

Gly-Q™ Glycan Analysis System

User Manual

Product Code: GQ2100-110, GQ2100-220
Version: AB



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

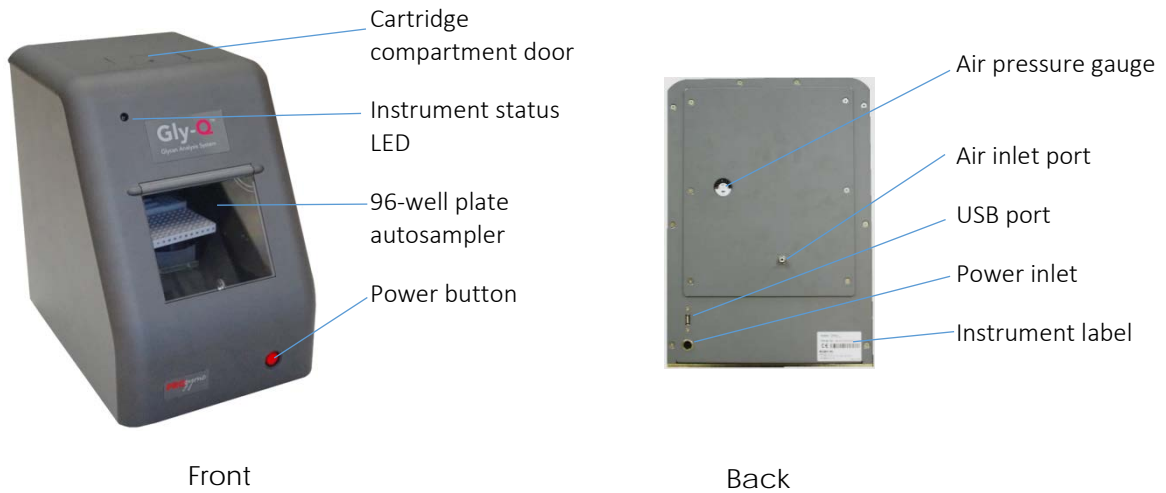
The Gly-Q System components are listed below and described in more details in the sections that follow:



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

2.2.1 Gly-Q Instrument

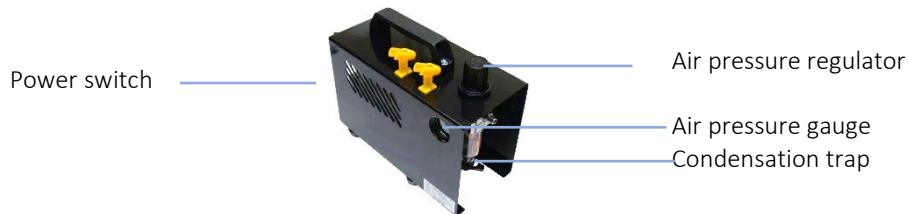
The Gly-Q Instrument is a compact unit comprised of a capillary gel electrophoresis core integrated with a high sensitivity fluorescence detector and 96-well autosampler. When used as part of a complete system, the instrument facilitates rapid separation, identification and relative quantitation of labeled 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 air pressure of the instrument.
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	A label detailing instrument serial number, CE marking and manufacturer information.

2.2.2 Gel Recharging Air Pump

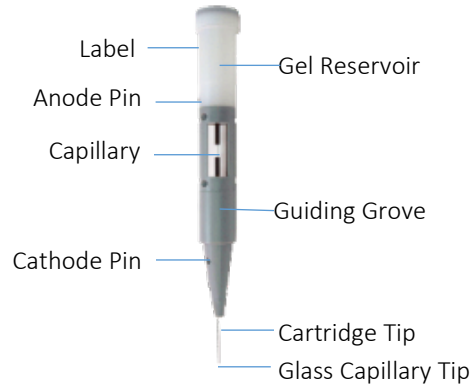
The gel recharging air pump connects to the instrument and provides air pressure for automated recharging of the capillary with gel between sample injections.



Feature	Description
Air pressure regulator	Air pressure regulator for adjusting air pump output.
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.

2.2.3 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: fragile, glass tip extends beyond cartridge metal 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 enables correct cartridge positioning within the instrument.
Cartridge tip	Metal end of the capillary.

2.2.4 Sample Preparation and Analysis Kits

Gly-X N-Glycan Rapid Release and Labelling Kits

Prozyme Gly-X sample preparation kits utilize a novel in-solution enzymatic deglycosylation followed by labelling 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 phase, the method is simple, rapid and suitable for automation.

