SCIEX
The Power of Precision

Fast Glycan Labeling and Analysis Kit

For the PA 800 Plus Pharmaceutical Analysis System

Application Guide

RUO-IDV-05-4092-D

May 2024

AB Sciex Pte. Ltd.
Blk33, #04-06 Marsiling Industrial Estate Road 3
Woodlands Central Industrial Estate, Singapore 739256

Contents

Contents

Fast Glycan Labeling and Analysis Kit

The Fast Glycan Labeling and Analysis kit uses capillary electrophoresis to separate and quantify oligosaccharides, also referred to as glycans, that are released from glycoproteins.

This kit contains the reagents and supplies that are required to label, separate, and identify oligosaccharides for 100 samples with the PA 800 Plus Pharmaceutical Analysis system.

This document gives instructions for sample preparation with the Fast Glycan Labeling and Analysis kit. It also gives instructions for data acquisition and data analysis with the PA 800 Plus software and Waters Waters Empower™ 3 (FR4) software.

Use the information in this application guide as a place to start. If required, change the injection time, voltage, injection type, or other parameters to find the best conditions for the requirements.

Note: For instructions about how to use the system safely, refer to the document: *Overview Guide*.

Note: For accurate results, we strongly recommend the use of the Fast Glycan Labeling and Analysis kit with a PA 800 Plus system that has been qualified with an *Operational Qualification*.

Safety

Refer to the safety data sheets (SDSs), which are available at sciex.com/tech-regulatory, for information about the correct handling of materials and reagents. Always follow standard laboratory safety guidelines. For information about hazardous substances, refer to the section: Hazardous Substance Information.

Intended Use

The Fast Glycan Labeling and Analysis kit is for laboratory use only.

Introduction

The Fast Glycan Labeling and Analysis kit is a widely-applicable sample preparation and analysis method for N-glycan profiling of glycoproteins. Carbohydrate profiling of therapeutic glycoproteins provides valuable data toward understanding the activity and efficacy of these molecules. Glycans strongly influence circulation half-life, immunogenicity, and receptor-binding activity as well as physicochemical and thermal stability of proteins of therapeutic interest. Identifying disease-related alterations to N-glycan structures can lead to the discovery of new biomarkers for early diagnostics.

The methodology uses enzymes to remove oligosaccharides from glycoproteins, and then labels the released oligosaccharides with a fluorophore. A single molecule of fluorophore binds to a single oligosaccharide molecule in a 1:1 stoichiometry. The labeled oligosaccharides are then separated by size in an N-CHO capillary and detected with laser-induced fluorescence (LIF).

Workflow

Table 1 Fast Glycan Labeling and Analysis Kit Workflow

Step	To Do This	Refer To
1	Prepare the reagents and stock solutions.	Prepare the Reagents and Stock Solutions
2	Denature the proteins.	Denature the Proteins
3	Release the N-Glycans.	Release the N-Glycans
4	Label the glycans.	Label the Released N-Glycans
5	Remove any excess dye.	Remove the Excess Dye from the Samples
6	Install the LIF detector.	Install the LIF Detector
7	Install the cartridge.	Install the Cartridge
6	Prepare the gel buffer and load the buffer trays.	Load the Buffer Trays
8	Load the sample tray.	Load the Sample Tray
9	Separate the glycans.	Run the Samples
10	Dispose of any waste.	Waste Disposal
11	Store the cartridge after use.	Stow the Cartridge
12	Analyze and identify the glycans.	Analyze and Identify the Glycans

Required Equipment and Materials

Note: For items with a reorder part number, sometimes the reorder quantity is different than the quantity in the kit.

Table 2 Fast Glycan Labeling and Analysis Kit (PN B94499PTO)

Component	Quantity	Reorder Part Number
BST-Bracketing Standard (10 pmol)	1	N/A

Table 2 Fast Glycan Labeling and Analysis Kit (PN B94499PTO) (continued)

Component	Quantity	Reorder Part Number
D1 reagent (50 µL)	5	N/A
D2 reagent (250 mM)	5	N/A
D3 reagent (1.5 mL)	1	N/A
D4 reagent (1.5 mL)	2	N/A
GU Ladder process control (5 mg)	1	N/A
HR-NCHO Glycan Separation Gel (56 mL)	1	N/A
IST-Internal Standard (5 mg)	1	N/A
L5-Catalyst (5 mg)	1	N/A
L6-Fluorophore (5 mg)	5	501309
M1 Magnetic Beads (22 mL)	1	N/A
Magnetic Stand	1	N/A
Pre-Assembled Capillary Cartridge	1	A55625

Table 3 Other Supplies from SCIEX

Component	Quantity	Part Number
CE Grade water (140 mL)	1	C48034
LIF Performance Test Mix (20 mL)	1	726022
PCR microvials (200 µL)	100	144709
Universal vial caps, blue	100	A62250
Universal vials	100	A62251

Table 4 Other Required Reagents or Supplies

Description	Vendor	Part Number
(Optional) Alpaqua Magnum FLX Magnet Plate	Alpaqua	A000400
1× phosphate buffered saline (PBS) (1 L)	Santa Cruz Biotechnology	sc-362182
1 M sodium cyanoborohydride in THF	MilliporeSigma	296813

Table 4 Other Required Reagents or Supplies (continued)

Description	Vendor	Part Number
2-mercaptoethanol	MilliporeSigma	M7154
Acetonitrile, HPLC-grade	Other Lab Supplier	Various
Peptide-N-glycosidase F enzyme (PNGase F)	New England Biolabs	P0704S

Storage Conditions

- Upon receipt, keep the Fast Glycan Labeling and Analysis kit at 2 °C to 8 °C.
- Keep the cartridge at ambient temperature.

Customer-Supplied Equipment and Supplies

- Powder-free gloves, neoprene or nitrile recommended
- Protective eyewear
- Laboratory coat
- Analytical balance
- Applicable centrifuge
- Microfuge tubes, 1.5 mL
- PCR tubes, 0.2 mL flat-cap
- Heat block capable of maintaining 60 °C
- Luer-Lok syringe, or equivalent, with a 22 gauge needle
- Pipettes and applicable tips
- Thermometer for measuring the heat block temperature
- Vortex mixer

Required Detector

A laser-induced fluorescence (LIF) detector with an excitation wavelength of 488 nm and a 520 nm emission filter is required.

Required Cartridge or Capillary

Note: Some versions of the Fast Glycan Labeling and Analysis kit include a pre-assembled cartridge. If required, the cartridge follows.

One of the following:

- Pre-assembled cartridge (PN A55625), with a total length of 30.2 cm and 50 µm inner diameter (i.d.)
- Capillary cartridge (PN 144738) and bare-fused silica capillaries, 50 µm inner diameter (i.d.) × 67 cm (PN 338451) trimmed to a total length of 30.2 cm and an effective length of 20 cm

Required Software

The GU Value software must be installed on the PA 800 Plus controller.

For systems that use 32 Karat software version 10.3 or higher, the GU Value software is included with the 32 Karat software.

For systems that use older versions of the 32 Karat software (before 10.3), the GU Value software must be downloaded and then installed. On a computer with Internet access, log in to sciex.com, and then go to https://sciex.com/products/capillary-electrophoresis/pa-800-plus-pharmaceutical-analysis-system. Search for `Fast Glycan software`, and then download the installation file that has the *Release Notes*, example data, and the GU Value software. For installation instructions, refer to the document: *Release Notes*.

Methods and Sequences

Note: This section applies to users with the PA 800 Plus system with the PA 800 Plus and 32 Karat software. If the system will be used with the Empower™ software, then the methods are different. Refer to Run the Samples with the Waters Empower™ Software.

For systems that use 32 Karat software version 10.3 or higher, the sequence and the following methods are installed on the PA 800 Plus controller at `C:\32Karat\projects\Fast Glycan\Method` and `C:\32Karat\projects\Fast Glycan\Sequence`. They are not available separately for download. The methods can also be created manually. Refer to the section: Methods.

- Methods:
  - `Fast Glycan Conditioning.met`: Conditions the capillary.
  - `Fast Glycan Separation.met`: Separates the sample with a pressure injection of the sample.
  - `Fast Glycan Shutdown.met`: Cleans the capillary at the end of a sequence and turns off the light source.

  - `Fast Glycan GU Ladder Separation.met`: Does a separation of the glucose ladder standard.
- Sequence file:
  - `HR-NCHO Fast Glycan.seq`: A sequence with the separation method that uses electrokinetic sample injection.

Prepare the Reagents and Stock Solutions

Note: The solutions in this section can be made ahead of time. Make sure that the solutions are stored correctly and are used before the solution aliquot expires.

Reconstitute the Bracketing Standard

1. Add 100 µL of CE Grade water to the BST vial, and then mix with a vortex mixer. This makes a 50 nM solution.
2. Make aliquots of the solution in 20 µL portions. When not in use, keep at –35 °C to –15 °C for a maximum of 6 months.

Reconstitute the IST-Internal Standard

1. Add 1,500 µL of CE Grade water to the IST vial, and then mix with a vortex mixer. This results in a 440 µM solution.
2. To minimize repeated freeze-thaw cycles, divide the solution into aliquots in 0.5 mL microfuge vials. Choose a volume applicable for the experimental design.

   A 20 µL aliquot is sufficient for a sequence of up to 96 samples.

   When not in use, keep at –35 °C to –15 °C for a maximum of 6 months.

Reconstitute the Fluorophore Label (L6)

- Add 240 µL of L5 to the L6 vial, and then mix the solution with a vortex mixer. When not in use, keep at –35 °C to –15 °C for a maximum of 3 months.

Prepare the Samples

Denature the Proteins

Note: The following instructions give quantities for 20 samples. For reference, quantities for 1 sample are also given. For experiments with a different number of samples, modify the quantities as applicable.

Prepare the Denaturation Solution

1. Reconstitute the D2 reagent.
   a. Add 50 µL of CE Grade water to the D2 vial.
   b. Mix the contents of the vial with a vortex mixer.

   The reconstituted D2 reagent can be kept at 4 °C for up to 24 hours.
2. Prepare a new 0.2 mL flat-cap PCR tube, and then label it *Denaturation Solution*.
3. Add the reagents specified in the following table to the *Denaturation Solution* tube, and then mix the contents of the vial with a vortex mixer. This procedure produces enough for 20 samples.

Reagent	For 1 Sample	For up to 20 Samples
D1	1 µL	20 µL
Reconstituted D2	1 µL	20 µL
D3	1 µL	20 µL
D4	5 µL	100 µL

   Discard the unused portion.

Denature the Proteins

1. Set the heat block to 60 °C.
2. Prepare 100 µg of glycoprotein sample in 10 µL of CE Grade water.

   Note: For highly-glycosylated samples, a smaller quantity of protein can be used.
3. Prepare the magnetic beads.
   a. Use a vortex mixer to mix the M1 vial at maximum speed until all of the beads are in solution.
   b. Add 200 µL of M1 to a new flat-cap 0.2 mL PCR tube, referred to as the *sample tube* in the rest of this document.
   c. Put the sample tube on a magnetic stand. After the beads are pulled to the side of the vial, use a pipette to slowly and carefully remove the supernatant from the bottom of the sample tube without touching the beads with the pipette tip.

      Tip! Make sure that only the supernatant is removed. To prevent the removal of beads, consider removing smaller volumes until the all of the supernatant is collected.

d. Remove the sample tube from the magnetic stand.

4. Add 100 µg of the glycoprotein sample, prepared in step 2, to the sample tube. Do not touch the beads with the pipette tip.
5. Use a vortex mixer to mix the sample tube for 10 seconds at maximum speed. Make sure that the magnetic beads are fully mixed with the sample.
6. Add 5 µL of the previously-prepared denaturation solution to the sample tube, mix briefly, and then incubate the solution for 8 minutes at 60 °C in the heat block to denature the sample. Refer to the section: Prepare the Denaturation Solution.

Note: Do not close the sample tube. The sample tube must remain open for optimal reaction performance.

Release and Label the N-Glycans

Note: This kit does not contain releasing enzymes, such as PNGase F. Multiple enzymatic and chemical procedures can be used to release oligosaccharides from proteins. To successfully label the released glycans, avoid destruction of the reducing end of the glycan by employing the proper deglycosylation method. The following is a suggested protocol for *N*-deglycosylation that uses *N*-glycosidase F (PNGase F).

Note: The following instructions give quantities for 20 samples. For reference, quantities for 1 sample are also given. For experiments with a different number of samples, modify the quantities as applicable.

Prepare the Digestion Solution

Note: Different quantities of enzyme can be used but the total reaction volume should be 14 µL per sample.

1. Prepare a new 0.2 mL flat-cap PCR tube, and then label it *Digestion Solution*.
2. Add the reagents specified in the following table to the *Digestion Solution* tube, and then mix the tube for 5 seconds at maximum speed with a vortex mixer. This procedure produces enough for 20 samples.

Table 5 Digestion Solution Reagents

Reagent	For 1 Sample	For up to 20 Samples
D4	12 µL	240 µL
PNGase F enzyme	2 µL	40 µL

Note: Digestion should be carried out with 5 mU of PNGase F, where one unit is defined as the amount of enzyme required to catalyze the release of N-linked oligosaccharides from 1 µmol of denatured ribonuclease B per minute at 37 °C, pH 7.5.

Discard the unused portion.

Note: For applications where the presence of other proteins might cause a problem, such as analysis with a mass spectrometer, immobilized PNGase F can be used. Immobilized PNGase F is available from Genovis. Refer to the instructions from the manufacturer, and to the document: M. Szigeti, J. Bodnar, D. Gjerde, Zs. Keresztessy, A. Szekrenyes, A. Guttman, "Rapid N-glycan release from glycoproteins by immobilized PNGase F microcolumns", *J.Chromatogr*. B 1032 (2016) 139-143).

Release the N-Glycans

1. Add 12 µL of the digestion solution prepared in the previous section to the sample tube, and then incubate the solution for 20 minutes at 60 ºC in the heat block. Refer to the section: Prepare the Digestion Solution.

   Note: Do not close the sample tube. The sample tube must remain open for optimal reaction performance.

2. After 20 minutes, close the sample tube, and then mix for 10 seconds at maximum speed with a vortex mixer.
3. Add 200 µL of acetonitrile to the sample tube, and then mix for 10 seconds at maximum speed with a vortex mixer.
4. Incubate for 1 minute at ambient temperature.
5. Spin the tube for 1 second to 2 seconds with a microcentrifuge to remove any solution that might be suspended from the cap.
6. Put the sample tube on a magnetic stand. After the beads are pulled to the side of the vial, use a pipette to slowly and carefully remove the supernatant from the bottom of the sample tube without touching the beads with the pipette tip.
7. Remove the sample tube from the magnetic stand.

Tips for Best Results

- To avoid aggregating the beads, use the magnets or stand only for the time specified in the sample clean-up and elution procedure.
- Always vigorously re-suspend the beads before adding acetonitrile. The beads are less susceptible to aggregation in aqueous solutions than in organic solutions.
- To prevent loss of beads that might be floating in the supernatant, always pipette from the bottom of the vial.

- After the solution is mixed at high speed with a vortex mixer, some sample might be suspended from the vial cap. To prevent sample loss, spin the tube for 1 second to 2 seconds in a centrifuge to remove any solution that might be suspended from the cap.

Prepare the Labeling Solution

DANGER! Toxic Chemical Hazard. Read the safety data sheet for 1 M sodium cyanoborohydride (in THF) before use.

Note: Flammable gases can be released when sodium cyanoborohydride touches water. Keep this chemical in dry conditions. To minimize exposure to possible sources of moisture, use a dry needle to remove chemical and add dry argon gas to the container.

Note: The labeling solution must be made fresh before use.

1. Prepare a new 0.2 mL flat-cap PCR tube, and then label it *Labeling Solution*.
2. In a fume hood, add the reagents specified in the following table to the *Labeling Solution* tube, and then mix the tube with a vortex mixer. This procedure produces enough for 20 samples.

   Tip! Use the syringe to remove the cyanoborohydride from the vial.

   Table 6 Labeling Solution Reagents

Reagent	For 1 Sample	For up to 20 Samples
Reconstituted L6	9 µL	180 µL
D4	3 µL	60 µL
1 M sodium cyanoborohydride (in THF)	1 µL	20 µL
Reconstituted IST	1 µL	20 µL

3. Spin the tube for 1 second to 2 seconds in a centrifuge to remove any solution that might be suspended from the cap
   Discard the unused portion.

Label the Released N-Glycans

1. Working in a fume hood, add 11 µL of the *Labeling Solution* to each sample tube. Refer to the section: Prepare the Labeling Solution.
2. Mix the sample tube for 10 seconds at maximum speed with a vortex mixer.

Make sure that the magnetic beads are well mixed with the labeling solution because the beads contain the glycans to be labeled. If the beads are not mixed, then mix the solution again.

3. Incubate the sample tube in the heat block for 20 minutes at 60 °C.

Note: Do not close the sample tube. The sample tube must remain open for optimal reaction performance.

Note: Discard the unused labeling solution.

