Agilent

Agilent Gly-Q Glycan Analysis System (formerly ProZyme)

User Manual

Notices

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Safety Notices

CAUTION

A CAUTION notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a CAUTION notice until the indicated conditions are fully understood and met.

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A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

Contents

Safety Information

The Agilent Gly-Q system (formerly ProZyme) is intended to be installed and operated by the end user. To operate the system safely please review the following information.

WARNING **Both the instrument and the gel recharging air pump must be connected to an appropriate power source. (See "System Specifications" on page 48.)**

WARNING **Do not operate the system if any of the power cords are damaged.**

WARNING **The Gly-Q system is based on capillary gel electrophoresis separation driven by high voltage. Do not remove covers. Do not perform any maintenance of the electrical components.**

CAUTION **The instrument autosampler rotates and moves during operation. Keep the instrument door closed while running to avoid potential pinching hazard. Do not put your hands into the autosampler chamber unless the instrument is put into either the "Load Reagents" position or the "Load Samples" position and the autosampler has stopped moving.**

CAUTION **Follow local biohazard material handling regulations when working with human or animal samples.**

Suggestion: Wear appropriate gloves when handling samples and reagents used with the system.

CAUTION **Always turn off the power to the gel recharging air pump when emptying the condensation trap.**

Introduction

The Agilent Gly-Q system (formerly ProZyme)

The Gly-Q System is designed as a complete system for rapid analysis of oligosaccharides such as glycans. The system is comprised of capillary gel electrophoresis core integrated with a high sensitivity fluorescence detector and 96-well autosampler, replaceable capillary gel cartridge, gel recharging pump, Gly-Q Manager software and Gly-X with InstantQ assay kits and standards.

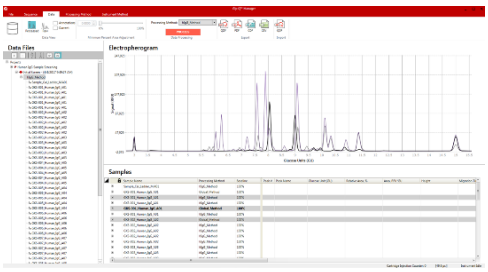
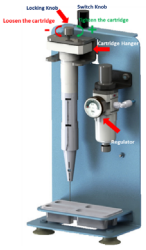
Description of system components

The Gly-Q System components are listed in **Table 1** and described in more details in the sections that follow.

Table 1 Agilent Gly-Q System components

	Agilent Gly-Q Instrument Product code GQ2000
	Agilent Gel recharging air pump (formerly ProZyme) Product code GQ2000-A
	Replaceable capillary gel cartridge
	Assay kit
	Standards and Controls

Table 1 Agilent Gly-Q System components (continued)

	Agilent Gly-Q manager software (formerly ProZyme)
	Cartridge priming station
	Agilent Gly-X manifold spacer (formerly ProZyme)

Gly-Q instrument

The Gly-Q Instrument is a compact unit comprised of a capillary gel electrophoresis core integrated with a high sensitivity fluorescence detector and 96-well autosampler. When used as part of a complete system, the instrument facilitates rapid separation, identification and relative quantitation of labeled oligosaccharides. See **"System Specifications"** on page 48 for instrument specifications.

Figure 1. Agilent Gly-Q instrument

Feature	Description
Instrument status LED	LED light serves as an indicator of the instrument status. The light blinks when high voltage is applied to the capillary.
96-well plate autosampler	The autosampler holds a 96-well microplate, system reagent vials and reagent trays. The autosampler moves and rotates during operation. The autosampler interior is protected by a hinged plastic door.
Power button	The power button turns the instrument On and Off
Air pressure gauge	Air pressure gauge displays inlet air pressure
Air inlet port	1/8 inch tube connector used for connecting instrument to gel recharging air pump
USB port	Computer port
Power inlet	Power supply port

Gel recharging air pump

The gel recharging air pump connects to the instrument and provides air pressure for automated recharging of the capillary with gel between sample injections. See **"System Specifications"** on page 48 for pump specifications.

Figure 2. Agilent Gel recharging air pump

Feature	Description
Air tubing (not shown)	1/8 inch tubing connects the pump to the instrument.
Power switch	Power switch turns the pump On and Off.
Condensation trap	Condensation trap collects condensate from air pump. This should be emptied weekly as routine maintenance.
Air outlet port	1/8 inch tube connector used for connecting gel recharging air pump to Instrument.
Power inlet (not shown on the back of the unit)	Power supply port.

Cartridge priming station

The cartridge priming station is designed to prepare the cartridge for first-time use before installing it into Gly-Q Instrument. The priming station can also be used to help troubleshoot cartridge clogging.

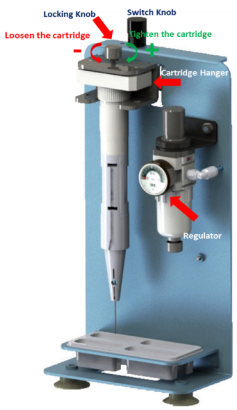


Figure 3. Cartridge priming station

Replaceable capillary gel cartridge

The replaceable capillary gel cartridge contains a 147-mm capillary mounted into the cartridge body with a gel matrix top reservoir and built-in electrode pins. The capillary is automatically refilled with gel matrix between sample runs.

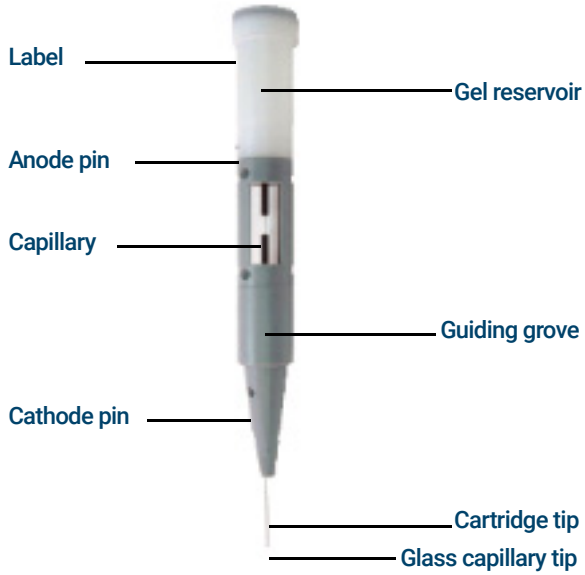


Figure 4. Replaceable capillary gel cartridge

Feature	Description
Capillary	Glass capillary. **Note:** fragile glass tip extends beyond metal cartridge tip
Anode pin	A pin that connects to a positively charged electrode
Cathode pin	A pin that connects to a negatively charged electrode
Label	Cartridge label with lot number and expiration date
Gel reservoir	Top reservoir containing separation gel
Guiding groove	Molded groove ensures correct cartridge positioning within the instrument
Cartridge tip	Metal tip of the cartridge

Sample preparation and Analysis kits

Agilent Gly-X N-Glycan rapid release and Labeling kits (formerly ProZyme)

Gly-X sample preparation kits use a novel in-solution enzymatic deglycosylation followed by labeling of released N-glycans with proprietary Agilent Instant dyes (formerly ProZyme). Gly-X with InstantPC (GX96-IPC) is designed for UHPLC and LC-MS applications. Gly-X with InstantQ (GX96-IPC) is specifically designed for CE separation on the Gly-Q System. Labeling (one minute) with InstantQ dye is followed by a simple 96-well vacuum plate-based clean up step. The samples are then ready for analysis on the Gly-Q Instrument. With the deglycosylation and labeling all carried out in solution, the method is simple, rapid, and suitable for automation.

Figure 5. Assay kit

Feature	Description
Gly-X Deglycosylation module for InstantQ Dye GX96-300	Reagents and consumables for rapid in-solution protein deglycosylation: • Gly-X Deglycosylation plate • Gly-X N-Glycanase • Gly-X Digestion buffer • Gly-X Denaturant • Gly-X Blocker (optional)
Gly-X InstantQ Labeling module GX96-301	Dye and solvents for instant labeling of released glycans: • InstantQ dye • InstantQ dye solvent • InstantQ activation reagent
Gly-X InstantQ Cleanup module GX96-302	Clean up plate, eluent and plastic consumables for sample clean-up before analysis: • InstantQ cleanup plate A, 96 wells • Gly-X collection plate, 96 wells • Waste tray for vacuum manifold • Gly-X Used-well sealing caps, black (for cleanup plate)
Gly-Q Cartridge module GQ103	Gly-Q Cartridge, separation buffer, mineral oil and reagent tray: • Gly-Q Cartridge • Gly-Q Separation buffer • Gly-Q Mineral oil • Gly-Q Reagent tray
Gly-Q Alignment standards module GKSQ-505	• Gly-Q Migration standards (GKSQ-500) • Gly-Q GU ladder (GKSQ-503)

NOTE **See the Agilent *Gly-X Rapid N-Glycan Release and Labeling with InstantQ Kit User Manual* (GX96-IQ) (formerly ProZyme) for more detailed product and protocol information.**

NOTE **Agilent GX100 Gly-X Manifold Spacer (formerly ProZyme) is required for the Agilent Gly-X Rapid Release and Labeling with InstantQ Dye Kit (formerly ProZyme).**

Glycan standards

Gly-Q alignment standards

Gly-Q glucose unit (GU) Ladder is generally the first run at the beginning of each sample sequence and produces >15 peaks in the electropherogram (see **Figure 6**). The Migration Standards (MS) coinjected with subsequent unknown samples will be aligned to the Maltodextrin (GU) ladder for glycan naming and alignment purposes.

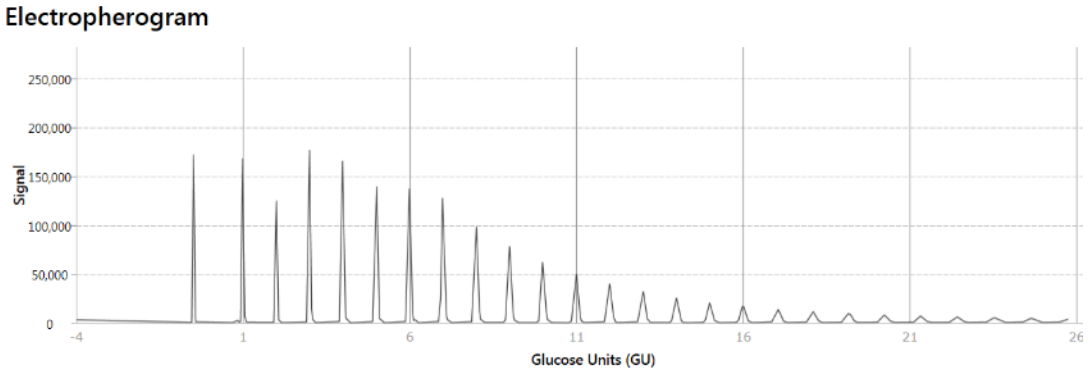


Figure 6. Electropherogram

Gly-Q Migration Standards (MS) are co-injected along with each unknown sample. A lower migration standard (LMS) and upper migration standard (UMS) corresponding to 3 and 15 glucose units respectively is used to align with the above described Maltodextrin (GU) ladder.

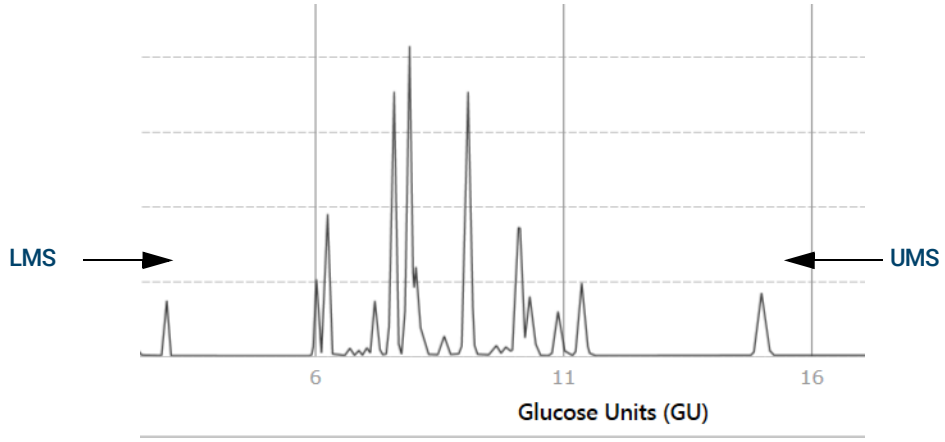


Figure 7. Gly-Q Migration standards

InstantQ Glycan standards

Standards are highly purified and well characterized individual oligosaccharides or mixed libraries of oligosaccharides. They are used for Gly-Q method calibration and validation. For the list of available InstantQ Standards and Controls, please refer to the following website links:

InstantQ Labeled Oligosaccharides

https://www.agilent.com/en/product/biopharma-hplc-analysis/glycan-analysis/glycobiology-standards-libraries/instantq-labeled-glycans

Gly-Q manager software

The Gly-Q Manager software controls Gly-Q instrument, collects and processes data, and reports results. The software features are organized into tabs and ribbon groups for easy intuitive navigation.

