Agilent AdvanceBio Sialic Acid Quantitation Kit

Streamlined sample processing

Advantages and Benefits

- Rapid and reliable total quantitation of sialic acid
- Broad dynamic range
- Compatible with fluorescence and UV plate reader detection
- Minimal sialic acid degradation
- User-friendly, high-throughput format
- Available in two sizes, for 48 or 96 samples
- Automation-friendly workflow

10 µL Glycoprotein
30 min Sialidase A Digest
Released Sialic Acid
60 min Conversion and Development
Read the Plate
Ex 530 nm / Em 590 nm (FLD) 530 nm (Absorbance

High Throughput, High Sensitivity Sialic Acid Quantitation

Sialic acid is critical in mediating the effectiveness of therapeutic glycoproteins. The presence (or absence) of sialic acid on the non-reducing terminal of *N*- or *O*-glycans can dramatically affect the pharmacokinetics of the protein, as well as its immunogenicity. Therefore, it is essential that sialic acid content on protein therapeutics be maintained, controlled, and monitored at the highest possible level. The AdvanceBio Total Sialic Acid quantitation kit (formerly ProZyme) represents a sensitive and high-throughput approach to sialic acid quantitation. The workflow is based on coupled enzyme reactions, converting enzymatically released sialic acid to hydrogen peroxide, which reacts stoichiometrically with a dye, generating an intense fluorescence and absorbance response. This approach allows enzymatic release, conversion, detection, and quantitation of sialic acid to be performed in a single well of a 96-well plate for fast and simple sample processing.

Workflow

Step 1: Release of Sialic Acid

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

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

N-Acetylneuraminic aldolase catalyzes the reversible reaction:

Sialic Acid $\longleftrightarrow$ Mannosamine + Pyruvic Acid

Then pyruvate oxidase catalyzes the reaction:

Pyruvic Acid $\longrightarrow$ Acetylphosphate + H_2O_2

H_2O_2 forms a 1:1 complex with the Dye to form a fluorescent Reporter Dye that may be read by fluorescence or absorbance detection.

Dye + H_2O_2 $\longrightarrow$ Reporter Dye

Learn more:
www.agilent.com/chem/glycananalysis

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Sample Preparation and Considerations

The AdvanceBio Total Sialic Acid quantitation kit is compatible with a variety of sample types including glycoproteins, glycopeptides, glycolipids, polysialic acids as well as whole cells. For optimal results, sample should be contained in water, PBS, or other similar low-molarity buffers.

The dynamic range of this assay is between 40 and 1,000 pmol sialic acid for fluorescence detection and 500 to 4,000 pmol sialic acid for absorbance detection. The kit is available in two formats suitable for generating 48 and 96 data points. Each sample requires six data points when performed in triplicate (sample and negative control) or four data points when performed in duplicate. Each set of samples requires a sialic acid standard curve to be generated alongside samples for reliable and appropriate sialic acid quantitation (Figure 1). Sialic acid standard (*N*-acetylneuraminic acid), supplied as a 100 µM stock solution, is consistent and reliable across three separate lots of material (Figure 2). All data presented here was collected on a BioTek Synergy H1 microplate reader with fluorescence detection.

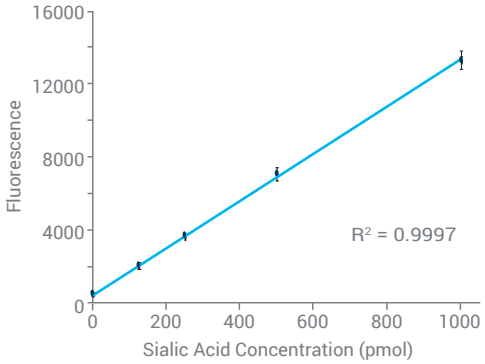

Figure 1. Agilent AdvanceBio Total Sialic Acid quantitation kit standard curve. A five-point standard curve corresponding to 0, 125, 250, 500, and 1,000 pmol sialic acid with an R2 value of 0.9997.

