

Gly-Q™ Glycan Analysis System

User Manual

Product Code: **GQ2100-110, GQ2100-220**

Version: AC



This product is intended for in vitro experimental research use only

Note: The following suggestions and data are based on information we believe to be reliable. They are offered in good faith, but without guarantee, as conditions and methods of use of our products are beyond our control. We recommend that the prospective user determine the suitability of our materials and suggestions before adopting them on a commercial scale. Suggestions for use of our products or the inclusion of descriptive material from patents and the citation of specific patents in this publication should not be understood as recommending the use of our products in violation of any patent or as permission to license to use any patents of ProZyme, Inc.

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SECTION 1: SAFETY INFORMATION

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



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.



Warning:

This instrument must be connected to an appropriate power source.



Warning:

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



Caution:

The instrument autosampler rotates and moves during operation. Keep the instrument door closed while running to avoid potential pinching hazard.

SECTION 2: INTRODUCTION

2.1 The Gly-Q System

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 InstantQ assay kits and standards.

2.2 Description of System Components

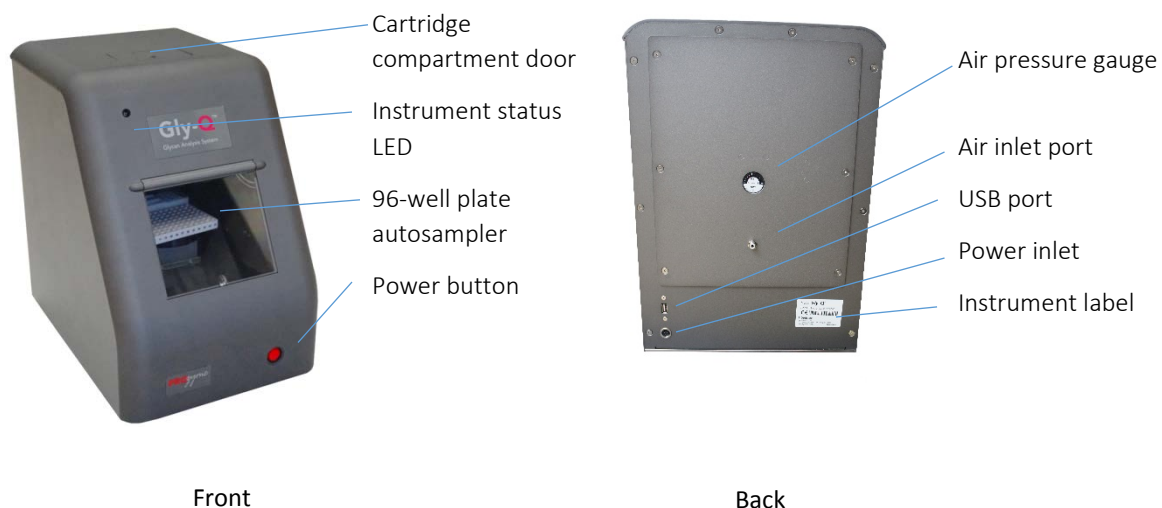
The Gly-Q System components are listed below and described in more details in the sections that follow:



- 1 Gly-Q Instrument
- 2 Gel Recharging Air Pump
- 3 Replaceable Capillary Gel Cartridge
- 4 Assay Kit
- 5 Standards and Controls
- 6 Gly-Q Manager Software
- 7 Cartridge Priming Station
- 8 Gly-X Manifold Spacer

2.2.1 Gly-Q Instrument

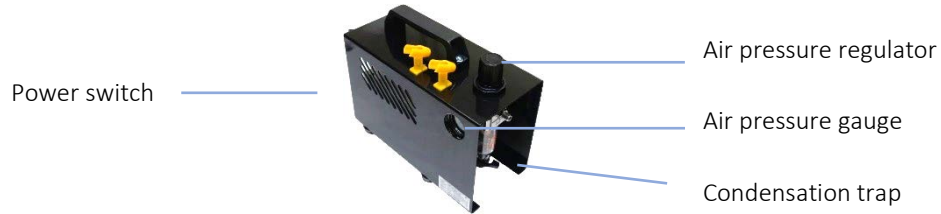
The Gly-Q Instrument is a compact unit comprised of a capillary gel electrophoresis core integrated with a high sensitivity fluorescence detector and 96-well autosampler. When used as part of a complete system, the instrument facilitates rapid separation, identification and relative quantitation of labeled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



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 main 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.
Instrument label	Instrument serial number & CE marking

2.2.2 Gel Recharging Air Pump

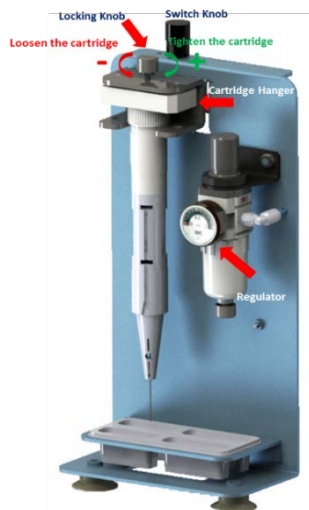
The gel recharging air pump connects to the instrument and provides air pressure for automated recharging of the capillary with gel between sample injections.



Feature	Description
Air pressure regulator	Regulator for adjusting air pump output pressure.
Air pressure gauge	Air pressure gauge displays air pump output.
Air tubing	1/8-inch tubing connects the pump to the instrument (not shown).
Power switch	Power switch turns the pump On and Off.
Condensation trap	Condensation trap collects condensate from air pump. This should be cleared weekly as routine maintenance.

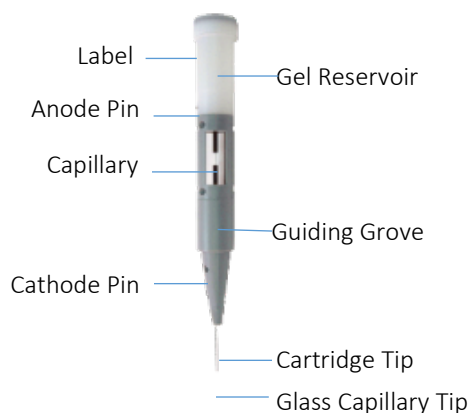
2.2.4 Cartridge Priming Station

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



2.2.3 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.



Feature	Description
Capillary	Glass capillary, Note: <i>fragile glass tip extends beyond metal cartridge tip</i>
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

2.2.4 Sample Preparation and Analysis Kits

Gly-X N-Glycan Rapid Release and Labeling Kits

ProZyme Gly-X sample preparation kits utilize a novel in-solution enzymatic deglycosylation followed by labeling of released N-glycans with proprietary ProZyme Instant dyes. Gly-X with InstantPC is designed for UHPLC and LC-MS applications. Gly-X with InstantQ is specifically designed for CE separation on the Gly-Q System. Labeling (1 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.