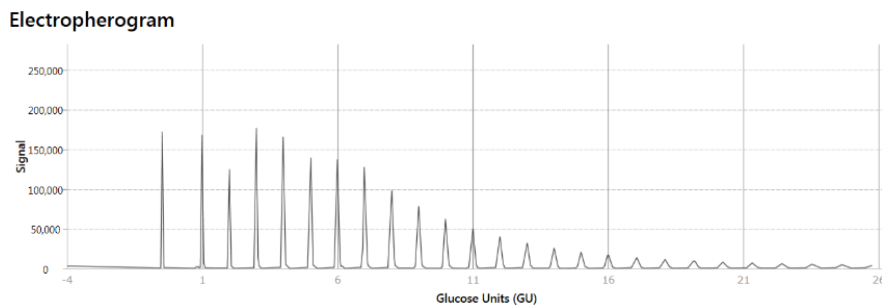


Feature	Description
Gly-X Deglycosylation Module for InstantQ Dye	Reagents and consumables for rapid in-solution protein deglycosylation: <ul style="list-style-type: none"> • Gly-X Deglycosylation Plate • Gly-X N-Glycanase • Gly-X Digestion Buffer • Gly-X Denaturant • Gly-X Blocker (optional)
Gly-X InstantQ Labeling Module	Dye and solvents for instant labelling 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 • InstantQ Manifold Spacer • InstantQ Collection Plate • Waste Tray for vacuum manifold • Plate Sealing Film
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

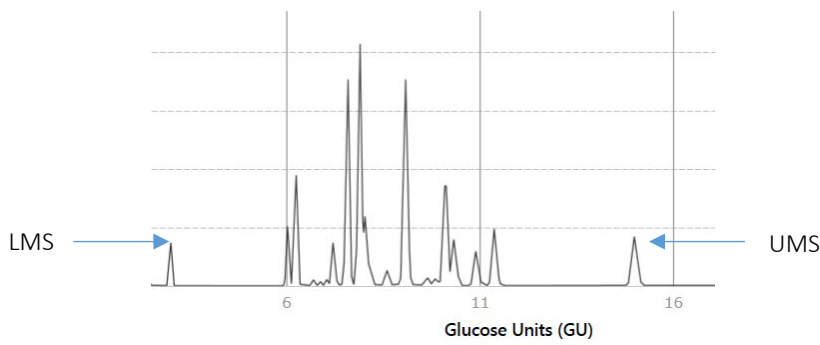
Note: See the *Gly-X Rapid N-Glycan Release and Labeling with InstantQ kit user manual* for more detailed product and protocol information.

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 produce >15 peaks in the electropherogram (see below). The Migration Standards (MS) from 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

- Unlabeled Oligosaccharides

prozyme.com/collections/glyko-standards-unlabeled-n-linked-oligosaccharides

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:

The screenshot displays the Gly-Q Manager software interface. At the top, there is a ribbon with tabs for File, Sequence, Data, Processing Method, and Instrument Method. Below the ribbon, the interface is divided into several sections:

- Autosampler Layout:** Shows a grid of sample wells (1-12) and controls for reagents and standards (MA, MB, MC, MD) with buttons for Park, Wash, Separation, and Clean.
- Electropherogram:** A graph showing Signal (RFU) on the y-axis (0 to 200,000) and Time (Seconds) on the x-axis (0 to 120). The signal starts at approximately 50,000 RFU, rises to a plateau of about 180,000 RFU by 24 seconds, and then shows a series of sharp peaks that decrease in intensity over time.
- Sequence Preview:** A table listing the sequence of samples to be run. The table has columns for Project Name, Run Name, Sample Name, Sample Type, Position Name, Injection, Instrument Method, Processing Method, Comment, Status, and Time.
- Instrument Status Bar:** Located at the bottom, it shows the current instrument method step (Separation & Detection), sequence progress, date and time (04/17/22 [ETA: 4/4/2017 2:10 PM]), cartridge injection count (0), and instrument status (Running).

Feature

Description

Sequence tab

Sequence tab enables:

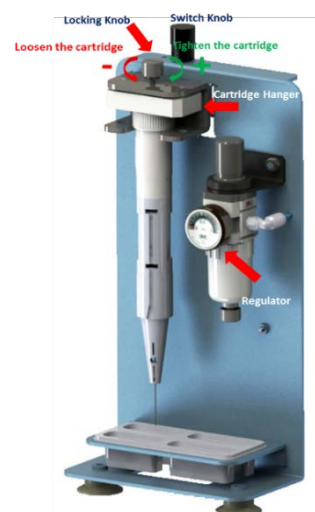
- Naming the project
- Naming the run
- Selection of the instrument method
- Selection of the processing method
- Creation or import of the sample set
- Export of the sample set
- Loading samples
- Loading reagents
- Starting and stopping the sequence
- Monitoring sequence progress
- Returning the cartridge to Park position

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.

