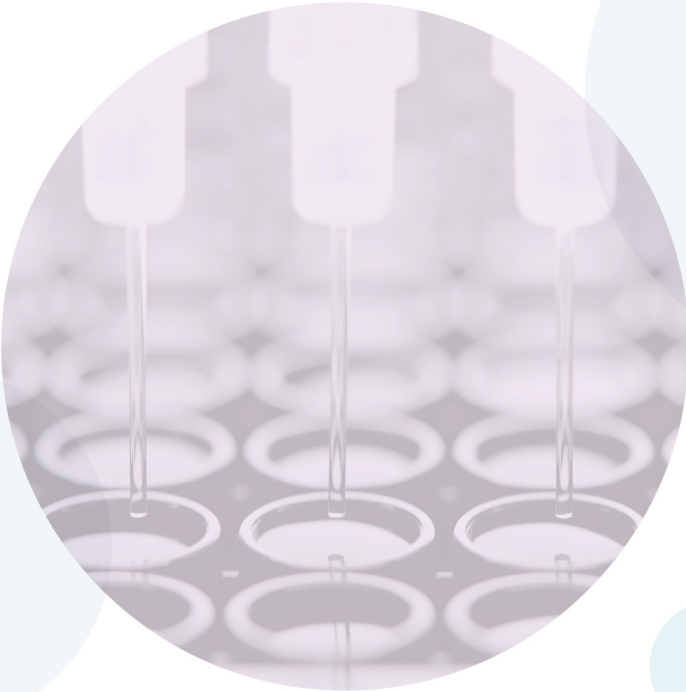


Sialic Acid (GlyS) Kit User Guide



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Sialic Acid (GlyS) Kit User Guide

Rev A

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Table of Contents

Chapter 1:	
Welcome	3
<i>Intended use</i>	4
<i>Introduction</i>	4
<i>Kit overview</i>	4
<i>Conventions and symbols used in this guide</i>	6
<i>ForteBio technical support</i>	6
Chapter 2:	
Getting Started	7
<i>Materials required</i>	8
<i>Storage and stability</i>	8
<i>Important procedural notes for optimal performance</i>	9
<i>Protocol Guidelines</i>	12
<i>Assay protocol: human mAb</i>	15
<i>For Octet RED96e, RED96, 384 and HTX (8, 16 and 96 channel systems)</i>	15
<i>Assay protocol: non-mAb</i>	20
<i>Assay protocol: Fc-fusion protein</i> ..	20
<i>Assay protocol for direct assay: purified glycoprotein</i>	21
Chapter 3:	
Analysis	23
<i>Glycan analysis</i>	24
<i>Loading Data Files</i>	24
<i>Viewing the Data</i>	24
	<i>Processing Data</i>
	25
	<i>Generating the Glycan Analysis Report</i>
	27
Chapter 4:	
Additional Assay Guidelines	31
<i>Control samples</i>	32
<i>Confirmation of signal by sialidase digestion assay</i>	32

CHAPTER 1:

Welcome

Intended use	4
Introduction	4
Kit overview	4
Conventions and symbols used in this guide	6
ForteBio technical support	6

Intended use

The Sialic Acid (GlyS) Kit is intended for relative screening of terminal sialic acid in crude or purified samples. It has been developed as a simpler and faster complement to HPLC/MS/LCMS methods with reduced hands-on time for customers who desire more information earlier in their workflow. The GlyS Kit provides relative screening and is not intended for absolute quantitation. This kit is for research use only and is not intended for diagnostic use.

Introduction

During cell line and drug development, post-translational modifications (PTMs) impact functional characteristics of the protein of interest (POI). These PTMs range from phosphorylation, glycosylation, methylation and many others. Glycosylation is a major PTM that has received of pharmacopeial attention due to its complexity and variability. Protein glycosylation requires the interaction of many enzymes and substrates, and it also depends on the cell line factors and culture conditions. These factors may induce significant variability in the POI. This variability can affect product safety and efficacy, more commonly referred to as critical quality attributes (CQA).

POI glycosylation patterns are considered a CQA due to different types of glycosylation having various impacts on the biological activity of a protein — protein folding, conformation, distribution, stability and activity. Protein glycosylation can affect *in vitro* stability (product shelf-life), isolation and purification steps (process consistency) and pharmacokinetics (half-life). Sialic acid is known to have an impact on the stability of a protein, and higher levels of sialic acid is often correlated with better stability. There is also literature showing the impact of sialic acid on clearance of drug molecules from a biological system.

ForteBio's Sialic Acid (GlyS) Kit provides a rapid and convenient method for relative screening of terminal sialic acid content in crude or purified samples. Sialic acid data can then be combined with titer data using Octet Data Analysis HT software version 11.1 or higher to funnel through POIs that are high producers and have desirable sialic acid content.

Kit overview

The GlyS Kit allows relative screening of terminal sialic acid content in crude or purified samples, and is comprised of GlyS biosensors and a reagent kit. GlyS biosensors are pre-immobilized with lectin that detects both N- and O-linked terminal sialic acid but has higher specificity towards O-linked terminal sialic acid. The reagent kit includes buffers for sample, antibody and detection substrate preparation.

The recommended workflow for the GlyS Kit is dependent on the type of sample to be analyzed. This user guide includes protocols for monoclonal antibody (mAb) samples, non-mAb samples, Fc-fusion proteins and purified samples. For purified samples, there

is no signal amplification required as the biosensor is binding only to the terminal sialic acid available on the protein of interest. However, for crude samples, since there is competition from sialic acid present on host cell glycoproteins (HCP) to bind to GlyS biosensors, the protocol provides necessary steps to selectively amplify signal from a POI's sialic acid and not from HCP. This selective amplification of signal ensures the signal is from the POI and not from HCP, and removes the need to purify samples. The GlyS kit can be used on any Octet system with different throughput experienced based on the system.

The glycan screening feature in Octet Data Analysis HT software version 11.1 and higher allows the combining of a POI's titer data and GlyS data. This results in a chart that shows combined information with titer (high/low producer) on the X-axis and relative sialic acid content (stability of POI) on the Y-axis. This feature enables decisions to be made faster based on CQA requirements. Details on how to use this analysis feature are covered in "Analysis" on page 23.

A list of Octet systems and channel capability for each are shown in Table 1-1. A 96-channel system provides highest throughput. A Sidekick station is recommended with an 8 or 16-channel system to increase throughput.

Table 1-1: Octet systems and channel capability

System	Number of Channels	Plate Types
Octet RED96/RED96e	8	96-well plates only
Octet QK ^e	8	96-well plates only
Octet RED384	8 or 16	96- and 384-well plates
Octet QK384	8 or 16	96- and 384-well plates
Octet HTX	8, 16, 32, 48 or 96	96- and 384-well plates

The workflow for relative screening of terminal sialic acid using the GlyS Kit with an Octet system is shown in Figure 1-1.