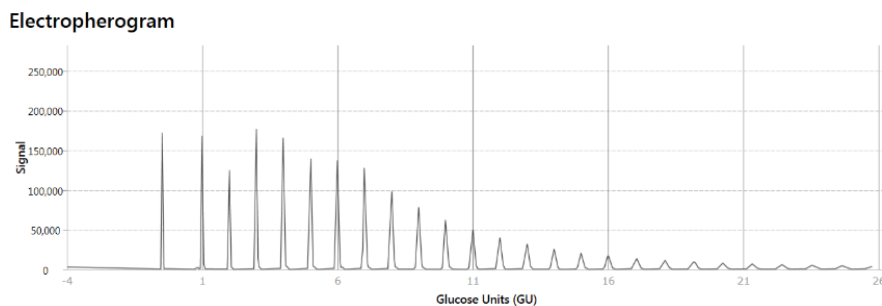
Feature	Description
Gly-X Deglycosylation Module for InstantQ Dye	Reagents and consumables for rapid in-solution protein deglycosylation: <ul style="list-style-type: none">• Gly-X Deglycosylation Plate• Gly-X N-Glycanase• Gly-X Digestion Buffer• Gly-X Denaturant• Gly-X Blocker (optional)
Gly-X InstantQ Labeling Module	Dye and solvents for instant labeling of released glycans: <ul style="list-style-type: none">• InstantQ Dye• InstantQ Dye Solvent• InstantQ Activation Reagent
Gly-X InstantQ Cleanup Module	Clean up plate, eluent and plastic consumables for sample clean-up before analysis: <ul style="list-style-type: none">• 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	Gly-Q Cartridge, Separation Buffer, mineral oil and Reagent Tray: <ul style="list-style-type: none">• Gly-Q Cartridge• Gly-Q Separation Buffer• Gly-Q Mineral Oil• Gly-Q Reagent Tray
GlyQ Alignment Standards Module	<ul style="list-style-type: none">• Gly-Q Migration Standards• Gly-Q GU Ladder

Note: See the *Gly-X Rapid N-Glycan Release and Labeling with InstantQ kit user manual* for more detailed product and protocol information.

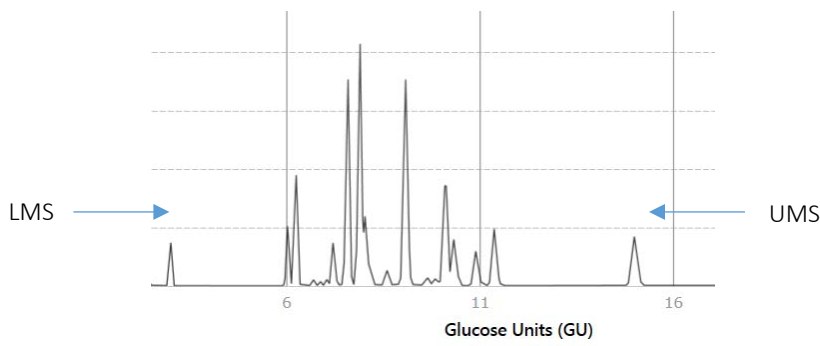
Note: GX100 Gly-X Manifold Spacer is required for the *Gly-X Rapid Release and Labeling with InstantQ Dye Kit*.

2.2.5.1 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 below). The Migration Standards (MS) co-injected with subsequent unknown samples will be aligned to the Maltodextrin (GU) ladder for glycan naming and alignment purposes.



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.



2.2.5.2 Glycan Standards

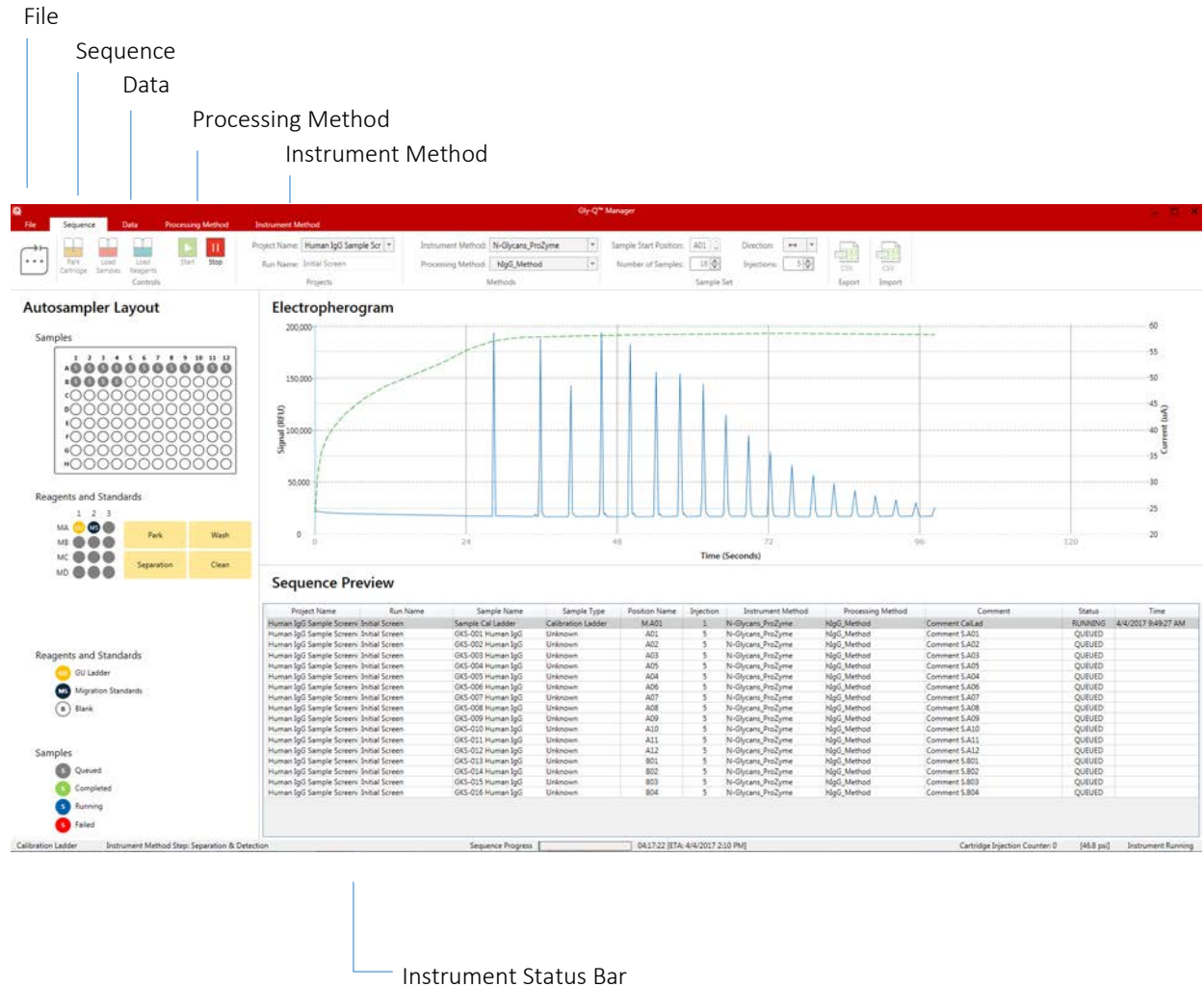
Standards are highly purified and well characterized individual oligosaccharides or mixed libraries of oligosaccharides. They are used for Gly-Q method calibration and validation. For the list of available InstantQ Standards and Controls, please refer to the following website links:

- InstantQ Labeled Oligosaccharides

prozyme.com/collections/instantq-standards

2.2.6 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:



Feature	Description
Data tab	Data tab enables: <ul style="list-style-type: none">• Data review• Integration adjustment• Data reprocessing• Data export/reporting
Processing Method Tab	Processing method tab enables: <ul style="list-style-type: none">• Processing method design

Instrument Method tab	Instrument method tab enables: <ul style="list-style-type: none"> • Instrument method design • Saving an Instrument Method
File tab	File tab enables and informs the user: <ul style="list-style-type: none"> • Serial Number of Instrument and Cartridge • Gly-Q Manager software version • Air Flow Check • Gel Flow Check
Instrument Status Bar	Instrument Status Bar informs the user: <ul style="list-style-type: none"> • Instrument connectivity • Progress of run • Estimated finish time of run

Note: See Section 4 for more detailed description of Gly-Q Manager functionality.

SECTION 3: INSTALLATION

3.1 Unpacking

Inspect shipping boxes. If you notice apparent damage, contact ProZyme Technical Support, (800)457-9444 (US & Canada); +1 510-683-6900; info@prozyme.com

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.

3.2 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 20-30 °C
- The optimal operating humidity should be within 40-95% RH range, non-condensing
- The optimum altitude for Gly-Q System operation is 20-2000 m

3.3 Power Requirements

The input voltage of Gly-Q Instrument is 24V DC, and the minimum current is 2A. A suitable power adapter with a cord has been included in the package (100~240V AC, 50-60Hz).

The Gly-Q System air pump is available in 110V and 220V formats.

3.4 Computer Requirements

- Windows 7, 8.1 or 10; 64-bit version
- 1 GHz or faster CPU
- Minimum 8 GB RAM
- 1 TB Hard Disk Drive; 500 GB Solid-State Drive preferred
- Minimum 100 GB free space on the disk drive
- Monitor and graphics card capable of Full HD (1920 x 1080)

3.5 System Set-up



- 1 Plug air pump, Gly-Q Instrument power adapter, computer and monitor into approved and properly grounded power outlets. A surge protector is recommended to minimize power fluctuations.
- 2 Plug power adapter low voltage line into Gly-Q Instrument power inlet.
- 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 1 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 ProZyme if you need assistance.

3.6 Software Installation



- ❶ Log into PC as Administrator.
- ❷ Download the latest version of Gly-Q Manager from the ProZyme website:

<http://prozyme.com/products/gly-q-manager-software-ggsw2000>

Note: Please contact ProZyme for password information

- ❸ Double click on the .exe file, click on Gly-Q Manager Setup.
- ❹ 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 won't delete data. All user data will be preserved during installation.

3.7 Cartridge Preparation

3.7.1 Components



❶ Capillary gel cartridge clam-shell container (keep this for storage between uses)

❷ Tape on cap (red, keep this for storage between uses)

❸ Pin used to puncture the cartridge cap (white) prior to initial use

❹ Gel reservoir. Store the tip of the cartridge in the gel reservoir inside the clam-shell container

3.7.2 Unpacking and Preparing the Cartridge

❶ Open gel-cartridge clam-shell container and remove the cartridge



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

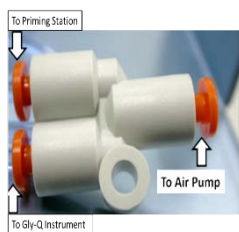


- 3 Carefully remove and pin from package. Insert pin into the hole of the cartridge cap and push it down to the end. Slight resistance should be encountered when performing this step.

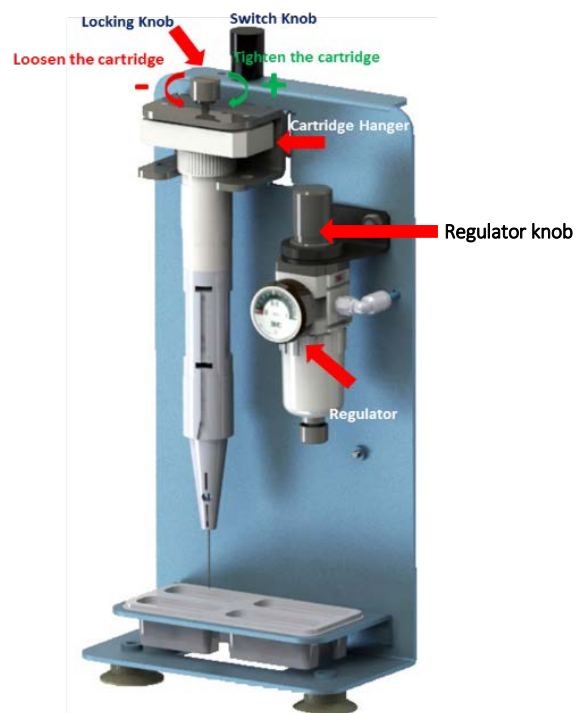


3.7.3 Priming the Cartridge

- 1 Turn off the Air Pump and use the illustration 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.
- 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 ~ 0.5 mPa.
- 7 Droplets of gel should be observed flowing at cartridge tip in ~ 30 -120 sec
- 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



3.7.4 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



Note: Cartridges can only be inserted in one orientation as shown in figure above.

- 3 Close 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 clamshell package can be added to most plastics recycling streams.

3.8 Ending a Run

1. After samples have been processed, the system will return the Cartridge to the Park position (see section 4.3, autosampler layout).
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 3 days. Ensure that the Park position in the buffer tray (Section 4.4.3) is filled with water to prevent the capillary tip from drying.

3.9 Vacuum Manifold Setup

ProZyme 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)



- ❶ Assemble vacuum manifold.
- ❷ Connect vacuum manifold and pump inlet with tubing via liquid trap reservoir.
- ❸ 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

SECTION 4: GLY-Q MANAGER SOFTWARE

4.1 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.