2.2.7 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.



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.

3.2 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 tubing. The airline from the air pump to the instrument is bisected by an air filter.

Note: After turning on air pump it should run sporadically 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.

3.5 Software Installation



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

<http://prozyme.com/products/gly-q-manager-software-gqsw2000>

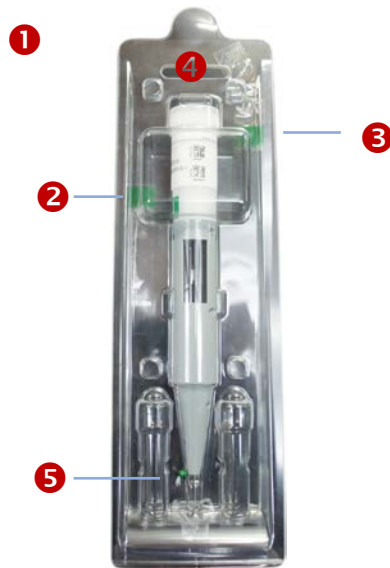
Note: Please contact ProZyme for password information

- 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 won't delete data. All user data will be preserved during installation.

3.6 Cartridge Preparation

3.6.1 Components



- ❶ Capillary gel cartridge clam-shell container (keep this for storage between uses).
- ❷ Tape (green) on body of cartridge
- ❸ Tape on cap (green, keep this for storage between uses).
- ❹ Top of the cartridge to be punctured prior to initial use.
- ❺ Pin in gel reservoir, used to puncture cartridge cap prior to first use. Store the pin in the gel reservoir inside the clam-shell container.

3.6.2 How to Unpack and Prime the Cartridge

Unpacking the Cartridge

- 1 Open gel-cartridge clam-shell container.



- 2 Peel off the tape.



- 3 Carefully remove cartridge and pin from protection gel.



- 4 Peel tape from the top of cartridge (retain tape for storage of cartridge between uses)

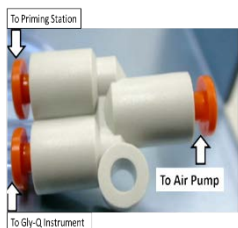


- 5 Insert pin into the hole of the cartridge cap and push it down to the end. Slight resistance should be encountered when performing this step.

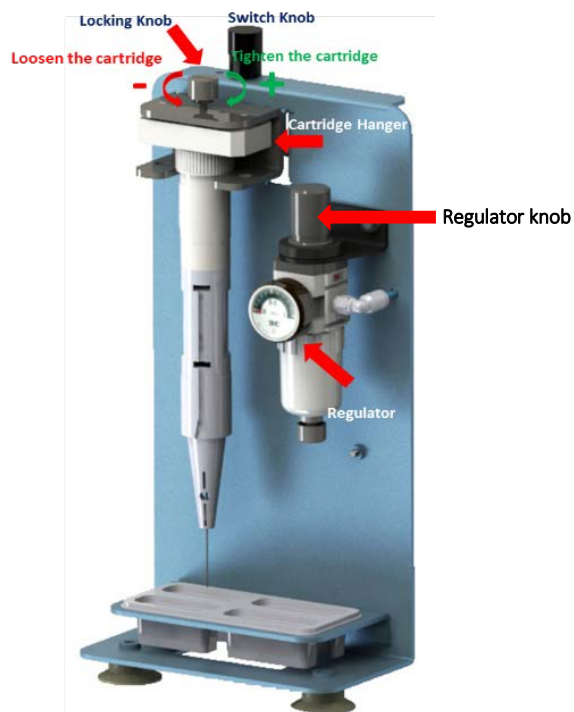


Priming the Cartridge

- 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



Inserting the Cartridge into the Instrument

- 1 Gently wipe the cartridge tip with wet 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.

- 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.

3.7 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)



- 1 Assemble vacuum manifold.
- 2 Connect vacuum manifold and pump inlet with tubing via 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

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.

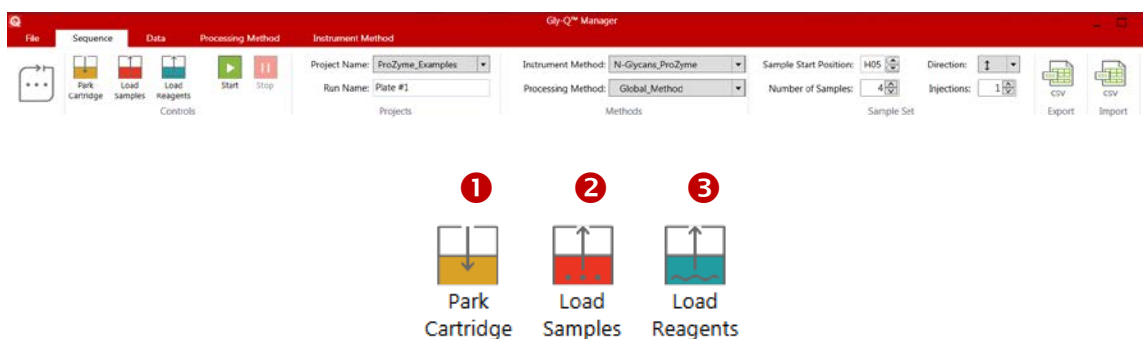
4.2 Controlling and Monitoring Gly-Q Instrument