Remove the Excess Dye from the Samples

Tip! Make sure that only the supernatant is removed. To prevent the removal of beads, consider removing smaller volumes until the all of the supernatant is collected.

Note: The following procedure uses a magnetic stand. If required, then another magnet can be used instead of the stand.

1. After the labeling reaction is complete, remove the sample tube from the heat block.
2. Rinse the labeled sample.
   a. Add 10 µL of D4 to the sample tube.
   b. Close the lid, and then mix for 10 seconds at maximum speed with a vortex mixer.
   c. Add 160 µL of acetonitrile to the sample tube.
   d. Close the lid, and then mix for 10 seconds at maximum speed with a vortex mixer.
   e. Let the tube incubate for 1 minute at ambient temperature.
   f. If required, then spin the sample tube in a centrifuge for 1 second to 2 seconds at maximum speed to remove any solution suspended from the lid.
   g. Put the sample tube on a magnetic stand. After the beads are pulled to the side of the vial, use a pipette to slowly and carefully remove the supernatant from the bottom of the sample tube without touching the beads with the pipette tip.
   h. Remove the sample tube from the magnetic stand.
3. Wash the sample.
   a. Add 20 µL of CE Grade water to the sample tube.
   b. Close the lid, and then mix for 10 seconds at maximum speed with a vortex mixer.
   c. Add 160 µL of acetonitrile to the sample tube.
   d. Close the lid, and then mix for 10 seconds at maximum speed with a vortex mixer.
   e. Let the tube incubate for 1 minute at ambient temperature.

f. If required, then spin the sample tube in a centrifuge for 1 second to 2 seconds to remove any solution suspended from the lid.

g. Put the sample tube on a magnetic stand. After the beads are pulled to the side of the vial, use a pipette to slowly and carefully remove the supernatant from the bottom of the sample tube without touching the beads with the pipette tip.

4. Do the wash again: Repeat step 3.

CAUTION: Potential Sample Loss. Do not wash the sample more than three times, even if the sample tube appears to contain dye. Additional washes might remove small glycans as well as the dye.

5. Remove the sample tube from the magnetic stand.
6. Add 100 µL of CE Grade water to the sample tube, and then mix for 10 seconds at maximum speed to elute the labeled glycans from the beads.
7. Put the sample tube on a magnetic stand. After the beads are pulled to the side of the vial, use a pipette to slowly and carefully remove the supernatant from the bottom of the sample tube without touching the beads with the pipette tip.
8. Put the supernatant in a 0.2 mL PCR vial.

   The supernatant contains the labeled and purified glycans.

9. Do one of the following:

   - Prepare and load the sample tray, and then start the run. Refer to the section: Load the Sample Tray.
   - Store the vials at –35 °C to –15 °C for a maximum of 1 month.

(Optional) Prepare and Label the GU-Glucose Ladder Standard

DANGER! Toxic Chemical Hazard. Read the safety data sheet for 1 M sodium cyanoborohydride (in THF) before use.

1. Reconstitute a portion of the GU-Glucose Ladder Standard.

   a. Add 5 mg of the GU-Glucose Ladder Standard to a 1.5 mL microfuge vial.

   b. Add 80 µL of CE Grade water to the vial, and then mix the contents of the tube with a vortex mixer until the GU-Glucose Ladder Standard is completely dissolved.

2. Add 2 µL of the GU solution to a 0.5 mL microfuge vial.

   Discard the remainder of the GU solution.

3. Add 9 µL of reconstituted L6 to the GU-Glucose Ladder Standard.
4. In a fume hood, add 1 µL of 1 M sodium cyanoborohydride (in THF) to the GU vial, and then put the cap on the vial.

5. Use a vortex mixer to mix the contents of the vial for 10 seconds at maximum speed.
6. Use a centrifuge to spin the vial for 1 second to 2 seconds to bring the solution to the bottom of the vial.
7. Open the vial cap, and then use the heat block to incubate the vial at 60 °C for 40 minutes.

   Note: Do not close the sample tube. The sample tube must remain open for optimal reaction performance.

   Tip! Measure the temperature of the heat block with a thermometer to make sure the temperature is correct.

   After incubation, the labeled GU-Glucose Ladder Standard is a dry yellow pellet.
8. Prepare the labeled GU ladder stock solution.
   a. Add 100 µL of CE Grade water to the solid, and then mix until the solid is completely dissolved.
   b. With a microcentrifuge, spin the vial for a few seconds to bring the solution to the bottom of the vial.
      This is the labeled GU ladder stock solution. When not in use, keep at –35 °C to –15 °C for a maximum of 6 months.
9. Add 200 µL of the diluted GU ladder stock solution in a microvial, and then put the vial in the sample tray. Refer to the section: Load the Sample Tray.

Prepare the PA 800 Plus System

Use the procedures in this section to prepare the PA 800 Plus system to acquire data.

Tip! To save time, turn on the light source 30 minutes before the start of the run to let it become warm.

Install the LIF Detector

1. Turn off the PA 800 Plus system.
2. Install the LIF detector. For detailed instructions, refer to the document: *Maintenance Guide*.
3. Turn on the system.

Clean the Electrodes, Insertion Levers, and Interface Block

CAUTION: Potential System Damage. Do not let the buffer crystallize on the electrodes, opening levers, capillary tips, and interface block. Salt crystals might cause broken capillaries, bent electrodes, jammed vials, or missed injections.

Clean the electrodes, opening levers, and interface block every week or when chemistries are changed. For detailed instructions, refer to the section: "Clean the Electrodes, Insertion Levers, and Interface Block" in the document: *Maintenance Guide*.

The separation buffer can evaporate resulting in salt deposits in the system unless regular and thorough cleaning is performed.

Install the Cartridge

1. Remove the cartridge from the box.
2. For a pre-assembled cartridge, remove the aperture from the cartridge, and then install the LIF aperture and probe guide. For detailed instructions, refer to the document: *Maintenance Guide*.
3. Install the cartridge in the PA 800 Plus system. For detailed instructions, refer to the document: *Maintenance Guide*.

   Tip! Turn on the laser, and then let it become warm for at least 30 minutes.

4. (Optional) Calibrate the LIF detector.

   Use the Calibration wizard, which is available from the Instrument Configuration dialog in the 32 Karat software. For detailed instructions, refer to the section: Calibrate the LIF Detector (Optional).

Condition the Capillary

1. Condition the capillary with the conditioning method (downloaded from the SCIEX website).
2. Before a new capillary is used, to rinse the capillary, do a 5 minute wash at 80 psi with CE Grade water.

Load the Buffer Trays

CAUTION: Potential System Damage. Do not fill any vial with more than 1.5 mL of liquid. Fill waste vials with 1.0 mL of liquid. Do not let more than 1.5 mL of liquid collect in waste vials. If a vial is filled with more than 1.5 mL of liquid, then the pressure system can be damaged.

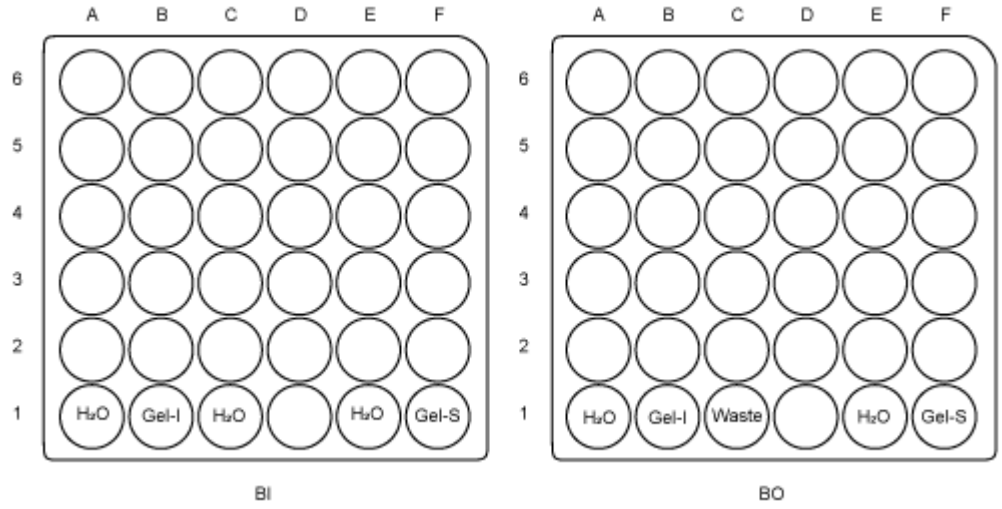
Note: To prevent air bubbles, do not shake or vigorously mix the separation gel. Air bubbles might cause issues with the separation.

Note: Do not reuse the vials or caps, because they might be contaminated with dried gel and other chemicals.

1. Use the layout in the follwing figure to put the vials in the buffer trays. Each row is sufficient for a minimum of 20 runs.

The vials of water in positions E1 in both trays are used for the `Fast Glycan Shutdown` method.

Figure 1 Buffer Tray Layout



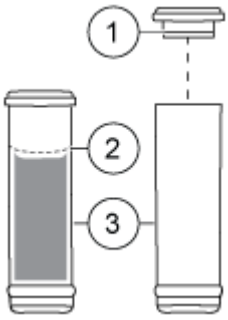
Note: During electrophoresis, the ionic strength of the buffer changes. The separation method is programed to increment the buffer vials after 20 runs to prevent ionic depletion.

2. Fill the vials shown in the following table, and then attach the cap. Refer to the following figure.

Table 7 Vials to Prepare

Label	No. of Vials	Vol./Vial (mL)	Reagent
Water	5	1.5	CE Grade water
Gel-I	2	1.5	HR-NCHO Glycan Separation Gel
Gel-S	2	1.5	HR-NCHO Glycan Separation Gel
Waste	1	1.0	CE Grade water

Figure 2 Universal Vial and Cap Setup



Item	Description
1	Universal vial cap
2	Maximum fill line
3	Universal vial

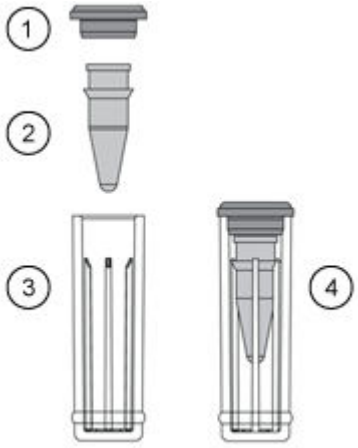
Load the Sample Tray

Note: Do not reuse the vials or caps, because they might be contaminated with dried gel and other chemicals.

Note: For workflows that have small sample volumes, SCIEX sells vials that are specially made for small volumes. For volumes between 5 µL and 50 µL, with a standard volume of 25 µL, use nanoVials. For volumes between 50 µL and 200 µL, with a standard volume of 100 µL, use microvials.

1. Prepare the BST-Bracketing Standard, and then put it in the sample tray.
   Refer to the section: Reconstitute the Bracketing Standard.
   a. Add 30 µL of CE Grade water to one aliquot of the reconstituted BST-Bracketing Standard.
   b. Mix the BST-Bracketing Standard.
   c. Put a microvial in a universal vial. Refer to the following figure.

Figure 3 Microvial in a Universal Vial



Item	Description
1	Universal vial cap
2	Microvial
3	Universal vial
4	Microvial inside a universal vial

d. Transfer all of the diluted BST-Bracketing Standard to the microvial.

e. Put a blue cap on the universal vial.

2. Add the samples to the microvials. For each sample:

   a. If required, gently thaw the sample.

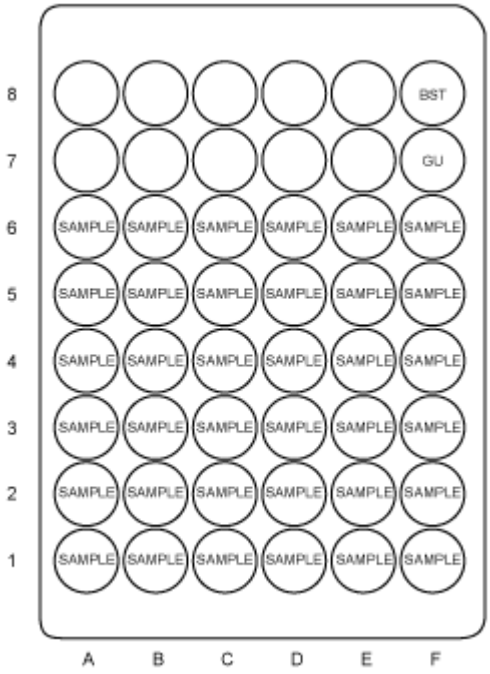
   b. Transfer between 50 µL to 200 µL of the sample to a microvial.

   c. Put a blue cap on the universal vial. Refer to the figure: Figure 3.

3. Put each universal vial in the sample tray. Refer to the figure: Figure 4.

Note: The maximum number of samples that can be put in the sample tray is 36.

Figure 4 Sample Tray Layout



4. (Optional) Load the GU-Glucose Ladder Standard.
   The GU-Glucose Ladder Standard serves as the process control.
   a. Put the microvial that contains the GU-Glucose Ladder Standard in a universal vial. Refer to the figure: Figure 3.
   b. Put a blue cap on the universal vial.
   c. Put the vial in position SI:F7 in the sample tray. Refer to the figure: Figure 4.

Run the Samples

Create the Example Instrument

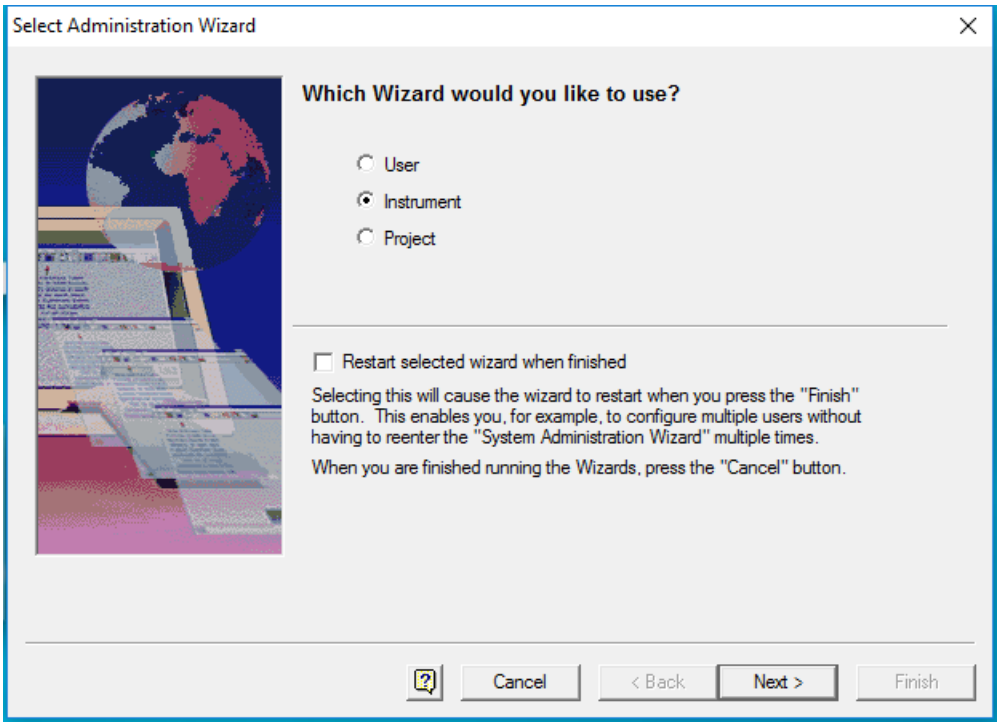
Note: The following procedure is required if the PA 800 Plus software will be used for data acquisition and analysis. If the 32 Karat software will be used instead, then this procedure is not required.

This procedure requires the user name and password for a user with administrative privileges for the 32 Karat software.

For detailed instructions, refer to the documents: *32 Karat Software Help* or *Methods Development Guide*.

1. Double-click the 32 Karat icon on the desktop.
2. Click **Tools** > **Enterprise Login**, type the user name and password, and then click **Login**.
3. Click **Tools** > **System Administration Wizard**.

Figure 5 Select Administration Wizard Window



4. Click **Instrument**, and then click **Next.**
5. Follow the instructions in the wizard to create the instrument. When prompted for the instrument name, type `Fast Glycan`.
   The PA 800 plus System Configuration dialog opens.
6. Click **LIF Detector**,  , and then click **OK**.
7. Do one of the following:
   - If the system is connected to the controller and it is turned on, then click **Auto Configuration**.
   - If the system is not connected to the controller or it is turned off, then in the **Configured modules** list, right-click **LIF Detector** and select **Open**. Make sure that the trays are configured correctly, and then click **OK**.
8. Click **OK**.
   The PA 800 plus System Configuration dialog closes.

