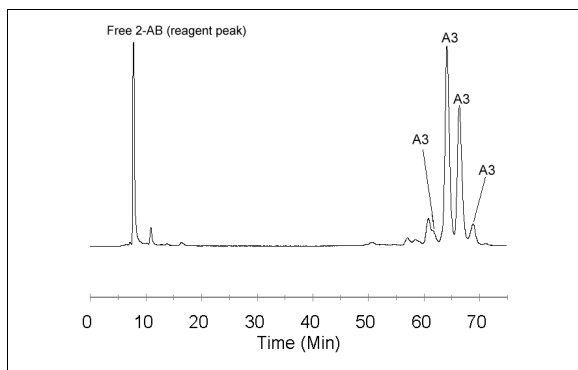


Figure 1 - HPLC results:

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.

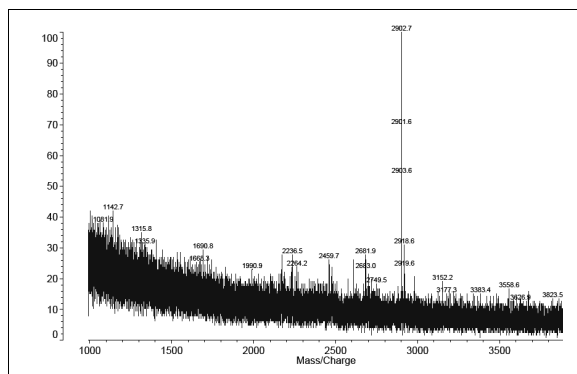


Figure 2 - MALDI-TOF of A3 [M + Na]⁺

Molecular Weight: 2880.6 (average)¹

Isolation: A3 complex-type N-linked oligosaccharide is typically released from a glycoprotein using N-Glycanase® or anhydrous hydrazine⁸, 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: HPLC², mass spectrometry^{3,4}, FACE⁵, ¹H-NMR⁶ and HPAEC-PAD⁷.

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.

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. 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.
6. Vliegenthart JFG, Dorland L and van Halbeek H. High-resolution, ¹H-nuclear magnetic resonance spectroscopy as a tool in the structural analysis of carbohydrates related to glycoproteins. *Adv Carb Chem Biochem* 1983 41: 209-374.
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.
8. Takasaki S, Mizouchi T and Kobata A. Hydrazinolysis of asparagine-linked sugar chains to produce free oligosaccharides. *Meth Enzymol* 1982; 83:263-8.

Recombinant PNGase F is covered by US Patent No 5,238,821 and EP Patent No 0472651 B1, which are exclusively licensed for manufacture by ProZyme, Inc.

Authorized Signature