



CERTIFICATE OF ANALYSIS

PRODUCT NAME: GLYKO[®] MONOSACCHARIDE STANDARD SET

PRODUCT CODE: GKRP-3500

LOT NUMBER: DP13A1601

PACK SIZE: 3 vials with 100 nmol of each monosaccharide per vial (qualitative standard for glycan identification)

PURITY: $\geq 90\%$ 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

QUALITY CONTROL:

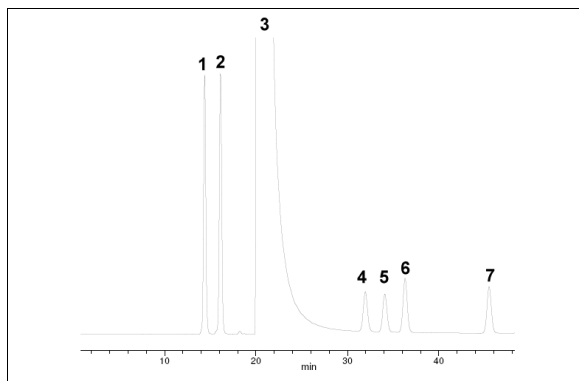


Figure 1 - HPLC of 2-AA labeled Monosaccharide Standard Set

- Peak 1 = D-Glucosamine
- Peak 2 = D-Galactosamine
- Peak 3 = Free 2-AA (reagent peak)
- Peak 4 = D-Galactose
- Peak 5 = D-Mannose
- Peak 6 = D-Glucose
- Peak 7 = L-Fucose

Preparation: The Monosaccharide Standard Set was prepared by mixing equimolar amounts of monosaccharide solutions which were prepared from monosaccharides dried to a constant weight.

Composition: An equimolar mixture of six monosaccharides: D-Galactose, D-Mannose, D-Glucose, L-Fucose, D-Glucosamine, and D-Galactosamine.

Analysis: The purity and structural integrity of the standard is assessed by HPLC¹. The Monosaccharide Standard Set is first labeled by reductive amination with 2-aminobenzoic acid (2-AA) using the Anumula method², followed by reverse phase HPLC analysis using a GlycoSep™ R column (GKI-4727). The Monosaccharide Standard Set gives a characteristic profile of six peaks correlating to each monosaccharide (Figure 1).

Applications: The monosaccharide standard set can be used as a qualitative standard for monosaccharide identification and quantitative standard for relative monosaccharide composition analysis in chromatographic applications. The set contains non-N-acetylated amino sugars (D-Glucosamine and D-Galactosamine) as acid hydrolysis of glycoconjugates results in deacetylation of N-acetyl-D-Glucosamine and N-acetyl-D-Galactosamine.

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 glucose.

Reconstitution: Use HPLC-grade water or an aqueous buffer to dissolve the glycan set (not to exceed 500 µl). From this stock solution, further dilutions may be made. Store the reconstituted glycan set at -20°C in working aliquots. Avoid multiple freeze/thaw cycles.

REFERENCES

1. Guile, G. R., Rudd, P. M., Wing, D. R., Prime, S. B. and R. A. Dwek. A rapid and high-resolution high-performance liquid chromatographic method for separating glycan mixtures and analyzing oligosaccharide profiles. **Anal Biochem** **240**: 210-226 (1996).
2. Anumula, KR. Quantitative determination of monosaccharides in glycoproteins by high-performance chromatography with highly sensitive fluorescence detection. **Anal biochem** **202(2)**: 275-83 (1994).

Authorized Signature