



CERTIFICATE OF ANALYSIS

PRODUCT NAME: GLYKO® TRI-SIALYLATED, GALACTOSYLATED, TRIANTENNARY

COMPLEX N-GLYCAN (A3)

PRODUCT CODE: GKC-335300

LOT NUMBER: DP07E1701a

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

PURITY: ~86% of glycan by HPLC (A3 resolves as 2 - 4 peaks due to

heterogeneity of sialic acid linkages)

Not recommended for MALDI-TOF applications

FORM: Dry solid. Supplied as an ammonium salt to prevent desialylation.

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

EXPIRATION: April 2012 (may be used for 1 year after reconstitution)

STRUCTURE:

Neu Ac $\alpha(2-3/6)$ Gal $\beta(1-3/4)$ GlcNAc $\beta(1-2)$ Man $\alpha(1-6)$ Man $\beta(1-4)$ GlcNAc $\beta(1-4)$ GlcNAc Neu Ac $\alpha(2-3/6)$ Gal $\beta(1-3/4)$ GlcNAc $\beta(1-2)$ Man $\alpha(1-3)$ Neu Ac $\alpha(2-3/6)$ Gal $\beta(1-3/4)$ GlcNAc $\beta(1-4)$

QUALITY CONTROL:

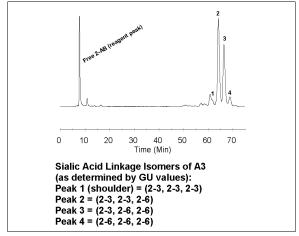


Figure 1 - HPLC of 2-AB labeled A3

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, and separated from peptide material using a combination of 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 [NeuAc α (2-3) or NeuAc α (2-6)] and the Gal residue linkage of one of the arms [Gal β (1-4) or Gal β (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 GlycoSepTM N HPLC column, which differ due to the presence of either one or two NeuAc α (2-3) 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

- Average molecular weight was calculated using the ExPASy GlycanMass calculator: http://us.expasy.org/tools/glycomod/glycanmass.html
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- Papac, D.I., A. Wong and A.J.S. Jones. Analysis of acidic oligosaccharides by matrix-assisted laser desorption/ionization time of flight mass spectrometry.
 Anal Chem 68: 3215-3223 (1996).
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- Vliegenthart, J.F.G., L. Dorland and H. van Halbeek. High-resolution, ¹H-nuclear magnetic resonance spectroscopy as a tool in the structural analysis of carbohydrates related to glycoproteins. Adv Carb Chem Biochem 41: 209-374 (1983).
- Townsend, R.R., M.R. Hardy, O. Hindsgaul and Y.C. Lee. High-performance anion-exchange chromatography of oligosaccharides using pellicular resins and pulsed amperometric detection. **Anal Biochem 174:** 459-470 (1988).
- 8. Takasaki, S., T. Mizouchi and A. Kobata. Hydrazinolysis of asparagine-linked sugar chains to produce free oligosaccharides. **Meth Enzymol 83:** 263- (1982).

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