Agilent AdvanceBio Sialic Acid Profiling and Quantitation Kit

Rapid quantitation of NANA and NGNA species

Advantages and Benefits

Detection:

- Workflow may be completed in approximately 5 hours including incubation periods
- Broad detection range of sialic acids, from 1 to 2000 pmol
- Picomolar sensitivity for proteins with low levels of sialylation

Format:

- 96-well plate format
- Pre-dispensed quantitative NANA and NGNA standards included with kit
- Qualitative Sialic Acid Reference Panel included with kit
- Automation-friendly

Complementary to the related AdvanceBio Total Sialic Acid quantitation kit (p/n GS48-SAQ and GS96-SAQ), which quantitates total sialic acid content using a plate reader, but does not differentiate between sialic acid species

Streamlined Profiling and Quantitation of Sialic Acids in Biotherapeutic Glycoproteins

Glycans are carbohydrates composed of monosaccharides arranged into many different possible oligosaccharide structures based on composition and linkage position. Sialic acid capping at the nonreducing terminal of *N*- or *O*-glycans can serve a key role in mediating the effectiveness of therapeutic glycoproteins. Depending on the molecule and the application, terminal sialic acid may reduce the rate of clearance, reduce antibody-dependent cellular cytotoxicity (ADCC) activity, or can be anti-inflammatory. Two common sialic acid species found in biotherapeutics are *N*-acetylneuraminic acid (NANA or Neu5Ac) and *N*-glycoylneuraminic acid (NGNA or Neu5Gc). Neu5Ac is generally the predominant species while Neu5Gc is not synthesized by humans and its presence on biotherapeutics can potentially be immunogenic. Therefore, it is essential to monitor both the absolute quantity of sialic acid and also the levels of different sialic acid species present in therapeutic glycoproteins.

The Agilent AdvanceBio Sialic Acid profiling and quantitation kit (p/n GS24-SAP) represents a sensitive, high-throughput approach to both sialic acid profiling and quantitation. Sialic acids are released from glycoproteins using acid hydrolysis, followed by derivatization with the fluorophore 1,2-diamino 4,5-methylenedioxybenzene (DMB), allowing for separation by reversed-phase (RP) liquid chromatography with fluorescence detection (FLD) and optional mass spec (MS) detection (Figure 1).

Learn more

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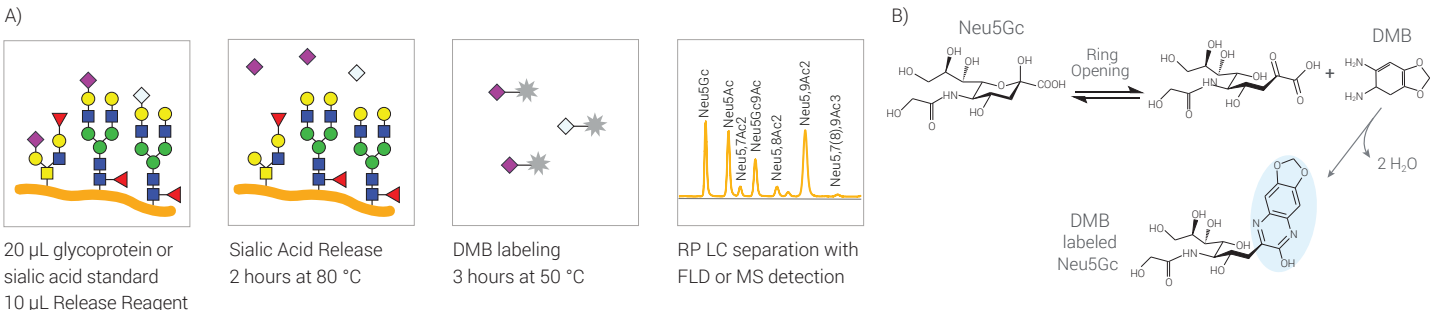


Figure 1. Sialic acid release and DMB labeling workflow A) Overview B) DMB labeling mechanism of sialic acid Neu5Gc.