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:



- 1** Park Cartridge moves cartridge into Park position for short term storage on the instrument (up to 3 days).
- 2** Load Samples control rotates the autosampler for access to the sample loading stage.
- 3** Load Reagents control rotates the autosampler. For access to load reagents including alignment ladders and bracketing 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 or select from the list of existing projects
- ❷ Name your run

Methods

Instrument Method: ❶
Processing Method: ❷

Methods

- ❶ Select Instrument Method
- 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: ❸
Number of Samples: ❷ Injections: ❹

Sample Set

- ❶ Define the position of the first sample
- ❷ Define the number of samples to be analyzed
- ❸ Define the direction the samples will be injected (↔ or ↕)
- 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

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

Sequence Preview

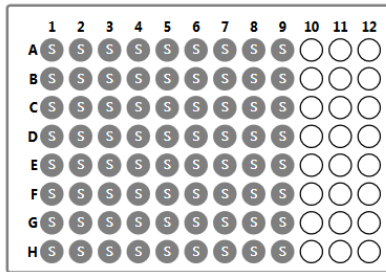
After a sequence is created, a table with a list of samples queued for analysis will be displayed on the main screen.

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	MA01	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		

Autosampler Layout

An autosampler layout on the left will show color coded positions for samples, reagents and standards.

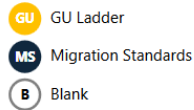
Samples



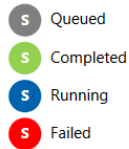
Reagents and Standards



Reagents and Standards



Samples



Reagent Tray for:

- (P) Park; 4 ml DI water + mineral oil to cover
- (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:



- 1 Sample name
- 2 Instrument method step
- 3 Well position
- 4 Sequence progress and estimated time of completion
- 5 Cartridge injection counter
- 6 System Pressure
- 7 Instrument status

4.4 Running a Sequence

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:



Monitoring Sequence Progress

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

The screenshot displays the Q10 Manager software interface. The top menu bar includes 'File', 'Sequence', 'Data', 'Processing Method', and 'Instrument Method'. The main window is divided into several sections:

- Autosampler Layout (3):** A grid of 12 sample wells. Well 1 is green (Completed), well 2 is blue (Running), well 3 is red (Failed), and wells 4-12 are grey (Queued).
- Electropherogram (2):** A chromatogram plot showing 'Signal (AU)' on the y-axis (0 to 250,000) and 'Time (Seconds)' on the x-axis (0 to 120). A sharp peak is visible at approximately 24 seconds.
- Sequence Preview (4):** A table listing sequence runs. The currently running sample (Sample 3) is highlighted in a darker gray.
- Sequence Progress Bar (1):** A progress bar at the bottom of the screen showing the current position (A20) and time remaining (03:39:38 [ETA: 44:00:17 2:10 PM]).

