

Total Sialic Acid Quantitation of Biotherapeutic Glycoproteins



Sialic acid analysis simplified and standardized

The composition of glycans present on biotherapeutic glycoproteins can affect immunogenicity, pharmacokinetics, and pharmacodynamics. Glycans are carbohydrates composed of monosaccharides arranged into many different possible oligosaccharide structures based on composition and linkage position. 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 forms commonly found in biotherapeutics are N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc). Neu5Ac is usually the predominant species, while Neu5Gc is not synthesized by humans and its presence on biotherapeutics can be immunogenic. Given the importance of sialic acids on biotherapeutic glycoproteins, it is essential to monitor the absolute quantity of sialic acid.

The AdvanceBio Total Sialic Acid Quantitation kit, used for rapid quantitation of free or released sialic acid from intact glycoproteins, offers several advantages:

- Samples ready for analysis in about 1.5 hours enable rapid and reliable quantitation of total sialic acid released from intact proteins by Sialidase A.
- Broad dynamic range of detection of sialic acid levels
- Compatible with plate readers providing measurement by fluorescence detection or absorbance
- Minimal (if any) degradation of sialic acid due to enzymatic release
- User-friendly, high-throughput 96-well microplate format suitable for generating 48 and 96 data points
- Automation-friendly

Total sialic acid quantitation solution designed and manufactured by Agilent

The Agilent AdvanceBio Total Sialic Acid Quantitation kits (GS48-SAQ and GS96-SAQ) provide all the reagents necessary to prepare sialic acid samples in about 1.5 hours. Analysis is quick and easy using a standard microplate reader¹ with measurement by absorbance or fluorescence.

In this guide, you'll find the list of consumables needed to start quantitating sialic acid in your sample. The products listed were used to assess the sialic acids present in rituximab (Rituxan, a monoclonal antibody or mAb), etanercept (Enbrel, an Fc fusion protein), cetuximab (Erbix, a monoclonal antibody), and the NISTmAb.² The study quantitated sialic acid using AdvanceBio Total Sialic Acid Quantitation kits, as well as profiled and quantitated sialic acids using AdvanceBio Sialic Acid Profiling & Quantitation kit (p/n GS24-SAP).^{3,4}

Total sialic acid analysis kit chemistry and workflow^{4,5}:

The kit uses a coupled enzyme reaction, converting enzymatically-released sialic acid to hydrogen peroxide, which stoichiometrically reacts with a dye, generating intense fluorescence or absorbance signal. This approach allows enzymatic release of sialic acid, conversion, detection and quantitation to be performed in a single well for fast and simple processing.

Step 1: Release of Sialic Acid (30 minutes)

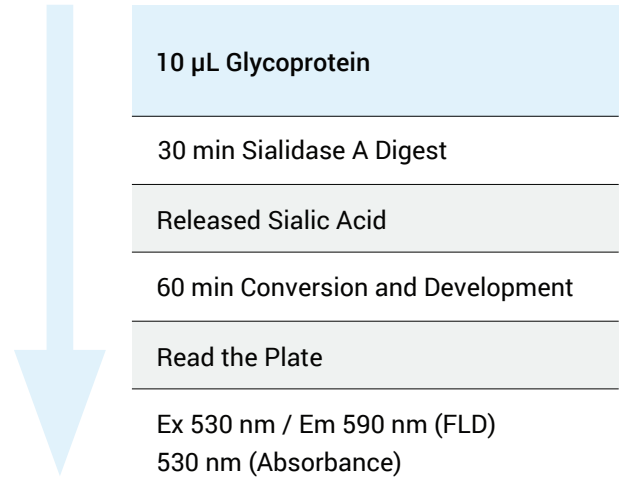
Glycoprotein + Sialidase A \rightarrow Sialic Acid + Desialylated Glycoprotein (minus sialic acid)

Step 2: Detection of Released Sialic Acid (60 minutes)

Sialic Acid + N-Acetylneuraminic aldolase \leftrightarrow Mannosamine + Pyruvic Acid

Pyruvic Acid + Pyruvate oxidase \rightarrow Acetylphosphate + H₂O₂

Dye + H₂O₂ \rightarrow Reporter Dye



For information on N-Glycan sample preparation and analysis see: N-Glycan Analysis: Better Together ([5994-1647EN](#))

Shown here are examples of linearity and reproducibility data for the included quantitative Neu5Ac standard supplied with Agilent AdvanceBio Total Sialic Acid Quantitation kits:

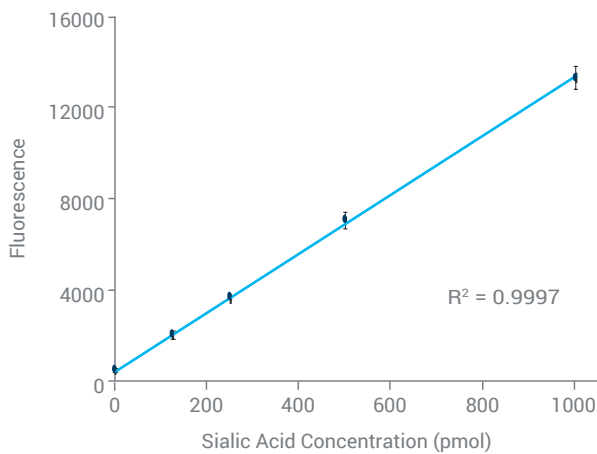


Figure 1. Agilent AdvanceBio Total Sialic Acid Quantitation kit standard curve. A five-point standard curve corresponding to 0, 125, 250, 500, and 1,000 pmol sialic acid with an R² value of 0.9997.