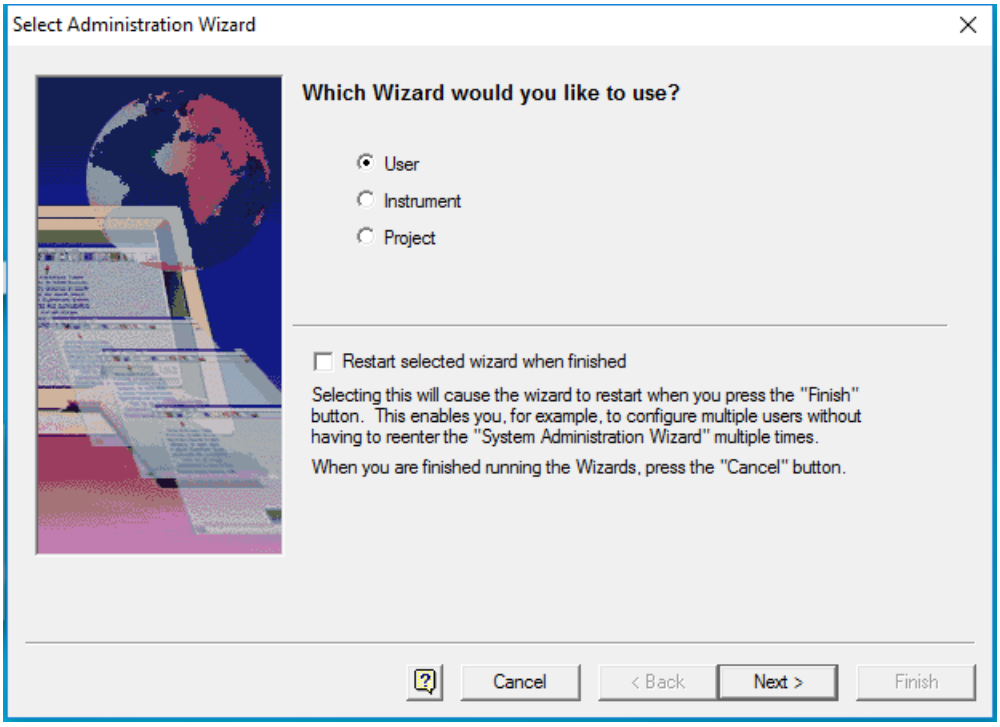
Create the Example Project

Note: The following procedure is required if the PA 800 Plus software will be used for data acquisition and analysis. If the 32 Karat software will be used instead, then this procedure is not required.

This procedure requires the user name and password for a user with administrative privileges for the 32 Karat software.

1. Double-click the 32 Karat software icon on the desktop.
   If the 32 Karat software is already open, close any instrument windows that are open.
2. Click **Tools** > **Enterprise Login**, type the user name and password, and then click **Login**.
3. Click **Tools** > **System Administration Wizard**.

Figure 6 Select Administration Wizard Window



4. Click **Project** and then click **Next.**
5. Follow the instructions in the wizard to create the project. When prompted for the project name, type `Fast Glycan`.
   Make sure to assign users to the project.

   For detailed instructions, refer to the documents: *32 Karat Software Help* or *System Administration Guide*.

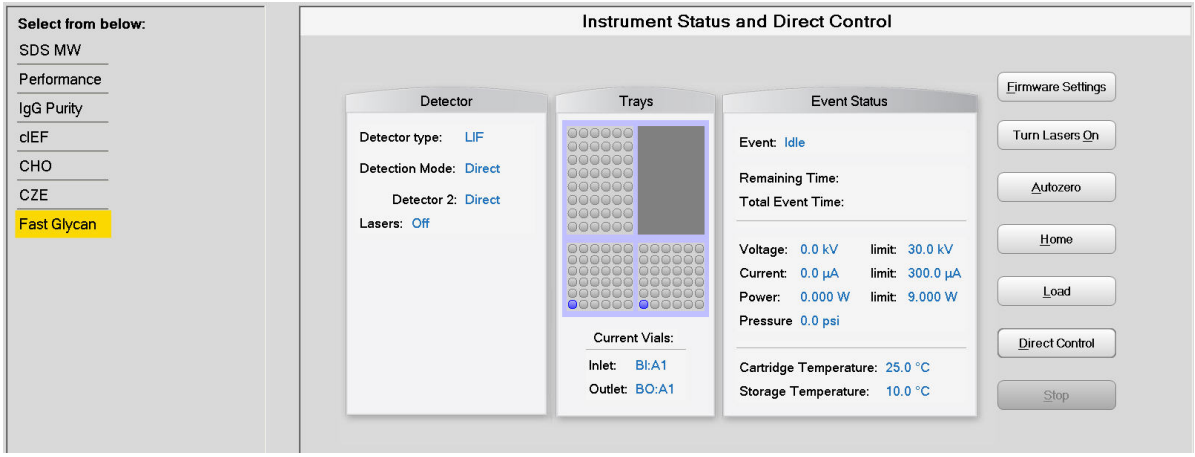
6. If required, then download the method and sequence files from the SCIEX website. Refer to the section: Methods and Sequences.
7. Copy the methods to the methods folder for the project. By default, this is `C:\32Karat\projects\Fast Glycan\Method`.
8. Copy the sequence to the sequence folder for the project. By default, this is `C:\32Karat\projects\Fast Glycan\Sequence`.

Create the Sequence and Start the Run

Note: For Waters Empower™ software users, refer to the section: Run the Samples with the Waters Empower™ Software.

1. Double-click the PA 800 Plus software icon on the desktop to open the PA 800 Plus software.
2. In the **PA 800 plus** window, click (**Run**) in the upper right corner.
3. Click the **Fast Glycan** instrument, and then click **Fast Glycan**. If system administration is enabled, then type a user name and password when prompted.
   The Instrument Status and Direct Control page opens.

Figure 7 Instrument Status and Direct Control Page



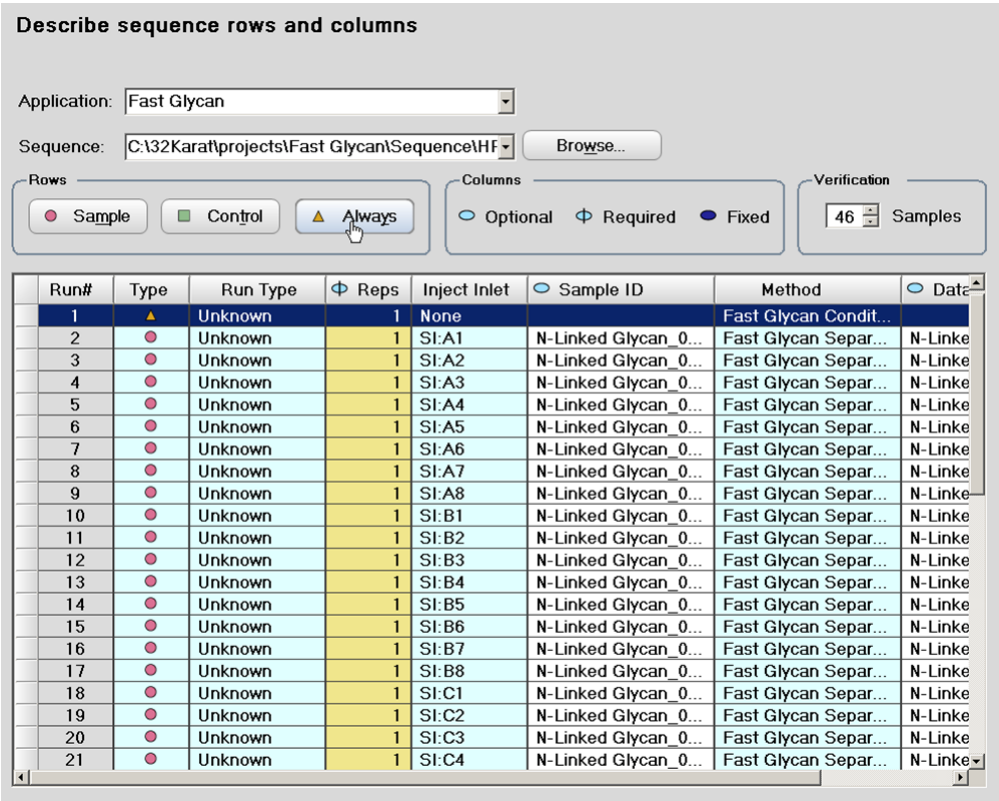
4. Click (**Describe**).
5. In the **Application** list, click **Fast Glycan**. In the **Sequence** list, click **Browse**, and then browse to the **Fast Glycan** sequence. If required, type a user name and password.
   The page updates to show the selected sequence and all of the rows in the sequence are designated as samples.
6. Set the type for the first and last rows in the sequence. The first row is for capillary conditioning and the last row is for system shutdown.

a. Click the first row (with the `Fast Glycan Conditioning` method) to select it, and then click Always (**Always**) in the **Rows** section.

b. Click the last row (with the `Fast Glycan Shutdown` method) to select it, and then click Always (**Always**) in the **Rows** section.

The icon in the **Type** column first and last rows in the sequence is now a triangle.

Figure 8 Describe sequence rows and columns Page: Conditioning Method Set to *Always*



Run#	Type	Run Type	Reps	Inject Inlet	Sample ID	Method	Date
1	▲	Unknown	1	None		Fast Glycan Condit...	
2	●	Unknown	1	SI:A1	N-Linked Glycan_0...	Fast Glycan Separ...	N-Linke
3	●	Unknown	1	SI:A2	N-Linked Glycan_0...	Fast Glycan Separ...	N-Linke
4	●	Unknown	1	SI:A3	N-Linked Glycan_0...	Fast Glycan Separ...	N-Linke
5	●	Unknown	1	SI:A4	N-Linked Glycan_0...	Fast Glycan Separ...	N-Linke
6	●	Unknown	1	SI:A5	N-Linked Glycan_0...	Fast Glycan Separ...	N-Linke
7	●	Unknown	1	SI:A6	N-Linked Glycan_0...	Fast Glycan Separ...	N-Linke
8	●	Unknown	1	SI:A7	N-Linked Glycan_0...	Fast Glycan Separ...	N-Linke
9	●	Unknown	1	SI:A8	N-Linked Glycan_0...	Fast Glycan Separ...	N-Linke
10	●	Unknown	1	SI:B1	N-Linked Glycan_0...	Fast Glycan Separ...	N-Linke
11	●	Unknown	1	SI:B2	N-Linked Glycan_0...	Fast Glycan Separ...	N-Linke
12	●	Unknown	1	SI:B3	N-Linked Glycan_0...	Fast Glycan Separ...	N-Linke
13	●	Unknown	1	SI:B4	N-Linked Glycan_0...	Fast Glycan Separ...	N-Linke
14	●	Unknown	1	SI:B5	N-Linked Glycan_0...	Fast Glycan Separ...	N-Linke
15	●	Unknown	1	SI:B6	N-Linked Glycan_0...	Fast Glycan Separ...	N-Linke
16	●	Unknown	1	SI:B7	N-Linked Glycan_0...	Fast Glycan Separ...	N-Linke
17	●	Unknown	1	SI:B8	N-Linked Glycan_0...	Fast Glycan Separ...	N-Linke
18	●	Unknown	1	SI:C1	N-Linked Glycan_0...	Fast Glycan Separ...	N-Linke
19	●	Unknown	1	SI:C2	N-Linked Glycan_0...	Fast Glycan Separ...	N-Linke
20	●	Unknown	1	SI:C3	N-Linked Glycan_0...	Fast Glycan Separ...	N-Linke
21	●	Unknown	1	SI:C4	N-Linked Glycan_0...	Fast Glycan Separ...	N-Linke

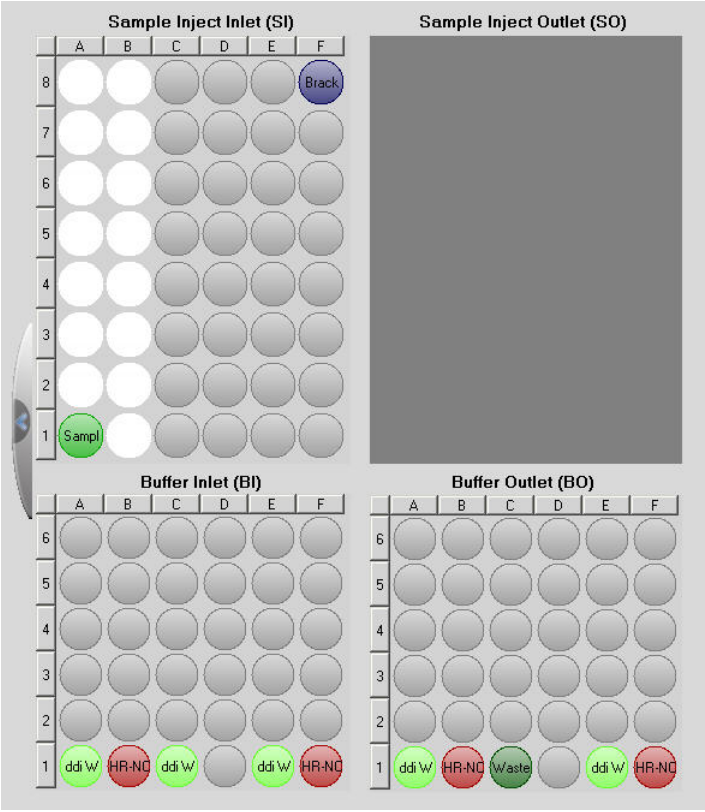
7. (Optional) For rows containing system suitability samples, click the row to select it, and then click Control (**Control**).

8. In the lower right corner of the window, click Save (**Save**), and then click Finish (**Finish**).

9. In the **Number of samples** field, click the arrow buttons to set the number of samples for the run.

As the number of samples changes, the images of the buffer and sample trays on the right are updated to show the correct number of vials and their locations for the run. For example, in Figure 9, one row of reagents is required for 16 samples.

Figure 9 Tray Map for Fast Glycan



10. If the buffer and sample trays have not been loaded, click Load (Load), load the buffer and sample trays in the PA 800 Plus system, and then close the door.

11. Click Next (**Next**), and then click **Yes - run now**.

Figure 10 Samples Loaded Prompt

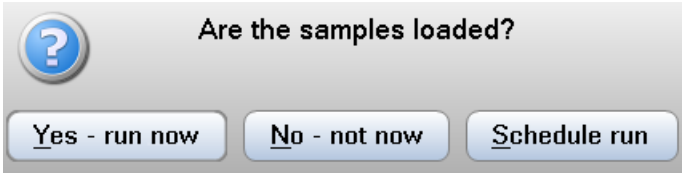
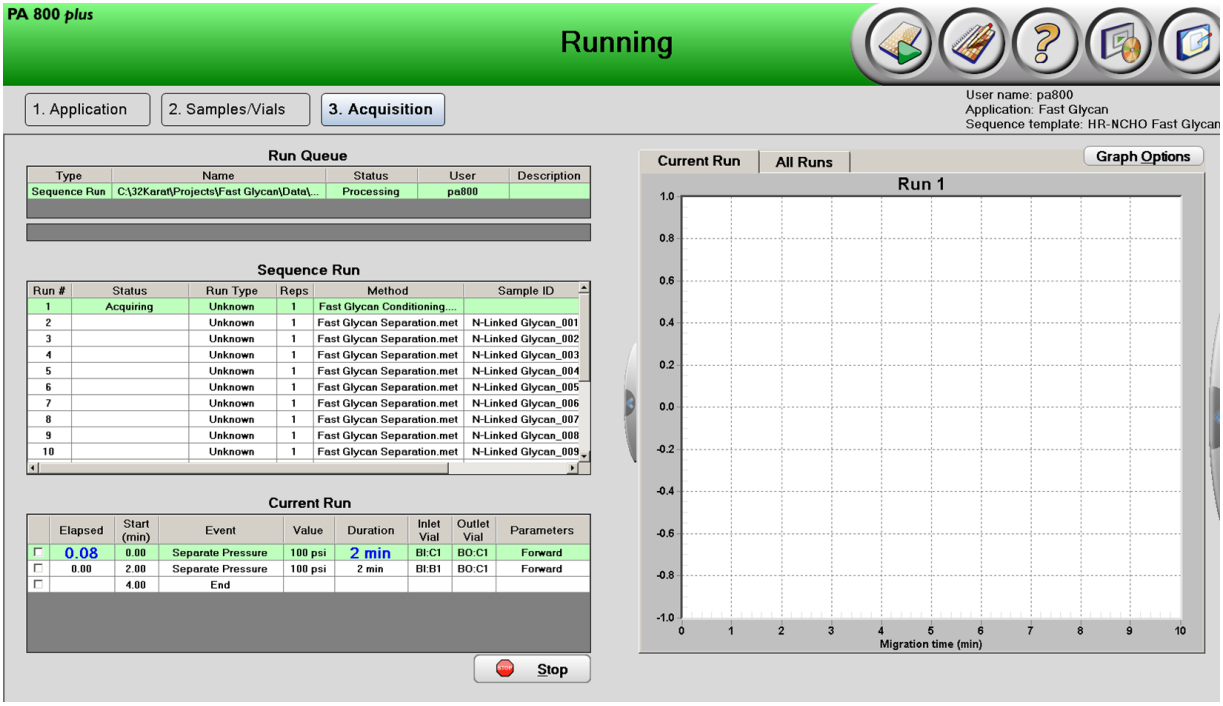


Figure 11 PA 800 Plus Software During Data Acquisition



Waste Disposal

WARNING! Biohazard or Toxic Chemical Hazard. Obey local directives to discard chemicals, cartridges, capillaries, vials and caps, and the remains of the prepared samples. They might contain regulated compounds and biohazardous agents.