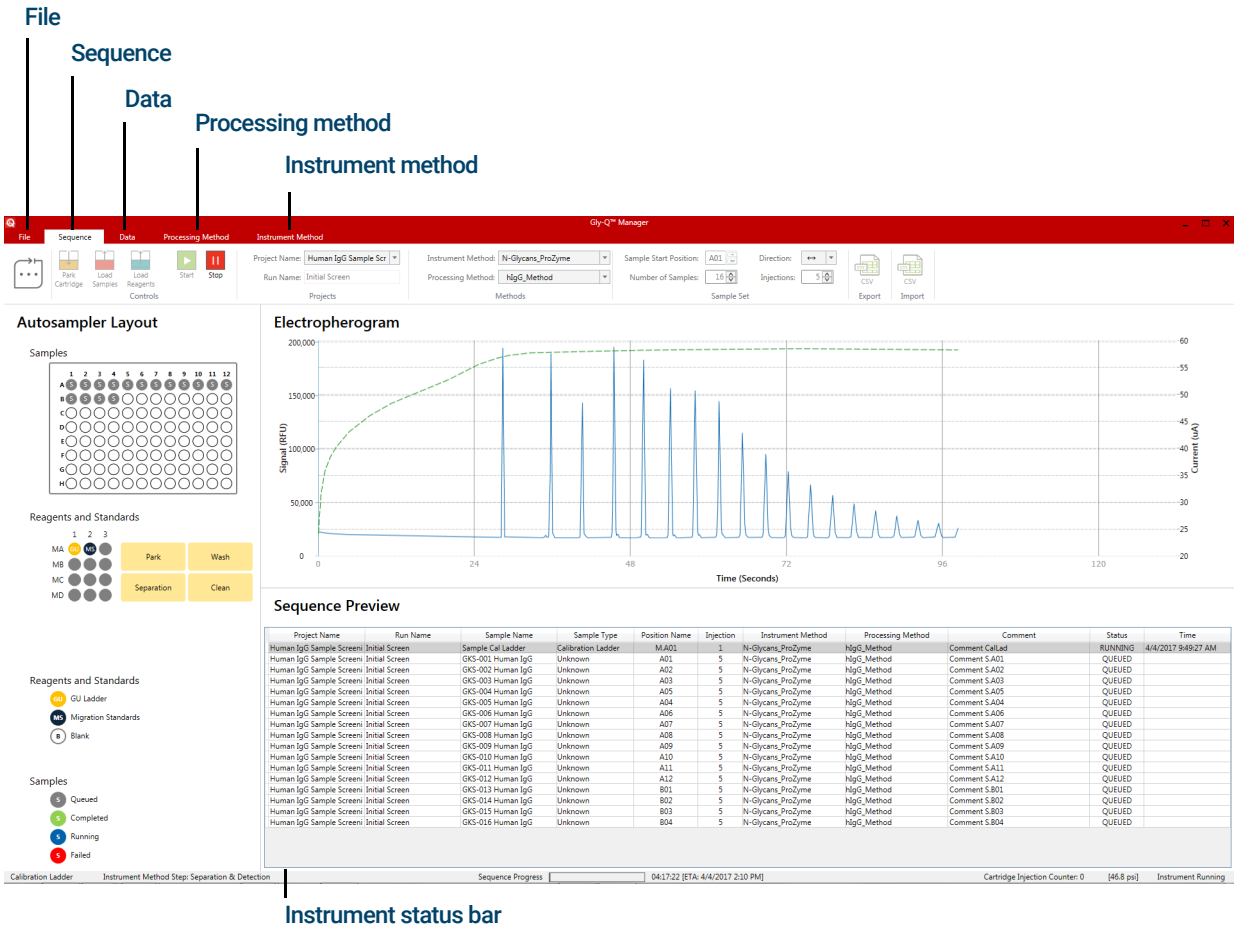


Figure 8. Gly-Q Manager software

Feature	Description
File tab	File tab enables and informs the user: • Serial number of Instrument and Cartridge • Gly-Q Manager software version • Air flow check • Gel flow check
Sequence tab	Sequence tab enables: • Access to the autosampler • Programming a sequence • Starting and Stopping a sequence • Monitoring sequence operations in real-time
Data tab	Data tab enables: • Data review • Integration adjustment • Data reprocessing • Data export/reporting
Processing method tab	Processing method tab enables: • Processing method design

Feature	Description
Instrument method tab	Instrument method tab enables: • Instrument method design • Saving an instrument method
Instrument status bar	Instrument status bar informs the user: • Instrument connectivity • Progress of run • Estimated finish time of run

NOTE

See "GLY-Q Manager Software" on page 22 for more detailed description of Gly-Q Manager functionality.

Installation

Unpacking

Inspect shipping boxes. If you notice apparent damage, contact Agilent Technical Support at **https://www.agilent.com/en/contact-us/page**.

Unpack the system components and place them on the laboratory bench.

NOTE

The Gly-Q Instrument contains sensitive optics that can be influenced by condensation. If the instrument was shipped in cold weather, do not remove the instrument from the shipping box immediately, allow it to warm up to room temperature.

System location

A suitable environment is important to ensure the best performance of the Gly-Q System. The recommended placement conditions are listed below:

- Flat and stable laboratory bench with no devices generating vibrations
- Clear space above the instrument to allow for capillary gel cartridge insertion
- Clear space behind the instrument to allow for air circulation, electrical and air tubing connections
- The ambient temperature should be stable and within a range of 20 to 30 °C
- The optimal operating humidity should be within 40 to 95% RH range, noncondensing
- The optimum altitude for Gly-Q System operation is 20 to 2,000 m

Power requirements

The input voltage of Gly-Q Instrument is 24 V DC and the maximum current is 1.25 A. A suitable power supply with a power cord is included in the package (100 to 240 V AC, 47 to 63 Hz). See **"System Specifications"** on page 48.

The input voltage of Gly-Q System gel recharging air pump is 12 V DC and the maximum current is 6.67 A. A suitable power supply with a power cord is included in the package (100 to 240 V AC, 47 to 63 Hz). See **"System Specifications"** on page 48.

Computer requirements

- Operating System — Windows 7, 8.1 or 10; 64-bit version
- Processor — 1 GHz or faster CPU
- Memory — Minimum 8 GB RAM
- Disk Drive — 1 TB Hard Disk Drive or 500 GB Solid-State Drive preferred
- Disk Space — Minimum 100 GB free space on drive (Solid-State Drive recommended)
- Monitor — Minimum resolution 1920 x 1080 (Full HD)
- Graphics Card — Supports Full HD (1920 x 1080 resolution)
- Port — USB 2.0 or greater

System setup

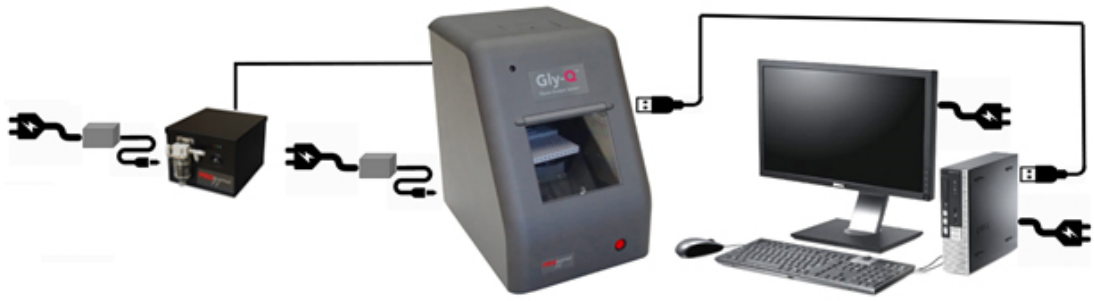


Figure 9. System setup

1. Plug the air pump power supply, Gly-Q Instrument power supply, computer and monitor into approved and properly grounded power outlets. A surge protector is suggested to minimize power fluctuations.
2. Plug each power supply low voltage line into the power inlet of the appropriate unit (Gly-Q Instrument power inlet and air pump power inlet). The two connectors are different to prevent using the wrong power supply.
3. Connect computer and Gly-Q Instrument with USB cable.
4. Connect air pump outlet and Gly-Q Instrument air inlet port with provided 1/8-inch tubing. The airline from the air pump to the instrument is bisected by an air filter.

NOTE

After turning on the air pump, it should run intermittently for less than one minute. If the air pump is running more consistently this is a sign of air pressure compromise that may affect performance. The user should check the air filter and tubing for possible leaks. Spare tubing and replacement air filter are shipped with the instrument. Contact Agilent if you need assistance.

Software installation

Figure 10. Agilent Gly-Q Manager setup window

1 Log into PC as Administrator.

2 Download the latest version of Gly-Q Manager from the Agilent website: https://www.agilent.com/en/products/capillary-electrophoresis-ce-ms/glycan-analyzer/gly-q.

NOTE **Please contact Agilent for password information.**

3 Double click on the .exe file, click on Gly-Q Manager setup.

4 Go through software installation steps.

NOTE **If you have an older version of Gly-Q Manager software installed on your computer, you will be asked to uninstall it prior to installing a newer version. Installing new version of Gly-Q Manager will not delete data. All user data will be preserved during installation.**

Cartridge preparation

Components

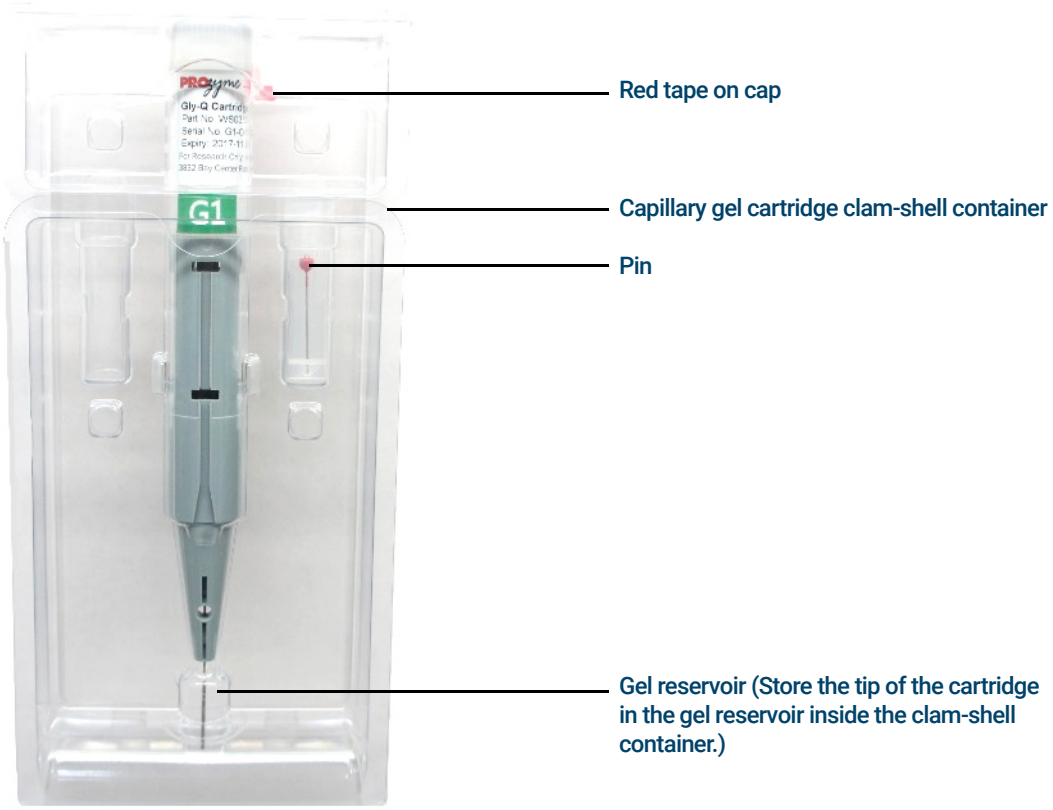


Figure 11. Components

Unpacking and Preparing the cartridge

1 Open gel-cartridge clam-shell container and remove the cartridge.

2 Peel off the tape from side and top of cartridge (retain top tape for storage of cartridge between uses).

3 Carefully remove and pin from package. Insert pin into the hole of the cartridge cap, and push it down to the end. Slight resistance should be encountered when performing this step.

Priming the cartridge

This procedure is to prime new cartridges. For subsequent runs, we recommend running a Gel Flow Check (**"File settings"** on page 44) each day before the cartridge is used.

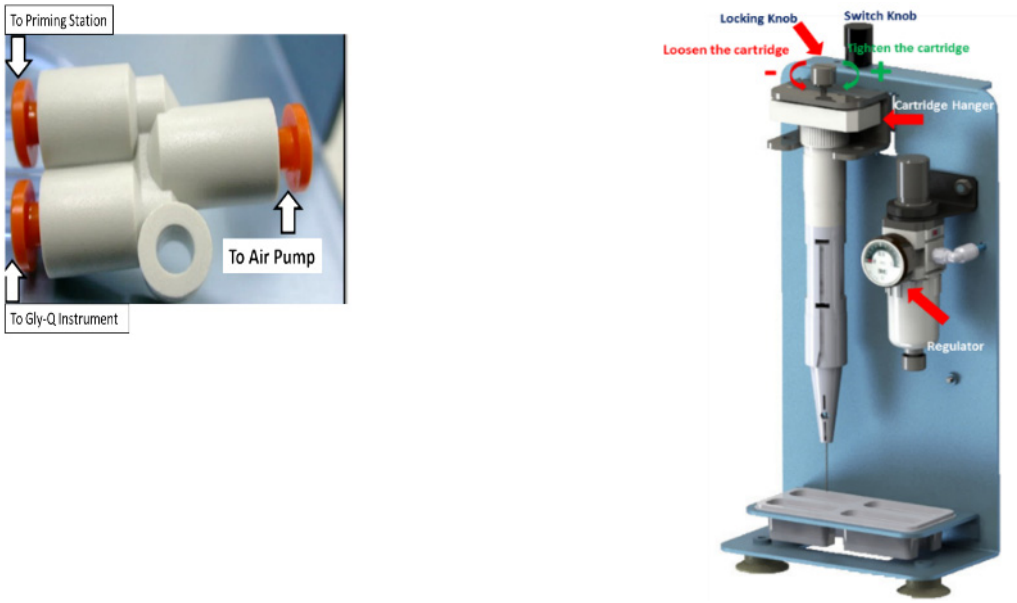
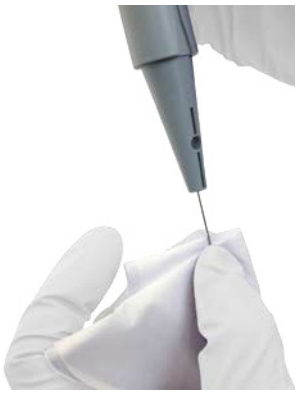


Figure 12. Priming station

1. Turn off the Air Pump, and use the illustration in **Figure 12** to reconnect the air tubing.
2. Put a buffer tray at the bottom of purge station.
3. Slide the cartridge into the Cartridge Hanger and secure by turning the Locking Knob as illustrated in **Figure 12**.
4. Push the knob on top of the regulator down.
5. Turn Air Pump ON.
6. Pull Regulator Knob UP and turn CLOCKWISE to open airflow until pressure reaches ~ 0.4 mPa.
7. Droplets of gel should be observed flowing at cartridge tip in ~ 30 to 120 seconds.
8. Turn the Regulator Knob COUNTERCLOCKWISE until the airflow is OFF.
9. Once the pressure returns to 0, push the Regulator Knob DOWN.
10. Slowly release the cartridge and remove from priming station.

Inserting the cartridge into the instrument

1 Gently wipe the cartridge tip with Kimwipe or similar tissue.



NOTE **The glass capillary extends beyond the end of the metal tip, and is fragile, handle with care, do not tap tip on bench or hard surfaces.**

2 After gently depressing cartridge door atop Gly-Q Instrument, insert cartridge and close door. Push the cartridge to the bottom.