Example Data: Total Sialic Acid Quantitation Results

Table 1. Examples of various glycoprotein starting concentrations, along with total quantity of protein that are compatible with AdvanceBio Sialic Acid quantitation kit.

	Fetuin	MabThera*	Enbrel	Zaltrap	Orencia	EPO alfa
Concentration (mg/mL)	0.25	10	0.25	0.5	0.5	1
Sample volume (µL)	10	10*	10	10	10	10
Sample mass (µg)	2.5	100	2.5	5	5	10
MW (kDa)	48	145	150	115	92	30.4
pmol protein	52	690	16.7	43	54	329

*For glycoproteins with lower levels of sialylation such as monoclonal antibodies, up to 30 µL sample may be used with the kit.

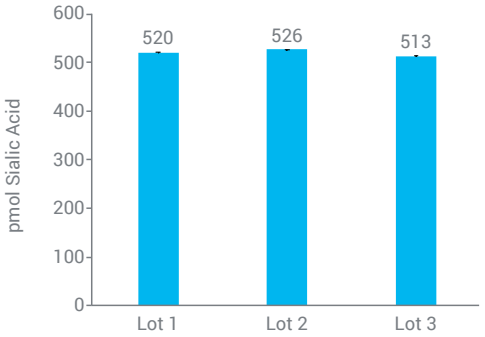

Figure 2. Neu5Ac sialic acid standard solution lot to lot comparison. 500 pmol sialic acid measurement.

Table 2. Representative total sialic acid quantitation for various glycoproteins. SA = sialic acid; N = 3.

	Fetuin	MabThera	Enbrel	Zaltrap	Orencia	EPO alfa
Starting concentration (mg/mL)	0.25	10	0.25	0.5	0.5	1
MW (kDa)	48	145	150	115	92	30.4
nmol SA/10 µL sample	0.58	0.08	0.55	0.45	0.59	0.16
nmol SA/mg protein	232.53	0.79	220.93	90.27	117.27	15.50
mol SA/mol protein	**11.16**	**0.11**	**33.14**	**10.38**	**10.79**	**0.47**
%CV (n=3)	0.55%	2.65%	1.81%	0.46%	0.60%	3.59%
SD (mol SA/mol protein)	0.06	0.00	0.60	0.05	0.06	0.02

Kit Components

The Agilent AdvanceBio Total Sialic Acid quantitation kit contains the following reagents and standards:

Module: AdvanceBio Total Sialic Acid Quantitation Kit GS48-SAQ and GS96-SAQ			
Component	**Units GS48-SAQ**	**Units GS96-SAQ**	**Storage**
100 µM *N*-acetylneuraminic acid (NANA, NeuAc) Sialic Acid Standard, 1 mL	1	2	–20 °C
Bovine Fetuin Control, 0.4 mg, lyophilized	1	2	–20 °C
SAQ Dye, lyophilized	1	2	–20 °C
Horseradish Peroxidase, lyophilized	1	2	-20 °C
DMSO	1	2	–20 °C – 4 °C
Conversion Reagent, lyophilized	1	2	–20 °C
SAQ Buffer A	1	2	–20 °C – 4 °C
SAQ Buffer B	1	2	–20 °C – 4 °C
SAQ Buffer C	1	2	–20 °C – 4 °C
Sialidase A	1	2	4 °C
96-Well Clear Bottom Microplate	1	2	–20 °C – RT
Sealing Film	1	2	–20 °C – RT

Ordering Information

Part number	Name
GS48-SAQ	Agilent AdvanceBio Total Sialic Acid quantitation kit, 48-ct
GS96-SAQ	Agilent AdvanceBio Total Sialic Acid quantitation kit, 96-ct

For the user manual, see Agilent publication number 5994-1277EN.

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agilent_inquiries@agilent.com

Europe
info_agilent@agilent.com

Asia Pacific
inquiry_lsca@agilent.com

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

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