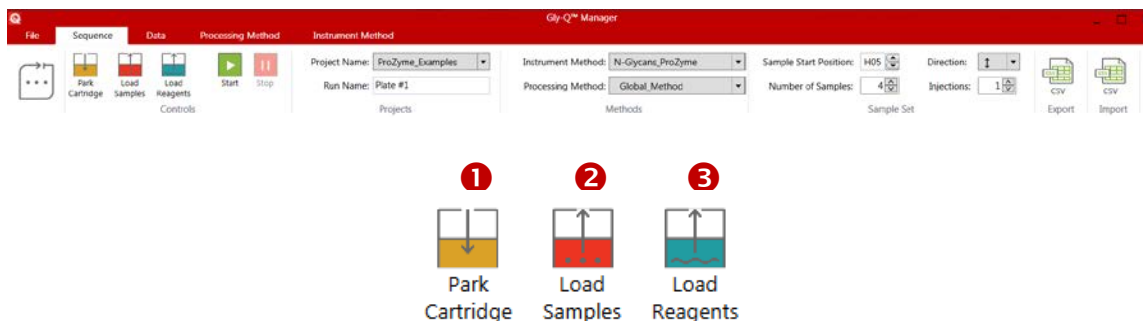
4.2 Controlling and Monitoring Gly-Q Instrument

To control and monitor the Gly-Q Instrument with Gly-Q Manager, the instrument must be turned on and connected to a computer via 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 via 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 via the Sequence tab:



- ❶ Park Cartridge moves cartridge into Park position for short term storage on the instrument (up to 3 days).
- ❷ Load Samples control rotates the autosampler for access to the sample loading stage.
- ❸ Load Reagents control rotates the autosampler for access to load reagents including GU Ladder and Migration Standards.

4.3 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 & Run

Project Name: ❶

Run Name: ❷

Projects

❶ Name your project by selecting “New Project” from the pull down, or select from the list of existing projects.

❷ Name your run

Methods

Instrument Method: ❶

Processing Method: ❷

Methods

❷ Select Processing Method

Note: Default Instrument Methods are recommended. See sections 4.6 and 4.7 on how to create Instrument Methods and Processing Methods.

Sample Set

Sample Start Position: ❶ Direction: ❷

Number of Samples: ❸ Injections: ❹

Sample Set

❶ Define the position of the first sample

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

❸ Define the number of samples to be analyzed

❹ Define the number of injections (number of replicates for each sample)

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 below)

4.3.1 Import & Export



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

4.3.2 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	hlgG	Unknown	H05	1	N-Glycans_ProZyme	Global_Method	hlgG		
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		

4.3.3 Autosampler Layout

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

Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	S	S	S	S	S	S	S	S	S			
B	S	S	S	S	S	S	S	S	S			
C	S	S	S	S	S	S	S	S	S			
D	S	S	S	S	S	S	S	S	S			
E	S	S	S	S	S	S	S	S	S			
F	S	S	S	S	S	S	S	S	S			
G	S	S	S	S	S	S	S	S	S			
H	S	S	S	S	S	S	S	S	S			

Reagents and Standards

	1	2	3		
MA	GU	MS		Park	Wash
MB					
MC					
MD				Separation	Clean



Reagents and Standards

- GU** GU Ladder
- MS** Migration Standards
- B** Blank

Samples

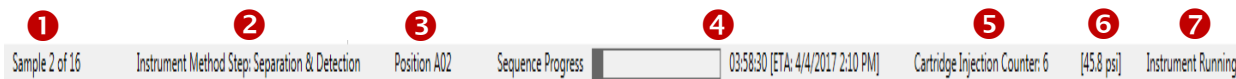
- S** Queued
- S** Completed
- S** Running
- S** Failed

Reagent Tray for:

- (P)** Park; 4 ml DI water + mineral oil to cover, optional
- (W)** Wash/Waste; 4 ml DI water
- (C)** Clean; 4 ml DI water
- (S)** Separation Buffer; 4 ml, included with Gly-Q Cartridge Module

Sequence Status Bar

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



- ❶ Sample name
- ❷ Instrument method step
- ❸ Well position
- ❹ Sequence progress and estimated time of completion
- ❺ Cartridge injection counter
- ❻ System Pressure
- ❼ Instrument status

4.4 Running a Sequence

4.4.1 Starting and Stopping a Sequence

After a sequence is created it can be run by clicking on Start button:

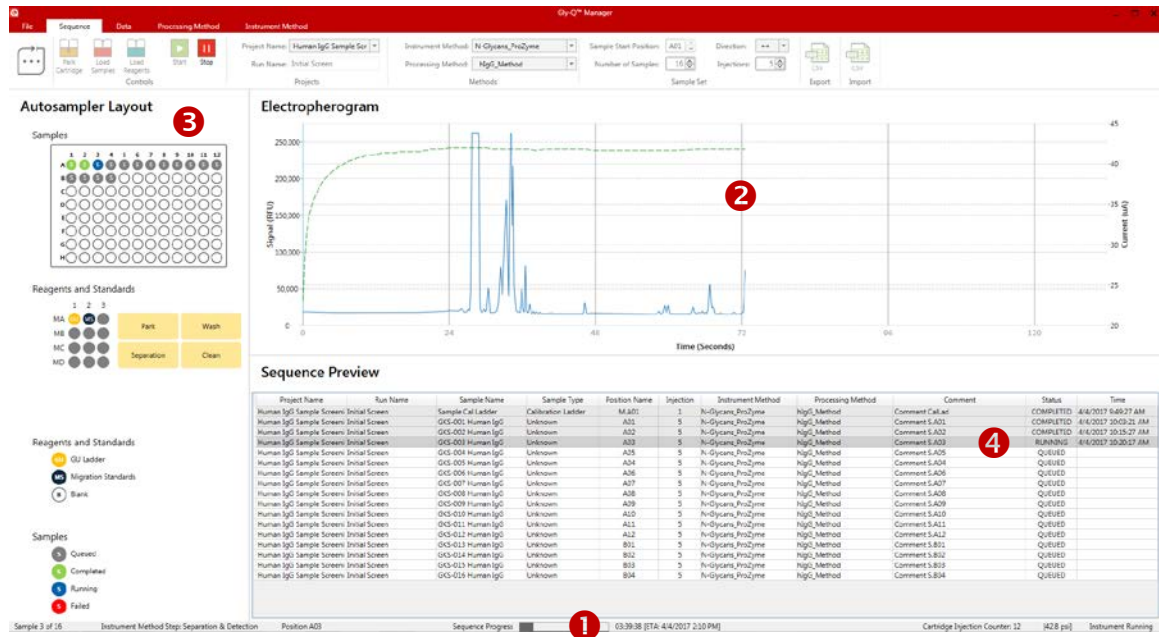


A sequence can be stopped by clicking on Stop button:



4.4.2 Monitoring Sequence Progress

Sequence progress will be displayed on 4 elements of the screen:



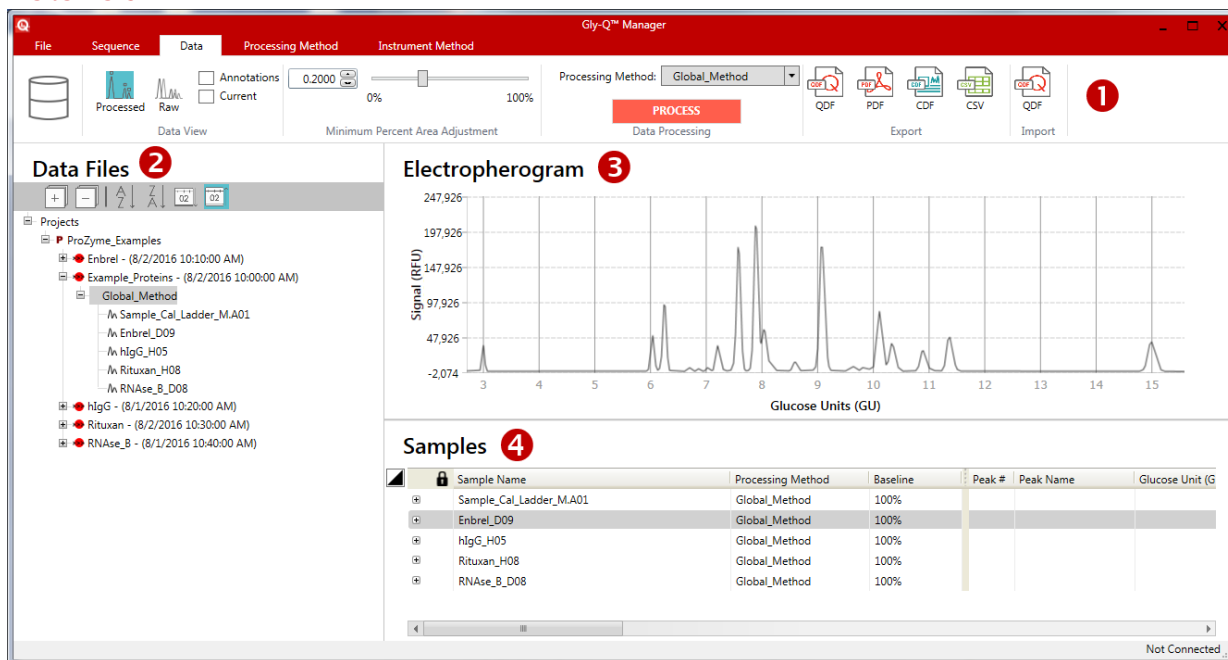
- 1 Sequence progress bar at the bottom of the screen will show time remaining for sequence completion.
- 2 The electropherogram chart will show real time detector signal.
- 3 The autosampler layout will show completed samples (green), failed samples (red), currently running sample (blue) and queued samples (grey).
- 4 The sequence progress table will be updated with currently running sample highlighted in darker gray.

4.5 Data Review, Export and Reporting

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 below.

1 Select the Data tab:

Data Tab

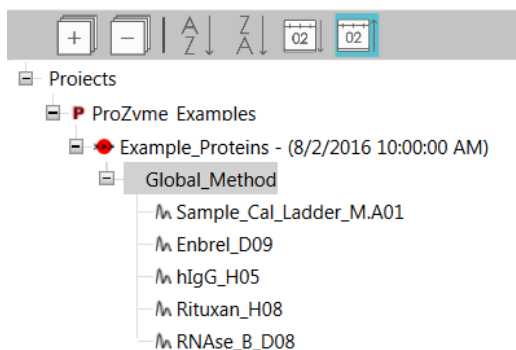


The Data tab includes the following 4 areas:

- 1 Data View Ribbon – this includes tools to adjust and to export data
- 2 Data Files tree – the data you wish to work on is selected here
- 3 Electropherogram – this is the data display which can include 1 or more samples
- 4 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.

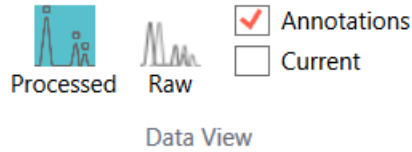
2 On the Data Files tree, select the sample set:

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:

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:

Samples

Sample Name	Processing Method	Baseline	Peak #	Peak Name	Glucose Unit (GU)	Relative Area, %
EnbreI_D09	Global_Method	100%				
hlgG_H05	Global_Method	100%				
Rituxan_H08	Global_Method	100%				
RNase_B_D08	Global_Method	100%				

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

Samples

Sample Name	Processing Method	Baseline	Peak #	Peak Name	Glucose Unit (GU)
Sample_Cal_Ladder_M.A01	Global_Method	100%			
Enbrel_D09	Global_Method	100%			
Enbrel_D09			1	Peak -0.8	-0.89
Enbrel_D09			2	Peak -0.8	-0.83
Enbrel_D09			3	Peak -0.7	-0.74
Enbrel_D09			4	Peak -0.6	-0.70
Enbrel_D09			5	Peak -0.3	-0.30
Enbrel_D09			6	Peak 0.1	0.14

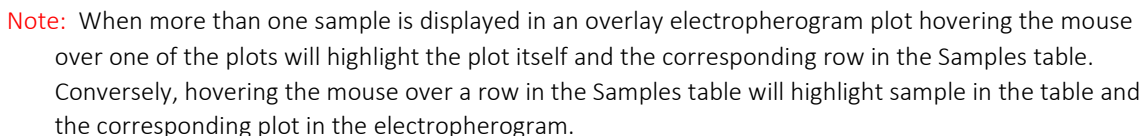
- ### Minimum Percent Area Adjustment



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 below. 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.

Selecting Multiple Samples

Example using "Control+Click" to select non-adjacent samples



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.

Samples

	Sample Name	Processing Method	Baseline
+	Sample_Cal_Ladder_M.A01	hIgG_Method	100%
+	GKS-001_Human_IgG_A01	Global_Method	100%
+	GKS-001_Human_IgG_A01	hIgG_Method	100%
+	GKS-001_Human_IgG_A01	hIgG_Method	100%
+	GKS-001_Human_IgG_A01	hIgG_Method	100%
+	GKS-001_Human_IgG_A01	hIgG_Method	100%
+	GKS-002_Human_IgG_A02	Global_Method	100%

A dialog opens to name the Virtual Data set.