Project Name	Run Name	Sample Name	Sample Type	Position Name	Injection	Instrument Method	Processing Method	Comment	Status	Time
Human IgG Sample Screens	Initial Screens	Sample Cal Leader	Calibration Leader	MA001	1	Hi-Cytom_Pro2Yme	hgl0_Method	Comment S.A01	COMPLETED	4/4/2017 5:40:37 AM
Human IgG Sample Screens	Initial Screens	GIS-001 Human IgG	Unknown	A01	5	Hi-Cytom_Pro2Yme	hgl0_Method	Comment S.A01	COMPLETED	4/4/2017 10:03:21 AM
Human IgG Sample Screens	Initial Screens	GIS-002 Human IgG	Unknown	A02	5	Hi-Cytom_Pro2Yme	hgl0_Method	Comment S.A03	COMPLETED	4/4/2017 10:15:27 AM
Human IgG Sample Screens	Initial Screens	GIS-003 Human IgG	Unknown	A03	5	Hi-Cytom_Pro2Yme	hgl0_Method	Comment S.A03	RUNNING	4/4/2017 10:20:47 AM
Human IgG Sample Screens	Initial Screens	GIS-004 Human IgG	Unknown	A04	5	Hi-Cytom_Pro2Yme	hgl0_Method	Comment S.A05	QUEUED	
Human IgG Sample Screens	Initial Screens	GIS-005 Human IgG	Unknown	A04	5	Hi-Cytom_Pro2Yme	hgl0_Method	Comment S.A04	QUEUED	
Human IgG Sample Screens	Initial Screens	GIS-006 Human IgG	Unknown	A06	5	Hi-Cytom_Pro2Yme	hgl0_Method	Comment S.A06	QUEUED	
Human IgG Sample Screens	Initial Screens	GIS-007 Human IgG	Unknown	A07	5	Hi-Cytom_Pro2Yme	hgl0_Method	Comment S.A07	QUEUED	
Human IgG Sample Screens	Initial Screens	GIS-008 Human IgG	Unknown	A08	5	Hi-Cytom_Pro2Yme	hgl0_Method	Comment S.A08	QUEUED	
Human IgG Sample Screens	Initial Screens	GIS-009 Human IgG	Unknown	A09	5	Hi-Cytom_Pro2Yme	hgl0_Method	Comment S.A09	QUEUED	
Human IgG Sample Screens	Initial Screens	GIS-010 Human IgG	Unknown	A10	5	Hi-Cytom_Pro2Yme	hgl0_Method	Comment S.A10	QUEUED	
Human IgG Sample Screens	Initial Screens	GIS-011 Human IgG	Unknown	A11	5	Hi-Cytom_Pro2Yme	hgl0_Method	Comment S.A11	QUEUED	
Human IgG Sample Screens	Initial Screens	GIS-012 Human IgG	Unknown	A12	5	Hi-Cytom_Pro2Yme	hgl0_Method	Comment S.A12	QUEUED	
Human IgG Sample Screens	Initial Screens	GIS-013 Human IgG	Unknown	B01	5	Hi-Cytom_Pro2Yme	hgl0_Method	Comment S.B01	QUEUED	
Human IgG Sample Screens	Initial Screens	GIS-014 Human IgG	Unknown	B02	5	Hi-Cytom_Pro2Yme	hgl0_Method	Comment S.B02	QUEUED	
Human IgG Sample Screens	Initial Screens	GIS-015 Human IgG	Unknown	B03	5	Hi-Cytom_Pro2Yme	hgl0_Method	Comment S.B03	QUEUED	
Human IgG Sample Screens	Initial Screens	GIS-016 Human IgG	Unknown	B04	5	Hi-Cytom_Pro2Yme	hgl0_Method	Comment S.B04	QUEUED	

- 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.

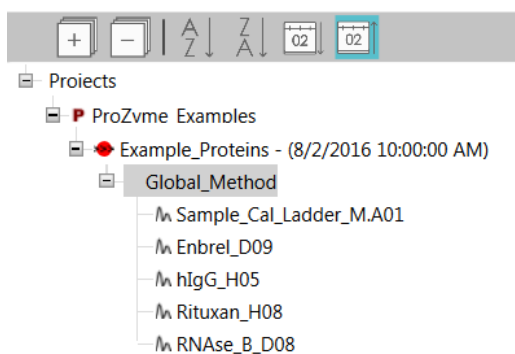
- 1 Select the Data tab:

Data Tab



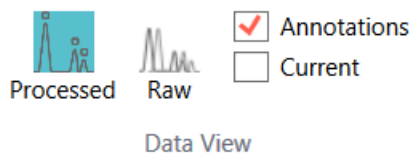
- 2 On the Data Files pan, select the sample set:

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 you want to view:

Samples

Sample Name	Processing Method	Baseline	Peak #	Peak Name	Glucose Unit (GU)	Relative Area, %
Enbrel_D09	Global_Method	100%				
hIgG_H05	Global_Method	100%				
Rituxan_H08	Global_Method	100%				
RNAse_B_D08	Global_Method	100%				

