

CERTIFICATE OF ANALYSIS

PRODUCT NAME: GLYKO® LACTO-N-HEXAOSE O-GLYCAN (LNH)

PRODUCT CODE: GKAD-00523

LOT NUMBER: DP08G2402

PACK SIZE: 500 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: May 2018, may be used for 1 year after reconstitution (extended

from prior exp. date based on re-assay)

RE-ASSAY DATE: May 2013

STRUCTURE:

Gal
$$\beta(1-4)$$
 GlcNAc $\beta(1-6)$

Gal $\beta(1-4)$ Glc

Gal $\beta(1-3)$ GlcNAc $\beta(1-3)$

QUALITY CONTROL:

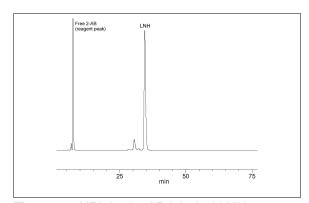


Figure 1 - HPLC of 2-AB labeled LNH

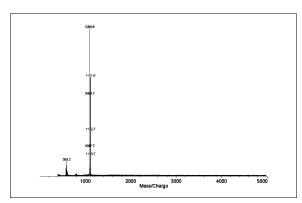


Figure 2 - MALDI-TOF of LNH [M + Na]⁺

Molecular Weight: 1073 (average)¹

Isolation: LNH O-linked oligosaccharide is typically found in human milk² and also can be chemically synthesized. Isolation and purification is achieved through a combination of chromatography techniques.

Structural Analysis: The purity and structural integrity of the glycan is assessed by one or more of the following techniques: HPLC³, mass spectrometry^{4,5}, ¹H-NMR⁶ and HPAE-PAD⁷.

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

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.

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.

REFERENCES

- Average molecular weight was calculated using ExPASy
 (http://us.expasy.org/tools/glycomod/glycanmass.html)
- 2. Kobata, A. & Ginsburg, V. (1972) Arch. Biochem. Biophys. **150**, 273.
- 3. Guile GR, Rudd PM, Wing DR, Prime SB, Dwek RA. A rapid and high-resolution high-performance liquid chromatographic method for separating glycan mixtures and analyzing oligosaccharide profiles. Anal Biochem. 1996;240:210-226.
- James DC, Jenkins N. Analysis of N-glycans by matrix-assisted laser desorption/ionization mass spectrometry; in A laboratory guide to glycoconjugate analysis. BioMethods 9 (Jackson P, Gallagher JT, ed). 1997;91-112.
- Papac DI, Wong A, Jones AJS. Analysis of acidic oligosaccharides by matrix-assisted laser desorption/ionization time of flight mass spectrometry. Anal Chem 68: 1996;3215-3223.
- Vliegenthart JFG, Dorland L, van Halbeek H. Highresolution, 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, Lee YC. High-performance anion-exchange chromatography of oligosaccharides using pellicular resins and pulsed amperometric detection. Anal Biochem 1988;174:459-470.

Authorized Signature