Stow the Cartridge

Stow the Cartridge Less Than 10 Days

1. Use the shutdown method to clean the capillary.
   The shutdown method fills the capillary with water.
2. Keep the cartridge up to 10 days in the system with the capillary tips immersed in vials of CE Grade water.

Stow the Cartridge More Than 10 Days

1. Use the shutdown method to clean the capillary.
2. Rinse the capillary with CE Grade water for 10 minutes at 100 psi.

3. Remove the cartridge from the system.
4. Put the cartridge in the cartridge storage box with the capillary tips immersed in vials of CE Grade water.
5. Keep the cartridge upright in the cartridge box at ambient temperature.

Prepare the Cartridge After Storage

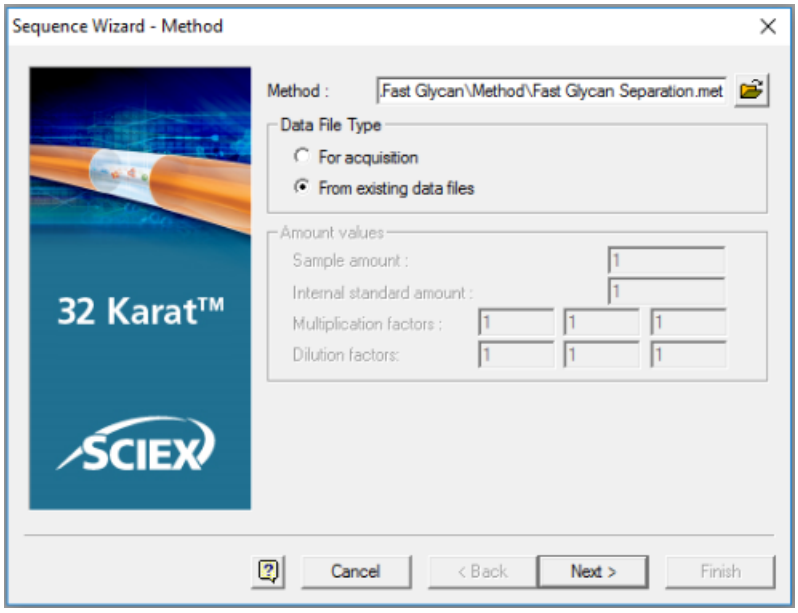
- If the cartridge has not been used for more than a day, then use the `Fast Glycan Conditioning.met` method to condition the capillary.

Analyze the Data

Analyze and Identify the Glycans

1. Open the 32 Karat software.
2. Right-click the **Fast Glycan** instrument, and then select **Open Offline** to open it.
3. Create a sequence with the Sequence Wizard.
   a. Click **File** > **Sequence** > **Sequence Wizard**.
   b. Click  (**Browse**), and then select the **`Fast Glycan Separation`** method.
   c. Click **From existing data files**.

Figure 12 Method Page

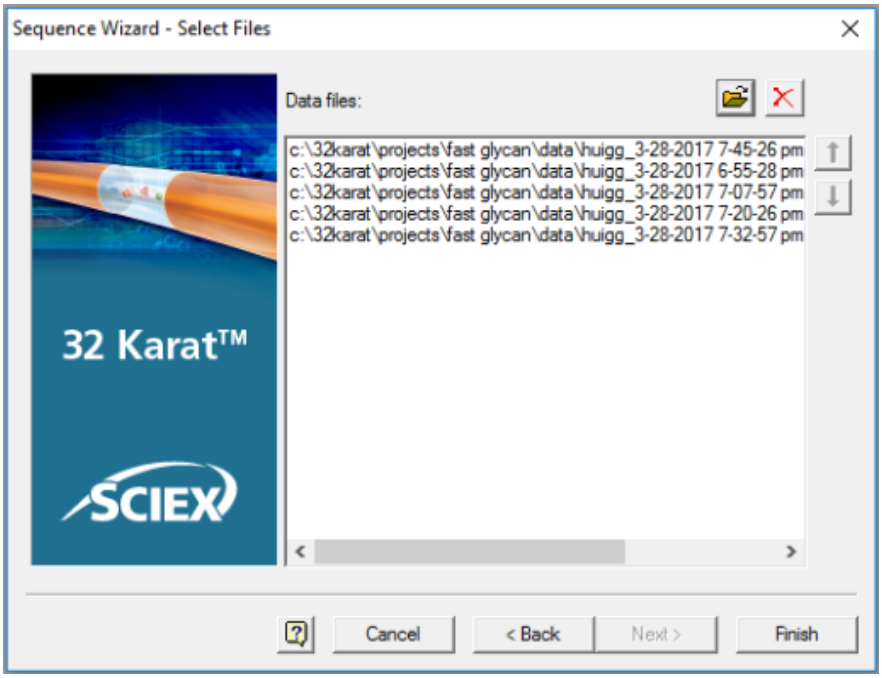


   d. Click **Next**.

e. In the **Select Files** page, click (**Browse**), and then navigate to the location of the data files.

f. For each file to be analyzed, click the file in the upper list, and then click **Add**. After all of the files to be analyzed are listed in the Data Files table, click **Open**.
The file names appear in the list in the Select Files page.

Figure 13 Select Files Page



g. Click **Finish**.

The wizard closes and a window with the sequence opens.

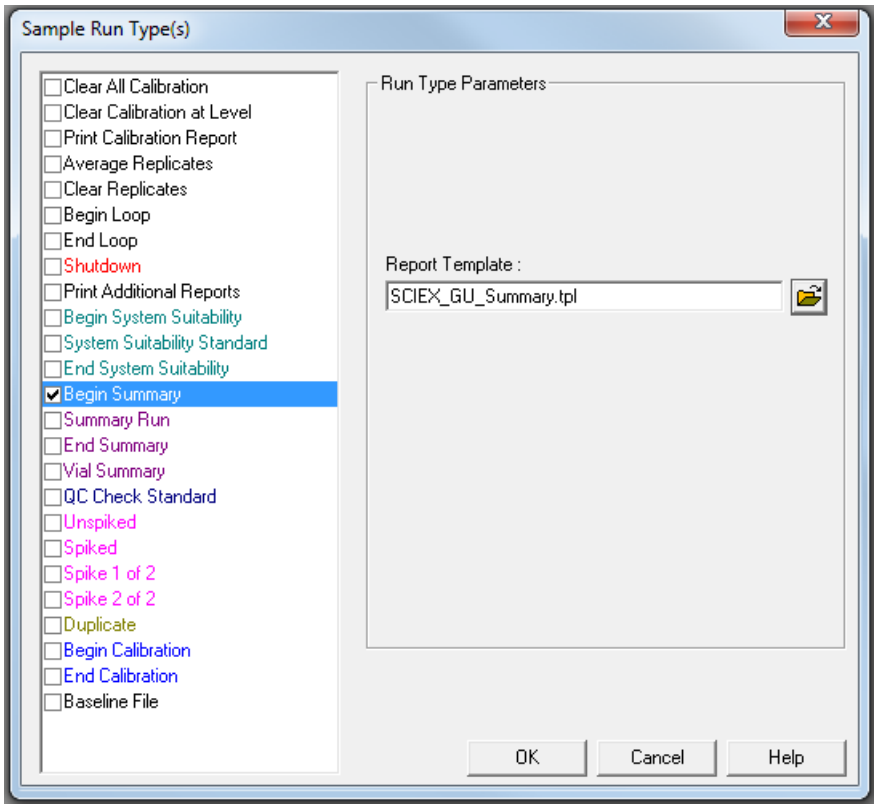
4. Click the **Run Type** column heading to select the column, and then right-click **Set Run Types** > **Summary**.
The **Run Type** column updates to show `Summary Begin` in the first row, `Summary End` in the last row, and `Summary Run` in all of the other rows.

5. In the first row of the sequence, click 

.
The Sample Run Type(s) dialog opens.

6. Make sure that **Begin Summary** is selected, and then click **OK**.

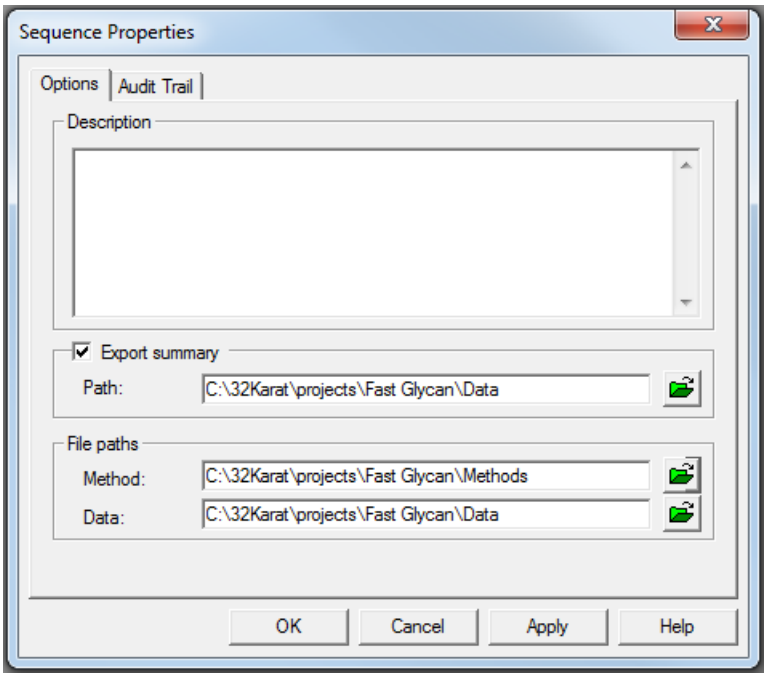
Figure 14 Sample Run Type(s) Dialog



7. Click **Sequence** > **Properties** .
   The Sequence Properties dialog opens.

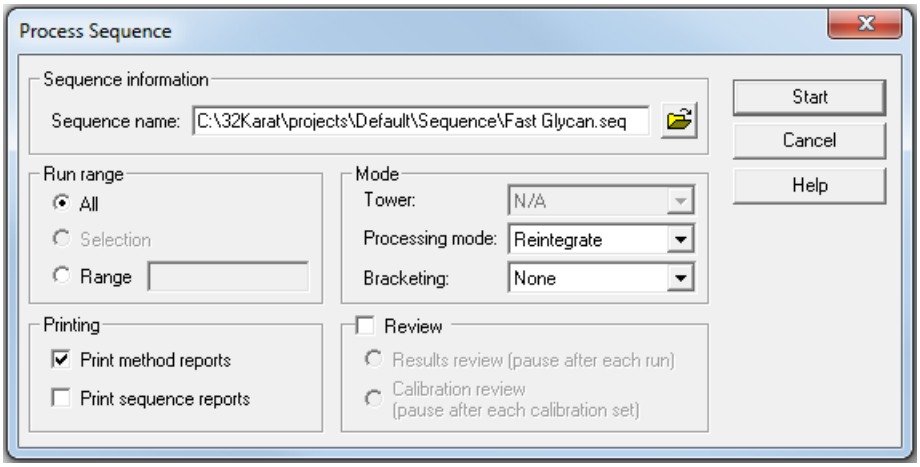
8. Select **Export summary**, click  (**Browse**), and then select the folder to save the results.

Figure 15 Sequence Properties Dialog



9. Click **File** > **Sequence** > **Save As**, and then type a name for the sequence.
10. Click **Sequence** > **Process**.
    The Process Sequence dialog opens.
11. Select **Print method reports**, and then click **Start**.

Figure 16 Process Sequence Dialog



The 32 Karat software processes the sequence, integrates the peaks, and then identifies the glycans. If there are many samples, the analysis can take some time. After the analysis is finished, several PDF reports are created:

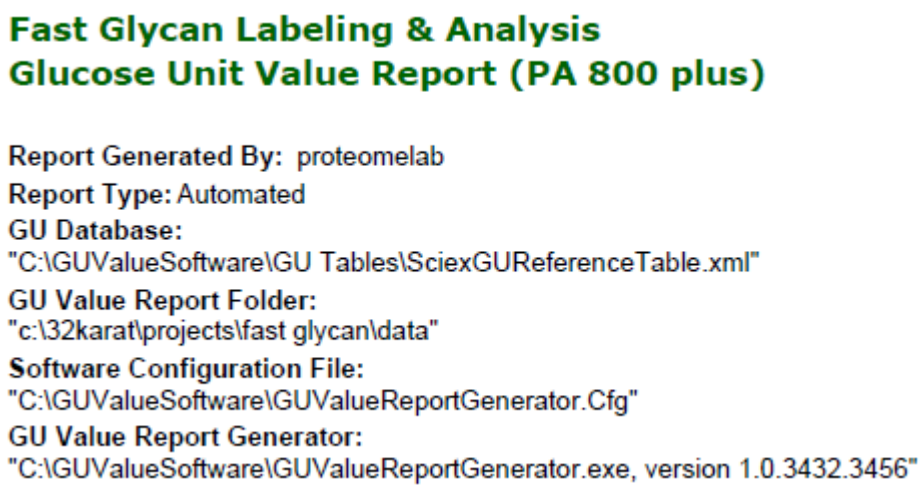
- The GU Value Report, which opens automatically at the end of the analysis. This report is saved to the folder specified in the **Data** folder in the Sequence Properties dialog and is named `GU Value Report v1.0_MMDDYYYY_HHMMSS.pdf`, where `MMDDYYYY` is the date and `HHMMSS` is the time that the file was created.
- For each data file in the sequence, a method report. The method report is saved to the folder specified in the Sciex PDF Writer Preferences dialog. The method reports are part of the GU Value Report.

GU Value Report

The following figures show the different sections of the GU Value Report. This report was created by analyzing the example data files.

At the top of the report is the report heading with general information about the analysis.

Figure 17 GU Value Report: Report Heading



After the heading is the sample index (not shown), a list of the data files that were analyzed when the sequence was processed.

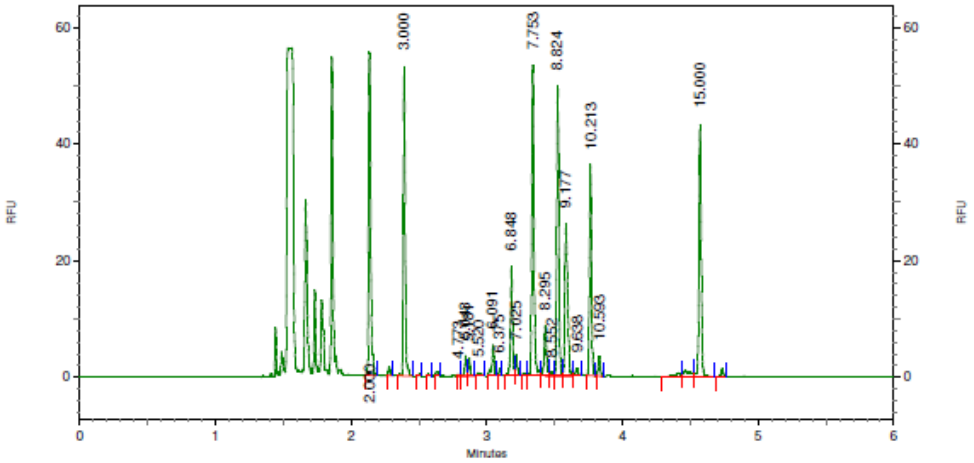
After the sample index is a series of method reports, one for each row in the sequence. The first part of the report gives information including sample file name, sample ID, location of the file, and the location and name of the method. Refer to the following figure: Figure 18.

Figure 18 Method Report: Heading and Electropherogram

SCIEX PA 800 plus – Fast Glycan Analysis Report

File Name c:\32karat\projects\fast glycan\data\huigg_3-28-2017 7-45-26 pm-rep5.dat
Sample ID: huIgG_A_M702617_247
Method: C:\32Karat\projects\Fast Glycan\Method\Fast Glycan Separation.met
Run Time: 3/28/2017 7:45:37 PM
Analysis: 5/10/2017 12:40:30 PM

Electropherogram trace:



The second part of the report is a table of the glycans identified by the analysis. The glycans are named with Oxford glycan IDs in the **Name** and **ProbableMatch** columns. Use the IDs to find the structure by referring to the Oxford and Composition Letter Translation Table at the end of the GU Value Report. Refer to the following figure: Figure 19.