New Data Set

Project Name:

Human IgG Sample Screening

Data Set Name:

VirtualDataSet

Session Name:

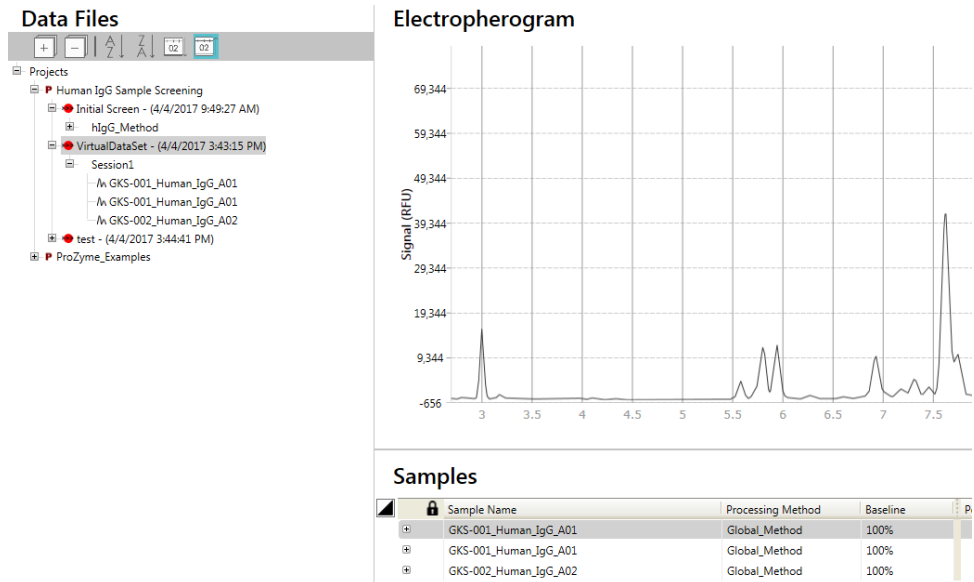
Session1

Calibration Ladder:

Create

Cancel

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.



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

Data Processing

Processing Method: Enbrel Method (B)

PROCESS

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 & Reporting



QDF

PDF

CDF

CSV

Export

- 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 header of the report can be customized in the File tab.
- 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 CSV export will create two files for selected samples: a file with peak table data and a file with XY data.

4.6 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 $\alpha(2,3)$ -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.
- hlgG_Method: N-glycans from human IgG, biantennary, some with $\alpha(2,6)$ -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 $\alpha(2,3)$ -linked in CHO vs. $\alpha(2,6)$ -linked in human IgG, and human IgG does not contain a significant

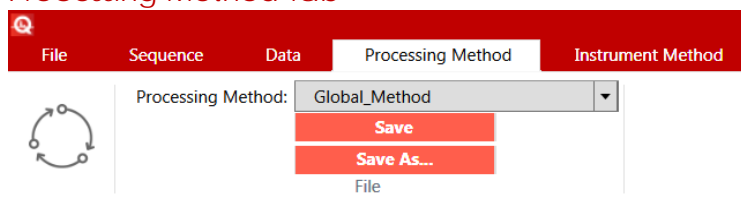
amount of high mannose type glycans.

- Rituxan_Method: N-glycans from Rituxan, monoclonal IgG made CHO. Biantennary, some high mannose.
- RNaseB_Method: high mannose type N-glycans, Man5 through Man9.

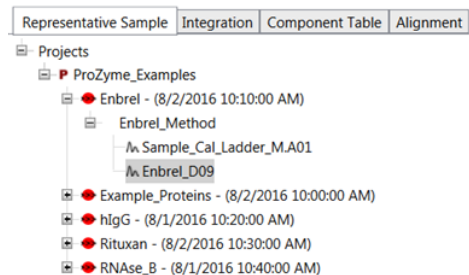
While these methods can be used as a starting point, each laboratory is expected to develop and validate a processing method that meets the needs of a specific application.

- 1 To create or modify a processing method, select the Processing Method tab and pick one of the existing processing methods from the drop-down menu:

Processing Method Tab



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



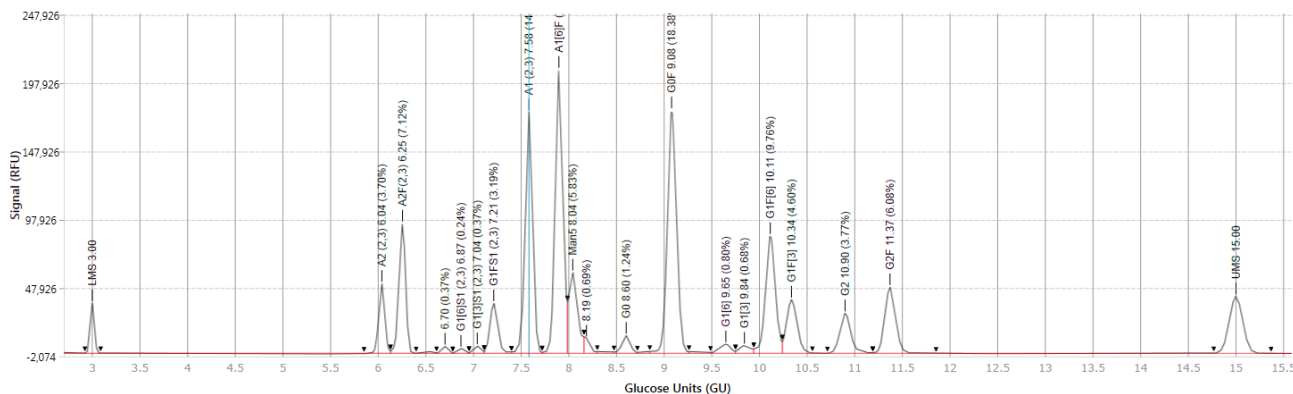
- 3 Open the peak table by clicking on  sign and review peak integration:

Samples Table

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

- 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.

Electropherogram



5 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 whether Detect Shoulders should be used. Checked will detect peak shoulders as separate peaks and is recommended, un-checked will identify peak with shoulders as a single peak.
- 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 a left window value and a right window value. Settings of 4 and 14 are recommended. If the window is set outside of the LMS and UMS, these Migration Standards will be omitted from the percent area calculations.

Representative Sample
Integration
Component Table
Alignment

Integration Parameters

Baseline Valley-to-Valley Modulation:

☒ Detect Shoulders

Minimum Percent Area:

Processed Data Range

Left Window: Right Window:

- 6 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.

Representative Sample Integration **Component Table** Alignment

☒ Glycan Naming

Left Window: 0.10 Right Window: 0.10

Component Table

Peak Name	Glucose Unit (GU)	Left	Right
Man5	8.062	0.100	0.100
Man6	9.011	0.100	0.100
Man7	9.952	0.065	0.100
Man8	10.847	0.075	0.100
Man9	11.445	0.070	0.100

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.

- 7 In the Alignment tab, define Lower and Upper Migration Standards, select Predictive UMS Search (checked is recommended), and check Dextran Ladder Rule:

Representative Sample Integration Component Table **Alignment**

Bracketing Standards

Peak Name	Glucose Unit (GU)
Lower Migration Standard	3
Upper Migration Standard	15
-- Not Set --	

Alignment Options

☒ Predictive UMS Search

Dextran Ladder Rule

☒ Always use latest ladder

☐ Use specific ladder:

- 8 Save the Processing method by clicking on Save or Save As.