Required Equipment and Sample Preparation Considerations

The AdvanceBio Sialic Acid profiling and quantitation kit is compatible with a variety of different sample types including glycoproteins, glycopeptides, glycolipids, polysialic acids and whole cells.

Depending on the sample type and the concentration, samples may require dilution for highly sialylated samples, while samples such as monoclonal antibodies with low levels of sialylation might require concentration by drying and resuspension in a smaller volume of DI water. The kit includes sufficient reagents to generate 24 data points following recommended usage. This enables six samples to be prepared in triplicate, or nine samples prepared in duplicate, see instruction manual for further details. The dynamic range of this assay is 1–2000 pmol sialic acid per sample well. Equipment to be provided by the user includes either a thermocycler, or heat blocks with lids capable of maintaining 50 °C and 80 °C for the required incubation steps. An analytical HPLC system (HPLC or UHPLC) with fluorescence detection is required for analysis, see instruction manual for more information.

Sialic Acid Profiling and Quantitation

- This workflow offers both qualitative identification (profiling) of sialic acid species using a sialic acid reference panel (SARP) with known sialic acid species (Figure 2) as well as absolute quantitation using Neu5Ac and Neu5Gc quantitative standards (Figure 3).
- Total sialic acid quantitation (Neu5Ac and Neu5Gc) results obtained with this kit are comparable with data obtainable with the AdvanceBio Total Sialic Acid quantitation kit (p/n GS48- or GS96-SAQ.) (Table 1).
- Quantitative Neu5Ac and Neu5Gc results are comparable to older DMB labeling workflows (GKK-407) (Table 2).

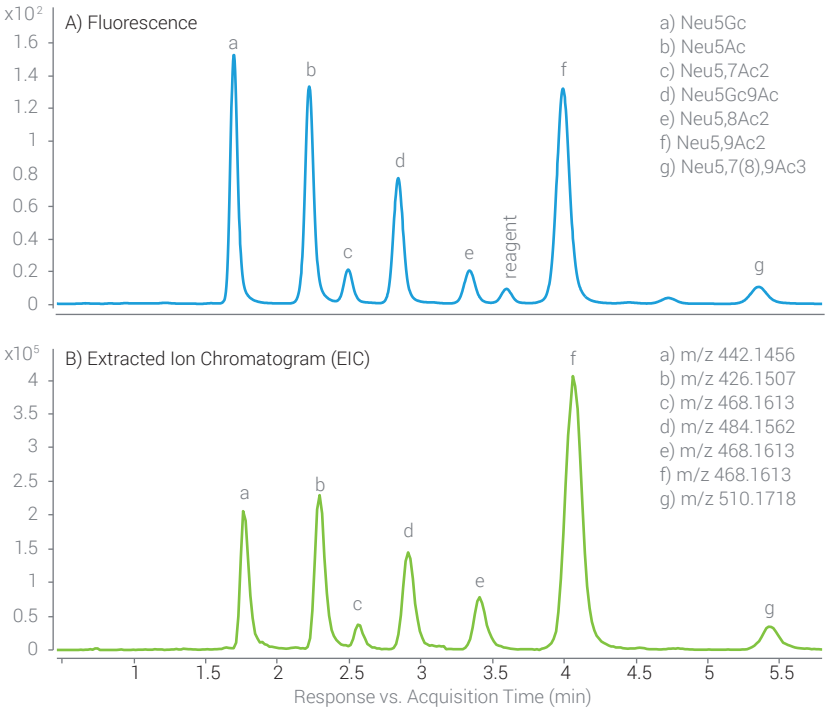
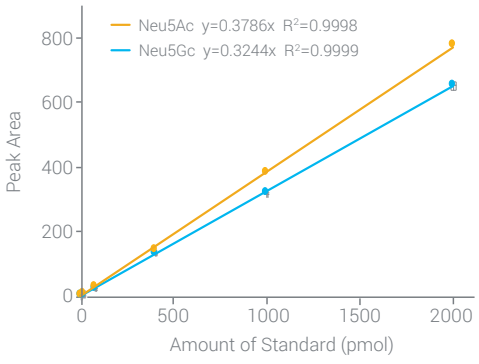


Figure 2. UHPLC chromatogram of DMB labeled SARP. A) Fluorescence B) Extracted ion chromatogram of DMB labeled sialic acid species, $[M+H]^{1+}$.