Figure 19 Method Report: Identified Glycans

LIF - Channel 1 Results Migration Time	VA	VA%	GU	Name	MonoMass	ProbableMatch
2.135	0.000	0.000	2.000	DP2		
2.392	0.000	0.000	3.000	DP3		
2.789	2724.961	0.113	4.773	A2G2S2	2222.780	
2.845	36271.666	1.505	5.043	FA2G2S2	2368.840	
2.871	30721.742	1.274	5.181	FA2BG2S2	2571.920	
2.938	7601.475	0.315	5.520	A2(6)G1S1	1769.630	
3.046	62716.607	2.602	6.091	FA2(3)G1S1	1915.690	
3.097	9481.278	0.393	6.375	A2G2S1	1931.690	
3.182	200047.241	8.298	6.848	FA2G2S1	2077.750	M5 | 1234.430
3.216	39367.982	1.633	7.025	FA2BG2S1	2280.830	
3.339	598218.983	24.814	7.753	FA2	1462.540	M6 | 1396.490
3.431	96008.937	3.982	8.295	FA2B	1665.620	
3.476	5834.606	0.242	8.552	M7[D2]	1558.540	
3.523	489986.233	20.325	8.824	FA2(6)G1	1624.600	

At the end of the report is the Oxford and Composition Letter Translation Table which shows structures for all of the glycans that the GU Value can identify. Refer to the following figures: Figure 20, Figure 21, and Figure 22.

Figure 20 Translation Table

Oxford and Composition Letter Nomenclature Translation Table

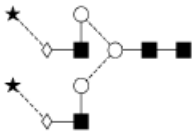
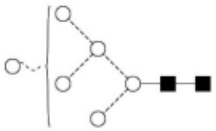
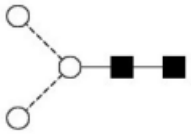
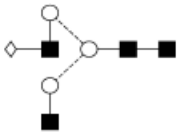
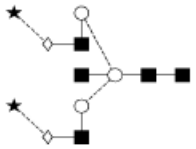
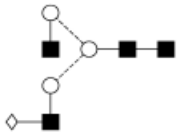
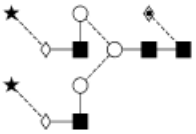
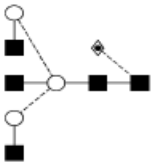
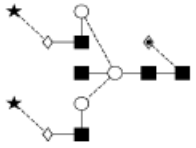
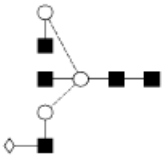
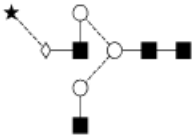
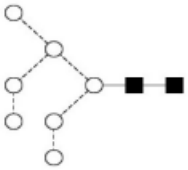
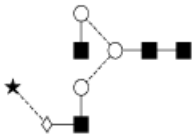
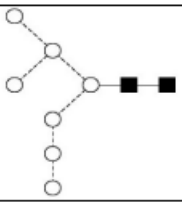
Oxford ID* (composition**)	Glycan Name	Glycan Structure	Oxford ID* (composition**)	Glycan Name	Glycan Structure
A2G2S2 (H5N4A2)	G2S2		M6 (H6N2)	Man-6	
M3 (H3N2)	Man-3		A2(6)G1 (H4N4)	G1	
A2BG2S2 (H5N5A2)	G2BS2		A2(3)G1 (H4N4)	G1’	
FA2G2S2 (H5N4F1A2)	G2FS2		FA2B (H3N5F1)	G0FB	
FA2BG2S2 (H5N5F1A2)	G2FBS2		A2B(3)G1 (H4N5)	G1’B	
A2(6)G1S1 (H4N4A1)	G1S1		M7[D2] (H7N2)	Man-7[D2]	
A2(3)G1S1 (H4N4A1)	G1’S1		M7[D3] (H7N2)	Man-7[D3]	

Figure 21 Translation Table (continued)

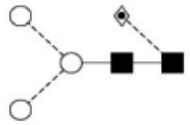
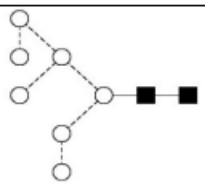
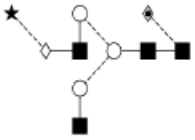
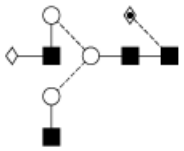
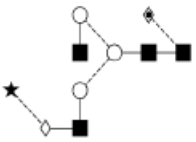
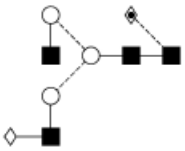
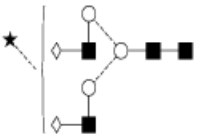
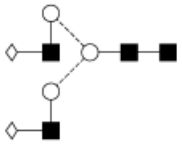
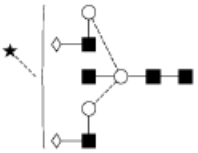
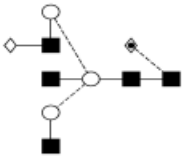
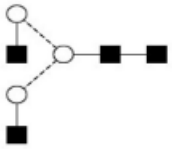
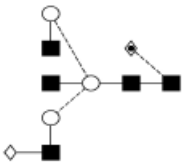
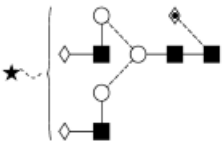
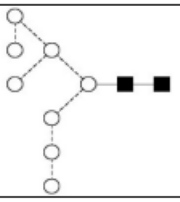
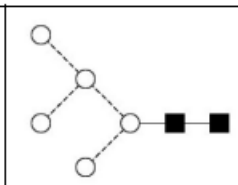
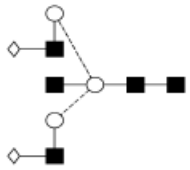
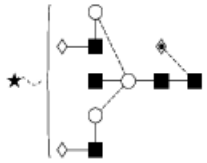
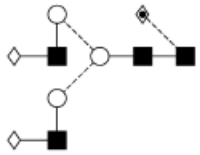
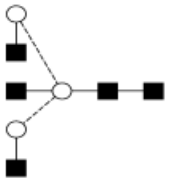
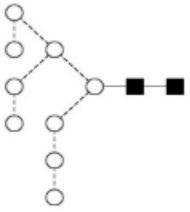
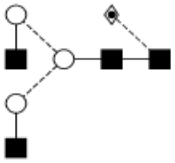
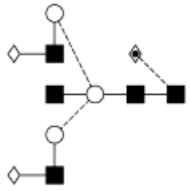
FM3 (H3N2F1)	Man-3F		M7[D1] (H7N2)	Man-7[D1]	
FA2(6)G1S1 (H4N4F1A1)	G1FS1		FA2(6)G1 (H4N4F1)	G1F	
FA2(3)G1S1 (H4N4F1A1)	G1'FS1		FA2(3)G1 (H4N4F1)	G1'F	
A2G2S1 (H5N4A1)	G2S1		A2G2 (H5N4)	G2	
A2BG2S1 (H5N5A1)	G2BS1		FA2B(6)G1 (H4N5F1)	G1FB	
A2 (H3N4)	G0		FA2B(3)G1 (H4N5F1)	G1'FB	
FA2G2S1 (H5N4F1A1)	G2FS1		M8[D1D3] (H8N2)	Man-8[D1D3]	

Figure 22 Translation Table (continued)

M5 (H5N2)	Man-5		A2BG2 (H5N5)	G2B	
FA2BG2S1 (H5N5F1A1)	G2FBS1		FA2G2 (H5N4F1)	G2F	
A2B (H3N5)	G0B		M9 (H9N2)	Man-9	
FA2 (H3N4F1)	G0F		FA2BG2 (H5N5F1)	G2BF	

*A2: trimannosil biantennary structure; M: mannose, F: fucose, B: bisecting GlcNAc; G: galactose; S: sialic acid
**H: hexose; N: N-Acetylhexosamine; F: fucose; A: sialic acid
Reference: Harvey DJ, Merry AH, Royle L, Campbell MP, Dwek RA, Rudd PM., Proposal for a standard system for drawing structural diagrams of N- and O-linked carbohydrates and related compounds., *Proteomics*. 2009 Aug;9(15):3796-801.

Troubleshooting

Symptom	Possible Cause	Corrective Action
No peaks	1. There are issues with the LIF detector. 2. The separation method is incorrect. 3. There is an air bubble at the bottom of the sample vial. 4. The capillary window or tip is broken. 5. The sample is missing or not in the correct position in the sample tray.	1. Make sure that the probe is connected correctly to the clamp bar on the LIF detector. Refer to the document: *Maintenance Guide*. 2. Open the separation method in the software, and then make sure that: • The voltage is correct. • **Reverse** polarity is selected. During the run, observe the amber LED on the PA 800 Plus System. It should be lit when the reverse polarity voltage is applied. 3. Use a centrifuge to spin the sample tube to make sure that there are no bubbles at the bottom. 4. Inspect the capillary window and tip. If either is broken, then replace the cartridge. If they are not broken, then: • Clean the probe aligner with a cotton swab dampened with CE Grade water. • Clean the aperture in a water bath. After cleaning, assemble the cartridge, and then run the samples again. 5. Make sure that the samples are in the correct locations in the sample tray.

Symptom	Possible Cause	Corrective Action
Low intensity peaks	1. The labeling reaction was not performed properly. 2. The PNGase enzyme activity was low. 3. The sample concentration is too low. 4. The deglycosylation was not complete.	1. Compare the peak intensity of the IST peak to the intensities of the DP2 and DP15 peaks from the bracketing standard. If the IST peak is lower than the DP2 and DP 15 peaks, prepare the sample again. Refer to the section: Release the N-Glycans. Make sure to: • Prepare fresh L6. • Make sure to add L5 to the L6 vial. • Make sure that the sodium cyanoborohydride solution is not cloudy and is free of precipitates. • Make sure that the labeling reaction incubates for 20 minutes and that the temperature of the heat block is 60 °C. 2. Examine the peak intensity for the IST peak. If it is greater than 50 RFU, then digest the sample again, and then add more PNGase F enzyme or use PNGase F from a different lot. Make sure to add D4 to both the denaturation and labeling solutions. 3. Make sure that the amount of protein is 100 µg (at a concentration 10 mg/mL). If the sample concentration is significantly lower, then concentrate the sample in a spin filter with a 10 kDa molecular weight cutoff (MWCO). 4. Make sure the that amount of protein is 100 µg. If it is acceptable, then prepare the sample again and increase the incubation time for the deglycosylation step, increase the quantity of enzyme, or use a new enzyme lot.
Saturated peak intensity	The sample concentration is too high.	Dilute the sample with water, and then run the sample again.

Symptom	Possible Cause	Corrective Action
Low current or no current	1. The capillary temperature is not correct. 2. The capillary window or tip is broken. 3. There are problems with the capillary. 4. The reagents are contaminated. 5. The buffer vials are not in the correct positions in the buffer trays.	1. Open the separation method in the software, and then make sure that the capillary temperature is correct. 2. Examine the capillary window and tip. If either is broken, then replace the cartridge. 3. Resolve other issues with the capillary: • Make sure that the capillary is not blocked. • Make sure that the aperture plug is seated in the cartridge and that the probe guide is attached securely to the plug. Refer to the document: *Maintenance Guide*. 4. Do not use vials or caps more than once. Fill clean vials with newly prepared reagents, cover the vials with clean caps, and then replace the vials in the tray. 5. Make sure that the buffer vials are in the correct position in the buffer tray.
Shifts in migration time between runs on the same day	The capillary was not properly equilibrated.	Use the conditioning method to equilibrate the capillary, and then run the samples again.
Shifts in migration time over an extended period of time with low current	The capillary is partially blocked or the surface is contaminated.	Rinse the capillary with CE Grade water at 75 psi for 2 minutes.

Symptom	Possible Cause	Corrective Action
Carryover	The vials or caps are contaminated.	Do not use vials or caps more than once. Replace the buffer vials with clean vials filled with buffer, attach clean caps to the vials, and then increment as required. Replace the water vials with clean vials filled with CE Grade water, attach clean caps to the vials, and then increment as required. Make sure that the waste vials contain 1.0 mL of water and have been put in the outlet buffer tray. Add one or more water dip steps to the time program after the sample injection step.
Spikes in electropherogram	Air is dissolved in the separation gel.	To remove bubbles from the gel buffer, do one or all of these: • Sonicate the buffer vials for 10 seconds to 20 seconds to remove air bubbles. • Use a centrifuge to spin the vial at 30 × *g* for 5 minutes to remove air bubbles. If air bubbles are still present, then prepare new gel buffer vials. Do not mix the buffer with a vortex mixer.
Extra peaks	The plasticware used during sample preparation or the sample vials are contaminated with materials that interact with L6-Fluorophore.	Use new microvials, especially for steps related to labeling.
Fewer small glycans (DP < 5) than expected	Small glycans were washed away during dye removal.	Do fewer wash steps to remove the dye.
Multiple **Save file as** dialogs appear during data analysis	The location of the Sciex PDF Writer software folder is incorrect.	Make sure that the folder location in the Preferences - PDF Writer dialog exists and that the other settings are correct. Refer to the document: *GU Value Software Release Notes*.

Symptom	Possible Cause	Corrective Action
The GU Value Report does not appear at the end of data analysis and the sequence summary	**Sciex PDF** is not selected as the printer for the instrument.	Make sure that the Sciex PDF Writer software is installed and configured correctly. Refer to the document: *GU Value Software Release Notes*.

Hazardous Substance Information A

The following information must be noted and the related safety measures must be obeyed. For more information, refer to the related safety data sheets. The safety data sheets are available on request or can be downloaded from our website, at sciex.com/tech-regulatory.

Hazard classification according to HCS 2012.

BST-Bracketing Standard

WARNING! May form combustible dust concentrations in air.

D1 Reagent

WARNING! Causes serious eye irritation and skin irritation.

D2 Reagent

DANGER! Toxic if swallowed. May cause allergy or asthma symptoms or breathing difficulties if inhaled. May cause an allergic skin irritation.

D3 Reagent

WARNING! Causes mild skin irritation.

HR-NCHO Separation Gel

WARNING! May be harmful if swallowed.

L5 Catalyst

DANGER! Causes severe skin burns and eye damage.

Other Reagents

These components are not classified as hazardous:

- D4 reagent
- G20-Glucose Ladder Standard
- IST-Internal Standard
- L6 fluorophore
- M1-Glycan Capture Beads

For reagents from other vendors, read the safety data sheet from the vendor before use.

Methods B

Note: The following information applies to users that use the PA 800 Plus system with the PA 800 Plus and 32 Karat software. If the system is used with the Waters Empower™ software, then the methods are different. Refer to the section: Run the Samples with the Waters Empower™ Software.

The Fast Glycan Labeling and Analysis application requires three methods.

A fourth method, to separate the GU ladder, is optional.

Note: The values on the Initial Conditions and LIF Detector Initial Conditions tabs are the same for all of the methods.