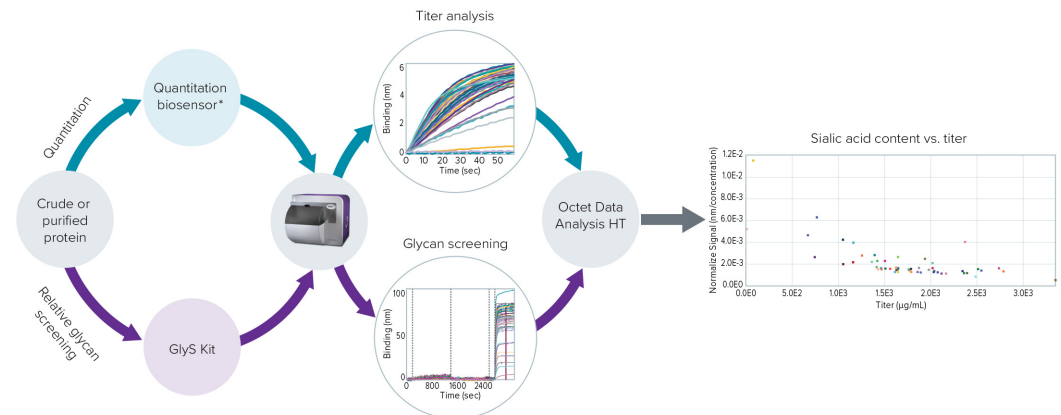


Figure 1-1: Sialic Acid (GlyS) Kit workflow on Octet system.

Conventions and symbols used in this guide



NOTE: Presents pertinent details on a topic. For example, general information, tips or alternate options.



IMPORTANT: Indicates the assay or procedure will not work if the guidelines provided are not properly followed.



WARNING & CAUTION: Informs the user that specific actions could cause irreversible consequences or damage. To prevent hazards, the manual should be read before operating the equipment.

ForteBio technical support

You can contact ForteBio technical support at:

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CHAPTER 2:

Getting Started

Materials required	8
Storage and stability	8
Important procedural notes for optimal performance	9
Protocol Guidelines	12
Assay protocol: human mAb	15
Assay protocol: non-mAb	20
Assay protocol: Fc-fusion protein	20
Assay protocol for direct assay: purified glycoprotein	21

Materials required

- Octet HTX, QK384, RED384, QK^e, RED96 or RED96e instrument with Octet Data Acquisition and Data Analysis software version 11.1 or higher
- A Sidekick station (ForteBio PN 30-5011) is highly recommended to improve throughput if using 8 or 16-channel instruments
- Black polypropylene 96-well or 384-well microplates (Greiner Bio-One 655209 or 781209) or 384 Tilted Well microplates.
- Sialic Acid (GlyS) Kit (ForteBio PN 18-5135) which includes the items listed in Table 2-1.
- PBS buffer without sodium azide
- Kinetics Buffer 10X (ForteBio PN 18-1105)
- Optional: Sialidase (Sigma N2876-25UN)
- Optional: Goat Anti-rabbitIgG, Secondary Detection Antibody (Jackson Immuno-research, 111-035-144 or equivalent)

Table 2-1: GlyS Kit contents

Item	Quantity
GlyS biosensors	1 tray of 96 biosensors
Glycan Buffer A	100 mL (2 x 50 mL)
Glycan Sample Prep Buffer	100 mL (2 x 50 mL)
Anti-human IgG Detection Antibody (50X)	0.5 mL
Glycan Detection Substrate (65X)	0.5 mL
Glycan Detection Buffer (40X)	0.72 mL
Glycan Wash Buffer	46 mL

Storage and stability

- GlyS biosensors should be stored at room temperature in the provided resealable bag with desiccant packet, away from direct sunlight. Do not refrigerate or freeze biosensors.
- All other reagents should be stored at 2-8 °C.
- Avoid exposure to higher temperature for all reagents (>25 °C).

- Store Glycan Detection Substrate and Glycan Detection Buffer in the dark. Prevent from long exposure to strong light.

Important procedural notes for optimal performance

- **Glycan Sample Prep Buffer.** All POIs should be diluted at least 1/10 (v/v) with Glycan Sample Prep Buffer for optimal glycan exposure to the biosensors. This buffer is specially formulated to relax folded glycoproteins so that terminal glycans can be accessible for binding to the GlyS biosensor.
- **Crude sample usage.** The GlyS Kit is designed to selectively amplify signal from the POI by using target secondary antibody (*i.e.* if the sample is human IgG, then an Anti-humanIgG detection antibody is used). This allows use of crude sample without any purification).
- **Assay interference.** HCPs in crude samples will directly compete with the POI for binding to the GlyS biosensor. Therefore, only glycoproteins with the same crude host cell conditions should be compared for meaningful results. It is important to design the experiment so that the following factors won't influence data interpretation:
 - Two different species of host cells (*i.e.* CHO cells and *E.coli* cells) will contain different host cell proteins with different levels of glycosylation. Thus, CHO HCP may compete with the POI differently than *E. coli* HCP and cause varying limitations for POI binding.
 - Crude samples from different stages of cell line development (CLD) may contain different amounts of HCPs. Thus, competition from samples which may contain lower amounts of HCP will be lower than that from samples which may contain higher amounts of HCP. It is recommended to test early and late-stage samples separately for a fair comparison.



NOTE: *Certain sample matrices (i.e. custom formulated media with varying amounts of sugars) can cause interference as well. It is important to test for assay interference as part of end-user validation.*

- **Reference (zero) sample.** A crude sample with similar HCP amounts but no POI expression is an ideal reference sample to measure background signal.
- **Assay Dynamic range.** GlyS amplification assay via secondary antibody will only pick up signals from the POI. However, presence of host cell glycoprotein will compress assay dynamic range depending on the amount of host cell glycoprotein present and/or the amount of sialylation of the host cell glycoproteins. Interference may cause non-linearity in the sample detection range. It is important to test for assay interference to determine the linear dynamic range of the sample as part of end-user validation.



NOTE: A signal shift of more than 60 nm is less accurate because the algorithm has a relatively less number of data points for calculation. If the binding signal nm shift is higher than 80 nm, it may exhibit a crashed signal pattern in the sensorgram. It is highly recommended to dilute samples to get a signal lower than 60 nm.

The optimal amount of starting glycoprotein should be determined by the end-user, depending on the level of sialylation and the method of detection used.



NOTE: We recommend performing a linearity test to determine the linear range (<60nm signal) of your POI. For example, dilutions ranging from 1/10, 1/100 and 1/1000 of your sample of interest with Glycan Sample Prep buffer should help determine the dilution required to remain in the linear range.

For mAb samples such as NIST human standard mAb, a dilution resulting in <10 µg/mL has shown to give reliable results. For non-mAb samples such as fetuin, a dilution resulting in <0.5 µg/mL has shown to give reliable results. For purified samples with high levels of sialic acid such as Erythropoietin (EPO) or fetuin, a direct binding assay with a dilution resulting in ~10-100 µg/mL has shown to give reliable results.

- **Binding specificity.** GlyS biosensors are pre-immobilized with a lectin that has high binding specificity towards O-linked terminal sialic acid in addition to other forms. Unlike typical antibody-antigen interactions, lectin-glycoprotein bindings are heterogenous and there may be non-specific binding. The preferred binding specificity of the pre-immobilized lectin on GlyS biosensors is shown in Figure 2-1 with it exhibiting highest preference towards the fifth structure.