Figure 13. Cartridge inserted in Gly-Q Instrument

NOTE **Cartridges can only be inserted in one orientation as shown in Figure 13.**

3 Close the Cartridge door.

NOTE **Dispose of spent cartridges by using a needle clipper, available with most sharps hazard systems to remove cartridge tip. The remaining cartridge is safely disposed of as trash. The clam-shell package can be added to most plastics recycling streams.**

Ending a run

1 After samples have been processed, the system will return the Cartridge to the Park position (see **"Autosampler layout"** on page 25).

2 Use the "Load Sample" and "Load Reagents" buttons to remove sample plate and reagent tray.

3 Store the cartridge.

   a Remove the cartridge from the instrument, and place tape over the cartridge cap (to cover the pin hole). Return it to the original clamshell packaging. The cartridge tip must be in contact with the gel block inside the package. Place inside the foil pouch, and store vertically at room temperature.

   b To store on the instrument: Press the **Park** button before shutting down the system. The cartridge can remain on the system for up to three days. Ensure that the Park position in the buffer tray (see **"Autosampler layout"** on page 25) is filled with water to prevent the capillary tip from drying.

Vacuum manifold setup

Agilent recommends the use of Millipore MultiScreen Vacuum Manifold (96-well) and Millipore High Output Pump (WP6211560 for 115 V/60 Hz or WP6222050 for 220 V/50 Hz).

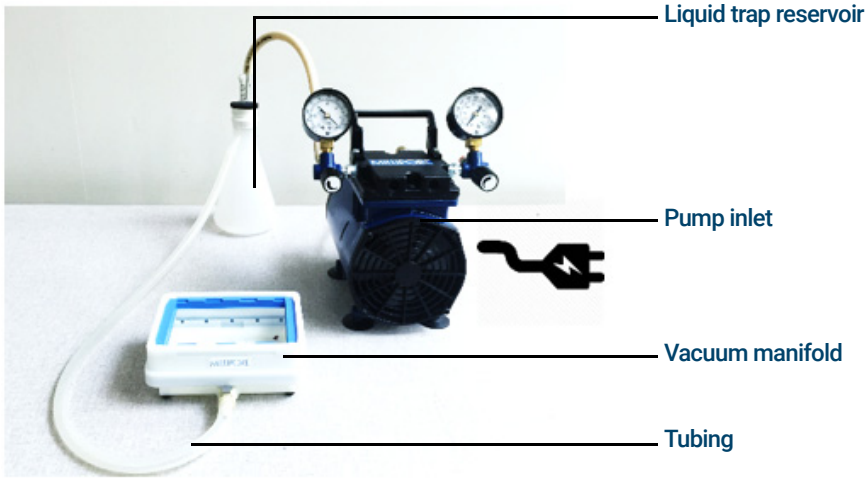


Figure 14. Vacuum manifold setup

1 Assemble the vacuum manifold.

2 Connect the vacuum manifold and pump inlet with tubing from the liquid trap reservoir.

3 Plug pump into an approved and properly grounded power outlet.

NOTE

For more detailed product information please refer to Millipore website:

http://www.emdmillipore.com/US/en/product/MultiScreen%E2%84%A2-Vacuum-Manifold-96-well,MM_NF-MAVM0960R#overview

https://www.emdmillipore.com/US/en/product/High-Output-Pump,-115-V60-Hz,MM_NF-WP6211560

GLY-Q Manager Software

Starting Gly-Q manager

To start the Gly-Q Manager, double-click the Gly-Q Manager icon on the desktop or select **Start -> Gly-Q Manager**.

Controlling and Monitoring Gly-Q instrument

To control and monitor the Gly-Q Instrument with Gly-Q Manager, the instrument must be turned on and connected to a computer with a USB cable.

The samples are always analyzed as part of a sequence using a predefined set of instrument commands stored in an Instrument Method and a predefined set of processing commands stored in a Processing Method.

When an analysis is running, you can monitor instrument operation through a status bar located at the bottom of the Gly-Q Manager screen, and observe real time detector signal and sequence progress on the autosampler layout and sequence progress table.

Direct instrument controls are accessible from the Sequence tab, see **Figure 15**.

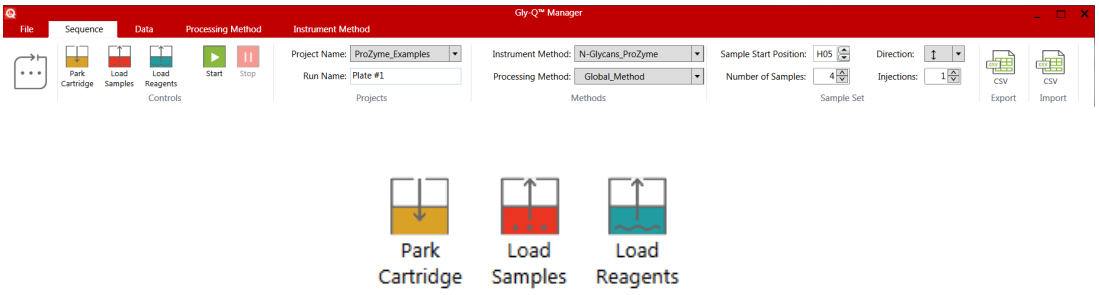


Figure 15. Sequence tab

Park Cartridge - Moves cartridge into Park position for short term storage on the instrument (up to three days).

Load Samples - Control rotates the autosampler for access to the sample loading stage.

Load Reagents - Control rotates the autosampler for access to load reagents including GU Ladder and Migration Standards.

Creating a sequence

A sequence defines how a group of samples is analyzed. Sequence can be created on the Sequence tab by defining the following fields:

Project and Run

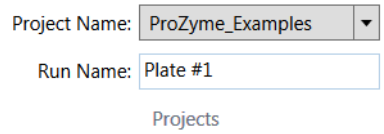


Figure 16. Project and Run options

1 Name your project by selecting **New Project** from the pull down, or select from the list of existing projects.

2 Name your run.

Instrument and Processing methods

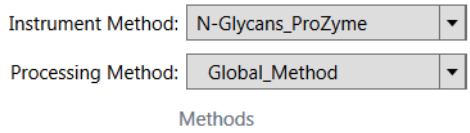


Figure 17. Instrument and Procession method options

1 Select Instrument Method.

2 Select Processing Method.

NOTE **Default Instrument Methods are recommended. See "Creating a processing method" on page 37 and "Creating an instrument method" on page 42 on how to create Instrument Methods and Processing Methods.**

Sample set

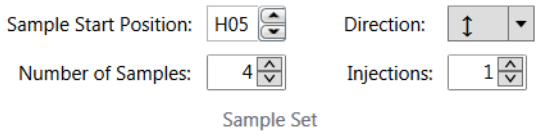


Figure 18. Sample set options

1 Define the position of the first sample.

2 Define the direction the samples will be injected (↔ or ↕).

3 Define the number of samples to be analyzed.

4 Define the number of injections (number of replicates for each sample).

NOTE **It is possible to return to a well periodically within a Sequence for repeat injections by specifying the well in the Position Name dropdown (see Figure 19). Be sure to edit the Sample Name if appropriate.**

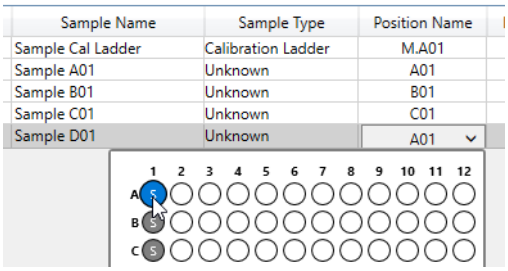


Figure 19. Position name dropdown

NOTE **Changes are saved automatically, and users are advised to double check these settings before starting a run. If needed, type sample names and comments into the sequence preview table or import sample set list from Excel, see import CSV in Figure 20.**

Import and Export

Figure 20. Import and Export CSV options

- Export feature allows for exporting a sequence in CSV (.xls) format
- Import feature allows for importing a sequence in CSV (.xls) format

Sequence preview

After a sequence is created, a table with a list of samples queued for analysis will be displayed on the main screen.

Project Name	Run Name	Sample Name	Sample Type	Position Name	Injection	Instrument Method	Processing Method	Comment	Status	Time
ProZyme_Examples	Plate #1	Sample_Cal_Ladder	Calibration Ladder	M.A01	1	N-Glycans_ProZyme	Global_Method	Sample_Cal_Ladder		
ProZyme_Examples	Plate #1	Enbrel	Unknown	D09	1	N-Glycans_ProZyme	Global_Method	Enbrel		
ProZyme_Examples	Plate #1	hIgG	Unknown	H05	1	N-Glycans_ProZyme	Global_Method	hIgG		
ProZyme_Examples	Plate #1	Rituxan	Unknown	H08	1	N-Glycans_ProZyme	Global_Method	Rituxan		
ProZyme_Examples	Plate #1	Rnase_B	Unknown	D08	1	N-Glycans_ProZyme	Global_Method	Rnase_B		

Figure 21. Samples table

Autosampler layout

The autosampler layout will show color coded positions for samples, reagents and standards.

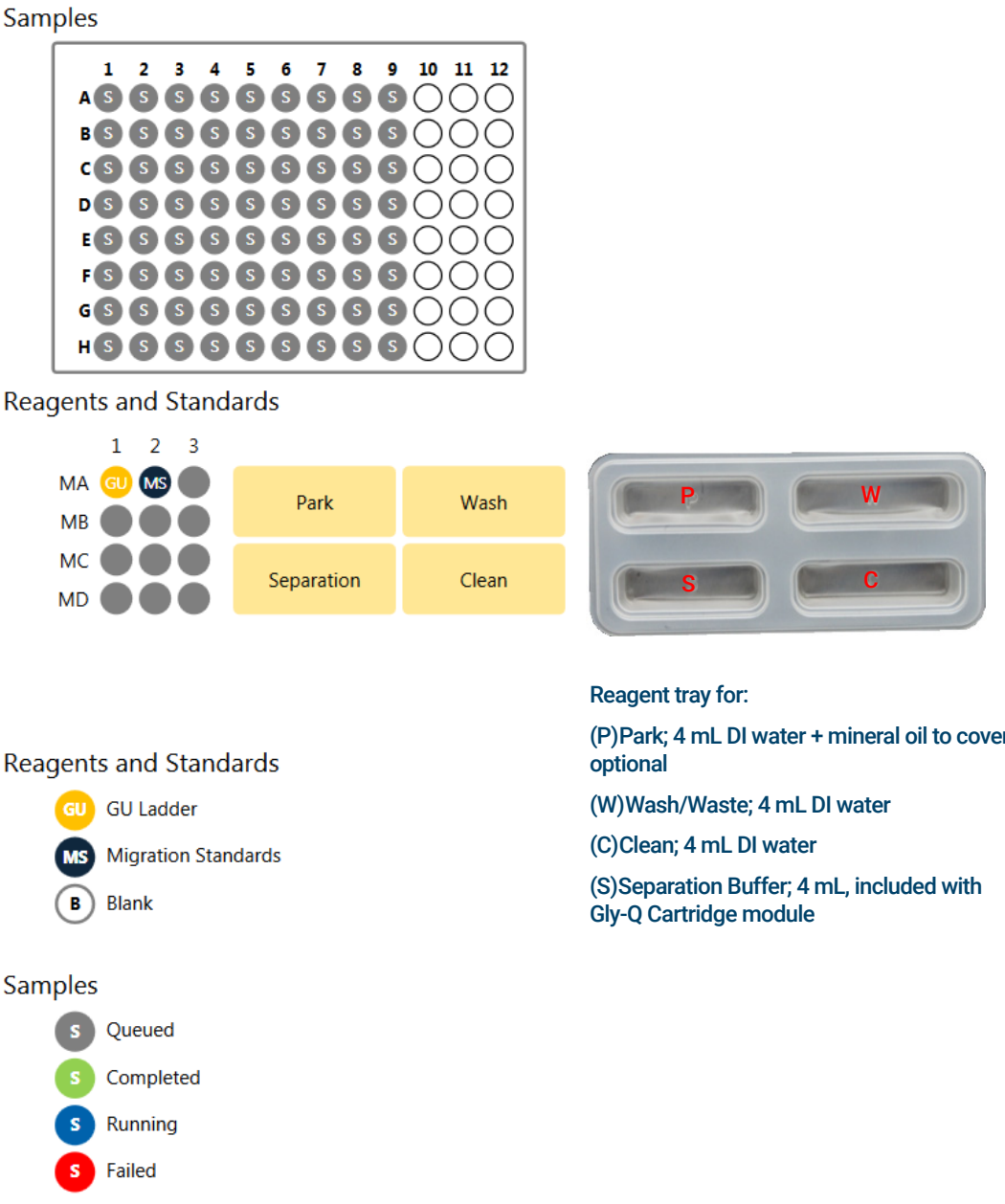


Figure 22. Autosampler layout

Sequence Status Bar

An instrument status bar at the bottom of the screen will display the instrument status.

A Sample name

B Instrument method step

C Well position

D Sequence progress and estimated time of completion

E Cartridge injection counter

F System Pressure

G Instrument status

Figure 23. Autosampler layout

Running a sequence

Starting and Stopping a sequence

After a sequence is created it can be run by clicking **Start**.

Figure 24. Start button

NOTE

If the system cannot read the information on the cartridge RFID tag the dialog shown in Figure 25 opens allowing the information to be entered manually. The information is on the cartridge and on the pouch used to store the cartridges between use. To activate the 'Run' button enter the cartridge serial number and use the 'date picker' control shown circled in blue to enter the expiration date.