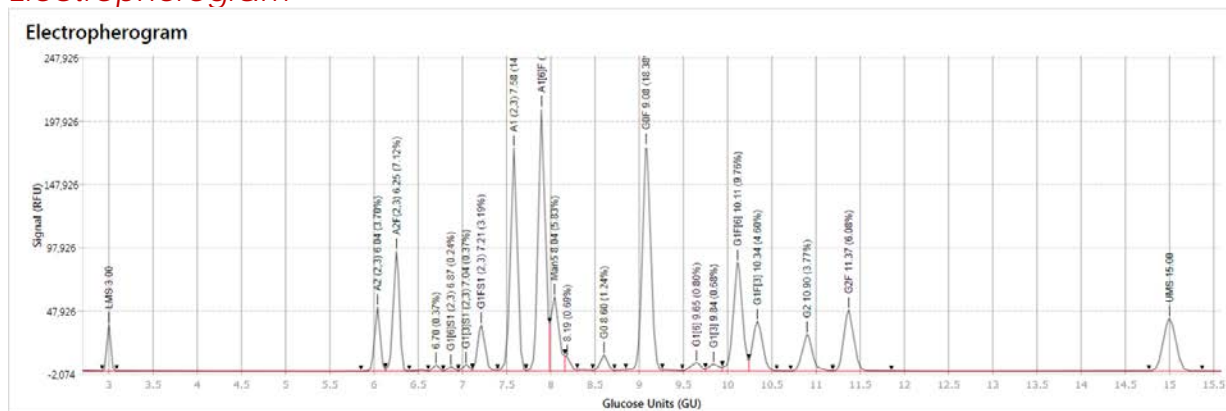
- 5 Open the peak table by clicking on **+** sign:

Peak Table

Sample Name	Processing Method	Baseline	Peak #	Peak Name	Glucose Unit (GU)	Relative Area, %
Enbrel_D09			13	Peak 1.1	1.09	0.00 %
Enbrel_D09			14	Peak 1.2	1.21	0.00 %
Enbrel_D09			15	Peak 1.4	1.39	0.00 %
Enbrel_D09			16	Peak 1.5	1.50	0.00 %
Enbrel_D09			17	Peak 1.6	1.62	0.00 %
Enbrel_D09			18	Peak 1.8	1.78	0.00 %
Enbrel_D09			19	Peak 2.0	2.03	0.00 %
Enbrel_D09			20	Peak 2.1	2.10	0.00 %
Enbrel_D09			21	Peak 2.3	2.26	0.00 %

- 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 unzoom.

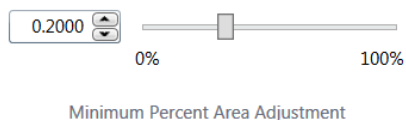
Electropherogram



Note: In some rare circumstances, the Lower Migration Standards (LMS) or Upper Migration Standards (UMS) may require manual adjustment. Please see the FAQs in Section 9 for further information.

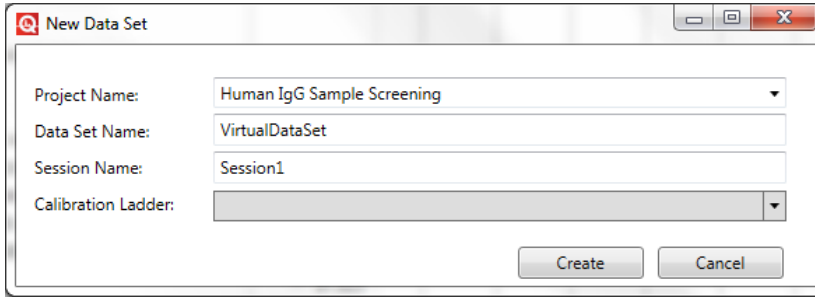
- 7 To make sure all peaks of interest are detected, adjust minimum peak area:

Minimum Percent Area Adjustment



Note: 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, see 7 below.

A dialog opens to name the Virtual Data set.



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.

Data Files

Electropherogram

Samples

Sample Name	Processing Method	Baseline
GKS-001_Human_IgG_A01	Global_Method	100%
GKS-001_Human_IgG_A01	Global_Method	100%
GKS-002_Human_IgG_A02	Global_Method	100%

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



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

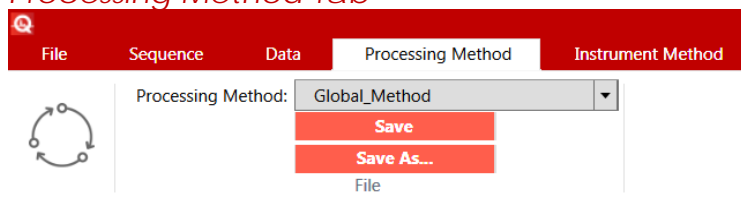
Processing method defines peak alignment, peak integration and peak calling. Gly-Q Manager installs equipped with several examples of processing methods.

- **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.

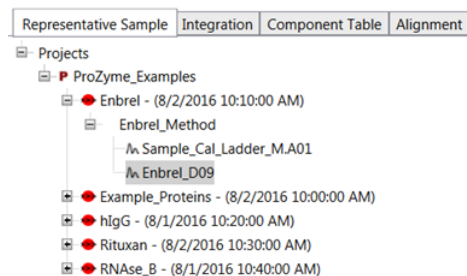
While these methods can be used as a starting point, each laboratory is expected to develop and validate a processing method that meets the needs of a specific application.