Conditioning Method

Figure B-1 Initial Conditions Tab

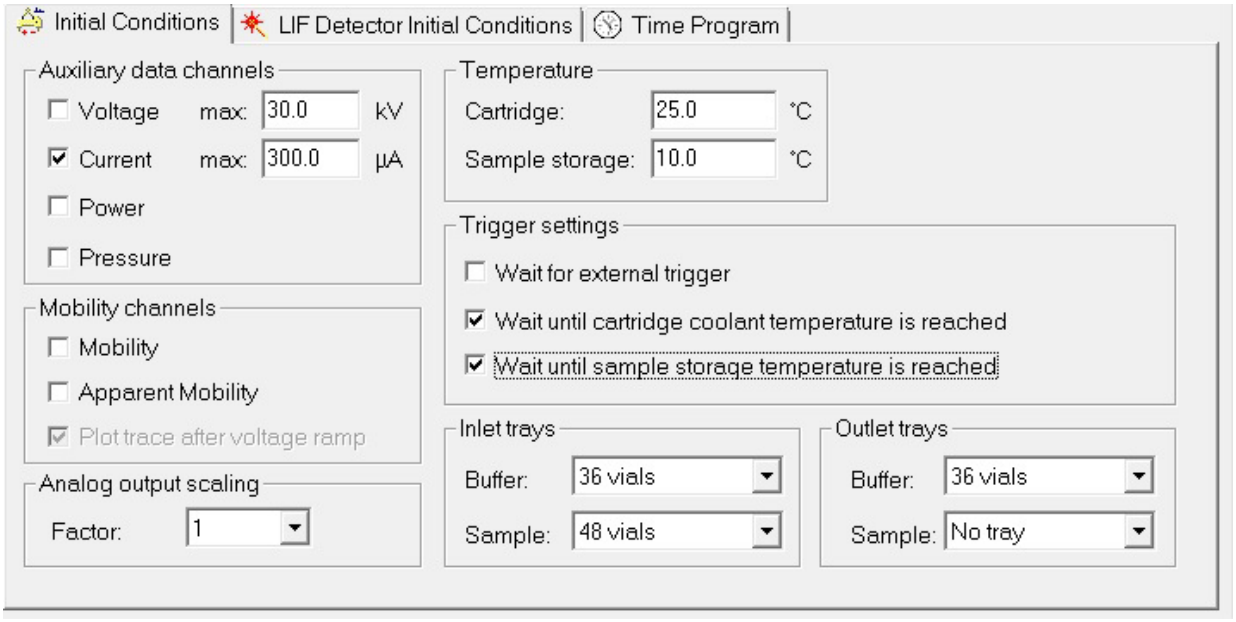


Figure B-2 LIF Detector Initial Conditions Tab

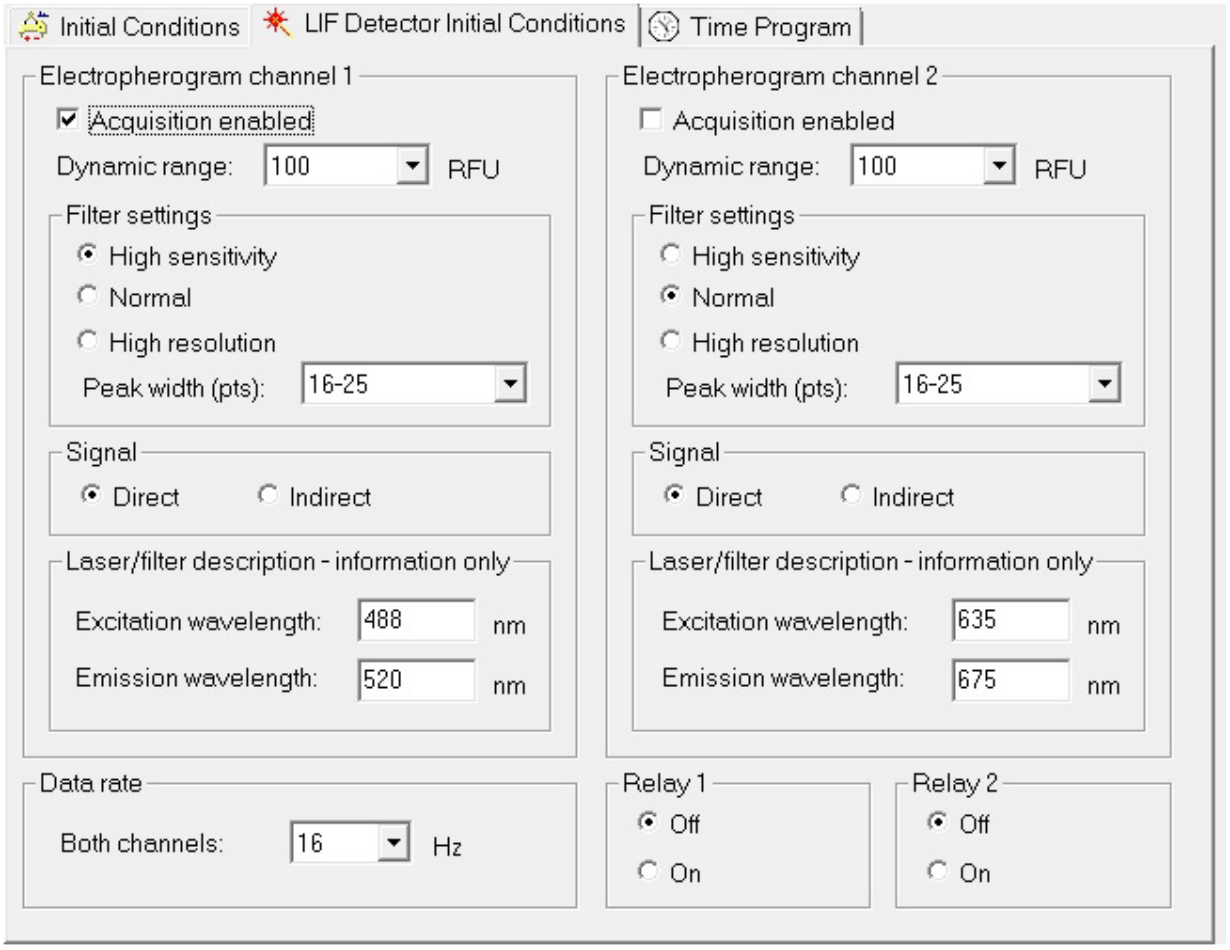
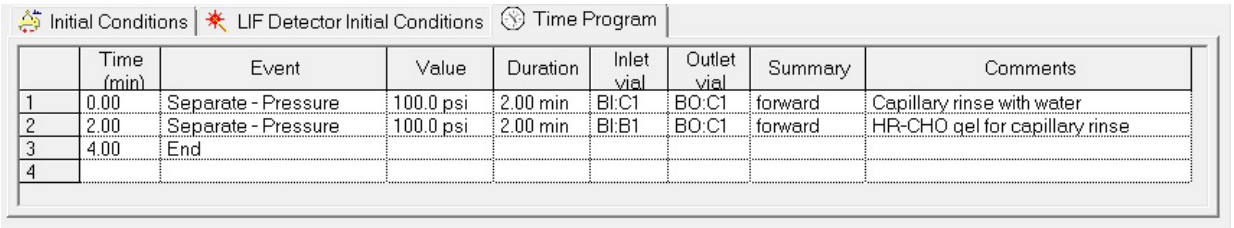


Figure B-3 Time Program Tab



	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1	0.00	Separate - Pressure	100.0 psi	2.00 min	BI:C1	BO:C1	forward	Capillary rinse with water
2	2.00	Separate - Pressure	100.0 psi	2.00 min	BI:B1	BO:C1	forward	HR-CHO gel for capillary rinse
3	4.00	End						
4								

Separation Method

Figure B-4 Initial Conditions Tab

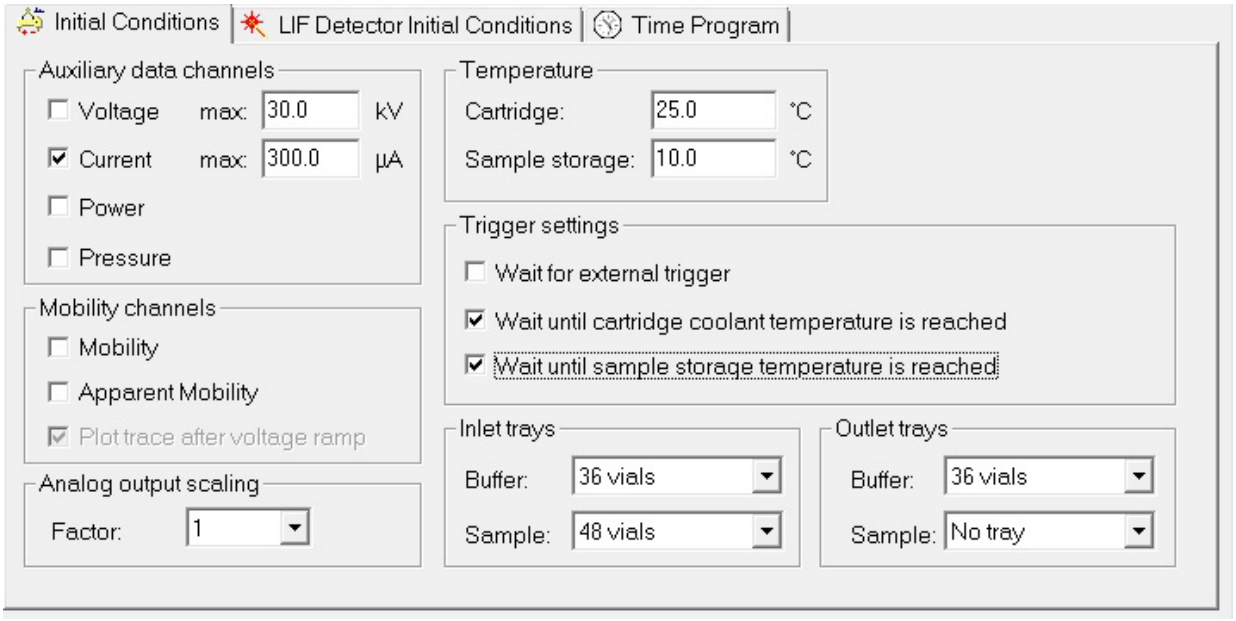


Figure B-5 LIF Detector Initial Conditions Tab

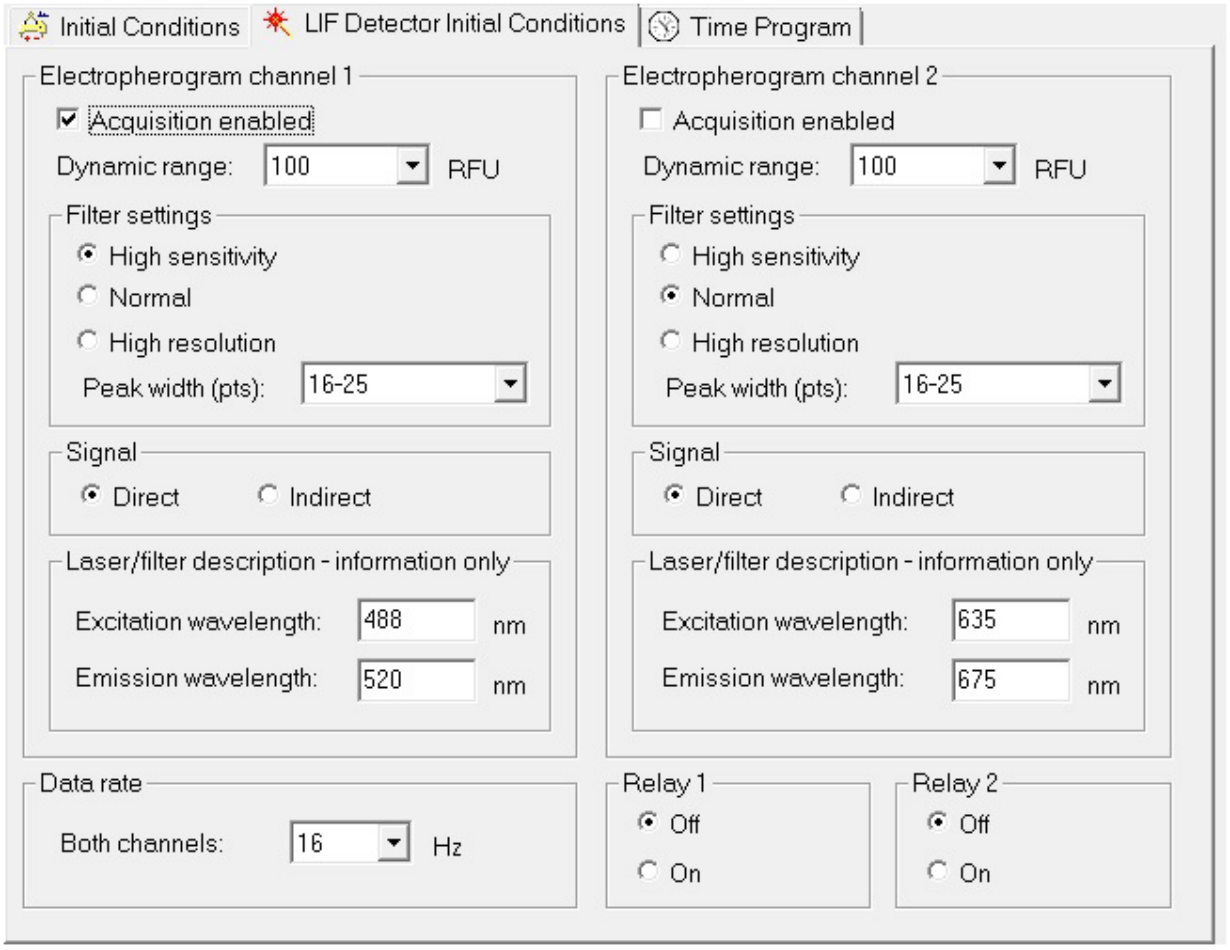
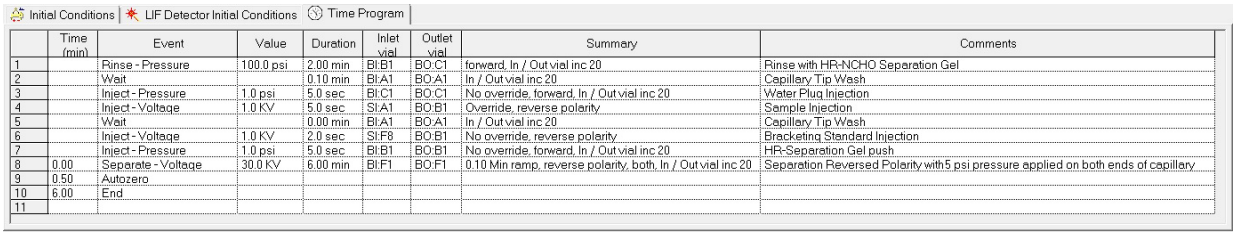


Figure B-6 Time Program Tab



Initial Conditions | LIF Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	100.0 psi	2.00 min	BI:B1	BO:C1	forward, In / Out vial inc 20	Rinse with HR-NCHO Separation Gel
2		Wait		0.10 min	BI:A1	BO:A1	In / Out vial inc 20	Capillary Tip Wash
3		Inject - Pressure	1.0 psi	5.0 sec	BI:C1	BO:C1	No override, forward, In / Out vial inc 20	Water Plug Injection
4		Inject - Voltage	1.0 KV	5.0 sec	SI:A1	BO:B1	Override, reverse polarity	Sample Injection
5		Wait		0.00 min	BI:A1	BO:A1	In / Out vial inc 20	Capillary Tip Wash
6		Inject - Voltage	1.0 KV	2.0 sec	SI:F8	BO:B1	No override, reverse polarity	Bracketing Standard Injection
7		Inject - Pressure	1.0 psi	5.0 sec	BI:B1	BO:B1	No override, forward, In / Out vial inc 20	HR-Separation Gel push
8	0.00	Separate - Voltage	30.0 KV	6.00 min	BI:F1	BO:F1	0.10 Min ramp, reverse polarity, both, In / Out vial inc 20	Separation Reversed Polarity with 5 psi pressure applied on both ends of capillary
9	0.50	Autozero						
10	6.00	End						
11								