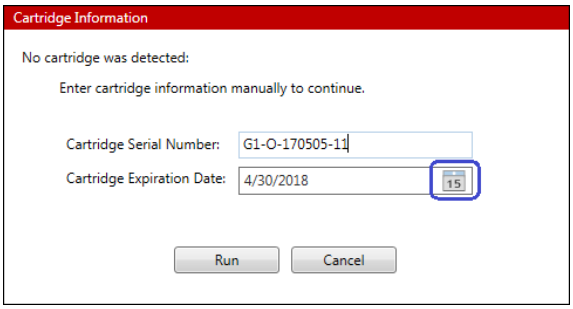


Figure 25. Cartridge information

NOTE **Make certain there is a cartridge installed in the instrument. If there is no cartridge in the instrument, then the system assumes the RFID tag is damaged and presents this dialog.**

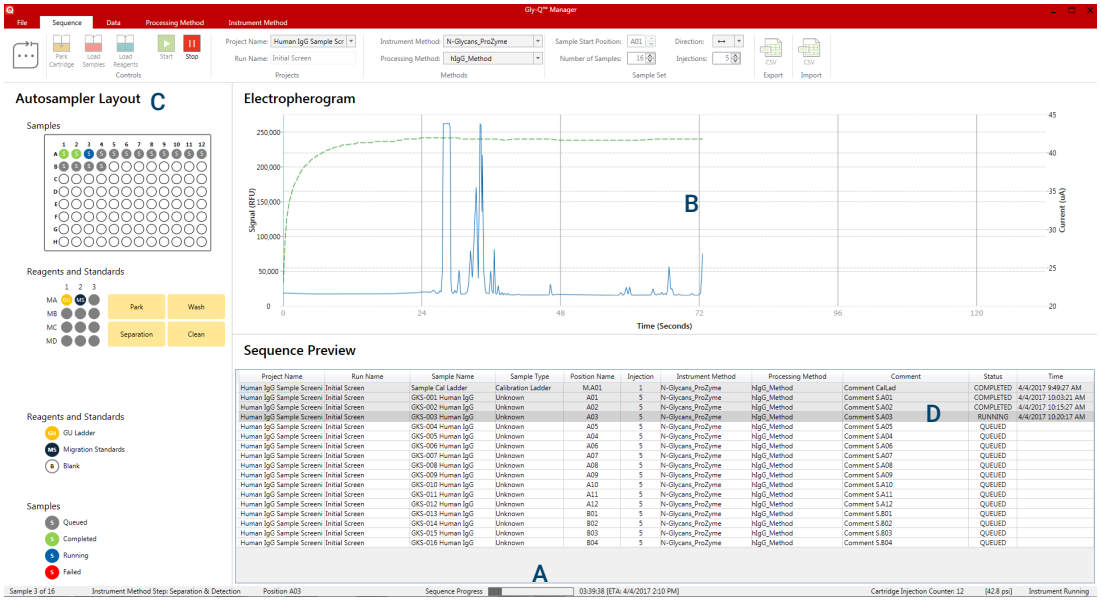
If a warning or error message regarding injection limits appears when starting a run refer to **"FAQs"** on page 53.

A sequence can be stopped by clicking **Stop**.

Figure 26. Stop button

Monitoring sequence progress

Sequence progress will be displayed on four elements of the screen.



A. Sequence progress bar at the bottom of the screen will show time remaining for sequence completion.

B. The electropherogram chart will show real time detector signal.

C. The autosampler layout will show completed samples (green), failed samples (red), currently running sample (blue) and queued samples (grey).

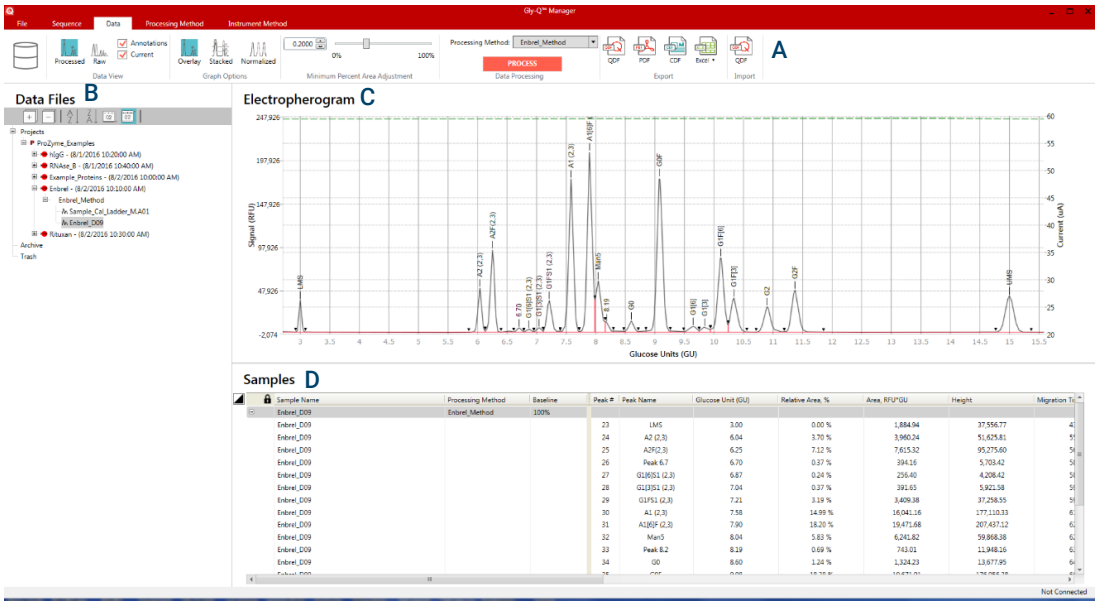
D. The sequence progress table will be updated with currently running sample highlighted in darker gray.

Figure 27. Four sequence screen elements

Data review, export, reporting, and management

After data for all samples in a sequence have been acquired and processed, it is useful to review electropherograms and peak tables before reporting the results. Peak integration may need to be adjusted before results are reported. This work is done on the Data tab shown **Figure 28**.

1 Select the Data tab.



The Data tab includes the following four areas:

A Data View Ribbon - this includes tools to adjust and to export data

B Data Files tree - the data you wish to work on is selected here

C Electropherogram - this is the data display which can include one or more samples

D Samples table - this is a list of the data that has been selected using the Data Files tree. This table is used to select the samples that will be displayed on the Electropherogram and to review the peak table for the selected samples.

Figure 28. Data tab

2 On the Data Files tree, select the sample set.

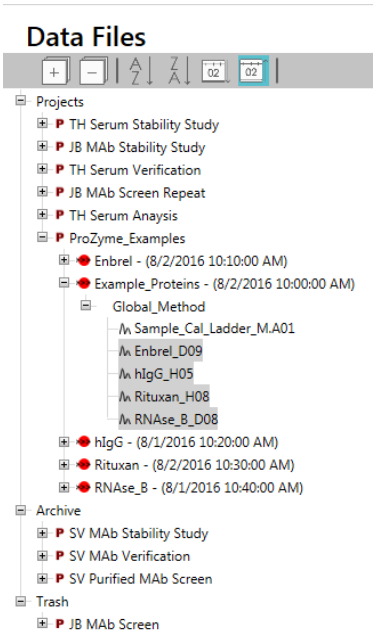


Figure 29. Data files

3 On the Data View ribbon, select processed or raw data view and decide to show or hide annotations and instrument current signal.

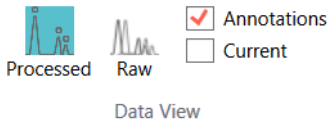


Figure 30. Data view

NOTE **Always start with processed data view with or without annotations. Raw data view and current are designed to aid in troubleshooting.**

4 On the Sample table, select the sample(s) you want to view.

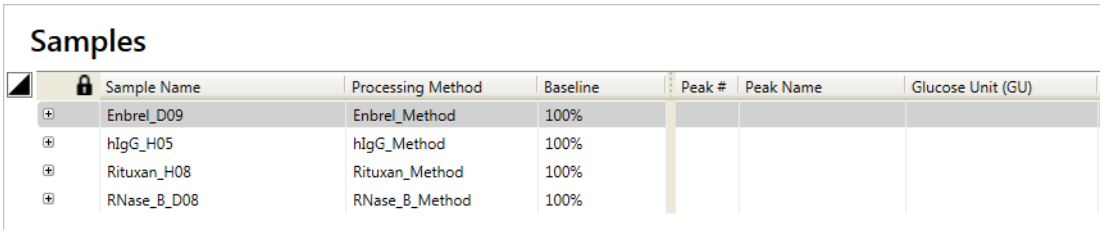


Figure 31. Sample table

5 Open the peak table for any or all of the samples selected by clicking on ⊞ sign before the sample name.

Sample Name	Processing Method	Baseline	Peak #	Peak Name	Glucose Unit (GU)	Relative Area, %
Sample_Cal_Ladder_M.A01	Enbrel_Method	100%				
Enbrel_D09	Enbrel_Method	100%				
Enbrel_D09			23	LMS	3.00	0.00 %
Enbrel_D09			24	A2 (2,3)	6.04	3.70 %
Enbrel_D09			25	A2F(2,3)	6.25	7.12 %
Enbrel_D09			26	Peak 6.7	6.70	0.37 %
Enbrel_D09			27	G1[6]S1 (2,3)	6.87	0.24 %
Enbrel_D09			28	G1[3]S1 (2,3)	7.04	0.37 %
Enbrel_D09			29	G1FS1 (2,3)	7.21	3.19 %
Enbrel_D09			30	A1 (2,3)	7.58	14.99 %
Enbrel_D09			31	A1[6]F (2,3)	7.90	18.20 %
Enbrel_D09			32	Man5	8.04	5.83 %

Figure 32.

NOTE **The Samples table will show Processed Peaks (within the processed data range, see "Integration parameters" on page 40) when viewing Processed Data, and will show Raw Peaks (all peaks) when viewing Raw Data.**

6 Review peak detection and integration by highlighting peak line in the peak table and reviewing peak start and end markers on electropherogram view. If needed, zoom in with a left mouse click/hold; zoom out with a right mouse click and selecting un-zoom.

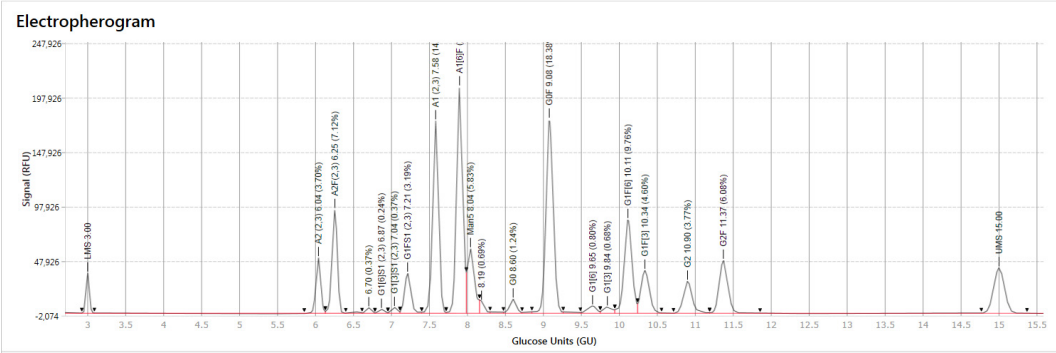


Figure 33. Electropherogram

NOTE **In rare circumstances, the Lower Migration Standards (LMS) or Upper Migration Standards (UMS) may require manual adjustment. It is important to view Raw Data when adjusting the LMS and/or the UMS. Please see "FAQs" on page 53 for further information.**

7 To make sure all peaks of interest are detected, adjust minimum peak area.

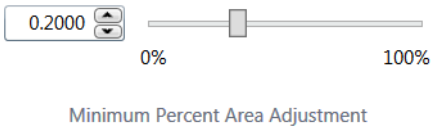


Figure 34. Minimum Percent Area Adjustment

NOTE **Default is set to 0.5%. This slider is only active when a single sample is selected in the Samples table. You cannot adjust the minimum percent area on more than one sample at a time.**

8 Reprocessing data. In some cases, one or more samples within a data set need to be reprocessed. To do this select the sample(s) to be reprocessed on the Samples table (NOT the Data Files tree). Multiple samples can be selected in the Samples table and all selected samples will be displayed in an overlay electropherogram plot as shown in **Figure 35**. This facilitates sample comparison, and allows reprocessing to be performed simultaneously on more than one sample. Use "Shift+Click" and/or "Control+Click" to select multiple samples as shown below.

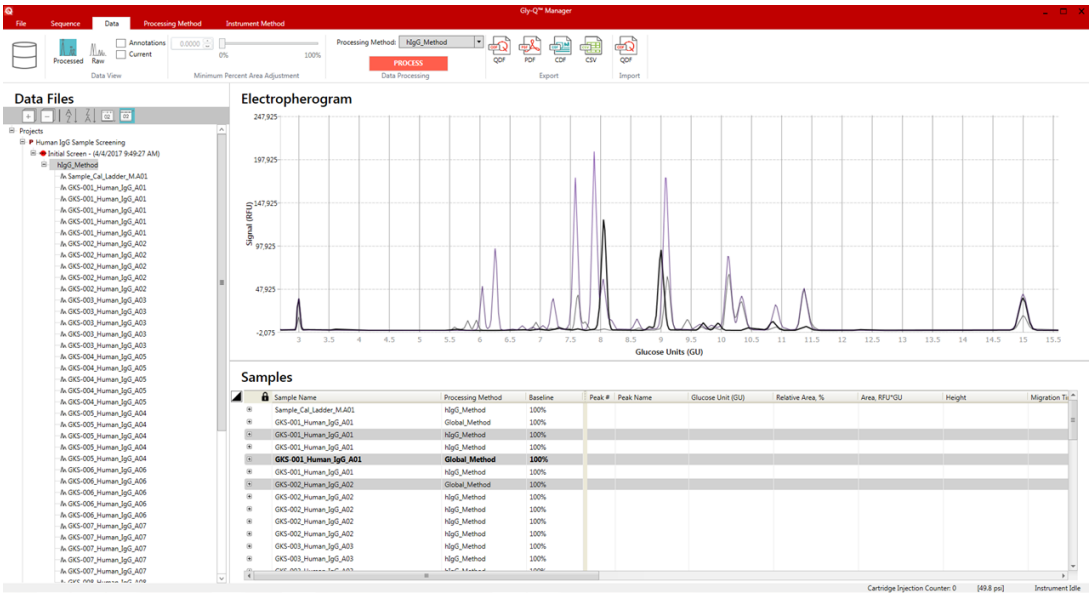


Figure 35. Selecting multiple samples using “Control+Click” to select non-adjacent samples

NOTE

When more than one sample is displayed in an overlay electropherogram plot hovering the mouse over one of the plots will highlight the plot itself and the corresponding row in the Samples table. Conversely, hovering the mouse over a row in the Samples table will highlight sample in the table and the corresponding plot in the electropherogram.

In some cases, data from different data sets must be reprocessed in an identical manner. Select data from multiple data sets in the Data Files tree and it will all be displayed in the Samples table. To reprocess them all together, you must combine them into a Virtual Data Set because reprocessing is restricted to data within a single data set. To create a Virtual Data set, first highlight the desired samples in the Sample table, and then right click on any of the selected samples and select **Create Virtual Data Set** in the pop-up menu.