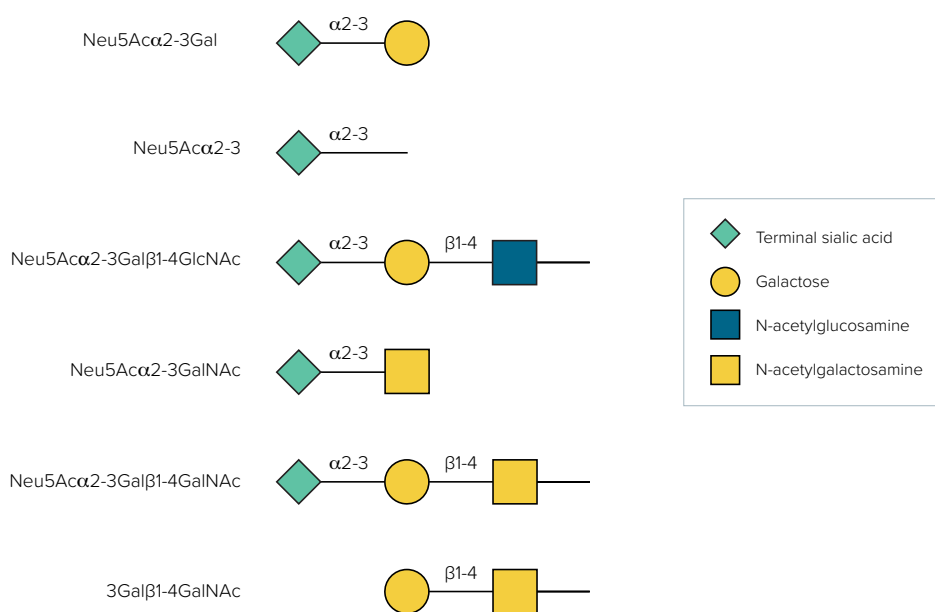


Figure 2-1: Preferred binding specificity of pre-immobilized lectin on GlyS biosensors towards different oligosaccharides.



NOTE: For meaningful comparison between early stage samples screened using the GlyS Kit and late stage samples characterized using HPLC/MS, it is recommended to use a total glycan digestion method which digests N- and O-linked glycans (e.g. acid hydrolysis) for glycan release during HPLC/MS validation to have comparable data with the GlyS Kit.

- **The GlyS Kit won't distinguish between NANA and NGNA forms of sialic acid.** The GlyS Kit is intended for relative screening (high/medium/low) of a POI's terminal sialic acid content in crude and purified samples only.
- **Sialidase Digestion Assay (Optional).** A sialidase digestion assay can be performed to validate binding specificity. A sialidase digestion workflow example is provided in “Additional Assay Guidelines” on page 31. As each biological sample's conditions are different, optimization of sialidase assay conditions according to the POI (amount of sample and enzyme to use, incubation time, etc.) is recommended.
- **Sidekick station (ForteBio PN 30-5011).** Use of a Sidekick station is recommended for this assay as it decreases total online assay time and improves throughput. A Sidekick station is not required when running this assay on the Octet HTX system, since all steps can be performed in high throughput (96-channel mode) on the instrument.

- **Re-use of reagents**
 - Avoid reusing reagents if possible
 - Glycan Detection Mix at working concentration can be re-used in a single experiment for multiple biosensor dips when using a Sidekick station.
 - Glycan Detection Substrate in a 96-well plate (200 μ L volume) can be re-used/dipped up to 10 times.
 - Glycan Detection Substrate in a 384-well plate (80 μ L volume) should be used only once.
 - We strongly recommend using Anti-hlgG Detection Antibody for a single dip only. This step is crucial for amplification of the POI and cross-well contamination from previous dip can interfere with binding and hence affect amplification if re-dipped.
 - Re-use of reagents when not using a Sidekick station is not recommended.
- **Assay signal variation from lot-to-lot.** It is recommended to use the same lot of GlyS biosensors for side by side comparison of POIs as the ranking is relative. The variation does not impact results or assay linear range. Refer to Certificate of Analysis (CoA) for lot-specific details.
- **Do not mix and match kits and biosensor trays.** Kit reagents are paired with a specific lot of biosensors to perform optimally. Lot numbers for individual components of a kit are printed on the CoA.
- **Glycan Detection Mix.** Please refer to the material Safety Data Sheet (SDS) for safety information on the Glycan Detection Substrate and the Glycan Detection Buffer. Dispose of used and unused reagents in accordance with all local, state and federal guidelines. Proper safety measures should also be taken when handling hazardous materials.

Protocol Guidelines

Prior to beginning an assay:

- Remove reagents from 4 °C and allow to equilibrate to room temperature on the bench top.
- Set out biosensors, microplates, and additional materials on a clean workplace.
- Confirm the Octet instrument has been powered on for at least 3 hours.
- Protect Glycan Detection Substrate and Glycan Detection Buffer from strong light.
- Please refer to the material safety data sheet (SDS) for safety information on the Glycan Detection Substrate which contains acetonitrile. Proper personal safety measures should be taken when handling hazardous materials.
- Use 200 μ L/well volumes for 96-well microplates and 80 μ L/well volumes for 384-well microplates.

It is important to know your instrument capacity (see Table 2-2 and Table 2-3). Depending on the capacity of your Octet system and the availability of a Sidekick system, assay steps can be performed on- or off-line depending on your throughput needs.

Table 2-2: Instrument capacity, 96 samples per run.

	96-channel mode (no Sidekick)		16 channel mode + Sidekick		8 channel mode + Sidekick	
Samples per run	96		96		96	
Workflow	Online/ Sidekick	Duration (mins)	Online/ Sidekick	Duration (mins)	Online/ Sidekick	Duration (mins)
Baseline (Glycan Sample Prep Buffer)	Online	3	Sidekick	3	Sidekick	3
User Sample	Online	20*	Sidekick	20*	Sidekick	20*
Detection Ab	Online	20*	Sidekick	20*	Sidekick	20*
Wash (Glycan Wash Buffer)	Online	3	Online	48	Online	96
Detection (Glycan Detection Mix)	Online	5	Online	48	Online	96
Total	51 min		91 min (1.5 hrs)		139 min (2.3 hrs)	

*20 min is the recommended incubation time to reach saturation. The duration can be modified in assay settings if the POI reaches saturation sooner, or a custom condition is desired.

Table 2-3: Instrument capacity, less than 96 samples per run.

	16-channel mode (no Sidekick), 384-well plate	16-channel mode (no Sidekick), 96-well plate	8 channel mode (no Sidekick), 96-well plate
Samples per run	64	32	16
Workflow	Online/ Sidekick	Online/ Sidekick	Online/ Sidekick
<ul style="list-style-type: none"> • Baseline (Glycan Sample Prep Buffer) • User Sample • Detection Ab • Wash (Glycan Wash Buffer) • Detection (Glycan Detection Mix) 	All steps online	All steps online	All steps online
Total	3.5 hrs	2 hrs	2 hrs

Assay protocol: human mAb

FOR OCTET RED96E, RED96, 384 AND HTX (8, 16 AND 96 CHANNEL SYSTEMS)

Option 1: No sidekick station

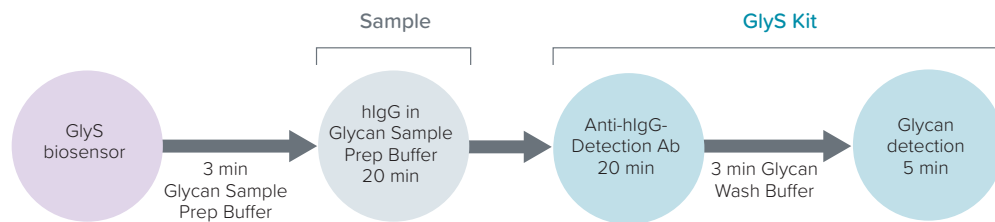


Figure 2-2: Human mAb assay workflow (no Sidekick station).

