



## CERTIFICATE OF ANALYSIS

PRODUCT NAME: GLYKO® SIALIC ACID REFERENCE PANEL

PRODUCT CODE: GKRP-2503

LOT NUMBER: P14G2503

PACK SIZE: 5 vials, 1.25 nmol/vial (qualitative standard for sialic acid identification)

PURITY: >90% sialic acid structures

FORM: Dry solid

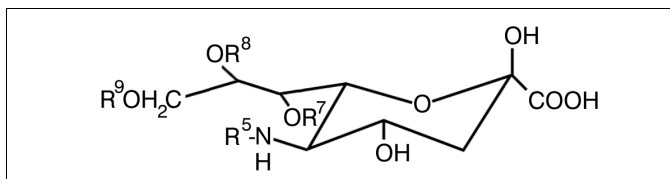
RECONSTITUTION: Use pure water or 1 mM acetic acid.

STORAGE: Store at -20°C in a desiccator

EXPIRATION: August 2019, 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).

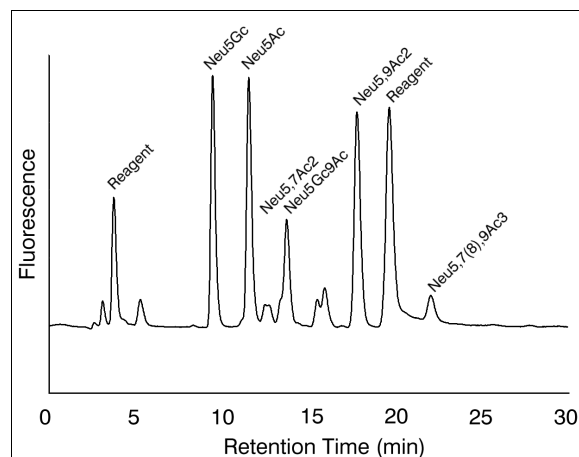
Flow rate: 0.5 - 0.9 ml/min.

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<sup>1,2</sup>. 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<sup>3,4,5</sup>:

- 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

1. Schauer R. Sialic Acids: Chemistry, Metabolism and Function. New York: Springer-Verlag; 1982. 344 p. (Cell Biology Monographs; 10).
2. Varki A. Diversity in the sialic acids. *Glycobiology*. 1992 Feb;2(1):25-40.
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.
5. Butor C, Diaz S, Varki A. High level O-acetylation of sialic acids on N-linked oligosaccharides of rat liver membranes. *J Biol Chem*. 1993 May 15;268(14):10197-206.

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Authorized Signature