CERTIFICATE OF ANALYSIS

PRODUCT NAME: GLYKO® ASIALO, GALACTOSYLATED, BIANTENNARY COMPLEX
                  N-GLYCAN, CORE-SUBSTITUTED WITH FUCOSE (NA2F)

PRODUCT CODE: GKC-024301

LOT NUMBER: DP08J0203e

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: September 2017, may be used for 1 year after

STRUCTURE:

$$\text{Gal } \beta(1-4) \text{ GlcNAc } \beta(1-2) \text{ Man } \alpha(1-6)$$
$$\text{Gal } \beta(1-4) \text{ GlcNAc } \beta(1-2) \text{ Man } \alpha(1-3)$$
$$\text{Man } \beta(1-4) \text{ GlcNAc } \beta(1-4) \text{ GlcNAc}$$
$$\text{Fuc } \alpha(1-6)$$

Quality Control:

Figure 1 - **HPLC results**:
NA2F 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 mass spec of NA2F $[M + Na]^+$

Molecular Weight: 1787.7 (average)[1]

Isolation: NA2F 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], mass spectrometry[3,4], FACE™[5], ^{1}H-NMR[6] and HPAE-PAD[7].

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 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

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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.

8. Takasaki S, Mizouchi T and Kobata A. Hydrazinolysis of asparagine-linked sugar chains to produce free oligosaccharides. Meth Enzymol 1982; 83:263-8.

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Authorized Signature