Table 2-4: Reagent preparation overview.

Reagent	Preparation
Human mAb (protein of interest)	1:10 or more dilution with glycan sample prep buffer
Glycan Sample Prep Buffer	1X
Anti-humanIgG Detection Anti-body (50X)	1:50 dilution with Sample Dilution Buffer
Glycan Detection Mix	See Glycan Detection Mix prep in Table 2-5
Glycan Wash Buffer	1X
Glycan Buffer A	1X

- 1. Prepare the sample plate.** You can choose either a 96-well plate (200 μ L volume/well), a 384-well plate (80 μ L volume/well) or a 384 tilted-well plate (40 μ L volume/well) depending on your sample availability.

NOTE: All buffers and diluents used in this assay should be azide-free.

- Pipette **Glycan Sample Prep Buffer** into the plate wells. Use 200 μ L for 96-well plates or 80 μ L for 384-well plates. The number of wells used should correspond to the number of samples being analyzed.

- b. Pipette your **human IgG samples** prepared in Glycan Sample Prep Buffer into the next column of wells. Use 200 μL for 96-well plates or 80 μL for 384-well plates.

The POIs should be diluted at least 1:10 using Glycan Sample Prep Buffer. This dilution is important to relax the glycoproteins so that terminal sialic acid can be accessible for binding to GlyS biosensor.

- c. Pipette **Anti-humanIgG Detection Antibody** prepared at 1:50 dilution in Glycan Buffer A into the next column of wells. Use 200 μL for 96-well plates or 80 μL for 384-well plates.
- d. Pipette **Glycan Wash Buffer** into the next column of wells. Use 200 μL for 96-well plates or 80 μL for 384-well plates.
- e. Prepare **Glycan Detection Mix**. Scale the Glycan Detection Mix volume up or down as needed based on your sample numbers. In 960 μL of PBS buffer, add 25 μL of Glycan Detection Buffer and 15 μL of Glycan Detection Substrate to make 1 mL of Glycan Detection Mix (Table 2-5).

NOTE: *The Glycan Detection Mix needs to be prepared fresh and we recommend it be used within 3-5 hours.*

Table 2-5: Glycan Detection Mix preparation.

Glycan Detection Mix (1 mL)*	Volume
PBS buffer	960 μL
Glycan Detection Buffer (40X)	25 μL
Glycan Detection Substrate, (65X)	15 μL
Total Glycan Detection Mix	1000 μL (1 mL)

*Scale the Glycan Detection Mix volume up or down as needed based on your sample numbers.

Please refer to the material safety data sheet (SDS) for safety information on the Glycan Detection Substrate and Glycan Detection Buffer. Dispose of used and unused reagents in accordance with all local, state and federal guidelines. Proper personnel safety measures should also be taken when handling hazardous materials.

- f. Pipette **Glycan Detection Mix** into the next column of wells. Use 200 μL for 96-well plates or 80 μL for 384-well plates. Examples of 384-well sample and reagent plate layouts are shown in Figure 2-3 and Figure 2-4.

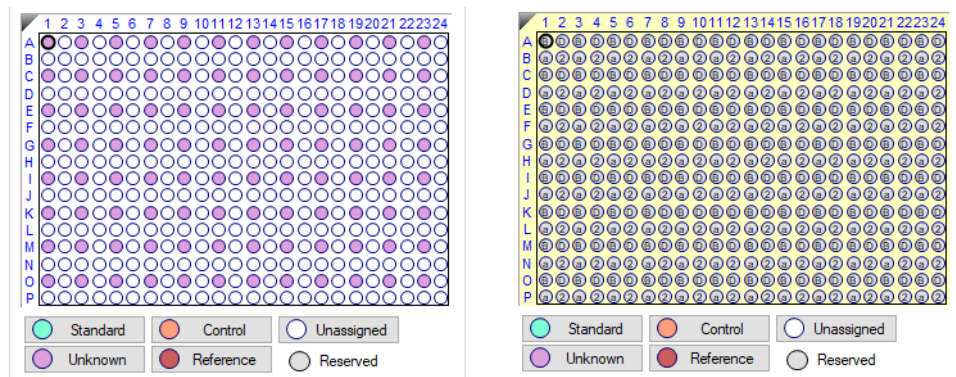


Figure 2-3: 96-channel mode using 384-well plates for the Octet HTX instrument. Left: sample plate. Right: reagent plate.

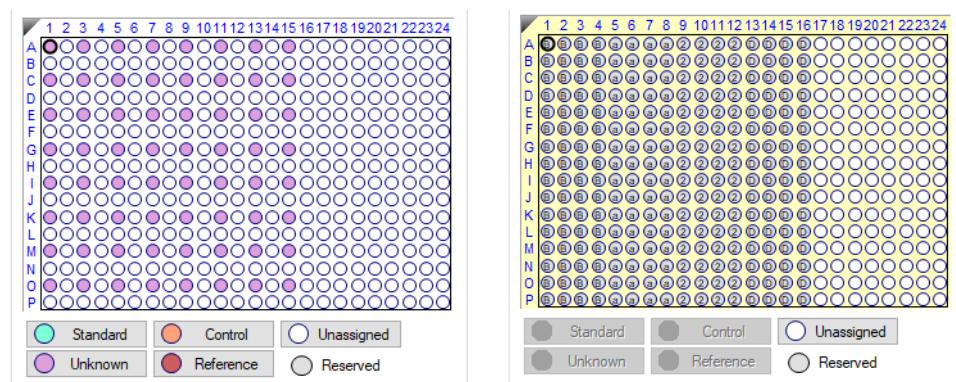


Figure 2-4: 16-channel mode using 384-well plates for Octet 384 and HTX instruments. Left: sample plate. Right: reagent plate.

2. **Pre-hydrate GlyS biosensors.** The number of GlyS biosensors used should correspond to the number of samples to be analyzed. There should be one full column of biosensors for each column (partial or full) of samples on the microplate.
 - a. Prepare a hydration plate by pipetting 200 μ L of Glycan Buffer A into each well of a 96-well plate. Well locations filled with Glycan Buffer A in the hydration plate should correspond to biosensor locations on biosensor tray.
 - b. Carefully disassemble the green biosensor tray from its blue holder tray
 - c. Place the pre-hydration plate into the blue holder tray.
 - d. Place the green tray with biosensors back on top of the blue holder tray such that the biosensors are dipping into the wells of the pre-hydration plate.
 - e. Let sit for at least 10 minutes.
3. **Run the assay.**

- a. Place the sample plate (detection plate if using the Sidekick station) and the GlyS biosensor tray assembly in the Octet instrument in the designated positions.
- b. In Octet Data Acquisition software, select **Advanced Quantitation** to view Sialic Acid Method files.
- c. Select the appropriate method file based on your instrument capacity and availability of a Sidekick station.
- d. In the Plate Definition tab, define **well location**, **Sample ID**, **concentration** and **dilution factor** for each of the samples
- e. Ensure the plate definition matches the actual sample and reagent positions on the plate(s). Modify assay settings and/or plate definition if necessary.
- f. Ensure the **Delay Start Time** box is checked in the Run Experiment Tab. A 600-second delay is recommended. A 300-second delay is required to enable the detection plate to equilibrate to 30 °C.
- g. Start the run. A full 96-well plate will take about 51 minutes to complete (including Sidekick station time).