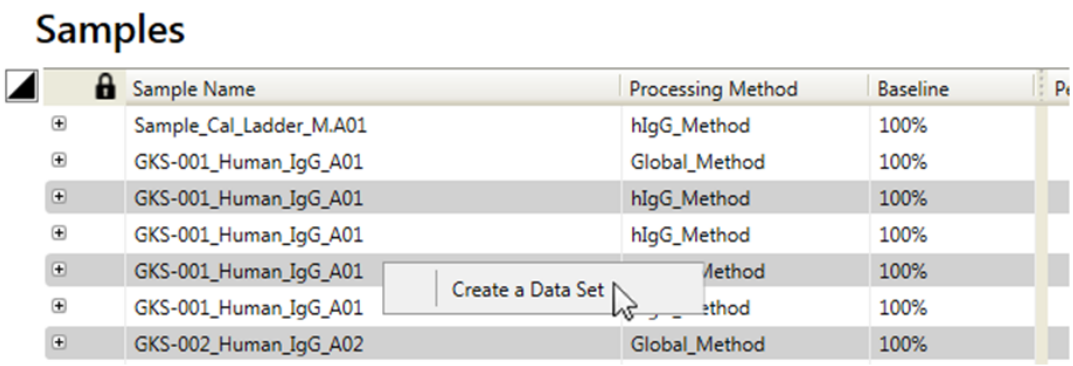


Figure 36. Create a Data set

A dialog opens to name the Virtual Data set.

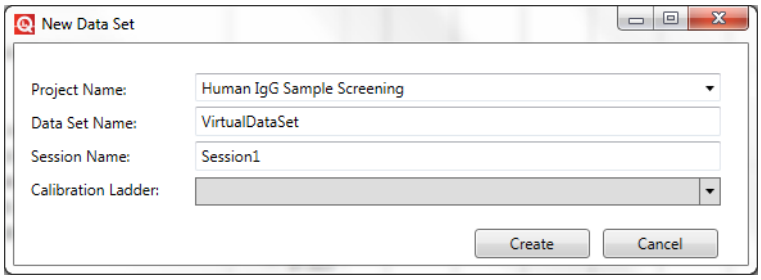


Figure 37. Virtual data set name

Select the desired project, enter the desired names, and if desired select a calibration ladder. Click on the **Create** button and the new Virtual Data Set will appear in the Data Files tree. Click on this data set, and only the samples included in the Virtual Data Set will be in the Samples table and only the first sample will be selected and displayed. Before proceeding use “Shift+Click” or “Control+Click” to select the other samples in the Virtual Data Set.

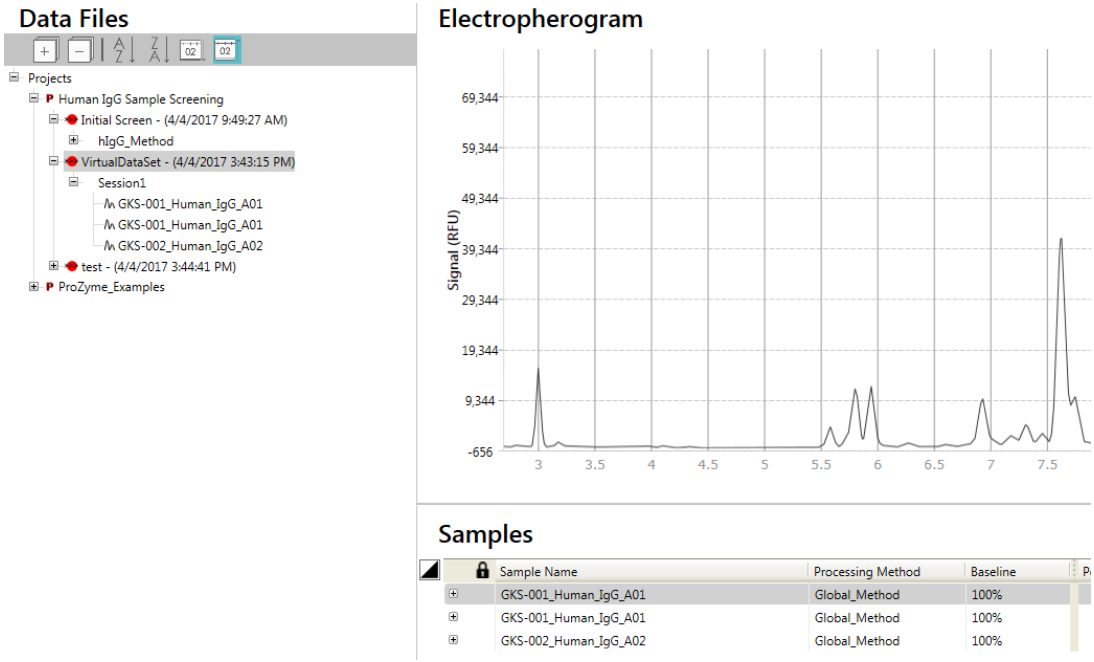


Figure 38. Virtual data set display

To reprocess the data selected in the Samples table, select the desired **Processing Method** and click **Process**.

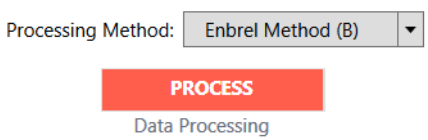


Figure 39. Data Processing

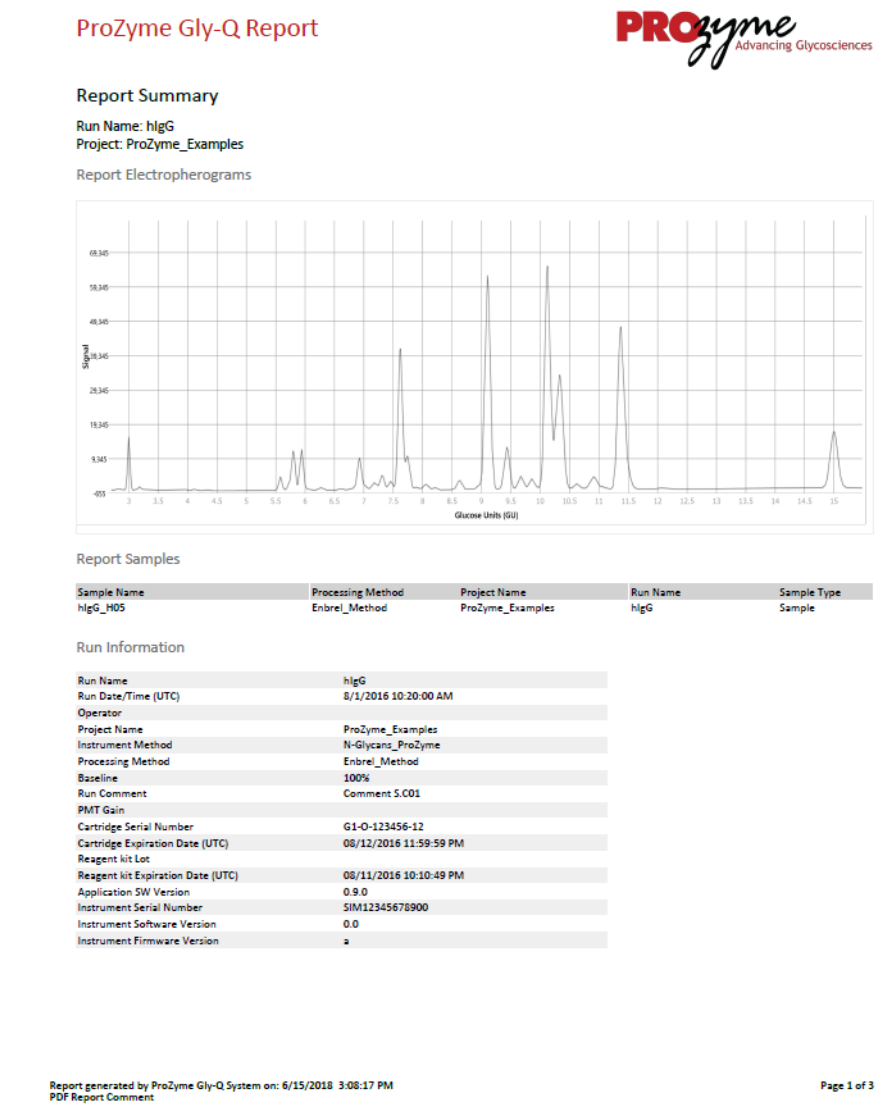
The new processing method will be displayed in the Processing Method column in the Samples table which is immediately to the right of the Sample Name.

9 After review, data can be exported in QDF, PDF, CDF or CSV formats.

Export and Reporting

Figure 40. Exporting and Reporting icons

- All Export and Reporting functions will include only the data currently selected in the Samples table and viewed on the Electropherogram at the time that any of the buttons shown in **Figure 40** are clicked.
- Selecting QDF export will create a file readable by Gly-Q Manager. This is particularly useful for sharing data with fellow researchers.
- Selecting PDF export will create an electronic report that can be printed. The report will include electropherograms and peak data tables for selected samples. The logo at the top of the report and the footer comment can both be customized in the File->Account tab.



ProZyme Gly-Q Report

PROzyme Advancing Glycosciences

Report Summary

Run Name: hIgG
Project: ProZyme_Examples

Report Electropherograms

Report Samples

Sample Name	Processing Method	Project Name	Run Name	Sample Type
hIgG_H05	Enbrel_Method	ProZyme_Examples	hIgG	Sample

Run Information

Run Name	hIgG
Run Date/Time (UTC)	8/1/2016 10:20:00 AM
Operator	
Project Name	ProZyme_Examples
Instrument Method	N-Glycans_ProZyme
Processing Method	Enbrel_Method
Baseline	100%
Run Comment	Comment S.C01
PMT Gain	
Cartridge Serial Number	G1-O-123456-12
Cartridge Expiration Date (UTC)	08/12/2016 11:59:59 PM
Reagent kit Lot	
Reagent kit Expiration Date (UTC)	08/11/2016 10:10:49 PM
Application SW Version	0.9.0
Instrument Serial Number	SIM12345678900
Instrument Software Version	0.0
Instrument Firmware Version	a

Report generated by ProZyme Gly-Q System on: 6/15/2018 3:08:17 PM
PDF Report Comment

Page 1 of 3

Figure 41. Gly-Q report

- Selecting CDF export will create a set of files in the industry standard AIA format that can be viewed by other analytical data packages such as Chromeleon and Empower.
- Selecting Excel export includes four options. Clicking on the button selects the first option. To select any of the other options you must click on the small black arrow within the button to pop up the selection list shown in **Figure 42**.

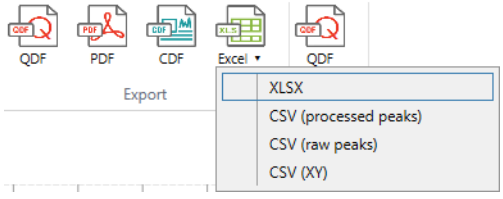


Figure 42. Excel export options

XLSX Report

This report is a native Excel File with multiple worksheets.

Summary - A list of the data included in the report with tabular data for all samples.

Run Information

Samples	Run Name	Project Name	Cartridge S/N
1 - 5	Lot R20180425	Lot Variation	G1-O-170505-66

Sample Information

Sample Number	Sample Name	Date/Time
1	Human_IgG_A01	05/01/2018 12:42 PM
2	Human_IgG_B01	05/01/2018 12:45 PM
3	Human_IgG_C01	05/01/2018 12:48 PM
4	Human_IgG_D01	05/01/2018 12:51 PM
5	Human_IgG_E01	05/01/2018 12:54 PM

hIgG_Method

			A2 (2,6)	A2F (2,6)	A2FB (2,6)	G1FS1 (2,6)	A1 (2,6)	A1F (2,6)	A1FB (2,6)	G0	G0F	G0FB	G1[6]	G1[3]	G1F[6]	G1F[3]	G1FB[6]	G2	G2F
		GU	5.57	5.8	5.92	6.92	7.31	7.59	7.71	8.64	9.12	9.45	9.68	9.87	10.1	10.4	10.4	10.9	11.4
1	Human_IgG_A01	%	1.41	1.96	2.4	2.63	1.32	9.16	2.48		18.7	4			20.3	14.3	14.3	1.58	19.8
2	Human_IgG_B01	%	1.8	2.43	2.53	2.32	1.44	9.04	2.2		19.1	3.89			20.5	14.2	14.2	1.54	19
3	Human_IgG_C01	%	1.64	2.24	2.31	2.41	1.44	8.97	2.65		19	3.92			20.5	14.1	14.1	1.78	19
4	Human_IgG_D01	%	1.81	2.19	2.46	2.45	1.39	8.9	2.32		19	3.92			20.2	14.5	14.5	1.81	19
5	Human_IgG_E01	%	1.72	2.49	2.48	2.36	1.31	9	2.3		19	3.95			20	14.7	14.7	1.6	19

Figure 43. XLSX report

Graphs - An overlay chart as seen on the screen when the report is created. This is a native Excel chart and can be adjusted within Excel. It also includes X and Y offset controls to stack and/or fan the traces.

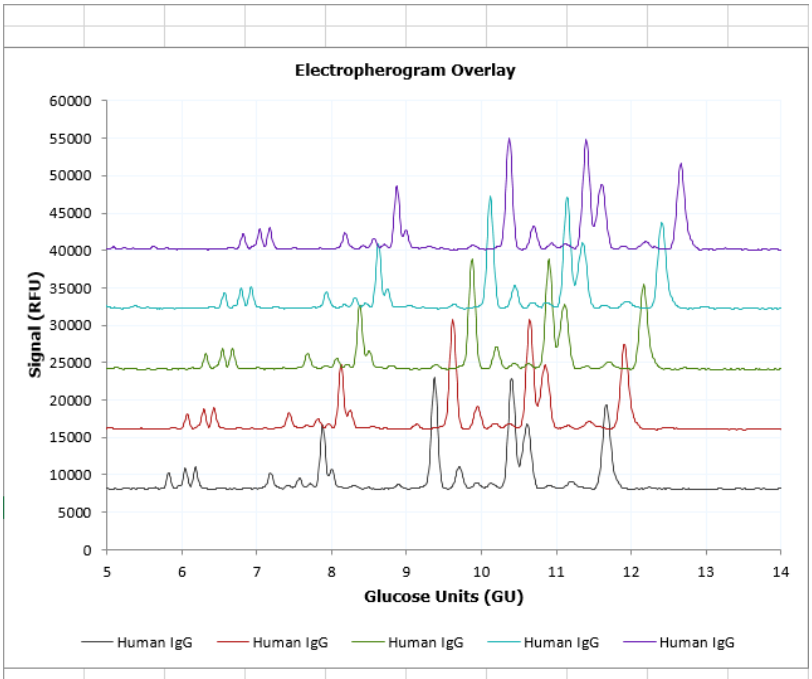
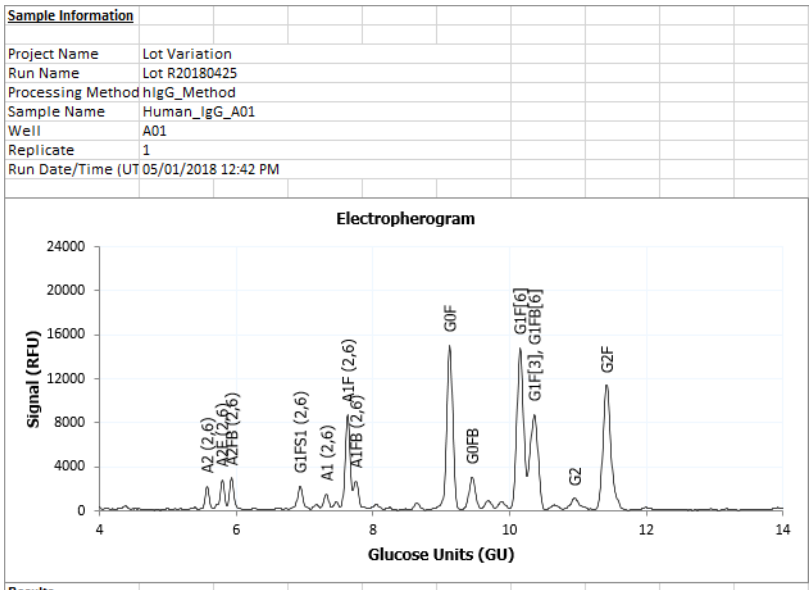


Figure 44. Graph report

Numbered worksheets - There will be one worksheet for every sample in the report and it includes a chart and peak table.