Save

Save As

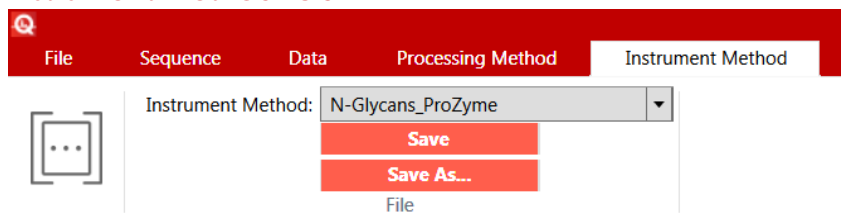
Note: After a processing method has been applied to a data set we recommend using 'Save As' if you wish to make changes to the method."

4.7 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.

- 1 To create or modify an instrument method, select the Instrument Method tab and pick one of the existing instrument methods from the drop-down menu:

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:

GU Ladder

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

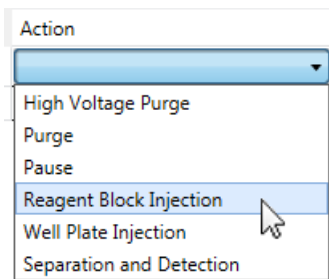
Samples

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

Shutdown

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

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 below to select a command:



- 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

- ③ Save the Instrument Method by clicking on Save or Save As.



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).*

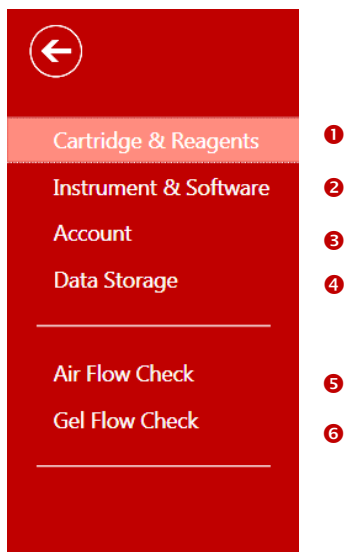
4.8 Ending a Run

4. After samples have been processed, the system will return the Cartridge to the Park position (see section 4.3, autosampler layout).
5. Use the “Load Sample” and “Load Reagents” buttons to remove sample plate and reagent tray.
6. 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 3 days. Fill the Park position of the buffer tray (Section 4.3.3) with water to prevent the cartridge tip from drying during storage on the instrument.
7. Samples may be stored, covered with sealing film, at -20°C for at least one month or up to 5 days at 4 °C.

4.9 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:



1 Cartridge and reagents settings:

Cartridge

Cartridge Serial Number: G1-O-123456-12
Cartridge Expiration Date: 9/20/2016
Runs Remaining: 196
Last Run Date: 9/19/2016

Reagents

Reagent Kit Lot Number:
Reagent Kit Expiration Date:

2 Instrument & software settings:


Instrument

Instrument Serial Number: 3185006595860000
Instrument Name: PROZYME

Software

Instrument FW Version: h
Instrument SW Version: 1.8
Application SW Version: 1.1.0.25

3 Account settings:

Company:	<input type="text" value="ProZyme, Inc."/>
Address:	<input type="text" value="3832 Bay Center Place, Hayward, CA 94545"/>
Phone:	<input type="text" value="+1 (800) 457-9444"/>
Email:	<input type="text" value="info@prozyme.com"/>
Logo:	<div><input type="button" value="Browse..."/></div>
<input type="button" value="Update"/>	

4 Data storage settings:

Database Root:	<input type="text" value="C:\ProgramData\ProZyme\GlycoManagerData"/>
----------------	--

5 Airflow check:

Air Flow Check

Follow this procedure to perform air flow check

1. Open the cartridge door and block the pressure nozzle with a tissue, as shown in the picture.
2. Click the Check button. The system will run for 30 seconds and will stop automatically.

Expected result: Air should be flowing through the opening in the cartridge door. If air is not flowing, check user manual for troubleshooting.



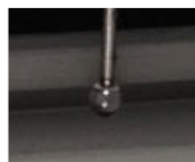
6 Gel Flow check:

Gel Flow Check

Follow this procedure to perform gel flow check

1. Insert the cartridge and close the cartridge door.
2. Click the Check button. The system will run for 60 seconds and will stop automatically.

Expected result: A droplet of gel should be visible on the capillary tip. If a droplet is not visible, check user manual for troubleshooting.



SECTION 5: CARE AND MAINTENANCE

Time Interval	Procedure
Weekly	Release condensation in the air pump <ul style="list-style-type: none">To release condensation, press the button in the bottom of the water cup 2-3 times to release the water in the cup.
Monthly	Air filter check <ul style="list-style-type: none">Turn off the air pump and release condensation in the water cup.Check the air filter. If the filter is damaged or wet, use a new filter to replace the old one. Replacement air filters are available from ProZyme.Turn the air pump on.Air flow can be assessed using the Air Flow Check functionality described in section 4.8, above.

SECTION 6: SYSTEM SPECIFICATIONS

General Technical Specifications

Environmental conditions	
Operating temperature	20-30°C
Operating humidity	40-95% RH, non-condensing
Operating altitude	20-2000 m
UL installation category	Category II

Instrument Specifications

Input voltage	100-240V, ~1A, 40-60Hz
Output voltage	1-15 kV
Light source	LED (310 nm)
Fluorescence detector	Photomultiplier tube, A/D resolution 18 bit, long pass filter ≥ 385 nm
Computer connection	USB
Physical dimensions (W x D x H)	30 x 38 x 40 cm
Weight	15 kg

Gel Recharging Air Pump Specifications

Input voltages	100-110V 220-240V
Output pressure range	1-100 psi

SECTION 7: 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.

ProZyme 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:

ProZyme 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 ProZyme Inc. through one (1) year after delivery of such purchased product to ProZyme'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 ProZyme Technical Support, (800)457-9444 (US & Canada); +1 510-683-6900; info@prozyme.com

Terms and conditions of sale may be found at: <http://www.prozyme.com/terms.html>

SECTION 8: INSTRUCTIONS FOR DEPOT REPAIR RETURNS

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

- ❶ Open the cartridge door and ensure there is no cartridge in the instrument.
- ❷ Check the sample and buffer tray to ensure there is no sample or fluid left in the instrument.
- ❸ Use 70% Ethanol (v/v) to clean the surface of the instrument for decontamination.
- ❹ Unplug the USB cable in the back.



- ❺ Unplug the power cable in the back.



Secure the cable node

Pull the connector header to unlock and detach the cable from instrument

- ❻ Remove the air tubing by simultaneously pressing the connector and remove the tubing rapidly



- ❼ Complete the ProZyme Decontamination Form (contact info@prozyme.com), pack the form with the instrument and contact the local distributor for shipping details

SECTION 9: 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?