Option 2: With a Sidekick Station

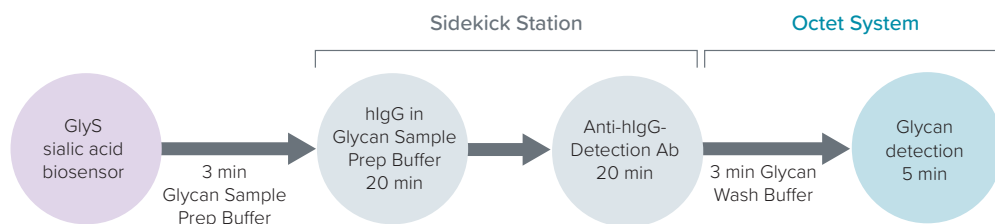


Figure 2-5: Human mAb assay workflow using a Sidekick station.

A Sidekick station is recommended for this assay as it decreases total online assay time and increases sample throughput for 8 and 16 channel systems. The Sidekick station is designed to mimic biosensor incubation in the Octet system at precise shaking speeds and temperatures.

NOTES:

Only 96-well plates can be used on the Sidekick station.

A Sidekick station is not required when running this assay on the Octet HTX system, since all steps can be performed on the instrument.

1. **Prepare samples and reagents.** See Step 1 in the human mAb assay protocol on page 15.

2. **Pre-hydrate GlyS biosensors using Glycan Buffer A.** Shake at 1000 rpm for 1 minute at 30 °C.
3. **Baseline.** Hydrate GlyS biosensors using Glycan Sample Prep Buffer. Shake at 1000 rpm for 3 minutes at 30 °C.
4. **Sample.** Capture sample of interest onto GlyS biosensors. Shake at 1000 rpm for 20 minutes at 30 °C.
5. **Detection Ab.** Perform the amplification step using Anti-hlgG Detection Antibody. Shake at 1000 rpm for 20 minutes at 30 °C.
6. **Detection plate.** Prepare Glycan Detection Mix and detection plate to run online on the Octet system.
 - a. Add 200 µL of Glycan Wash Buffer into Column 1, wells A-H, of a black, flat-bottom 96-well plate.
 - b. Add 200 µL of Glycan Detection Mix into Column 2, wells A-H, of the same plate.
7. **Transition from Sidekick station to Octet system.**
 - a. Once the Anti-hlgG Detection Antibody incubation is complete, remove the tray of GlyS biosensors from the Sidekick station.
 - b. Place the pre-hydration plate (step 1) containing Glycan Buffer A into the blue biosensor tray holder in the GlyS biosensor tray, and re-assemble the tray accessories by placing the GlyS biosensor tray on top so that the biosensor tips are soaking in Glycan Buffer A.
8. **Run detection steps on Octet system.** A full 96-well sample plate will take 1.5 hours using 8-channel mode or 48 minutes using 16-channel mode to complete.

Assay protocol: non-mAb

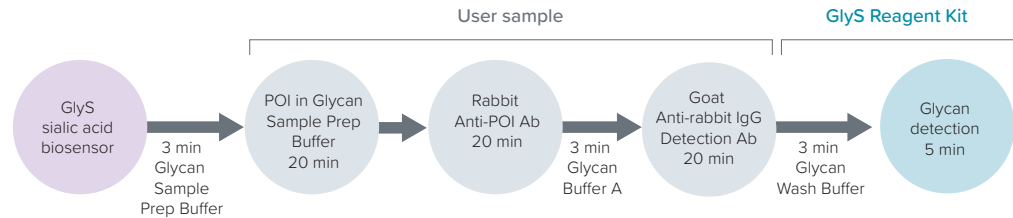


Figure 2-6: Non-mAb assay workflow.

Recommendations for this assay:

- An antibody against POI is required.
- A polyclonal antibody from rabbit works well in most cases.
- As a detection antibody, Goat-AntiRabbitIgG Detection Antibody (Jackson ImmunoResearch, PN111-035-144 or equivalent; requires to be HRP-labeled) can be used at a 1:50 dilution (~20 µg/mL) in Glycan Buffer A.
- Follow the “Assay protocol: human mAb” on page 15 with the exception of adding antibody against the POI on the sample plate and a 20 minute incubation time for the same in assay parameters.

Assay protocol: Fc-fusion protein

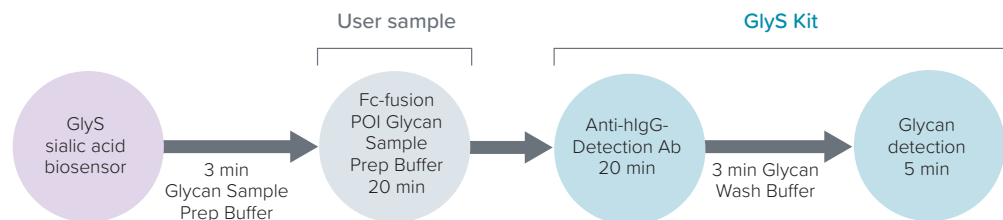


Figure 2-7: Fc-fusion protein assay workflow.

Recommendations for this assay:

- If the Fc-tag is from a non-human source, use an appropriate amplification Ab specific to the non-human Fc-tag. If the Fc-tag is from a human source, use the “Assay protocol: human mAb” on page 15.

- Since the glycosylation level will be different for each therapeutic protein under different conditions, the assay conditions and workflow should be adjusted as part of end-user assay optimization.

Assay protocol for direct assay: purified glycoprotein

A direct assay can be used for purified POIs. In a direct assay, no amplification steps are required. If the protein's level of glycosylation is low, the amplification assay workflow is recommended.

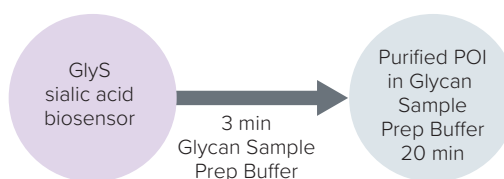


Figure 2-8: Direct assay workflow.

Table 2-6: Reagent preparation overview.

Reagent	Preparation
Glycan Buffer A	1X
Glycan Sample Prep Buffer	1X
Purified protein of interest	1:10 or more dilution with Glycan Sample Prep Buffer

- 1. Prepare the sample plate.**
 - a. Pipette 200 μ L of **Glycan Buffer A** into the wells of 96-well plate. The number of wells used should correspond to the number of samples being analyzed.
 - b. Pipette 200 μ L of **Glycan Sample Prep Buffer** into the next column of wells.
 - c. Pipette 200 μ L of **purified POI** prepared in Glycan Sample Prep Buffer into the next column of wells. At least a 1:10 v/v dilution of POI in Glycan Sample Prep Buffer is required.
- 2. Prepare the biosensor tray.** Biosensor locations should correspond to filled POI wells in the sample plate (one biosensor for each POI well in the sample plate).
 - a. Prepare a pre-hydration plate by pipetting 200 μ L of Glycan Buffer A into each well of a 96-well plate. Well locations filled with Glycan Buffer A in the pre-hydration plate should correspond to biosensor locations in biosensor tray.