Sample Information	
Project Name	Lot Variation
Run Name	Lot R20180425
Processing Method	hIgG_Method
Sample Name	Human_IgG_A01
Well	A01
Replicate	1
Run Date/Time (UT	05/01/2018 12:42 PM

Results

Peak	GU	Area %	Area	Height	Time (sec)
A2 (2,6)	5.57	1.41	114.25	2222.36	59.82
A2F (2,6)	5.79	1.96	159.18	2838.58	60.78
A2FB (2,6)	5.93	2.4	194.57	3007.11	61.35
G1FS1 (2,6)	6.93	2.63	213.06	2287.41	65.51
A1 (2,6)	7.32	1.32	106.78	1530.1	67.1
A1F (2,6)	7.63	9.16	742.47	8722.92	68.36
A1FB (2,6)	7.75	2.48	201.06	2681.63	68.84
G0F	9.12	18.68	1513.43	15031.01	74.32
G0FB	9.45	4	324.22	3102.32	75.61
G1F[6]	10.15	20.31	1645.82	14869.75	78.36
G1F[3], G1FB[6]	10.36	14.31	1159.71	8743.09	79.17
G2	10.95	1.58	128.4	1140.27	81.43
G2F	11.42	19.76	1600.93	11394.21	83.18

Figure 45. Numbered worksheet report

Results - A worksheet identical to the CSV (processed peaks) report described below. PLEASE DO NOT EDIT THIS WORKSHEET.

XY - A worksheet identical to the CSV(XY) report described below. PLEASE DO NOT EDIT THIS WORKSHEET.

Graph Calculations - Calculations made to configure the other worksheets in the report. PLEASE DO NOT EDIT THIS WORKSHEET.

CSV (processed peaks) Report

This report includes all of the information from the selected samples in a comma-separated-values (CSV) format which can be read by Excel and many other programs. When opened within Excel the pivot table function can be used to perform custom data analysis. This report includes only the peaks for each sample that are within the processed data range, see **"Integration parameters"** on page 40.

CSV (raw peaks) Report

This report is identical to the previous report except that it includes all peaks in the samples. This makes the report much larger and should be selected only when the peaks outside of the processed data range are of interest.

CSV (XY) Report

This report includes the electropherogram trace data for all of the selected samples in the same CSV format. This raw data can be used to create custom graphs in Excel and many other programs.

Data Management: Archive and Delete

Over time the number of projects increases hence it becomes more difficult to find the data you are looking for. To simplify searching for data in the database you can archive and delete projects.

The data files tree is shown in three pictures in **Figure 46**. On the left the selected project is under **Projects** which is the active database. In the center the selected project is under **Archive** which is used to hold valuable projects that will not be accessed frequently outside of the active database. On the right the selected project is under **Trash** which is used to place projects which are no longer needed.

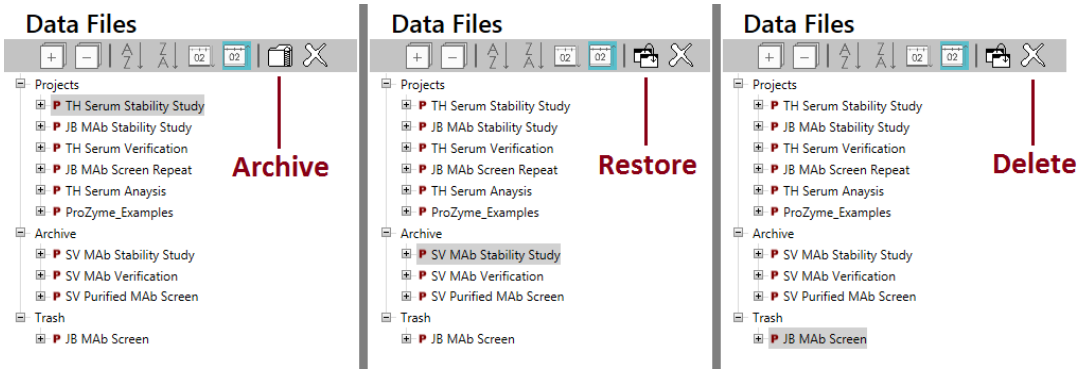


Figure 46. Data management options

The three buttons used for data management are shown in **Figure 46**.

- The **Archive** button is used to move projects from the active database into the archive.
- The **Restore** button is used to move projects from either the archive or the trash into the active database.
- When the selected project(s) is in the active database or the archive the **Delete** button moves them to the trash.
- When the selected project(s) is in the trash the **Delete** button will remove the project entirely.

NOTE **The functions of these buttons can also be accessed by using right-clicking on the selected project(s).**

Creating a processing method

Processing method defines peak alignment, peak integration and peak calling. Gly-Q Manager installs equipped with several examples of processing methods.

- **Enbrel_Method**: contains GU values for InstantQ-labeled N-glycans from Enbrel (Fc fusion protein) produced in CHO. Mainly biantennary glycans, some with α(2,3)-linked sialic acid, with some Man5.
- **Global_Method**: GU values for a broad range of N-glycans, combining the data from all processing methods listed.

- **hIgG_Method**: N-glycans from human IgG, biantennary, some with α(2,6)-linked sialic acid, some with bisecting GlcNAc. For glycoproteins produced in CHO use the Enbrel or Rituxan processing methods. Glycoproteins made in CHO generally lack bisecting GlcNAc N-glycans, sialic acid is α(2,3)-linked in CHO versus α(2,6)-linked in human IgG, and human IgG does not contain a significant amount of high mannose type glycans.
- **Rituxan_Method**: N-glycans from Rituxan, monoclonal IgG made CHO. Biantennary, some high mannose.
- **RNaseB_Method**: high mannose type N-glycans, Man5 through Man9.

While these methods can be used as a starting point, each laboratory is expected to develop and validate a processing method that meets the needs of a specific application.

To create or modify a processing method:

1 Select the **Processing Method** tab, and pick one of the existing processing methods from the drop-down menu.

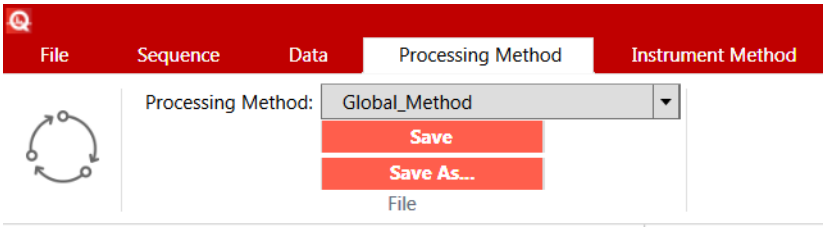


Figure 47. Processing Method tab

2 In the **Representative Sample** tab, select a sample that most closely matches the samples you plan to process.

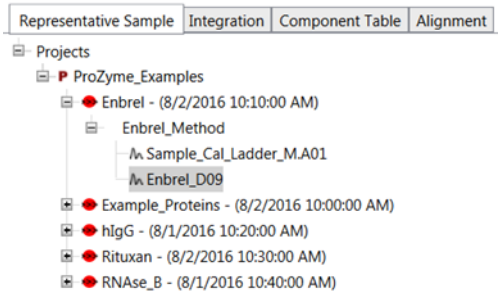


Figure 48. Representative sample selection

NOTE

To use a ladder injection to analyze data from other runs ladder injections can be 'added to the global ladder pool' by right-clicking on a ladder injection, see "Alignment parameters" on page 41.

3 Open the peak table by clicking on + sign and review peak integration.

Sample Name	Peak #	Peak Name	Glucose Unit (GU)	Relative Area, %	Area, RFU*GU
Enbrel_D09	10	LMS	3.00	0.00 %	1,894.77
Enbrel_D09	11	A2 (2,3)	6.04	3.79 %	3,970.41
Enbrel_D09	12	A2F(2,3)	6.25	7.27 %	7,613.10
Enbrel_D09	13	Peak 6.7	6.70	0.37 %	392.25
Enbrel_D09	14	G1[6]S1 (2,3)	6.87	0.24 %	255.80
Enbrel_D09	15	G1[3]S1 (2,3)	7.04	0.37 %	392.46
Enbrel_D09	16	G1FS1 (2,3)	7.21	3.22 %	3,369.04
Enbrel_D09	17	A1 (2,3)	7.58	15.21 %	15,921.63
Enbrel_D09	18	A1[6]F (2,3)	7.90	18.47 %	19,339.74
Enbrel_D09	19	Man5	8.04	6.48 %	6,783.51

Figure 49. Samples table

4 Review peak detection and integration by highlighting peak line in the peak table and reviewing peak start and end markers on Electropherogram view. If needed, zoom in by clicking and dragging a rectangle around the area of interest; zoom out with a right mouse click and select un-zoom.

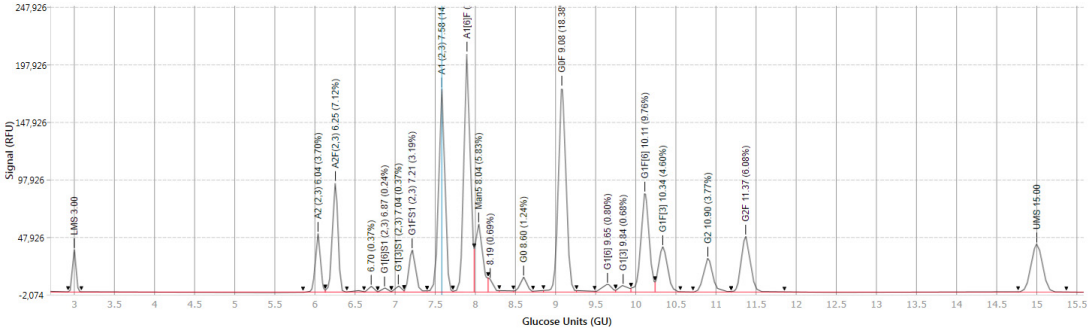


Figure 50. Electropherogram

Integration parameters

In the Integration Parameters Tab there are four parameters available.

- Select the **Baseline Valley-to-Valley Modulation** level. A setting of 100% is recommended, 0% is a flat baseline, and 50% is half way between these settings.
- Select the **Shoulder Detection** level. The '**Default**' setting detects peak shoulders as separate peaks and is recommended. The '**none**' setting turns off shoulder detection and identifies peak with shoulders as a single peak. The '**aggressive**' setting changes the parameters defining peak shoulders and detects more shoulder peaks but is rarely needed.
- Adjust the **Minimum Percent/Area** using the slider or by entering a number directly until all peaks of interest on the representative sample electropherogram are displayed.
- Select the **Processed Data Range** by entering left and right window values. Settings of 4 and 14 are recommended. Only peaks within the processed data range are included in sample analysis. Although not recommended, if the processed data range is increased and includes the LMS and/or the UMS, these Migration Standards will be omitted from the percent area calculations.

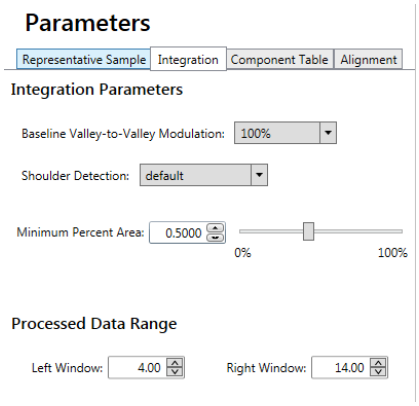


Figure 51. Integration parameters

In the **Component Table** tab, check the **Glycan Naming** checkbox, define **Peak Names**, **GU Units**, and **Left** and **Right** windows. The empty row at the bottom of the table can be used to add Glycans to the table.

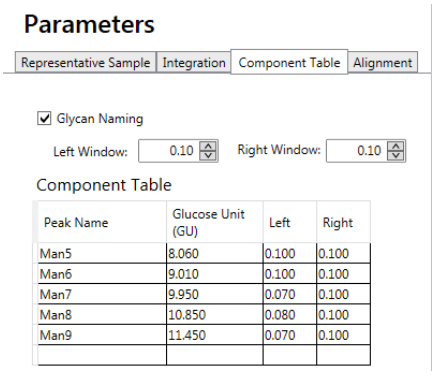


Figure 52. Component table

NOTE **The Left Window and Right Window value controls will simultaneously change all values in the table that are currently set to the value displayed in the controls. Values in the table that are not the same as the values in the controls will not be changed.**

Alignment parameters

In the Alignment tab parameters are set in three sections.

- **Migration Standards**: Define the Lower and Upper Migration standards which are normally 3 and 15 respectively. The 'Standard' LMS Search Method and checking Predictive UMS Search are both recommended however the 'allow relatively small peaks' setting for the LMS Search Method and/or unchecking the Predictive UMS Search may be useful if the migration standard peaks are small relative to the sample peaks.
- **Optional Alignment Controls**: This parameter was introduced in version 1.3, and should be used with caution. When samples are known to include a specific glycan that is included in the Component Table this setting can be used to identify the glycan and alignment will be adjusted using the known GU value of this glycan. The Peak Name drop down is used to identify the specific glycan and the 'Tallest' Search Mode setting is recommended. This should only be used when the glycan is present in all samples being analyzed, there are no other peaks near the specified glycan that could cause miss-identification of the correct peak, and the GU value for the glycan in the Component Table is correct. If any of these things are not true then analysis of all peaks will be adversely affected.
- **Dextran Ladder Rule**: All runs should start with a Calibration Ladder injection which will be the latest (most recent) ladder and this option is recommended. If there is a problem with the ladder injection within a specific run then the software includes ladder data that will be used. The Use Specific Ladder setting allows you to use a ladder injection from a different experiment. This is rarely necessary and to use a ladder it must be included in the Global Ladder Pool, see **Representative Sample** tab on **page 38**. The drop down selection allows any ladder in this pool to be specified.

