

CERTIFICATE OF ANALYSIS

PRODUCT NAME: GLYKO® 2-AB-(GLUCOSE HOMOPOLYMER)
 PRODUCT CODE: GKSB-503
 LOT NUMBER: P03G0210
 PACK SIZE: 200 pmol
 PURITY: >90% (Specification: ≥90%)
 FORM: Dried by centrifugal evaporation from aqueous solution.
 STORAGE: Store at -20°C before and after reconstitution
 EXPIRATION: June 08
 STRUCTURE: Variable length mixture of polymerized glucose units linked $\alpha(1-6)$, with 2-AB derivatization of the reducing termini (schematically summarized below).
 Glc-2-AB
 Glc $\alpha(1-6)$ Glc-2-AB
 Glc $\alpha(1-6)$ [Glc $\alpha(1-6)$]_n Glc-2-AB (where $0 \leq n \leq 21$ or more)

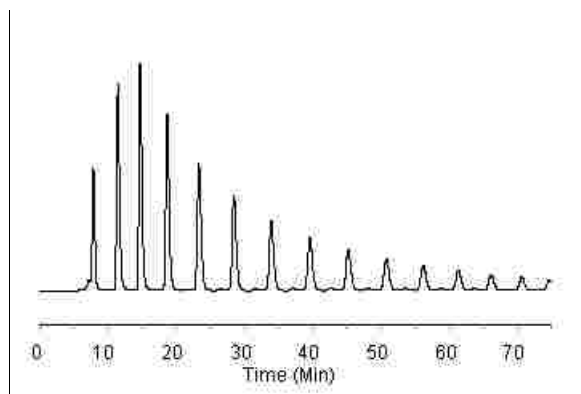


Figure 1 - Separation on GlycoSep N HPLC column (2nd peak is GU1, later peaks are successively larger)

Preparation: Glucose homopolymer standard ‘ladders’ are prepared by partial acid hydrolysis of dextran. The ladder is then labeled with 2-aminobenzamide (2-AB) by reductive amination¹. HPLC analysis of labeled glucose homopolymers gives a characteristic ladder profile with an initial non-glucose peak followed by a series of glucose polymer peaks from 1-mer to at least 23-mers. However, the number of peaks observed is dependent on the running conditions employed.

Structural Analysis: The purity and structural integrity of the glycan is assessed by HPLC (Figure 1).

Nomenclature: Homopolymers of glucose derived by hydrolysis of dextran are referred to by the number of glucose monomers that each contains. Thus, glucose unit 2 is a dimer of two glucose monosaccharides and is abbreviated as GU2. This corresponds to DP2 where the “degree of polymerization” nomenclature is used as in other homopolymer series.

Application: As a calibration standard for the GlycoSep™ N HPLC column.

Directions for Use: Reconstitute with a known volume of either HPLC grade water or the starting buffer used for the HPLC run (chromatography running conditions for GlycoSep HPLC columns are given in the protocol manuals shipped with the columns). The amount of 2-AB-(Glucose Homopolymer) injected on a GlycoSep column is typically up to 50 pmol of total polymer (*e.g.* reconstitute the contents of the vial in 40 µl starting buffer and inject 10 µl per run).

Handling: The labeled oligosaccharide is shipped as a dried solid. Allow the unopened vial to reach ambient temperature and tap unopened 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.

Ensure that any glass, plasticware or solvents used are free of glycosidases and environmental carbohydrates.

Minimize exposure to elevated temperatures or extremes of pH.

REFERENCES

1. Bigge *et al.* **Analytical Biochemistry** 230: 229-238 (1995).

Authorized Signature

CERTIFICATE OF ANALYSIS

PRODUCT NAME: GLYKO[®] 2-AB-(GLUCOSE HOMOPOLYMER)
PRODUCT CODE: GKSB-503
LOT NUMBER: P03G0209
PACK SIZE: 200 pmol
PURITY: >90% (Specification: ≥90%)
FORM: Dried by centrifugal evaporation from aqueous solution.
STORAGE: Store at -20°C before and after reconstitution
EXPIRATION: June 08 (may be used for 1 year after reconstitution)
STRUCTURE: A mixture of α(1-6)-linked glucose oligosaccharides with variable number of monomeric glucose units (1-23 or more). The reducing termini are derivatized with 2-AB (schematically summarized below).
 Glc-2-AB
 Glc α(1-6) Glc-2-AB
 Glc α(1-6) [Glc α(1-6)]_n Glc-2-AB (where 0 ≤ n ≤ 21 or more)

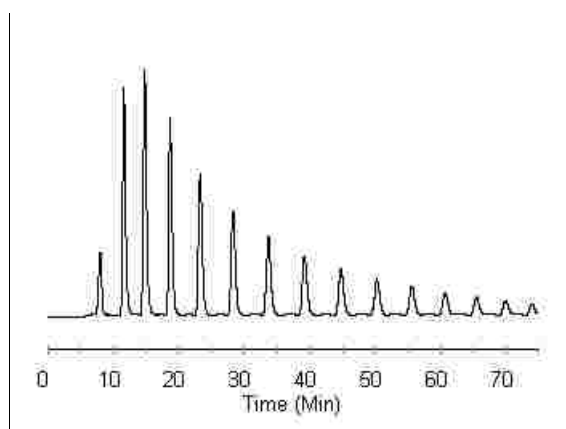


Figure 1: Separation on GlycoSep™ N HPLC column (2nd peak is GU1, later peaks are successively large)

Preparation: Glucose homopolymer standard ‘ladders’ are prepared by partial acid hydrolysis of dextran. The ladder is then labeled with 2-aminobenzamide (2-AB) by reductive amination¹. HPLC analysis of 2-AB-(Glucose Homopolymer) gives a characteristic profile with an initial non-glucose peak followed by a series of glucose polymer peaks from 1-mer to at least 23-mers. However, the number of peaks observed is dependent on the running conditions employed.

Structural Analysis: The purity and structural integrity of the glycan is assessed by HPLC (Figure 1).

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