- b. Carefully disassemble the green biosensor tray from its blue holder tray.
- c. Place the pre-hydration plate into the blue holder tray.
- d. Place the green tray with biosensors back on top of the blue holder tray such that the biosensors are dipping into the wells of the pre-hydration plate.
- e. Let sit for at least 10 minutes.

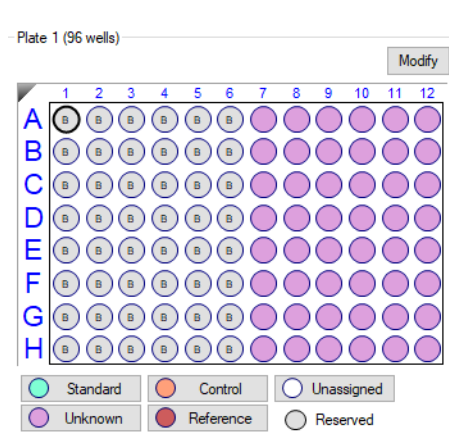


Figure 2-9: Example sample plate layout for purified POI direct assay.

3. Run the assay.

- a. In the Plate Definition tab, define **well location**, **Sample ID**, **concentration**, and **dilution factor** for each of the samples.
- b. Place the sample plate and GlyS biosensor tray assembly in the Octet instrument in their designated positions.
- c. In Octet Data Acquisition software, select **Advanced Quantitation** to view Sialic Acid Method files.
- d. Select the appropriate method file based on your instrument capacity.
- e. In the Plate Definition tab, define **well location**, **Sample ID**, **concentration**, and **dilution factor** for each of the samples if not done previously.
- f. Ensure the plate definition matches the actual sample and reagent positions on the plate(s). Modify assay settings and/or plate definition if necessary.
- g. Ensure the **Delay Start Time** box is checked in the Run Experiment Tab. A 600-second delay is recommended. A 300-second delay is required to enable the sample plate to equilibrate to 30 °C.
- h. Start the run. A full 96-well plate of samples will take about 51 minutes to complete.

CHAPTER 3:
Analysis

Glycan analysis 24

Glycan analysis

LOADING DATA FILES

You can preview data in Octet Data Analysis HT software by clicking on the experiment data file in the Experiment Explorer window (Figure 3-1).

To load data for analysis, select the folder with your glycan data in the Experiment Explorer window. Both glycan experiment and Protein A titer data files should be overlaid together in the software as shown in Figure 3-1. We currently do not support stand-alone glycan experiments where titer information is not available. To load this type of experiment file, double click the titer data file or drag it into the Segment window. Then select the lectin data file in the Experiment Explorer window and right-click to overlay the data onto the titer data file.

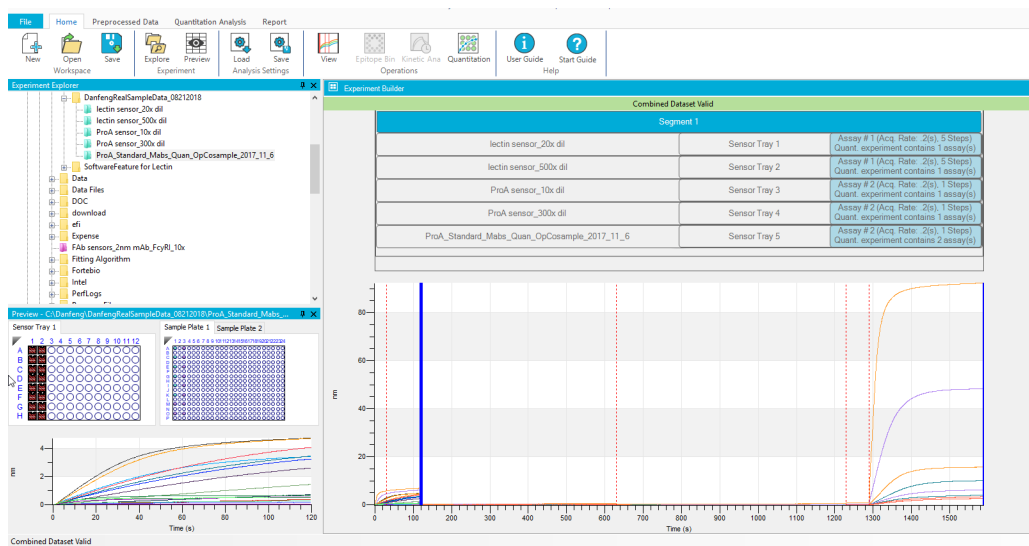


Figure 3-1: Loading Experiment Data

VIEWING THE DATA

1. Click on the **Preprocessed Data** tab, and view Sialic Acid or Titer data by selecting **Assay #1** or **Assay #2** (Figure 3-2).



NOTE: Sensor type, sample ID and dilution factor can be corrected in the Preprocess Data tab by selecting the wells on the sample or sample plate, right-clicking and selecting **Edit Sample Info** or **Edit Sensor Info**.

2. You can do all reference subtractions by first assigning the reference wells and then right-clicking and selecting the type of subtraction: **Subtract reference in selected wells** or **Subtract reference by column**.

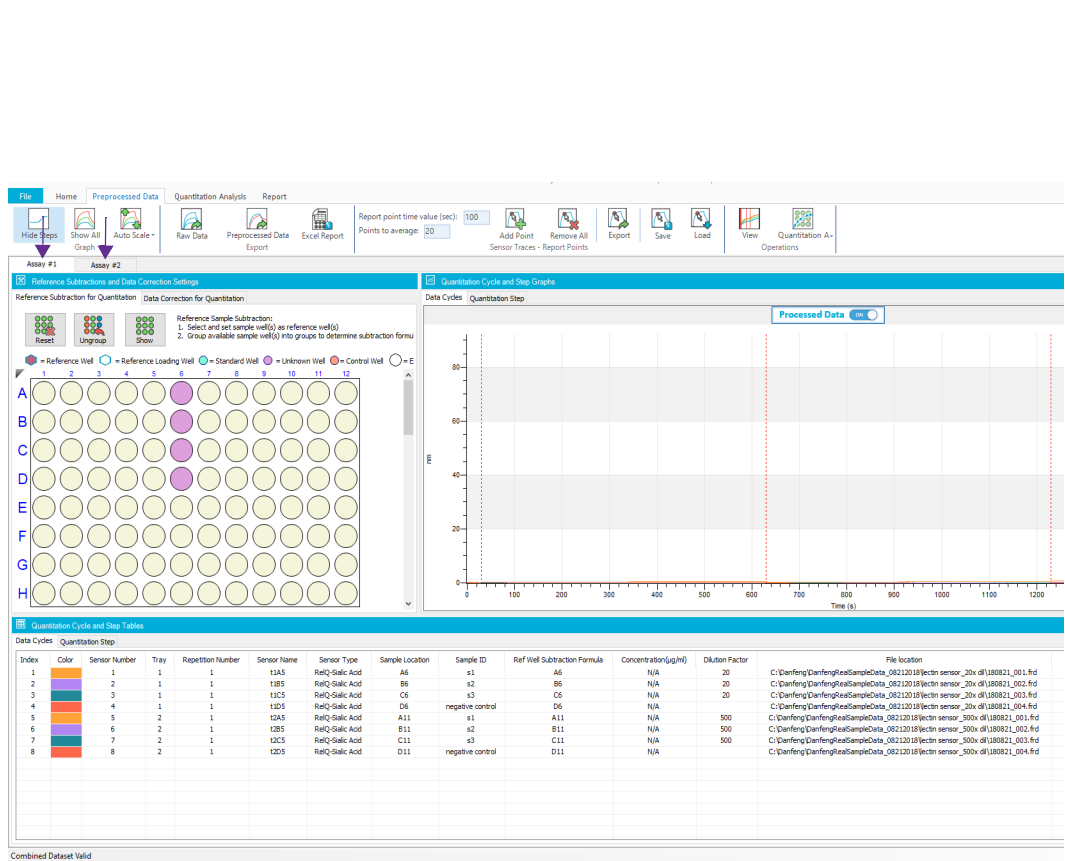



Figure 3-2: Viewing Sialic Acid and Titer Data

PROCESSING DATA

1. Click on the **Quantitation Analysis** tab.
2. Click on the **Titer Data** tab.
3. In the Standard Curve Equation window, select either 4-parameter logistic (4PL) weighted or unweighted, or 5-parameter logistic (5PL) weight or unweighted fit, and then the source for standard curve.