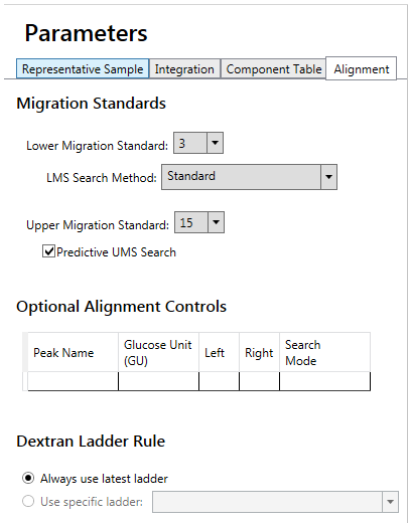


Figure 53. Dextran ladder rule

5 Save the Processing method by clicking on **Save** or **Save As**.

Figure 54. Save options

NOTE **After a processing method has been applied to a data set we recommend using 'Save As' if you wish to make changes to the method.**

Creating an instrument method

Instrument method defines a set of instrument commands needed to execute the sequence. Gly-Q Manager ships with several examples of instrument methods that can be modified and saved by the user.

To create or modify an instrument method:

1 Select the **Instrument Method** tab and pick one of the existing instrument methods from the drop-down menu.

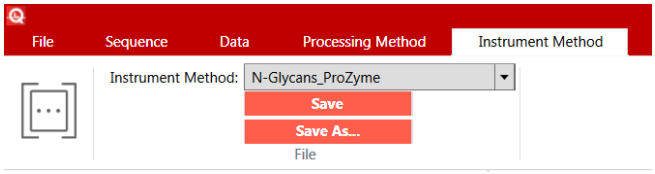


Figure 55. Instrument Method tab

2 Modify instrument commands for Calibration Ladder, Unknown Sample, and End of Sequence steps by selecting **Action**, **High Voltage**, **Duration**, and Autosampler **Position**.

Action	High Voltage	Duration, Sec	Position	Sampling Interval, Sec
High Voltage Purge	4.00	120.00	Wash	0.04
Pause		2.00	Clean	
Reagent Block Injection	2.00	2.00	MA01	0.04
Pause		2.00	Clean	
Separation and Detection	10.00	120.00	Separation	0.04

Figure 56. Calibration Ladder

Action	High Voltage	Duration, Sec	Position	Sampling Interval, Sec
High Voltage Purge	4.00	10.00	Wash	0.04
Pause		2.00	Clean	
Reagent Block Injection	2.00	2.00	MA02	0.04
Pause		2.00	Clean	
Well Plate Injection	2.00	2.00	Sample	0.04
Pause		2.00	Clean	
Separation and Detection	10.00	120.00	Separation	0.04

Figure 57. Samples

Action	High Voltage	Duration, Sec	Position	Sampling Interval, Sec
High Voltage Purge	4.00	90.00	Wash	0.04

Figure 58. End of Sequence

The following instrument commands can be used to create an instrument method. Click on the box in the Action column in any row three times to bring up the pop-up menu in **Figure 59** to select a command.

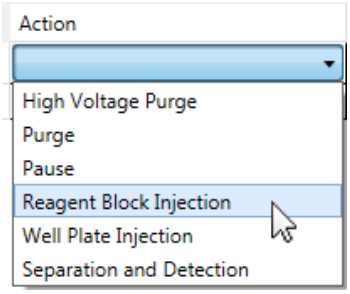


Figure 59. Action menu

- **High Voltage Purge**: both high voltage and air pressure driven capillary purge
- **Purge**: air pressure only driven capillary purge
- **Pause**: pausing between steps
- **Reagent Block Injection**: injection from dedicated reagent wells and troughs on the autosampler
- **Well Plate Injection**: injection from a 96-well plate
- **Separation and Detection**: running the sample and collecting data

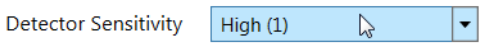


Figure 60. Detector Sensitivity setting

By default, **Detector Sensitivity** is set to High for standard instrument methods. Sensitivity can be turned down to either Medium of Low if saturated peaks are observed within the Processed Data Range, see **"Integration parameters"** on page 40.

3 Save the Instrument method by clicking on **Save** or **Save As**.

Figure 61. Save options

NOTE **Instrument Methods that have already been used in a sequence cannot be overwritten and can only be saved under a different name (Save As option).**

Ending a run

1 After samples have been processed, the system will return the Cartridge to the Park position (see **"Autosampler layout"** on page 25).

2 Use the "Load Sample" and "Load Reagents" buttons to remove sample plate and reagent tray.

3 Store the cartridge.

   a Remove the cartridge form the instrument and place tape over the cartridge cap (to cover the pin hole). Return it to the original clamshell packaging. The cartridge tip must be in contact with the gel block inside the package. Place inside the foil pouch and store vertically at room temperature.

   b To store on the instrument: Press the Park button before shutting down the system. The cartridge can remain on the system for up to three days. Fill the Park position of the buffer tray (**"Autosampler layout"** on page 25) with water to prevent the cartridge tip from drying during storage on the instrument.

4 Samples may be stored, covered with sealing film, at -20 °C for at least on month or up to five days at 4 °C.

File settings

File settings capture instrument serial number, cartridge and reagents lot information, define custom fields on data reports, perform Air and Gel flow checks.

To update system settings, select the **File** tab.

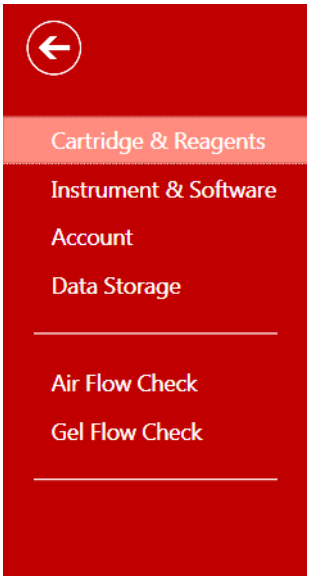


Figure 62. File tab

Cartridge and reagents settings

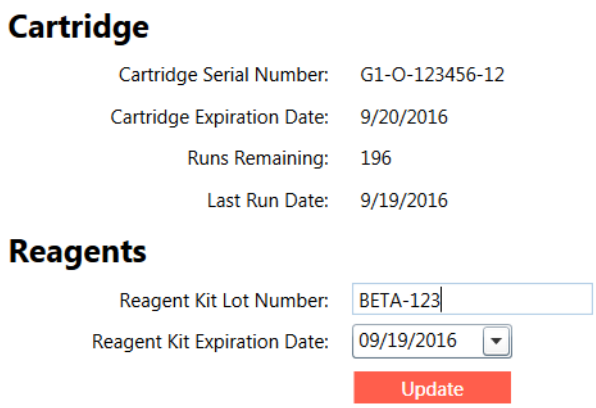


Figure 63. Cartridge and Reagents settings

Instrument and software settings

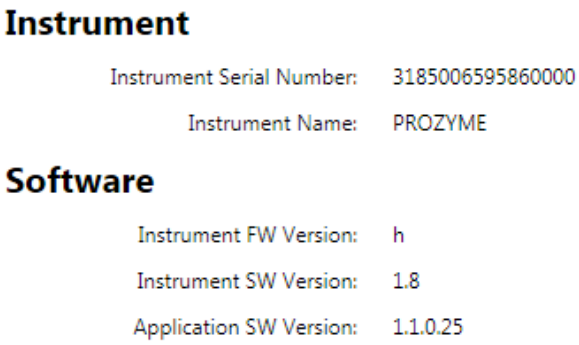


Figure 64. Instrument and Software settings

Account settings

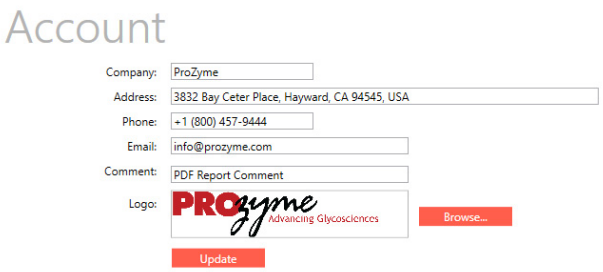


Figure 65. Account settings

NOTE **The Logo and Comment fields will appear in exported PDF reports.**

Data storage settings

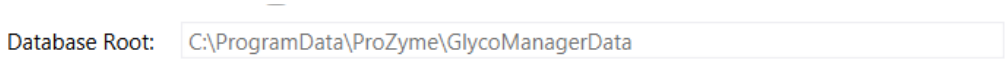


Figure 66. Data storage settings

NOTE **This setting is for information only and cannot be changed.**

Air flow check

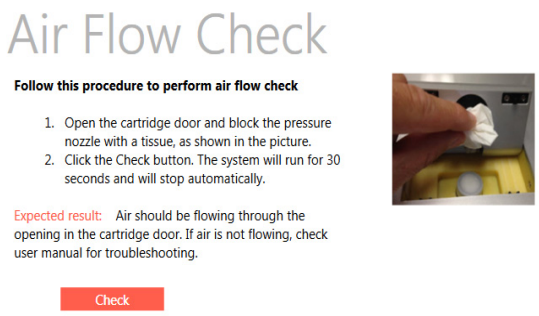


Figure 67. Air flow check

Gel flow check

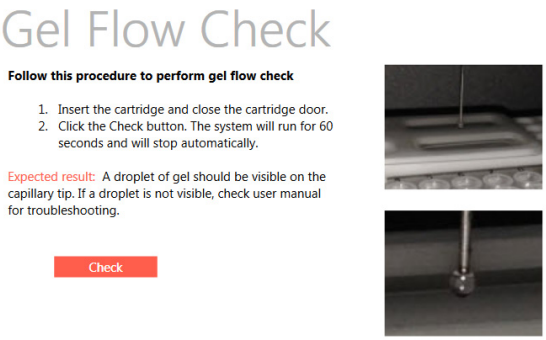


Figure 68. Gel flow check

Care and Maintenance

Time Interval	Procedure
Cleaning	Please clean the instrument with dry cloth
During Operation	Check the pressure on the software status bar to ensure that it is between 45 and 60 psi.
Weekly	Check the condensation trap on the gel recharging air pump for condensation. If there is condensation in the trap it should be drained • Turn off the power to the air pump. • Loosen the nut on the bottom of the condensation trap to drain the water from the trap. • When the trap is empty, tighten the nut to seal the condensation trap. • Turn on the power to the air pump; it will run briefly to pressurize the system and then turn off..
Monthly	In addition to emptying the condensation trap as described above, check the air filter: • Turn off the power to the air pump. • Loosen the nut on the bottom of the condensation trap to drain the water from the trap. • When the trap is empty, tighten the nut to seal the condensation trap. • Check the air filter. If the filter is damaged or wet, use a new filter to replace the old one. Replacement air filters are supplied with the instrument and are available from Agilent. • Turn on the power to the air pump; it will run briefly to pressurize the system and then turn off. • Check the air flow using the Air Flow Check functionality described in **"Ending a run"** on page 21.

System Specifications

General technical specifications

Environmental conditions	
Operating temperature	20 to 30 °C
Operating humidity	40 to 95% RH, noncondensing
Operating altitude	20 to 2,000 m
Conditions of transportation and storage	
Temperature	-30 to +60 °C
Humidity	20 to 80% RH, noncondensing
UL installation category	Category II

Instrument specifications

Input voltage	24 V DC 1.25 A Maximum
Output voltage	1 to 10 kV
Light source	LED (310 nm)
Fluorescence detector	Photomultiplier tube, A/D resolution 18-bit, long pass filter >385 nm
Computer connection	USB 2.0 or greater
Physical dimensions (L x W x H)	38 cm (L) x 30 cm (W) x 40 cm (H)
Weight	15 kg
Inlet air pressure	50 to 75 psi
Power supply	SINPRO MPU32B-108
Power supply input voltage	100 to 240 V AC, 47-63 Hz Suggestion: Use a surge protector outlet for the instrument and the computer to avoid electromagnetic interferences and damages to the inner electrical devices.

Gel recharging air pump specifications

Input voltages	12 V DC 6.67 A Maximum
Output pressure range	50 to 60 PSI
Dimensions	16.5 cm(L) * 12.5 cm(W) * 10.5 cm(H)
Weight	1.4 Kgs
Power supply	MEAN WELL GSM90B12-P1M
Power supply input voltage	100 to 240 V AC, 47-63 Hz Suggestion: Use a surge protector outlet for the instrument and the computer to avoid electromagnetic interferences and damages to the inner electrical devices.

Warranty Statement

Limitation of Liability

Gly-Q Analyzer, software, and all related reagents are designed for the use of electrophoresis analysis in general bio-chemistry laboratory for Research Use Only.

BEFORE ATTEMPTING TO OPERATE THE INSTRUMENT, READ ALL PRODUCT MANUALS AND FOLLOW THE INSTRUCTIONS.

Agilent assumes no liability whatsoever for any personal injury, property damage, or other loss resulting from not complying or familiar with the manuals, or improper operation of the devices.

Warranty statement

Agilent warrants all purchased products (other than extended warranties) against damage, defects in materials or workmanship, and failure to conform to the Quality Specifications, for the period commencing upon receipt of each purchased product by Agilent Technologies, Inc. through one year after delivery of such purchased product to Agilent's customers.

If, during the warranty period, any purchased product is discovered to be defective by reason of materials, workmanship or failure to meet the Quality Specifications, please contact Agilent Technical Support at **https://www.agilent.com/en/contact-us/page**.

Terms and conditions of sale may be found at: **www.agilent.com**

Instructions for Depot Repair Returns

To return the instrument for repairs, follow the steps below to clean surface and detached connectors and tubing.

1 Open the cartridge door and ensure there is no cartridge in the instrument.

2 Check the sample and buffer tray to ensure there is no sample or fluid left in the instrument.

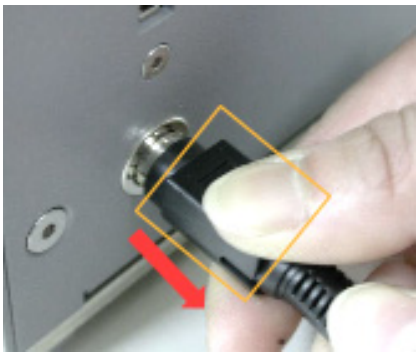
3 Turn off the power to the instrument and turn off the power to the gel recharging air pump.