- 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



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



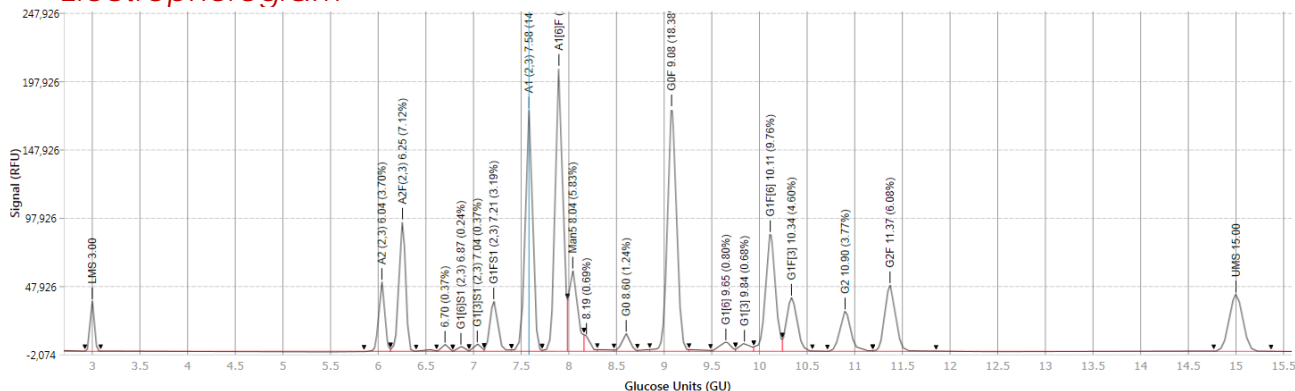
- Open the peak table by clicking on **+** sign and review peak integration:

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	G1F51 (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

- 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 select unzoom.

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 detected.
- Select the Processed Data Range by entering a left window value and a right window value. Settings of 4 and 14 are recommended. The left window should not be set below 3 and the right Window should not be set above 15 as these are the values of the bracketing standard.

Representative Sample Integration Component Table Alignment

Integration Parameters

Baseline Valley-to-Valley Modulation: 100%

Detect Shoulders

Minimum Percent Area: 0.2000

Processed Data Range

Left Window: 4.00 Right Window: 14.00

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.

- 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:

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

Save

Save As

Note: Processing methods that have already been applied to the data sets cannot be overwritten and can only be saved under a different name (Save As option).

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.

- 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

File Sequence Data Processing Method Instrument Method

Instrument Method: N-Glycans_ProZyme

Save

Save As...

File

- 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

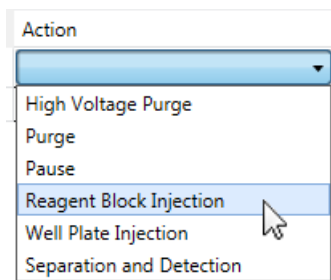
Samples

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

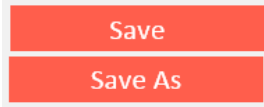
Action	High Voltage	Duration, Sec	Position	Sampling Interval, Sec
High Voltage Purge	4.00	90.00	Wash	0.04

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

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

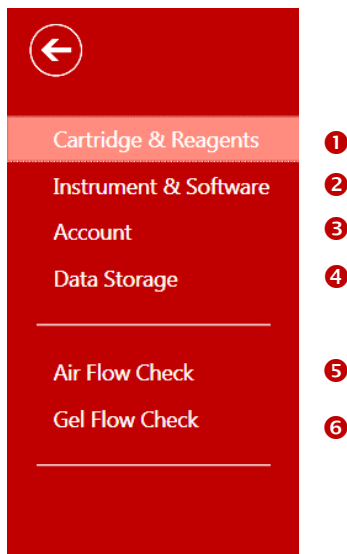


Note: Instrument Methods that have already been used in a sequence cannot be overwritten and can only be saved under a different name (Save As option).

4.8 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:



- 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:

Address:

Phone:

Email:

Logo:

4 Data storage settings:

Database Root:

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.

Check



SECTION 5: CARE AND MAINTENANCE

Time Interval	Procedure
Weekly	<p>Release condensation in the air pump</p> <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	<p>Air filter check</p> <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. 2 replacement air filters are supplied with the units and replacement air filters are available from ProZyme.• Turn the air pump on.• Nominally the air filter should be replaced every 3 months of regular use. 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	20-25°C
Operating humidity	40-95% RH, non-condensing
Operating attitude	20-2000 m
<hr/>	
UL installation category	Category II
<hr/>	

Instrument Specifications

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

Gel Recharging Air Pump Specifications

Input voltages	100-110V 220-240V
<hr/>	
Output pressure range	1-100 psi
<hr/>	

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.

