

CERTIFICATE OF ANALYSIS

PRODUCT NAME: GLYKO® GLUCOSE HOMOPOLYMER STANDARD FOR INSTANTAB™

PRODUCT CODE: GKIB-503

LOT NUMBER: DP18K0701a

PACK SIZE: 200 pmol (qualitative standard for glycan identification)

FORM: Dry solid

STORAGE: Store in the dark at -20°C or below before and after reconstitution.

EXPIRATION: May 2024, may be used for 1 year after reconstitution

STRUCTURE: A mixture of $\alpha(1-6)$ -linked glucose oligosaccharides with variable number of monomeric glucose units (1 - 15 or more). The reducing termini are derivatized with the fluorescent dye.

QUALITY CONTROL:

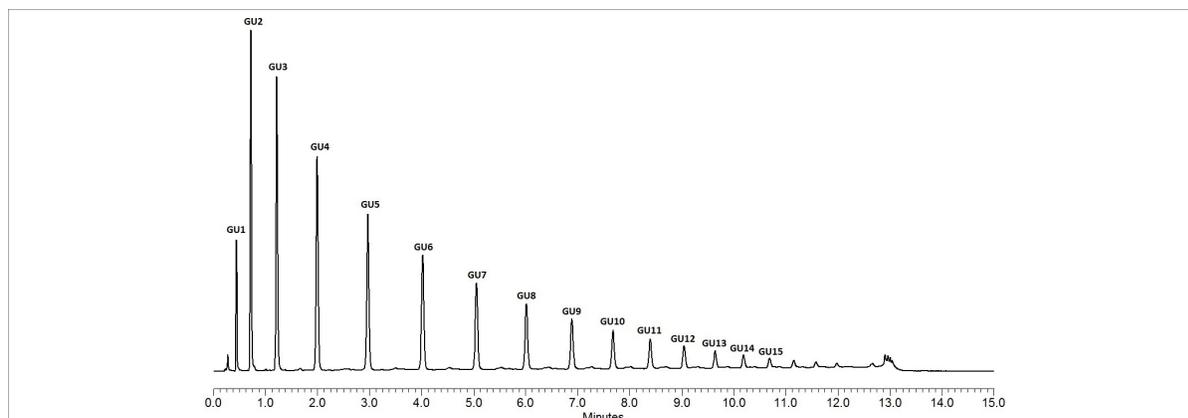


Figure 1 - UPLC® results: UPLC running conditions are described on the following page.

UPLC Running Conditions: 8 pmol of Glucose Homopolymer Standard for InstantAB 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:
 $\lambda_{\text{ex}} = 278 \text{ nm}$
 $\lambda_{\text{em}} = 344 \text{ nm}$

Application: As a calibration standard for HILIC HPLC¹ and UPLC².

Preparation: Glucose homopolymer standard 'ladders' are prepared by partial acid hydrolysis of dextran³. The ladder is then labeled and purified from excess labeling reagents. Liquid chromatographic analysis of Glucose Homopolymer Standard for InstantAB gives a characteristic profile with an initial non-glucose peak followed by a series of glucose polymer peaks from 1-mer to at least 15-mers. However, the number of peaks observed is dependent on the running conditions employed.

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.

Handling: The labeled 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 used are free of glycosidases and carbohydrate contaminants.

Minimize exposure to elevated temperatures or extremes of pH.

Reconstitution: Use ultra-pure water or an aqueous buffer to dissolve the glycan. Store the reconstituted glycan at -20°C in working aliquots. Avoid multiple freeze/thaw cycles.

Directions For Use: The amount of the labeled ladder standard injected on a UPLC column is typically 3 - 10 pmol of total glycan. For our Quality Control testing, one vial was dissolved in 30 µl of water and 1 µl injected on the ACQUITY column. For larger injection volumes or other LC systems we recommend further dilution as necessary for compatibility with your mobile phase. For suggested methods see Rapid UPLC Methods for Screening Labeled N-Glycans at:

www.prozyme.com/protocols/

REFERENCES

1. Guile GR, Rudd PM, Wing DR, Prime SB and Dwek RA. A rapid and high-resolution high-performance liquid chromatographic method for separating glycan mixtures and analyzing oligosaccharide profiles. *Anal Biochem* 1996 Sep 5;240(2):210-226.
2. Ahn J, Bones J, Yu YQ, Rudd PM and Gilar M. Separation of 2-aminobenzamide labeled glycans using hydrophilic interaction chromatography columns packed with 1.7 µm sorbent. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2010 Feb 1;878(3-4):403-8.
3. Yamashita, K., T. Mizuochi and A. Kobata. Analysis of oligosaccharides by gel filtration. *Meth Enzymol* 1982; 83:105-126.

Authorized Signature