Shutdown Method

Figure B-7 Initial Conditions Tab

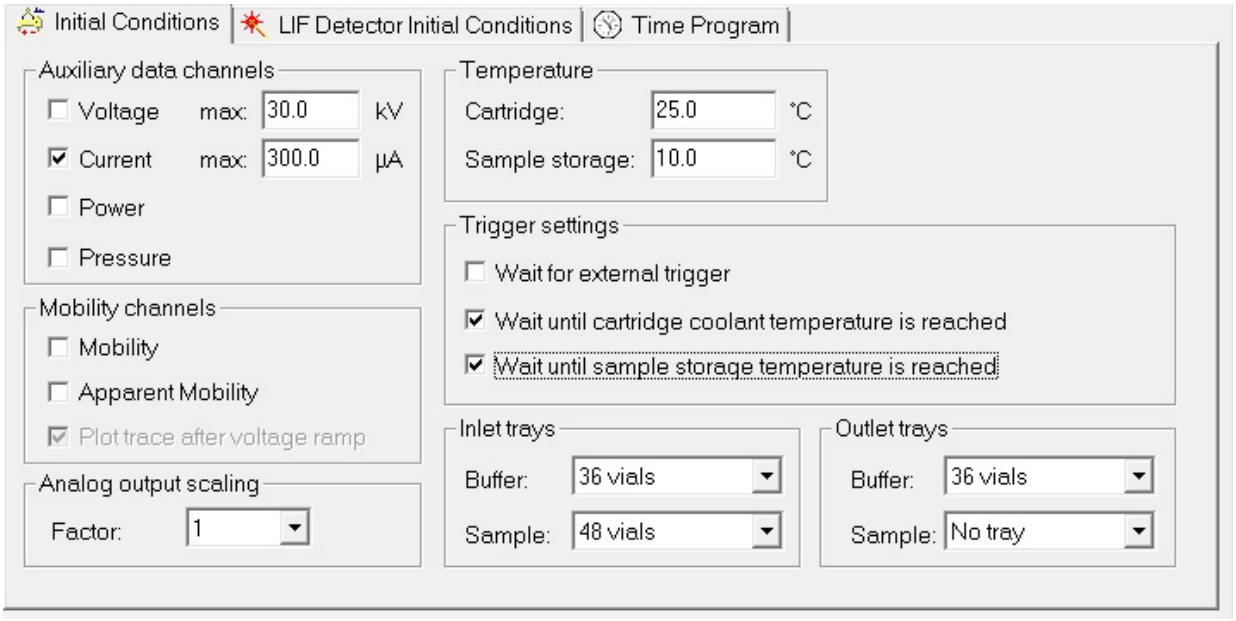


Figure B-8 LIF Detector Initial Conditions Tab

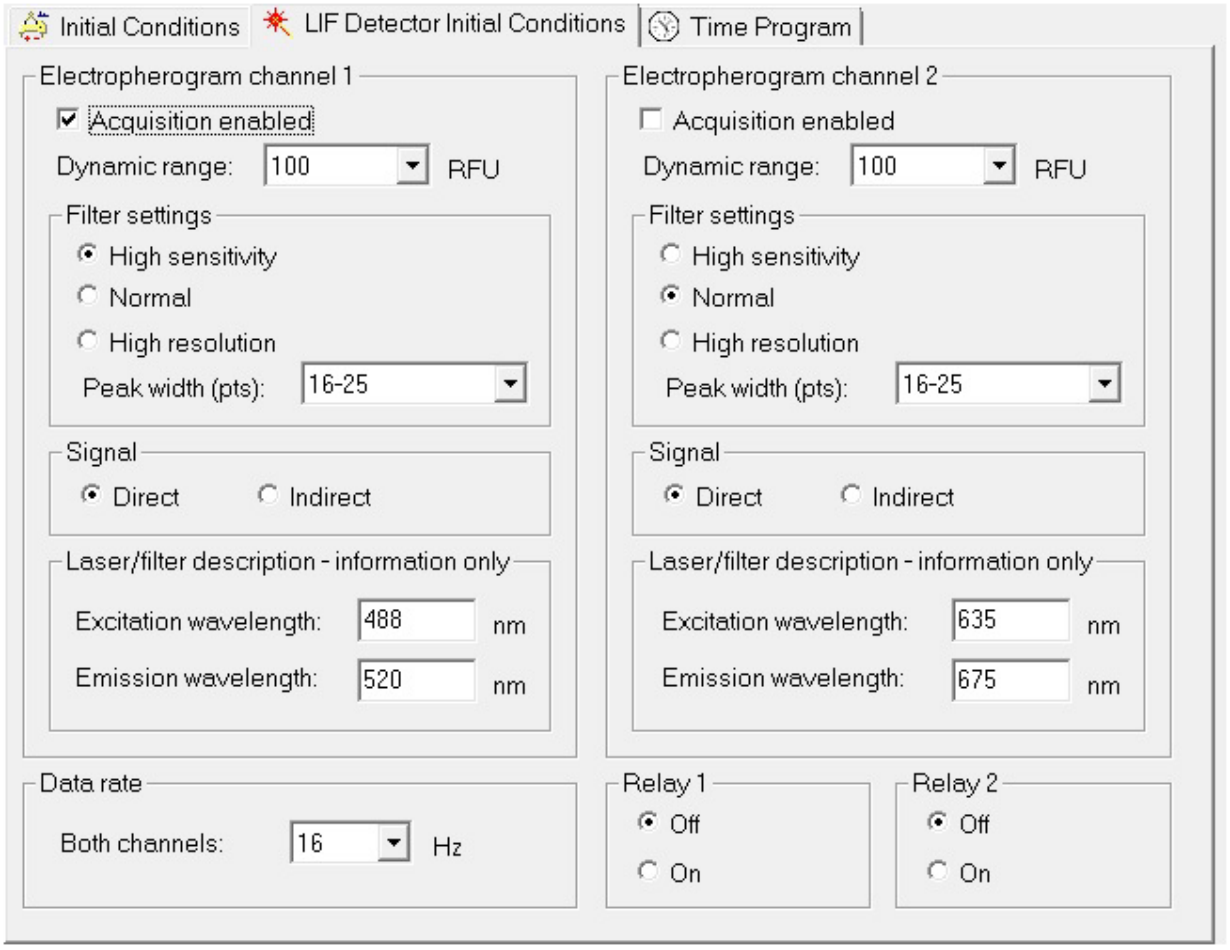
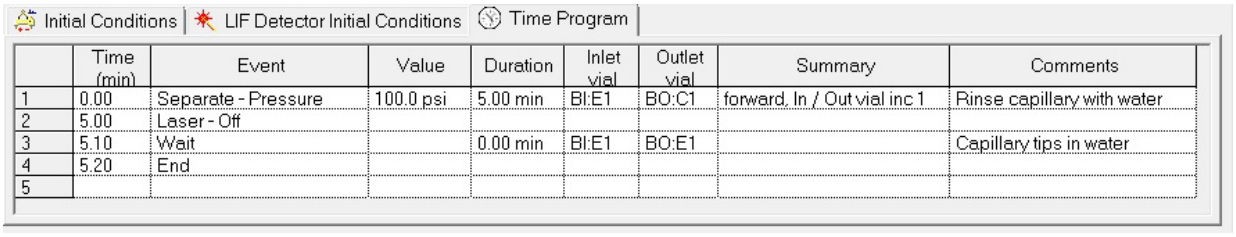


Figure B-9 Time Program Tab



	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1	0.00	Separate - Pressure	100.0 psi	5.00 min	BI:E1	BO:C1	forward, In / Out vial inc 1	Rinse capillary with water
2	5.00	Laser - Off						
3	5.10	Wait		0.00 min	BI:E1	BO:E1		Capillary tips in water
4	5.20	End						
5								

Calibrate the LIF Detector (Optional) C

This procedure is optional. If there is a requirement for consistency from system to system or cartridge to cartridge, then do this procedure. For information about the calibration, refer to the section: "About Automatic Calibration" in the document: *Maintenance Guide*.

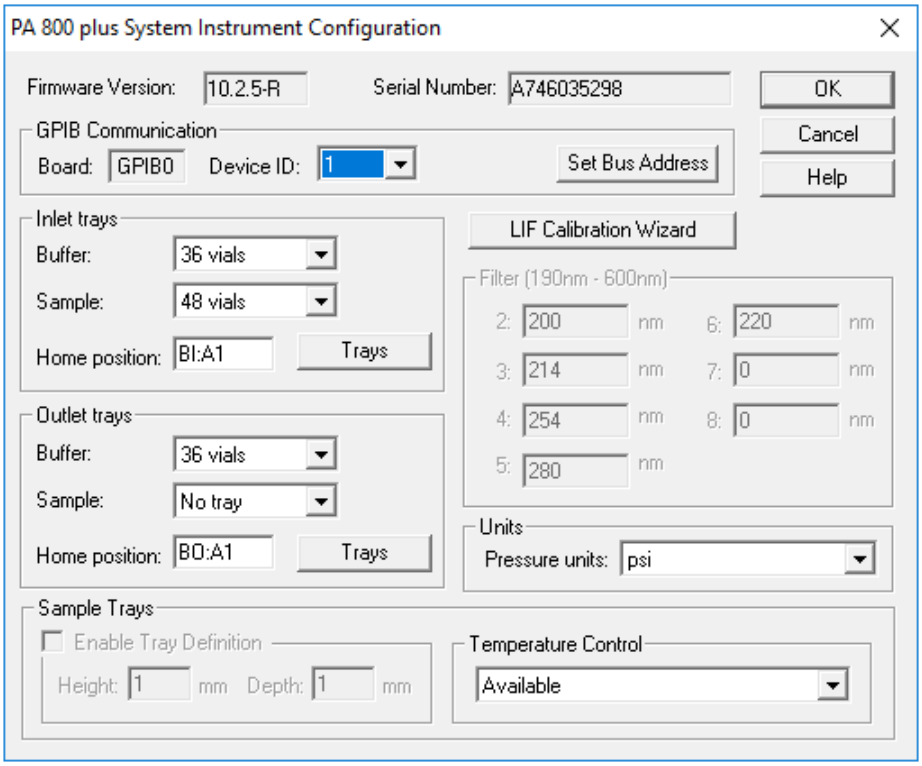
Calibrate the LIF detector after the LIF detector is installed, after a different cartridge is installed, or after a new capillary is installed in the cartridge.

Note: The following procedure technically does normalization, not calibration. Normalization uses a measured quality, such as the fluorescence of the LIF Performance Test Mix. Calibration uses an external standard. Because the software user interface uses the term *calibration*, that term is used in this guide.

Required Materials
• LIF Performance Test Mix • CE Grade water

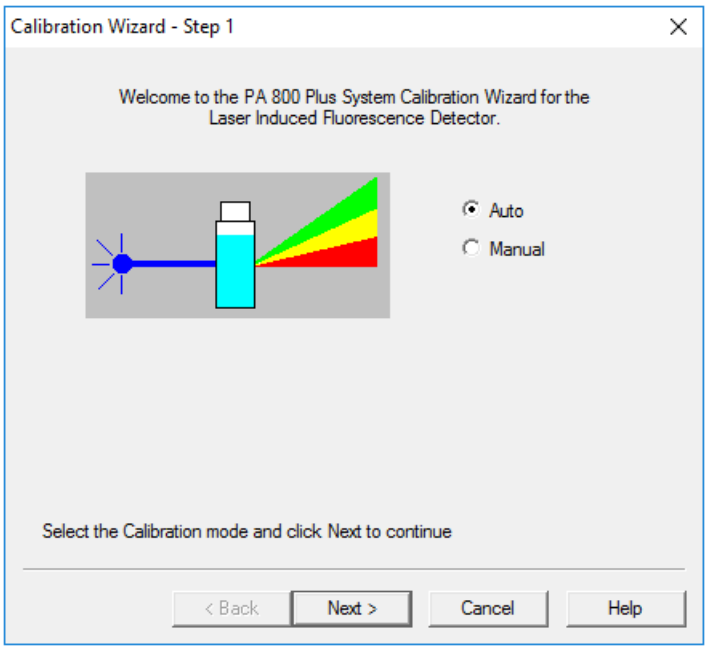
1. Turn on the PA 800 Plus system.
2. Open the 32 Karat software.
   The 32 Karat Software Enterprise window opens.
3. Open the LIF instrument, open the Direct Control window, and then turn on the laser.
4. In the 32 Karat Software Enterprise window, click **Tools** > **Enterprise Login**, and then log on as a user with Administrative privileges.
5. Right-click the **Fast Glycan** instrument icon, and then click **Configure** > **Instrument**.
   The Instrument Configuration dialog opens.
6. Click **Configure**.
   The PA 800 plus Configuration dialog opens.
7. In the right pane, click the **LIF Detector** icon, and then right-click and click **Open**.

Figure C-1 PA 800 plus System Instrument Configuration Dialog for LIF Detectors



8. Click **LIF Calibration Wizard**.
9. Do the calibration:
   a. Click **Auto**, and then click **Next**.

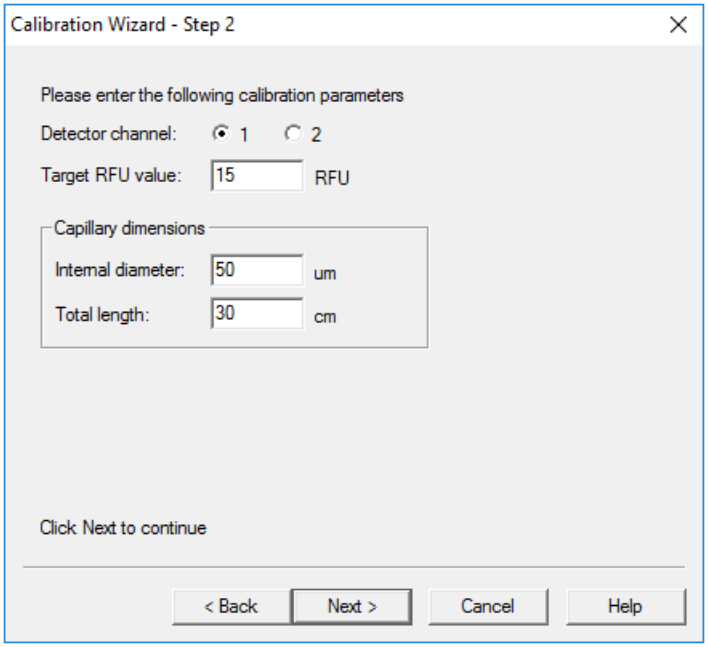
Figure C-2 Calibration Wizard - Step 1



b. In the **Target RFU** field, type `15`.

c. Make sure that the values in the Capillary dimensions section are correct, and then click **Next**.

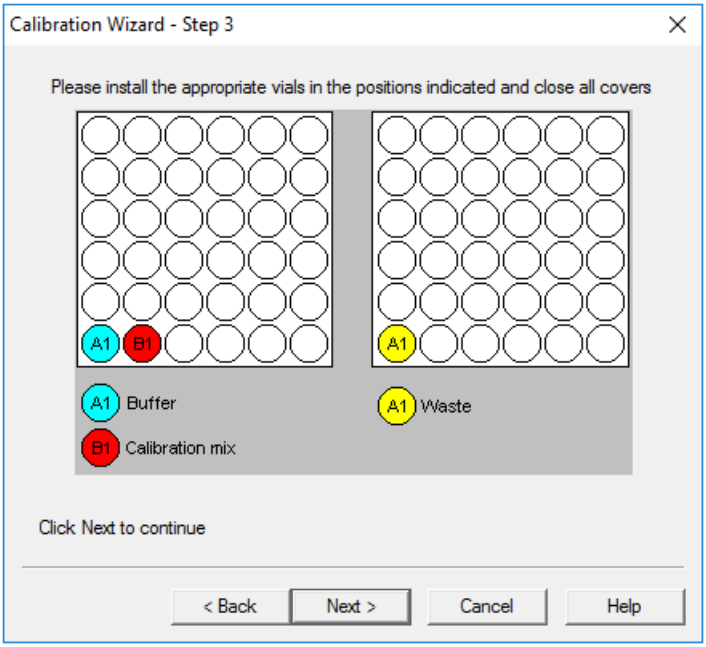
Figure C-3 Calibration Wizard - Step 2



10. Put a universal vial in position A1 in the buffer outlet tray.
11. Put universal vials in positions A1 and B1 in the buffer inlet tray.

Note: To prevent splashing, put the empty vials in the tray, and then add liquid and attach the caps.

Figure C-4 Calibration Wizard - Step 3



12. Fill the vials, and then put caps on the vials:
    - Inlet buffer tray position A1: 1.5 mL of CE Grade water
    - Inlet buffer tray position B1: 1.5 mL of LIF Performance Test Mix
    - Outlet buffer tray position A1: 1.0 mL of CE Grade water
13. Click **Next**.
    The 32 Karat software does the calibration. When the calibration is complete, the Calibration Wizard - Step 4 window opens.

    If the message `No step change detected` is shown, then the detector cannot detect the solution. For troubleshooting procedures, refer to the section: No Step Change Detected.
14. Close all of the dialogs and windows.

Troubleshoot the LIF Detector Calibration

CCF Values for LIF Detector Calibration

Issue	Action

Reported CCF value is less than 0.1
 	
or
 	
System performance is not satisfactory
	• Make sure that the correct capillary was used, and that it is not broken.
	• Make sure that the laser output for the laser in use on the system is correct.
	• Make sure that the correct filters are installed in the LIF detector:
	  • Excitation: 488 nm
	  • Emission: 520 nm
	• Replace the test mix, buffer, and capillary, and then do the calibration again. If the issue continues, then contact SCIEX Technical Support at sciex.com/request-support.

Reported CCF value is between 0.1 and 10.0	There is no issue with the system. Run a standard and make sure that the system performance is satisfactory.

Reported CCF value is more than 10
 	
or
 	
System performance is not satisfactory
	• Make sure that the laser output for the laser in use on the system is correct.
	• Make sure that the correct filters are installed in the LIF detector:
	  • Excitation: 488 nm
	  • Emission: 520 nm
	• Replace the test mix, buffer, and capillary, and then do the calibration again. If the issue continues, then contact SCIEX Technical Support at sciex.com/request-support.

No Step Change Detected

The LIF calibration compares detector signals from a nonfluorescent solution and a known fluorescent solution. When a rinse with nonfluorescent solution is done and then followed by a rinse with fluorescent solution, the first part of the detector signal should be near zero and the second part should be near the target fluorescent value. This detector output is in the shape of a step and is referred to as a *step change*. If a step change is not seen, then the applicable solutions are not passing the detector or the detector cannot detect the solutions.

1. Make sure that the switch on the right side of the laser is in the ON position.

2. Make sure that the laser that was supplied with the system is connected and the LASER ON light is illuminated.
3. To make sure that the solution goes through the capillary, from buffer inlet position A1 to an empty buffer vial in outlet position B1, use Direct Control to do a pressure rinse with CE Grade water at 20 psi for 5 minutes.
4. When the rinse starts, open the sample cover. Look at the outlet end of the capillary in position B1.
   - If there are droplets on the outlet end of the capillary, then do step 6.
   - If there are no droplets on the outlet end of the capillary, then the capillary is blocked or the system has a pressure failure. Continue with the next step.
5. Replace the capillary, and then do the pressure rinse again.
   - If there are still no droplets on the outlet end of the capillary, then contact SCIEX Technical Support at sciex.com/request-support.
   - If there are droplets on the outlet end of the capillary, then the detection system is the only possible cause. Continue with the next step.
6. Make sure that the correct filters are installed in the LIF detector.
7. If no step change is detected, then do the calibration procedure again. Refer to the section: Calibrate the LIF Detector (Optional).

   If the calibration procedure has been done more than 3 times, then manually set the calibration correction factor (CCF) to 1.0, and then calibrate the LIF detector again.

   If the LIF detector calibration continues to fail, then contact SCIEX Technical Support at sciex.com/request-support.

Run the Samples with the Waters Empower™ Software

D

This section gives instructions on data acquisition while the Waters Empower™ software is used.