- 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 Use 70% Ethanol (v/v) to clean the surface of the instrument for decontamination.
- 4 Unplug the USB cable in the back.



- 5 Unplug the power cable in the back.



Secure the cable node



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

- 6 Remove the air tubing by simultaneously inverting the connector and remove the tubing rapidly



- 7 Pack 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?

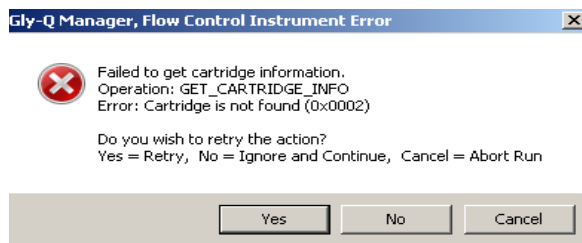
A. Insure that the USB cable is intact and installed correctly.

Insure that the instrument is turned on.

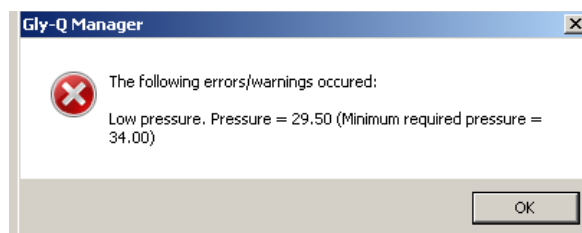
If 1&2 above are confirmed, reboot the instrument and computer. Occasionally communication can be lost if a prolonged period of inactivity is incurred.

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



A. Insure 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?

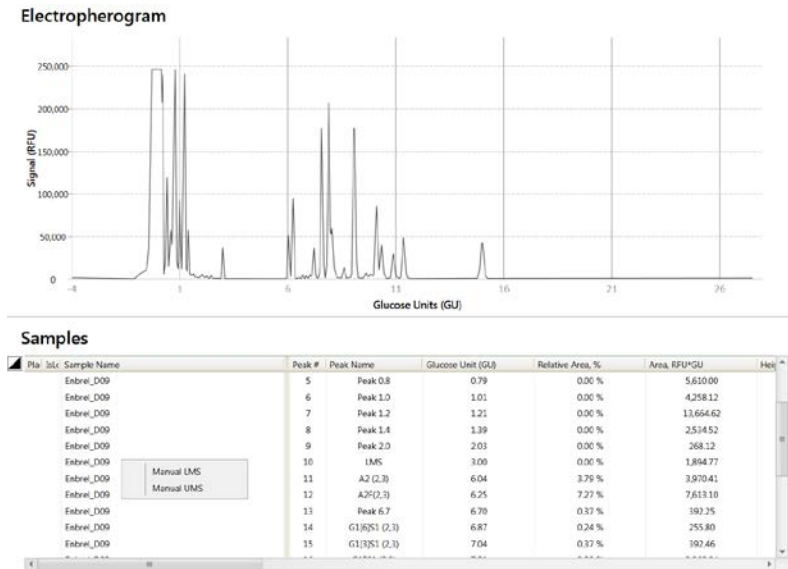
A. The air pump should run for ~ 30 seconds sporadically throughout an analysis session. If the pump is running for greater than 1 minute regularly throughout the run this could be an indication of air flow compromise. Again, the user should check integrity of airlines, air filters and perform the Air 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	IdL	Sample Name	Peak #	Peak Name	Glucose Unit (GU)	Relative Area, %	Area, RFU*GU	Height
		Enbrel_D09	5	Peak 1.8	1.77	0.00 %	5,640.01	
		Enbrel_D09	6	Peak 2.0	1.99	0.00 %	4,204.74	
		Enbrel_D09	7	Peak 2.2	2.19	0.00 %	13,394.81	
		Enbrel_D09	8	Peak 2.4	2.37	0.00 %	2,467.94	
		Enbrel_D09	9	LMS (Manual)	3.00	0.00 %	272.32	
		Enbrel_D09	10	Peak 4.0	4.02	2.88 %	2,811.66	
		Enbrel_D09	11	G1(3)S1 (2,3)	7.07	3.86 %	3,736.32	
		Enbrel_D09	12	G1(5)S1 (2,3)	7.27	7.34 %	7,110.81	
		Enbrel_D09	13	Peak 7.7	7.69	0.37 %	382.26	
		Enbrel_D09	14	A1(6)F (2,3)	7.84	0.24 %	235.64	
		Enbrel_D09	15	A1(6)F (2,3), Man5	8.00	0.37 %	360.88	

SECTION 10: LEGAL NOTICES

10.1 Trademarks and 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 ProZymes'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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