Sialic Acid	LOD (pmol)	LOQ (pmol)
Neu5Gc	0.012	0.040
Neu5Ac	0.016	0.053

Figure 3. Neu5Gc and Neu5Ac calibration curves, n=2. Limit of Detection (LOD) and Limit of Quantitation (LOQ) for Neu5G and Neu5Ac are shown in the table.

Table 1. Total sialic acid (Neu5Ac and Neu5Gc) with the Agilent AdvanceBio Sialic Acid profiling and quantitation kit (p/n GS24-SAP) in comparison to values obtained with the Agilent AdvanceBio Total Sialic Acid quantitation kit (p/n GS48-SAQ), n=3.

	AdvanceBio Sialic Acid Profiling and Quantitation Kit		AdvanceBio Total Sialic Acid Quantitation Kit	
	pmol/µg	%CV	pmol/µg	%CV
Rituxan	0.62	4.17%	0.47	5.04%
Enbrel	220	1.65%	210	12.34%
Erbitux	3.80	7.26%	3.49	0.69%
Fetuin	226	4.45%	232	7.39%

Table 2. Quantitation of Neu5Gc and Neu5Ac (pmol/µg) using the Agilent AdvanceBio Sialic Acid profiling and quantitation kit (p/n GS24-SAP) in comparison to the values obtained with the Glyko Signal DMB Sialic Acid labeling kit (p/n GKK-407) using additional Neu5Ac quantitative standard, n=3.
ND = not detected.

		AdvanceBio Sialic Acid Profiling and Quantitation Kit		GKK-407	
Glycoprotein	Sialic Acid	pmol/µg	%CV	pmol/µg	%CV
Rituxan	Neu5Gc	0.02	1.76%	ND	-
	Neu5Ac	0.60	4.25%	0.58	1.12%
Enbrel	Neu5Ac	223	2.92%	226	3.57%
Erbitux	Neu5Gc	3.68	1.02%	ND	-
	Neu5Ac	0.12	4.46%	ND	-
Fetuin	Neu5Gc	4.78	4.90%	ND	-
	Neu5Ac	222	4.44%	201	1.47%

Ordering Information

Part number	Name
GS24-SAP	Agilent AdvanceBio Sialic Acid profiling and quantitation kit, 24-ct

Kit Components

The Agilent AdvanceBio Sialic Acid profiling and quantitation kit contains the following reagents and standards for absolute quantitation and sialic acid identification.

Module: AdvanceBio Sialic Acid Profiling and Quantitation Kit GS24-SAP		
Component	**Units**	**Storage**
100 µM *N*-acetylneuraminic acid (NANA, NeuAc) Sialic Acid Standard, 200 µL	1	–20 °C
100 µM *N*-glycolylneuraminic acid (NGNA, NeuGc) Sialic Acid Standard, 200 µL	1	–20 °C
Sialic Acid Reference Panel, lyophilized	1	–20 °C
Vial A: Labeling Diluent, 300 µL	1	–20 °C
Vial B: Reductant	2	–20 °C
Vial C: DMB Dye	2	–20 °C
Vial D: Release Reagent, 300 µL	1	–20 °C
Cap Strips	6	–20 °C – RT
96-Well Reaction Plate	1	–20 °C – RT

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For more information on AdvanceBio sialic acid quantitation please visit:
http://www.agilent.us/chem/sialic_acid_quantitation

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