A separate standard curve can also be loaded by clicking  **(Load Standard)** (Figure 3-3).

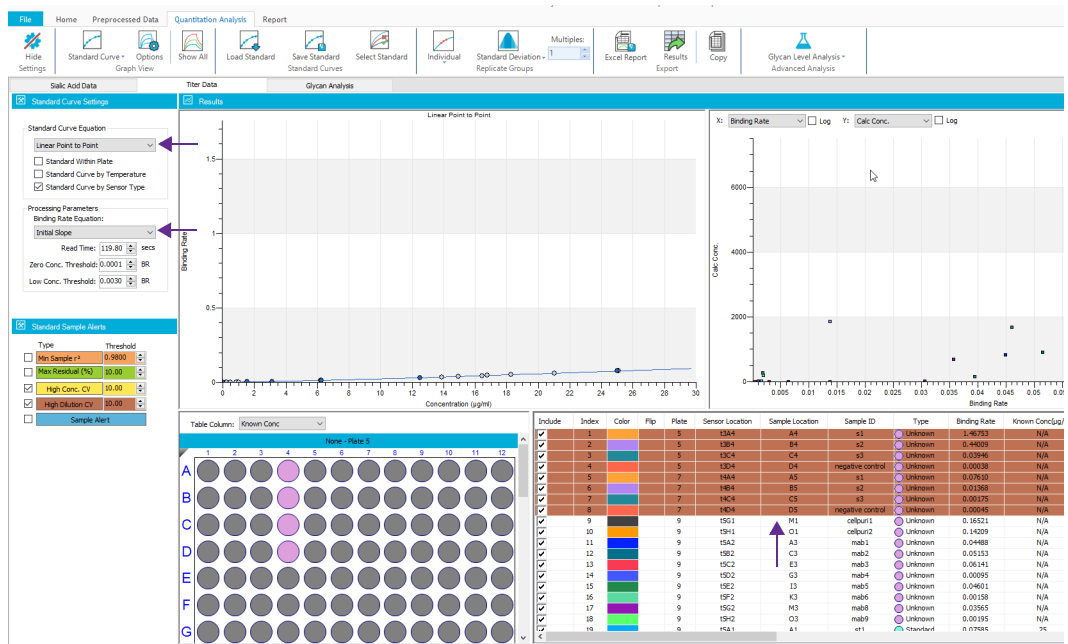



Figure 3-3: Selecting the Binding Rate Equation, Source for Standard Curve and Titer Concentration Data

4. Select **R equilibrium** as the Binding Rate Equation.
5. Pick the titer concentration data with desired dilution factors by checking or unchecking the **Include** box next to the rows in the table (Figure 3-3).
6. Click  (**Results**) to export the Protein A titer data.
7. Click on the **Glycan Analysis** tab.
8. Pick the glycan analysis data with desired dilution factors by checking or unchecking the box next to the rows in the table (Figure 3-4). The top graph on the right shows the titer information for all the samples, the middle graph is the Sialic Acid content of the samples and the bottom graph is the normalized binding signal (sialic acid content divided by the titer info for each sample).

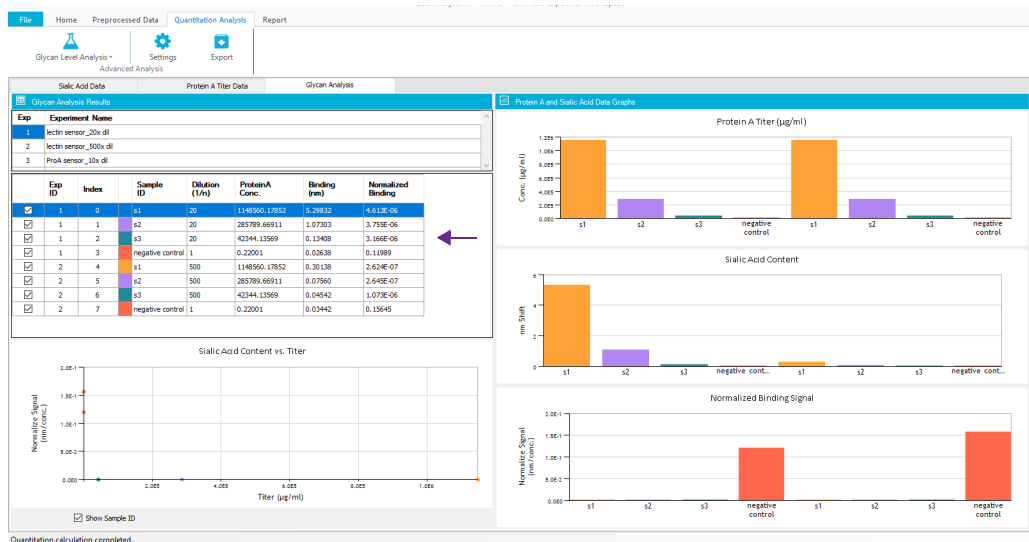



Figure 3-4: Picking Glycan Analysis Data

- Click  (Settings) if a sample had multiple dilutions in the quantitation assay and one of the dilutions needs to be excluded (Figure 3-4). You can then exclude data in the Raw concentration data table (Figure 3-5).

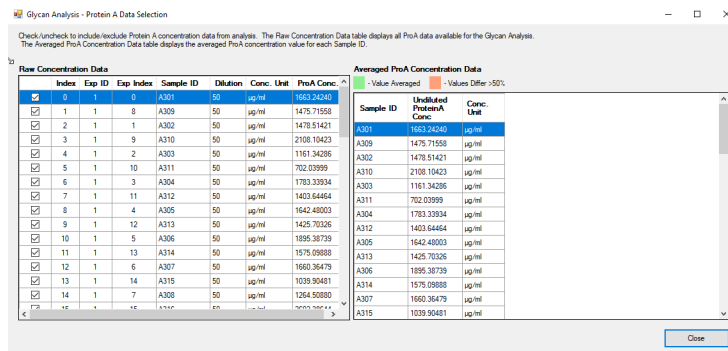



Figure 3-5: Raw Concentration Data

- Click the **Export** button to export the glycan analysis data.

GENERATING THE GLYCAN ANALYSIS REPORT

- In the Report tab, click  (Text) to record lot number and expiration date for the GlyS (Sialic Acid) (Figure 3-6).

