

CERTIFICATE OF ANALYSIS

PRODUCT NAME: GLYKO® GLUCOSE HOMOPOLYMER STANDARD

PRODUCT CODE: GKI-4503

LOT NUMBER: DP14A1601

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

FORM: Dry solid

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

EXPIRATION: September 2023, may be used for 1 year after reconstitution

(extended from prior exp. date based on re-assay)

RE-ASSAY DATE: September 2018

STRUCTURE: A mixture of $\alpha(1-6)$ -linked glucose oligosaccharides with variable

number of monomeric glucose units (1 - 23 or more).

QUALITY CONTROL:

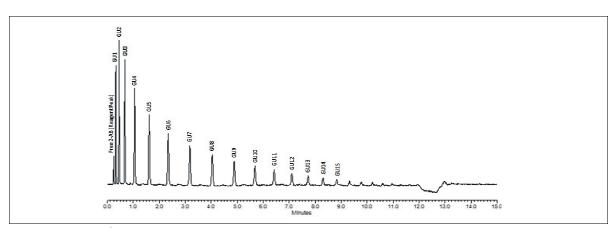


Figure 1 - UPLC® of of 2-AB labeled glucose homopolymer standard: UPLC running conditions are described on the following page.

UPLC Running Conditions: 12 pmol of 2-AB-labeled glucose homopolymer standard is injected on an ACQUITY[®] UPLC BEH Glycan column (1.7 μm, 2.1 x 100 mm) under the conditions listed below:

Time (min)	%ACN	%Buffer	Flow Rate
0	75	25	1
12	52.5	47.5	1
12.1	40	60	0.5
12.5	40	60	0.5
12.6	75	25	0.5
12.7	75	25	1
15	75	25	1

ACN: acetonitrile

Buffer: 100 mM ammonium formate,

pH 4.4

Flow rate: above, in ml/min

Temperature: 60°C Max Pressure: 15,000 psi

Fluorescence Detection:

 $\lambda_{\rm ex}$ = 330 nm $\lambda_{\rm em}$ = 420 nm

Isolation: The Glucose Homopolymer Standard was prepared by partial acid hydrolysis of dextran¹. The standard preparation gives a characteristic ladder profile of oligosaccharides from monomer to at least 23-mers of glucose.

Nomenclature: Homopolymers of glucose derived by partial hydrolysis of dextran are named according to the number of glucose monomers that each chain 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.

Structural Analysis: The purity and structural integrity of the glycan is assessed by one or more of the following techniques: HPLC², mass spectrometry³,⁴, FACE™⁵, ¹H-NMR⁶ and HPAEC-PAD7. HPLC or UPLC analysis yields a characteristic profile with an initial non-glucose peak followed by a series of glucose oligomer peaks; the number of peaks observed is dependent on the running conditions employed.

Application: As a calibration standard for HILIC HPLC and UPLC⁸.

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.

Reconstitution: Use HPLC-grade water or an aqueous buffer to dissolve the glycan. Recommended dilution is to $1 \mu g/\mu l$. Store the reconstituted glycan at -20°C in working aliquots. Avoid multiple freeze/thaw cycles.

REFERENCES

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- 7. Townsend RR, Hardy MR, Hindsgaul O and Lee YC. Highperformance anion-exchange chromatography of oligosaccharides using pellicular resins and pulsed amperometric detection. Anal Biochem 1988 Nov 1;174(2):459-70.
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