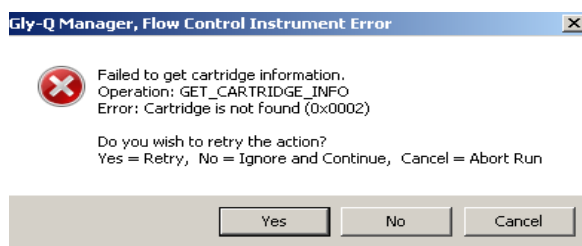
A. Ensure that the USB cable is intact and installed correctly.

Ensure that the instrument is turned on.

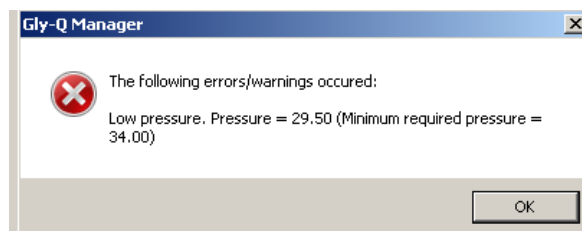
If 1&2 above are confirmed, reboot the instrument and computer. Occasionally communication can be lost if a prolonged period of inactivity is incurred.

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. How do I remedy the following error messages?



A. Ensure that the cartridge is installed correctly as per section 3.6.2, above.



A. Ensure that airline connections are intact and air filter is current and functional. The Airline Check in section 4.8 is another way to check this parameter.

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

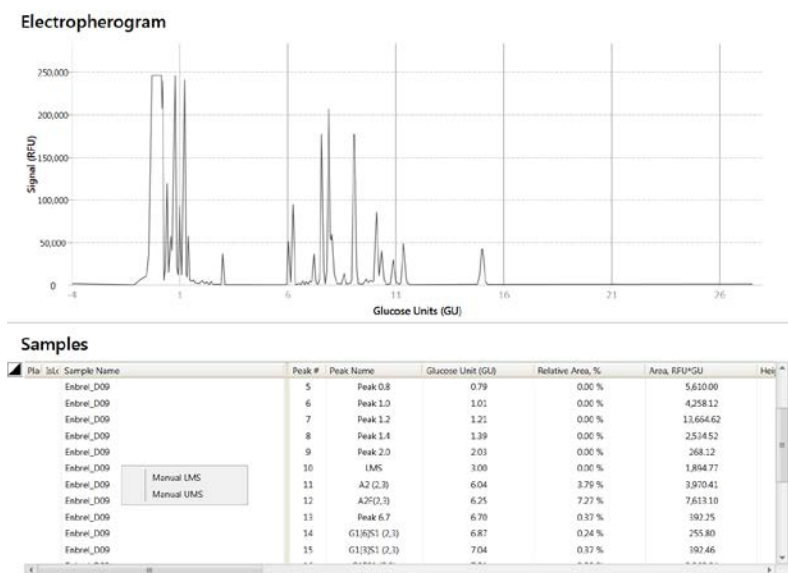
A. The air pump should run for ~ 30 seconds sporadically throughout an analysis session. If the pump is running for greater than 1 minute regularly throughout the run this could be an indication of air flow compromise. Again, the user should check integrity of airlines, air filters and perform the Air Flow function described in Section 4.8, above.

Q. When is the Gel Flow Check function used?

A. The Gel Flow check in section 4.8 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 clog is detected the user is advised to prime the cartridge as described in the cartridge preparation section, 3.6.2.

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

A. Within the Data Tab, Sample window, 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. GlyQ Manager further helps facilitate this process by enabling visualization with the corresponding electropherogram in the window above the Sample. Please see below for an example.



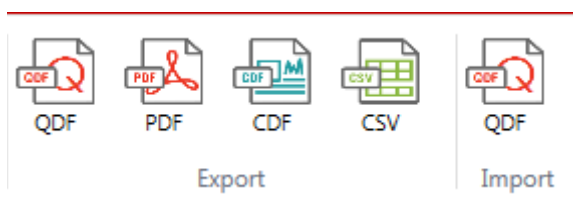
Manually designated LMS or UMS will be named accordingly in bold print, see below:

Samples

Plat	Idx	Sample Name	Peak #	Peak Name	Glucose Unit (GU)	Relative Area, %	Area, RFU*GU	Height
		Enbret_D09	5	Peak 1.8	1.77	0.00 %	5,640.01	
		Enbret_D09	6	Peak 2.0	1.99	0.00 %	4,204.74	
		Enbret_D09	7	Peak 2.2	2.19	0.00 %	13,394.81	
		Enbret_D09	8	Peak 2.4	2.37	0.00 %	2,467.94	
		Enbret_D09	9	LMS (Manual)	3.00	0.00 %	272.32	
		Enbret_D09	10	Peak 4.0	4.02	2.08 %	2,011.66	
		Enbret_D09	11	G1J3S1 (2,3)	7.07	3.86 %	3,736.32	
		Enbret_D09	12	G1FS1 (2,3)	7.27	7.34 %	7,110.81	
		Enbret_D09	13	Peak 7.7	7.69	0.37 %	362.26	
		Enbret_D09	14	A1J6F (2,3)	7.84	0.24 %	235.64	
		Enbret_D09	15	A1J6F (2,3), Man5	8.00	0.37 %	360.88	

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 of 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 programs that support the CVS format such as Microsoft Excel you can use the data export function with the CVS data file format.



SECTION 10: LEGAL NOTICES

10.1 Trademarks & Tradenames

ProZyme, Gly-Q, Gly-X, N-Glycanase, and InstantQ are trademarks or registered trademarks of ProZyme, Inc., in the United States and other countries. All other trademarks are the property of their respective owners.

Failure of a mark to appear on this page does not mean that ProZyme does not use the mark nor does it mean that the product is not actively marketed nor is not significant within its relevant market.

10.2 ProZyme Virtual Patent Marking

Visit <http://www.prozyme.com/patents> for a list of ProZyme products and patents.

SECTION 11: RESOURCES AND REFERENCES

Visit ProZyme's website for additional information, downloadable posters, publications and technotes.

<http://prozyme.com/pages/resources>

SECTION 12: TECHNICAL ASSISTANCE

ProZyme is committed to developing rapid, automatable methods for glycan analysis. Call us to discuss products in development.

If you have any questions or experience difficulties regarding any aspect of our products, please contact us:

TOLL FREE (800) 457-9444 (US & CANADA)

PHONE (510) 638-6900

FAX (510) 638-6919

E-MAIL info@prozyme.com

WEB www.prozyme.com

ProZyme values customer opinions and considers customers an important source for information regarding advanced or specialized uses of our products. We encourage you to contact us. We welcome your suggestions about product performance or new applications and techniques.



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