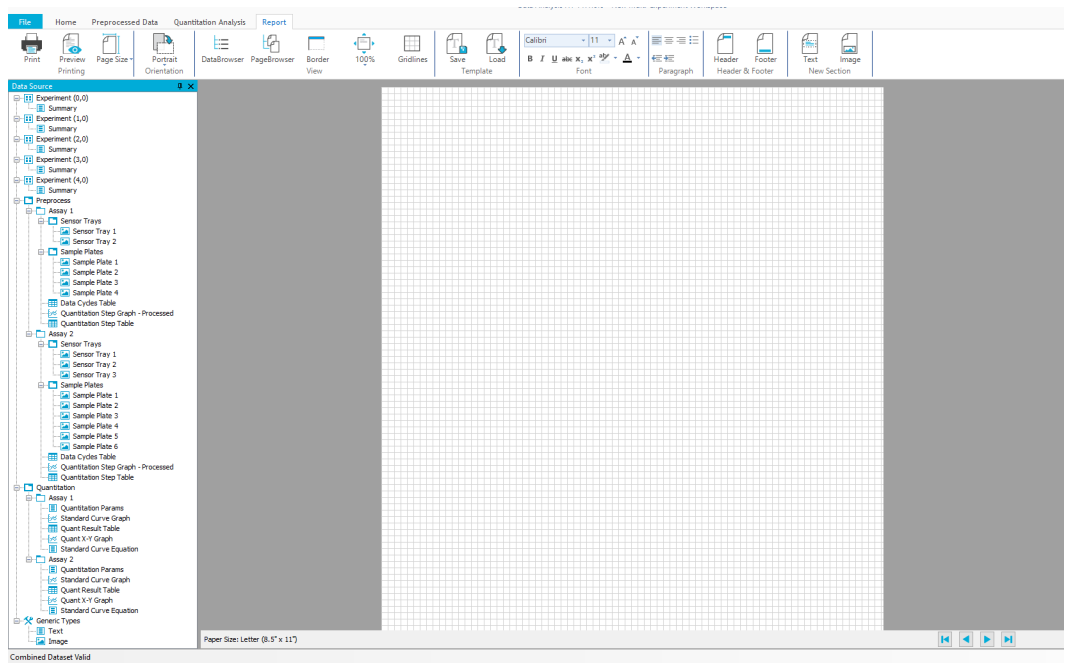
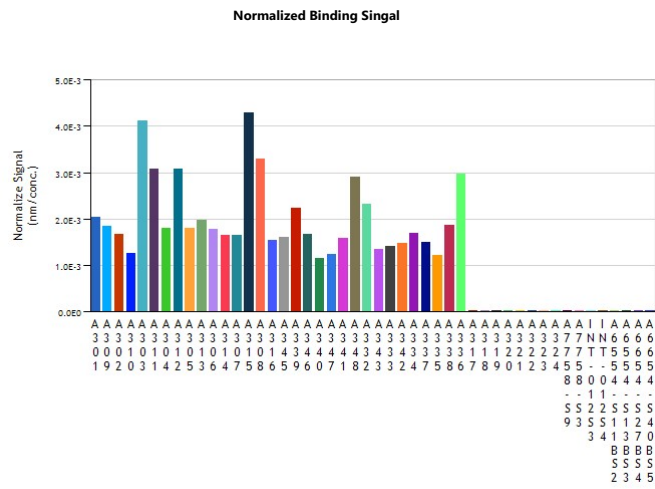
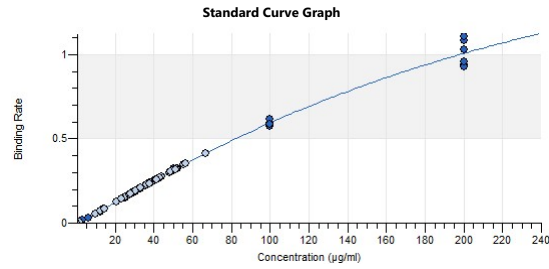


Figure 3-6: Generating the Glycan Analysis Report

2. Drag the information from the Data Source window to the Report grid to complete the report. An example is shown in Figure 3-7.

Sensor Type: GlyS Biosensor
 Lot Number: 489375
 Expiration Date: 5/2/2019



CHAPTER 4:

Additional Assay Guidelines

Control samples	32
Confirmation of signal by sialidase digestion assay	32

Control samples

Fetuin (non-mAb) and NIST (mAb-RM8671, NIH) are recommended as controls to confirm signal from the GlyS Kit. Both fetuin and NIST are well-studied and characterized for their sialic acid content and have publicly available literature. These should only be used as controls and not as standards for absolute quantitation; the GlyS Kit is intended for relative screening only.

Recommended concentrations for fetuin are 500 µg/mL and 7 µg/mL. Follow the “Assay protocol for direct assay: purified glycoprotein” on page 21 for fetuin.

Recommended concentrations for NIST are <10 µg/mL. Follow the “Assay protocol: human mAb” on page 15 for NIST.

Confirmation of signal by sialidase digestion assay

NOTE: Sialidase (N2876 Sigma; 25U powder) stock can be prepared by dissolving the powder in 1 mL ddH₂O. This will make 25 U/mL stock. Aliquot small volumes in Eppendorf tubes and store in -20 °C.

Add 10 µL of 25 U/mL sialidase stock to your POI for 96-well sample plate or 4 µL of 25 U/mL sialidase stock to your POI for a 384-well sample plate. Incubate at 37 °C for 1 hour. Digested samples should show no signal or reduced signal compared to undigested samples. An example of a sialidase digestion assay is shown in Figure 4-1. The non-treated sample exhibits a strong binding signal using GlyS Kit. However, the sialidase treated sample exhibits a diminished binding signal, confirming the binding specificity of GlyS Kit towards terminal sialic acid.

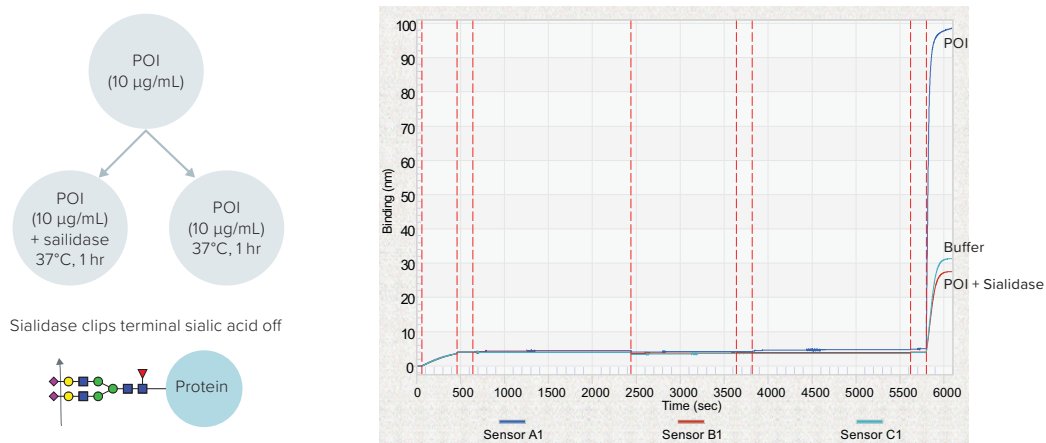


Figure 4-1: Sialidase digestion workflow and sialidase digestion data example.