4 To cut off the power disconnect the power plug to the instrument and the power plug to the gel recharging air pump.

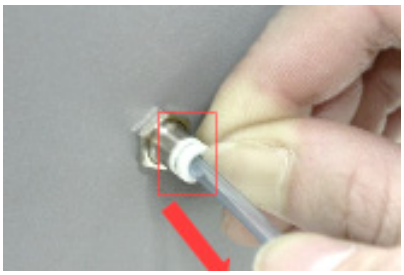
5 Unplug the USB cable in the back.

6 Unplug the power cable in the back.

7 Secure the cable node. Pull the connector header to unlock and detach the cable from instrument.



8 Remove the air tubing by simultaneously pressing the connector and remove the tubing rapidly.



9 Clean the outside of the instrument with dry cloth.

10 Print and fill out the Decontamination Certificate, **Figure 69** on page 52. Pack the form with the instrument and contact Agilent or your local distributor for shipping details. The User Manual is available at http://agilent.com/products/gq2100.

Decontamination Certificate

Please complete the form carefully and attach it to the item or instrument being returned.

Return authorization number _____

Company name _____

Contact person _____

Contact phone number _____

Instrument model _____

Instrument serial number _____

List accessories returned with the instrument _____

☐ No decontamination required

This instrument, and accessories if applicable, has not been in contact with any biological, chemical, or radioactive material that might cause infection or injury.

☐ Decontamination required

This instrument, and accessories if applicable, has been in contact with biological, chemical, or radioactive material and must be decontaminated prior to return. Please provide details.

Material	Yes/No	Description
Chemical		
Virus		
Bacteria		
Radioisotope		

Describe decontamination procedure:

_____

_____

I hereby certify that this instrument, and accessories if applicable, is free of any biological, chemical, or radioactive contamination.

Date: _____

Name: _____

Signature: _____

Figure 69. Decontamination certificate

FAQs

Q. Why does Gly-Q Manager not allow me to edit the Sequence Table I am preparing?

A. Be sure you have entered a Run Name before editing the Sequence Table.

Q. Does the Gly-Q instrument require a minimum volume for analysis?

A. The Gly-Q instrument requires a volume of at least 30 µL in a 96 well plate well for a run.

Q. Gly-Q Manager does not seem to be communicating to the instrument. How can this be fixed?

A. Ensure that the USB cable is intact and installed correctly.

Ensure that the instrument is turned on.

If 1 and 2 above are confirmed, reboot the instrument and computer. Occasionally communication can be lost if a prolonged period of inactivity is incurred.

Gly-Q Manager installs a Device Driver. If the driver is not installed properly instrument communication will not occur. Check with your IT professional to insure your computer is configured to accept Device Driver installation.

Q. What should I do about injection count warning or error messages?

A. The Gly-Q cartridges have both an expiration date and a limit on the number of injections that can be done using each cartridge. When the cartridge is old or has been used for many injections the performance may deteriorate. Cartridges have an absolute limit of 250 injections and a recommended limit of 200 injections.

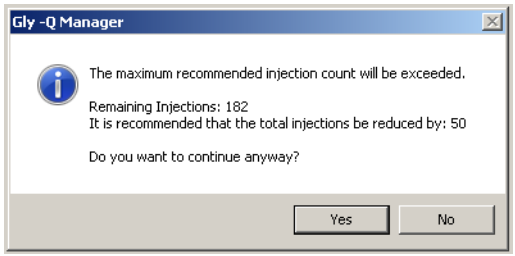


Figure 70. Warning Message - Exceeding the Recommended Injection Limit of 200

In this case the run can be started but there it is possible that the cartridge performance will degrade during the run. This is unlikely and in most cases the run will be completed successfully.

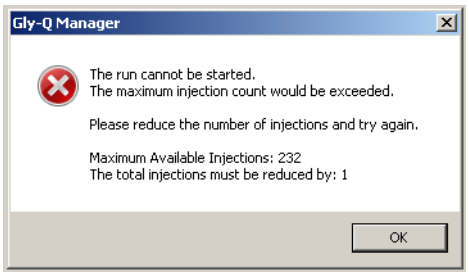


Figure 71. Error Message - Exceeding the Absolute Injection Limit of 250

In this case the run cannot be started until you reduce the number of injections in the sequence.

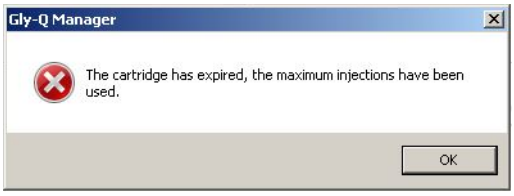


Figure 72. Error Message - Cartridge has Expired

In this case you must use a different cartridge to perform the run.

Q. How do I resolve the "Cannot Get Cartridge Information" error message?

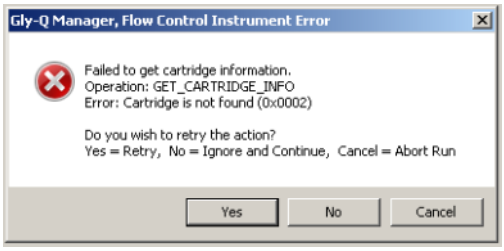


Figure 73. Error message - Cannot Get Cartridge Information

A. This message indicates that there is a problem reading the information on the cartridge RFID tag. In most cases the system will allow you to enter the cartridge information manually (see **"Starting and Stopping a sequence"** on page 26) but in this case manual entry is no longer working. If this happens you must contact Agilent technical support for assistance.

NOTE **If this happens you will be able to use the system with any cartridge that has a working RFID tag.**

Q. How do I resolve the "Low Pressure" error message?

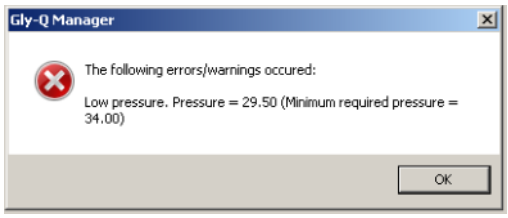


Figure 74. Error message - Low Pressure

A. Ensure that pump is turned on. If the pump is on then check that the airline connections are intact and that the air filter is current and functional. The Airline Check in **"Ending a run"** on page 21 is another way to check this parameter.

Q. The Air Pump runs quite frequently. Is this normal?

A. The air pump should run for ~ 30 seconds sporadically throughout an analysis session. If the pump is running for greater than one minute regularly throughout the run this could be an indication of air flow compromise. Again, the user should check integrity of airlines, air filters and perform the Air Flow function described in **"Ending a run"** on page 21.

Q. When is the Gel Flow Check function used?

A. The Gel Flow Check in **"File settings"** on page 44 is used to insure gel flow in a cartridge is optimal. If a cartridge is stored improperly the capillary may become clogged and consequently functionality may also be affected. The Gel Flow Check can be used to determine presence of a potential clog. If a blockage is detected the user is advised to prime the cartridge as described in the cartridge preparation **"Priming the cartridge"** on page 19. If priming does not resolve this, proceed to the next FAQ.

Q. Peaks are migrating later than usual and have poor shape. How do I resolve this?

A. Check the Current trace by clicking the Current box on the data ribbon (see **"Data review, export, reporting, and management"** on page 27). If the current is low or erratic, it may indicate a blocked cartridge.

It is recommended that the Gel Flow Check function under the 'File' menu in Gly-Q Manager be used whenever a cartridge is inserted into the Gly-Q System before performing a run (**"File settings"** on page 44). Since the capillary has a small inner diameter (0.75 µm) the tip of the capillary can become clogged and the Gel Flow Check may fail (no gel droplet observed). When this occurs the brief procedure below will often unblock the cartridge tip and restore normal operation.

1 Remove the cartridge from the system and follow the procedure in **"Priming the cartridge"** on page 19.

NOTE **If the air line is already connected to the purge stand there is no need to turn off the air pump.**

2 If droplets of gel are visible in **step 7** of the Priming procedure, the cartridge has been unblocked by Priming as gel is flowing, and the cartridge can be returned to the Gly-Q system. There is no need to repeat the Gel Flow Check when the cartridge in inserted into the system. If the gel does not flow, continue to **step 3**.

3 The next step is to perform a priming procedure with the capillary tip submerged in hot water (80 °C). This should be done with deionized or distilled water.

NOTE **If a thermometer is not available the water can be boiled and then allowed to stand for about five minutes before continuing. The temperature does not need to be exact but boiling water should not be used immediately.**

4 Fill the buffer tray in the purge stand with the hot water and then turn the black 'Switch Knob' at the top of the purge stand to lower the cartridge until the capillary tip is submerged in the hot water.

5 Adjust the 'Regulator Knob' to increase the pressure until it reaches approximately 0.4 mPa.

6 Leave the capillary tip submerged in the hot water under pressure for 10 minutes.

7 Turn the black 'Switch Knob' to raise the cartridge until the capillary tip is no longer submerged. Leave the pressure at ~0.4 mPa.

8 Wait for 120 seconds (two minutes) and examine the capillary tip. If gel is visible, the cartridge is no longer clogged and can be returned to the Gly-Q system. There is no need to repeat the Gel Flow Check when the cartridge is inserted into the system. If the gel does not flow then prepare another aliquot of hot water and repeat steps **4** through **8** a second time. Do not use the same aliquot of hot water as it will no longer be hot after being in the buffer tray for more than 10 minutes. If the cartridge is still clogged after the second purge in hot water then the cartridge cannot be unblocked and you must use a different cartridge.

Q. The Lower Migration Standard (LMS) and/or Upper Migration Standard (UMS) were not found or aligned correctly. What can I do?

A. The LMS and UMS can be identified manually when needed. Select the data on the Data Tab and set the Data View to Raw so all peaks in the sample will be included. In the Samples table, expand the sample to list all peaks as shown in the illustration below. Right click on the sequence that corresponds to the LMS/UMS and designate it as such. Gly-Q Manager further helps facilitate this process by enabling visualization with the corresponding electropherogram in the window above the Sample. Please see **Figure 75** for an example.

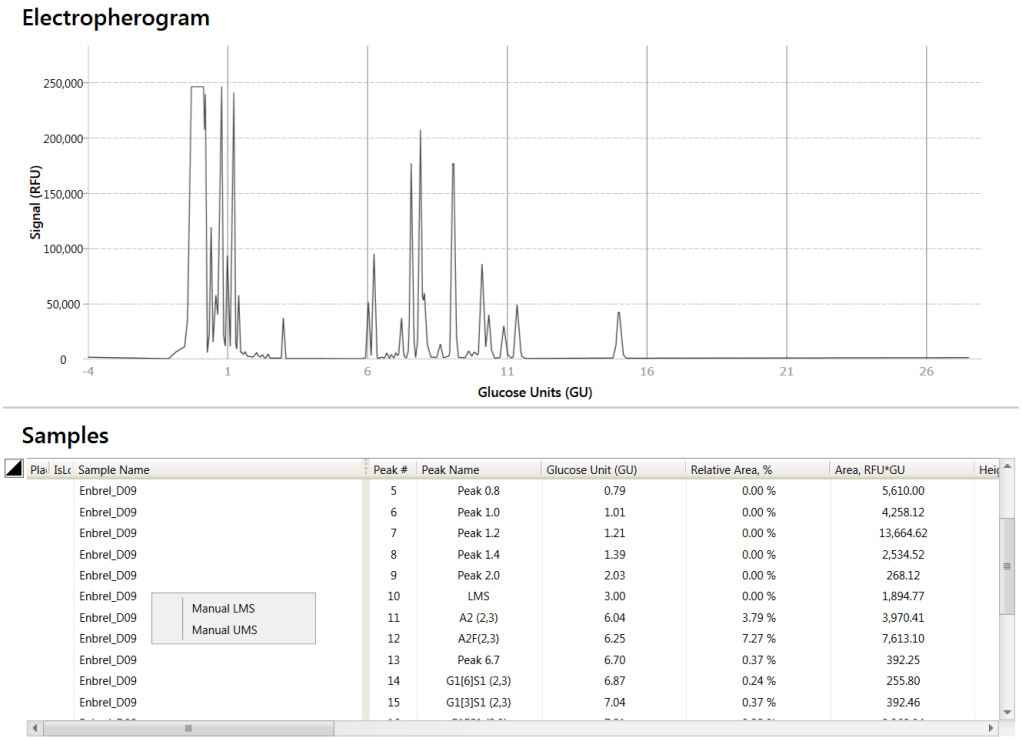


Figure 75.

Manually designated LMS or UMS will be named accordingly in bold print, see **Figure 76**:

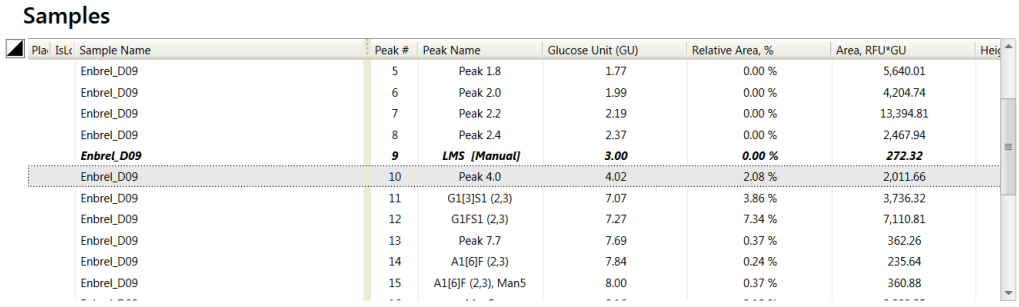


Figure 76.

Q. When collecting data, why can it not be saved in real time on a network or to a cloud location?

A. The system collects data in real time and is designed to save data on the local hard drive to prevent data loss if the network or internet connections are down. Once data has been collected there are a number or options available to move data to other computers. You can move data to a different computer running Gly-Q Manager using the data export and data import functions using the QDF data file format. If you want to analyze data using programs that support the industry standard AIA format such as Chromeleon or Empower you can use the data export function with the CDF data file format. If you want to analyze data using Excel you can export to a native Excel file or to CSV (comma-separated-values) files (use the arrow on the Excel Export button to select CSV file options). CSV files are also supported by a variety of other programs.

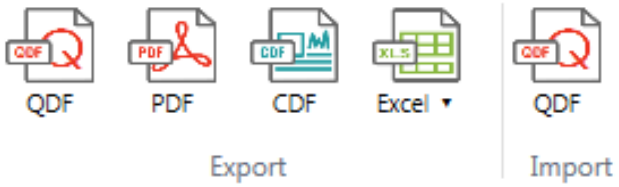


Figure 77.

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Virtual patent marking

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