



CERTIFICATE OF ANALYSIS

| | |
|-----------------|---|
| PRODUCT NAME: | GLYKO® SIALIC ACID REFERENCE PANEL |
| PRODUCT CODE: | GKRP-2503 |
| LOT NUMBER: | P14L0801 |
| PACK SIZE: | 5 vials, 1.25 nmol/vial (qualitative standard for sialic acid identification) |
| PURITY: | >90% sialic acid structures |
| FORM: | Dry solid |
| RECONSTITUTION: | Use pure water or 1 mM acetic acid. |
| STORAGE: | Store at -20°C in a desiccator |
| EXPIRATION: | January 2020, may be used for 2 months after reconstitution |

STRUCTURE:

The Sialic Acid Reference Panel contains a dried mixture of six sialic acid derivatives (see table below) in various amounts totaling ~1.25 nmol/vial.

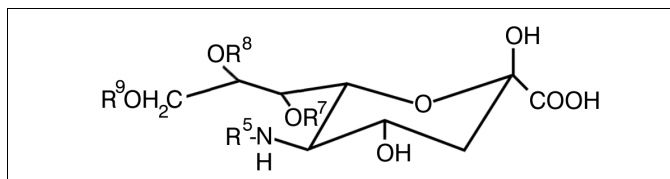


Figure 1 - Sialic Acid Reference Panel

| R= | Position: | | | |
|----------------|-----------|---------|------|----|
| | 5 | 7 | 8 | 9 |
| Neu5Ac | Ac | H | H | H |
| Neu5Gc | Gc | H | H | H |
| Neu5,7Ac2 | Ac | Ac | H | H |
| Neu5Gc9Ac | Gc | H | H | Ac |
| Neu5,9Ac2 | Ac | H | H | Ac |
| Neu5,7(8),9Ac3 | Ac | (Ac) or | (Ac) | Ac |

where Ac = Acetyl, Gc = Glycolyl

QUALITY CONTROL:

The Sialic Acid Reference Panel was labeled with DMB (1,2-diamino-4,5-methylene-dioxybenzene) and analyzed using reverse-phase HPLC on a GlycoSep™ R column (see Figure 2 for a typical result):

Solvent: Acetonitrile:methanol:water (9:7:84). In some cases it may be convenient either to change the proportion of acetonitrile in the solvent or to run a gradient of acetonitrile (from 5 to 10% v/v).

Flow rate: 0.5 - 0.9 ml/min.

Fluorescence detection:

$$\lambda_{\text{ex}} = 373 \text{ nm}$$

$$\lambda_{\text{em}} = 448 \text{ nm}$$

While differences in retention times may occur with different columns, flow rate, solvent or laboratory conditions, the elution order of DMB-sialic acids is consistent.

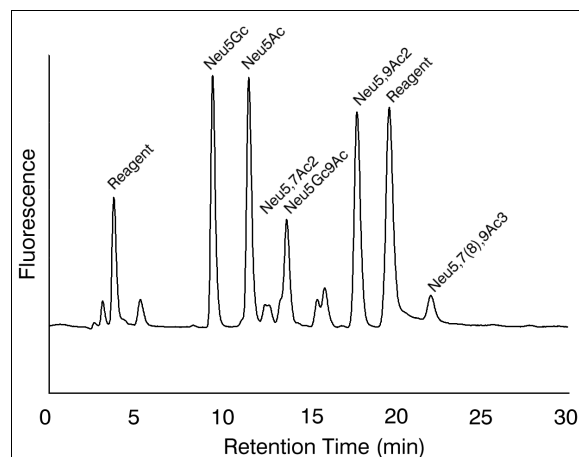


Figure 2 - Typical Profile of DMB-labeled Sialic Acid Reference Panel

BACKGROUND

Sialic acids are a family of 9-carbon carboxylated 2-keto sugars found on many glycans associated with glycoproteins^{1,2}. Over 25 sialic acid derivatives have been identified in nature and have been shown to play a major role in a variety of biological functions.

Sialic acids released from glycoconjugates have been identified by a variety of analytical techniques including TLC, GC, GC-MS, FAB-MS and HPLC. Of these techniques, derivatization of sialic acids with DMB with subsequent separation by reverse-phase HPLC offers many advantages^{3,4,5}:

- High sensitivity and specificity
- Good resolution of O-acetylated derivatives
- Minimal artifacts due to O-acetyl ester migration from the 7- to the 9-position

Use of Standards: The Sialic Acid Reference Panel may be used for comparison with unknown samples in a variety of analytical techniques. One such method is fluorescent tagging using the Glyko Signal™ DMB Labeling Kit (Cat # GKK-407) followed by reverse phase HPLC using a GlycoSep column (Cat # GKI-4727). Also useful as a mixed qualitative standard for TLC, GC, GC-MS and FAB-MS fractionation procedures.

Handling: The Sialic Acid Reference Panel 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.

May be stored at 2-8°C or -20°C, pH 3.0 - 6.0 upon reconstitution; however there is a possibility of migration of the O-acetyl groups from the 7-position to the 9-position and some breakdown of sialic acid structures.

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; high temperatures or low pH will cause degradation of the sialic acids.

REFERENCES

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3. Hara S, Yamaguchi M, Takemori Y, Furuhashi K, Ogura H, Nakamura M. Determination of mono-O-acetylated N-acetylneuraminic acids in human and rat sera by fluorometric high-performance liquid chromatography. Anal Biochem. 1989 May 15;179(1):162-6.
4. Manzi AE, Diaz S, Varki A. High-pressure liquid chromatography of sialic acids on a pellicular resin anion-exchange column with pulsed amperometric detection: a comparison of six systems. Anal Biochem. 1990 Jul;188(1):20-32.
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Authorized Signature