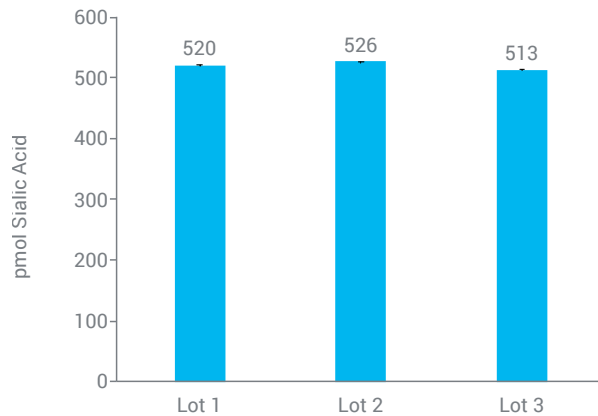


Figure 2. Neu5Ac sialic acid standard solution lot to lot comparison. 500 pmol sialic acid measurement.

Getting started with AdvanceBio Total Sialic Acid Quantitation kits

Sialic acid sample considerations

- Samples that can be measured by the kit include glycoproteins, glycopeptides, glycolipids, polysialic acids, or whole cells.
- The dynamic range of this assay is 40 to 1,000 pmol (fluorescence detection) and 500 to 4,000 pmol (absorbance detection). Sample concentration may need to be adjusted to assure the signal falls within the range.
- Some samples may contain free sialic acid. A negative control (sample blank) containing sample and all reaction components except for Sialidase A should be included in each run to account for free sialic acid signal in samples.
- Low levels of intrinsic glycoprotein fluorescence (or absorbance) will not interfere with sialic acid quantitation, as the negative control will be subtracted before determining the amount of sialic acid.
- Samples should be in water, PBS, or a similar buffer. Ideally the samples should not be in a high molarity buffer to ensure the correct reaction pH.
- Factors that can result in incomplete sialidase digestion include: too much sample in the reaction, insufficient incubation time or temperature, or situations when the sialic acid is sterically hindered from Sialidase A.
- Some O-acetylated sialic acids may be poor substrates for the N-acetylneuraminic aldolase and may not give an accurate value for the sialic acid content. The presence of O-acetyl groups can be confirmed by DMB derivatization followed by HPLC analysis using the AdvanceBio Sialic Acid Profiling and Quantitation kit (p/n GS24-SAP)^{3,4}. If present, de-O-acetylation of the sample can be carried out by mild base hydrolysis before treatment with the converting enzymes.

Incubation hardware

During the AdvanceBio Total Sialic Acid Quantitation sample preparation, the samples are enzymatically released in 30 minutes at 37°C. Conversion and color development occurs during a 60-minute incubation at 37 °C. For heating the samples in the 96-well plate provided, we recommend using a laboratory oven or block heater capable of 37 °C incubation. A heat block with a flat surface to accept a 96-well skirted plate (for example, VWR 13259-295 Modular Heating Block for Titer Plate) is also required. Alternatively, a plate reader with 37 °C temperature control may be used (no need for the heater and block).

Incubation Hardware (non-Agilent)	Part No.
Dry Block Heater, 4 Block (Qty 2) (Troemner)	HB4DG
Modular Heating Block for Titer plate (VWR)	13259-295

Easy selection and ordering information

To order items listed in the tables below from the Agilent online store, add items to your Favorite Products list by clicking on the MyList link in the header. Then, enter the quantities for the products you need, Add to Cart, and proceed to checkout. Your list will remain under Favorite Products for your use with future orders.

If this is your first time using Favorite Products, you will be asked to enter your email address for account verification. If you have an existing Agilent account, you will be able to log in. However, if you don't have a registered Agilent account, you will need to register for one. This feature is valid only in regions that are e-commerce enabled. All items can also be ordered online by clicking on the individual part numbers or through your regular sales and distributor channels.

MyList 1 of AdvanceBio Total Sialic Acid Quantitation consumables

Description	Part No.
Sample Preparation	
AdvanceBio Total Sialic Acid Quantitation kit, 48-ct	GS48-SAQ
AdvanceBio Total Sialic Acid Quantitation kit, 96-ct	GS96-SAQ
Standards	
Bovine Fetuin, heat-treated (0.4 mg)*	WS0021
Agilent-NISTmAb, 1 x 25 µL	5191-5744
Agilent-NISTmAb, 4 x 25 µL	5191-5745
Optional items for Agilent PlateLoc Thermal Microplate Sealer†	
Peelable aluminum plate seal	24210-001

* Not available for online sale. Contact your Agilent Representative for ordering information.

† Learn more about the Agilent PlateLoc Thermal Microplate sealer.

References

1. Agilent BioTek plate readers:
 - a. Synergy H1 Hybrid Multi-Mode reader
 - b. Synergy Neo2 Hybrid Multi-Mode Reader
 - c. Synergy LX Multi-Mode Reader
2. An Improved Workflow for Profiling and Quantitation of Sialic Acids in Biotherapeutics (5994-2352EN).
3. Sialic Acid Analysis of Biotherapeutic Glycoproteins using AdvanceBio Sialic Acid Profiling and Quantitation Kit and LC/FLD/MS, (5994-4201EN).
4. Agilent AdvanceBio Sialic Acid Profiling and Quantitation Kit Flyer (5994-2788EN).
5. Agilent AdvanceBio Total Sialic Acid Quantitation Kit (5994-1227EN).

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