Data analysis to determine the glucose unit (GU) values is not available in the Waters Empower™ software. It is up to the customer to decide how to perform data analysis. Either:

- Contact SCIEX for help with creating custom calculations for GU values, and then contact Waters for help with setting up the calculations in the Waters Empower™ software.
- In the Waters Empower™ software, create an export method, and then export the data to the AIA format. Open the 32 Karat and the GU Value softwares, and then analyze the exported data. Refer to the section: Create an Export Method.

Tip! Add the export method to the method set for automatic export after data acquisition. Refer to the documentation that comes with the Waters Empower™ software.

Create the Instrument Methods

Note: The validated instrument methods are included on the PA 800 Plus Empower™ Driver DVD. The methods can be imported instead of being creating manually. Refer to the section: Import the Instrument Methods. If the methods are missing, then use the following instructions to create them.

Three instrument methods are required:

- FAST GLYCAN_CONDITIONING
- FAST GLYCAN_SEPARATION
- FAST GLYCAN_SHUTDOWN

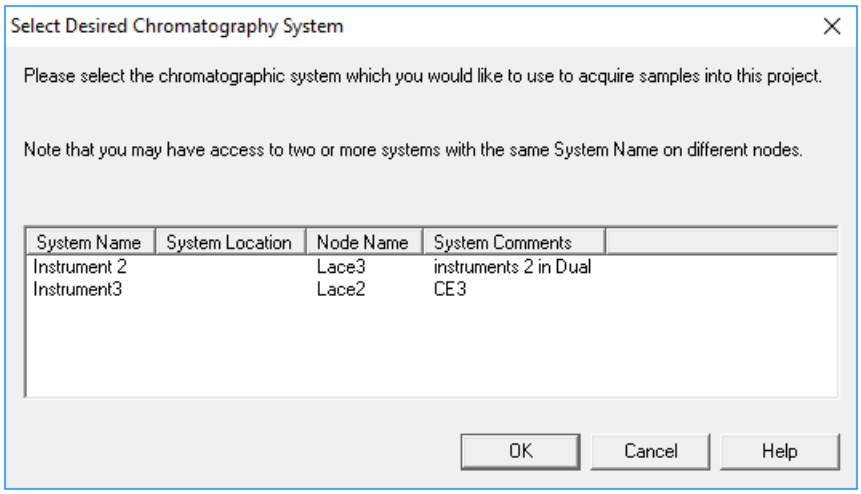
A fourth instrument method, GU LADDER_SEPARATION, is optional and is only needed if the glucose ladder is included as a sample.

Note: The values on the General and Detector tabs are the same for all of the methods.

Note: Pressure values can be shown in millibar (mbar) or pounds per square inch (psi). The registry setting for the Waters Empower™ software controls which value is used. The default unit is millibar. To change the units, refer to the document: *PA 800 Plus Empower™ Driver Release Notes.*

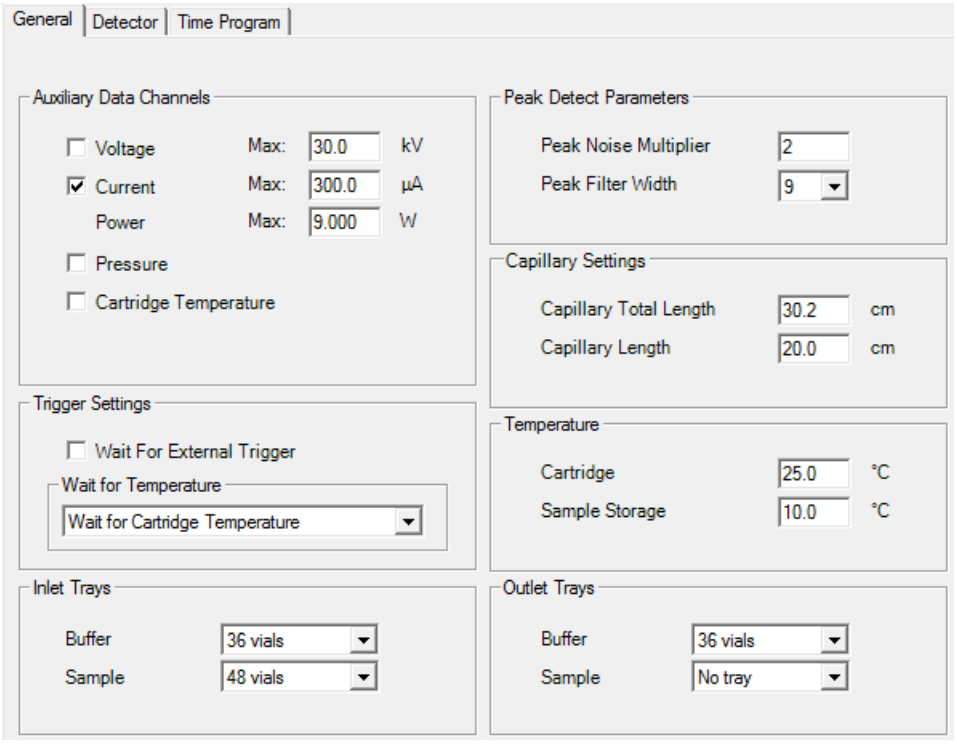
1. In the Waters Empower™ Software Project window, click **File** > **New Method** > **Instrument Method**.

Figure D-1 Select Desired Chromatography System Dialog



2. Click the system to be used, and then click **OK**.
   Make sure that the instrument is configured with an LIF detector.
   The Instrument Method Editor opens.
3. Set the parameters on the General tab.

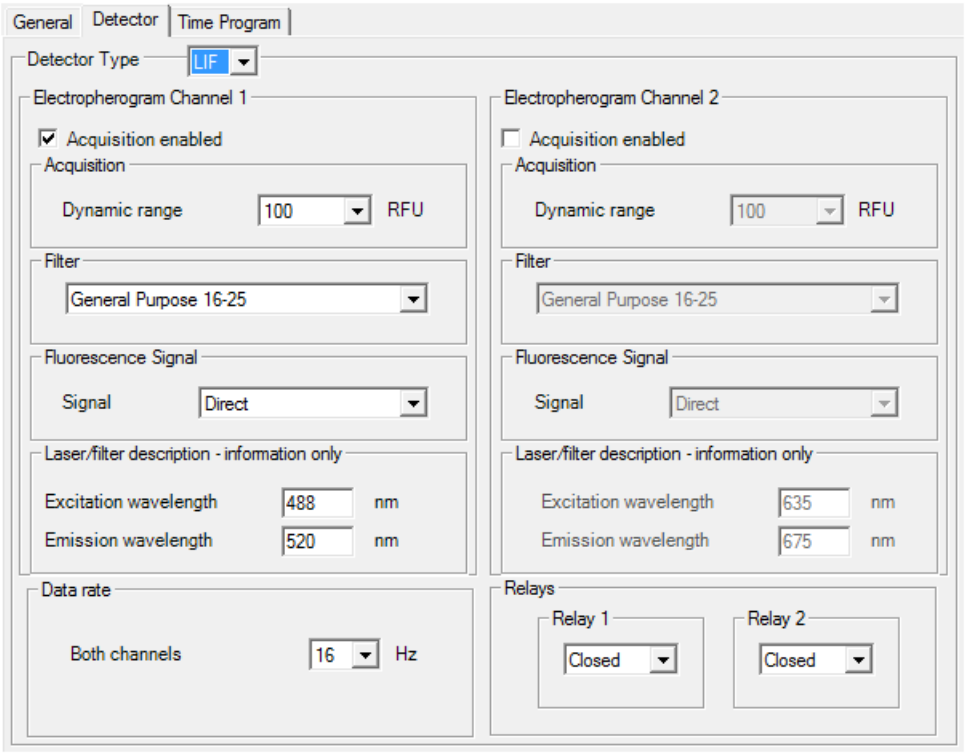
Figure D-2 General Parameters for FAST GLYCAN_CONDITIONING Instrument Method



4. Open the Detector tab, click **LIF** in the **Detector Type** list, and then set the parameters.

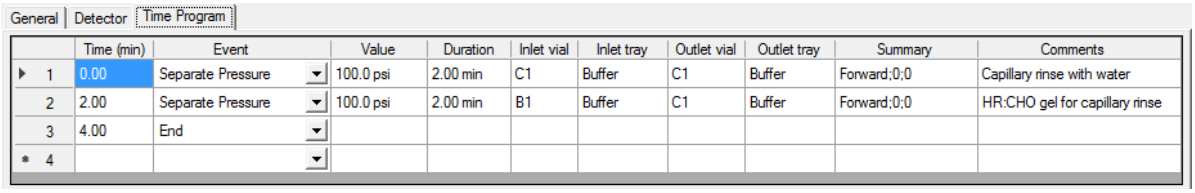
Note: For 3D data, in the Electropherogram Channel Data section, set the **Data Rate** to **On**.

Figure D-3 Detector Parameters for FAST GLYCAN_CONDITIONING Instrument Method



5. Add the events in the following figure to the time program.

Figure D-4 Time Program for FAST GLYCAN_CONDITIONING Instrument Method



	Time (min)	Event	Value	Duration	Inlet vial	Inlet tray	Outlet vial	Outlet tray	Summary	Comments
1	0.00	Separate Pressure	100.0 psi	2.00 min	C1	Buffer	C1	Buffer	Forward;0;0	Capillary rinse with water
2	2.00	Separate Pressure	100.0 psi	2.00 min	B1	Buffer	C1	Buffer	Forward;0;0	HR:CHO gel for capillary rinse
3	4.00	End								
* 4										

Note: If the system uses mbar as the units for pressure, then type the following:

- For the pressure in the **Separate Pressure** events (steps 1 and 2), type `6894.8`.

6. Save the instrument method.
   a. Click **File** > **Save**.
      The Save current Instrument Method dialog opens.
   b. Type `FAST GLYCAN_CONDITIONING` in the **Name** field.

c. (Optional) Type information in the **Method Comments** field.

d. If prompted, type the Waters Empower™ software password for the current user in the **Password** field, and then click **Save**.

The instrument method is saved to the current project.

7. Create the separation instrument method.

a. Set the parameters on the General tab. Refer to the figure: Figure D-2.

b. Set the parameters on the Detector tab. Refer to the figure: Figure D-3.

c. Add the events in the following figure to the time program.

Note: For the pressure in the **Separate Voltage Pressure** event (step 8), type `5`.

Figure D-5 Time Program for FAST GLYCAN_SEPARATION Instrument Method

General | Detector | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Inlet tray	Outlet vial	Outlet tray	Summary	Comments
1		Rinse Pressure	100.0 psi	2.00 min	B1	Buffer	C1	Buffer	Forward;20;20	Rinse with NR-CHO Separation Gel
2		Wait		0.10	A1	Buffer	A1	Buffer	20;20	Capillary Tip Wash
3		Inject Pressure	1.0 psi	5.0 s	C1	Buffer	C1	Buffer	Forward;20;20	Water Plug Injection
4		Inject Voltage	1.0 kV	5.0 s	A0	Sample List	B1	Buffer	Reverse (-);0;20	Sample Injection
5		Wait		0.00	A1	Buffer	A1	Buffer	20;20	Capillary Tip Wash
6		Inject Voltage	1.0 kV	2.0 s	F8	Sample	B1	Buffer	Reverse (-);0;0	Bracketing Standard Injection
7		Inject Pressure	1.0 psi	5.0 s	B1	Buffer	B1	Buffer	Forward;20;20	HR-Separation Gel push
8	0.00	Separate Voltage Pressure	30.0 kV	6.00 min	F1	Buffer	F1	Buffer	Reverse (-);0.1;Simultaneous;0;0	
9	0.50	Autozero								0.5 MIN
10	6.00	Stop Data								
11	6.00	End								
* 12										

Note: If the system uses mbar as the units for pressure, then type the following:

- For the pressure in the **Rinse Pressure** event (step 1), type `6894.8`.
- For the pressure in the **Inject Pressure** events (steps 3 and 6), type `68.9`.
- For the pressure in the **Separate Voltage Pressure** event (step 8), type `34.7`.

d. Save the method as `FAST GLYCAN_SEPARATION`.

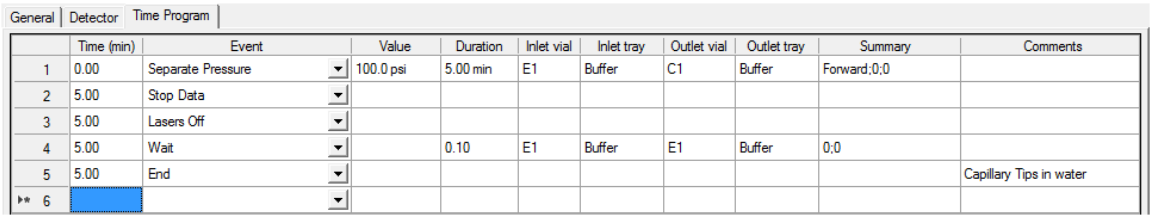
8. Create the shutdown instrument method.

a. Set the parameters on the General tab. Refer to the figure: Figure D-2.

b. Set the parameters on the Detector tab. Refer to the figure: Figure D-3.

c. Add the events in the following figure to the time program.

Figure D-6 Time Program for FAST GLYCAN_SHUTDOWN Instrument Method



General | Detector | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Inlet tray	Outlet vial	Outlet tray	Summary	Comments
1	0.00	Separate Pressure	100.0 psi	5.00 min	E1	Buffer	C1	Buffer	Forward;0;0	
2	5.00	Stop Data								
3	5.00	Lasers Off								
4	5.00	Wait		0.10	E1	Buffer	E1	Buffer	0;0	
5	5.00	End								Capillary Tips in water
▸* 6										

Note: If the system uses mbar as the units for pressure, for the pressure in the **Separate Pressure** event (step 1), type `6894.8`.

d. Save the method as `FAST GLYCAN_SHUTDOWN`.

9. (Optional) Create the separation instrument method for the glucose ladder.

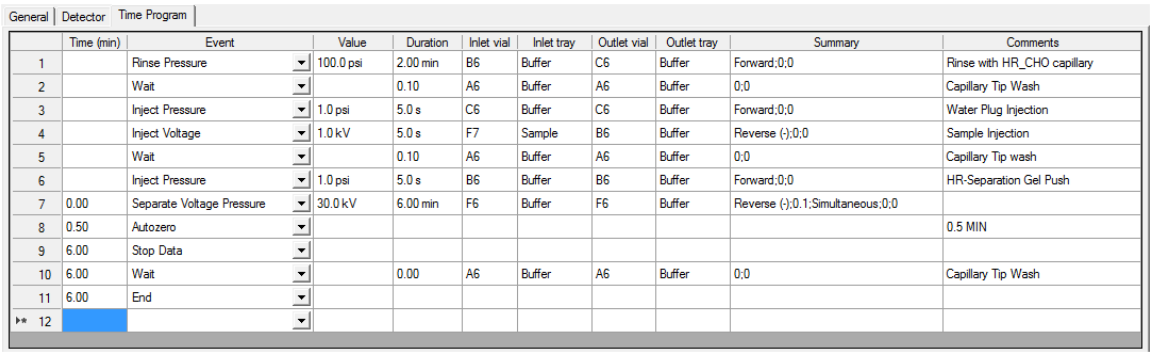
a. Set the parameters on the General tab. Refer to Figure D-2.

b. Set the parameters on the Detector tab. Refer to Figure D-3.

c. Add the events in the following figure to the time program.

Note: For the pressure in the **Separate Voltage Pressure** event (step 7), type `0.5`.

Figure D-7 Time Program for GU LADDER_SEPARATION Instrument Method



General | Detector | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Inlet tray	Outlet vial	Outlet tray	Summary	Comments
1		Rinse Pressure	100.0 psi	2.00 min	B6	Buffer	C6	Buffer	Forward;0;0	Rinse with HR_CHO capillary
2		Wait		0.10	A6	Buffer	A6	Buffer	0;0	Capillary Tip Wash
3		Inject Pressure	1.0 psi	5.0 s	C6	Buffer	C6	Buffer	Forward;0;0	Water Plug Injection
4		Inject Voltage	1.0 kV	5.0 s	F7	Sample	B6	Buffer	Reverse (-);0;0	Sample Injection
5		Wait		0.10	A6	Buffer	A6	Buffer	0;0	Capillary Tip wash
6		Inject Pressure	1.0 psi	5.0 s	B6	Buffer	B6	Buffer	Forward;0;0	HR-Separation Gel Push
7	0.00	Separate Voltage Pressure	30.0 kV	6.00 min	F6	Buffer	F6	Buffer	Reverse (-);0.1;Simultaneous;0;0	
8	0.50	Autozero								0.5 MIN
9	6.00	Stop Data								
10	6.00	Wait		0.00	A6	Buffer	A6	Buffer	0;0	Capillary Tip Wash
11	6.00	End								
▸* 12										

Note: If the system uses mbar as the units for pressure, then type the following:

- For the pressure in the **Rinse Pressure** event (step 1), type `6894.8`.
- For the pressure in the **Inject Pressure** events (steps 3 and 6), type `68.9`.
- For the pressure in the **Separate Voltage Pressure** event (step 7), type `3.5`.

d. Save the method as `GU LADDER_SEPARATION`.

Create the Method Sets

Three method sets are required:

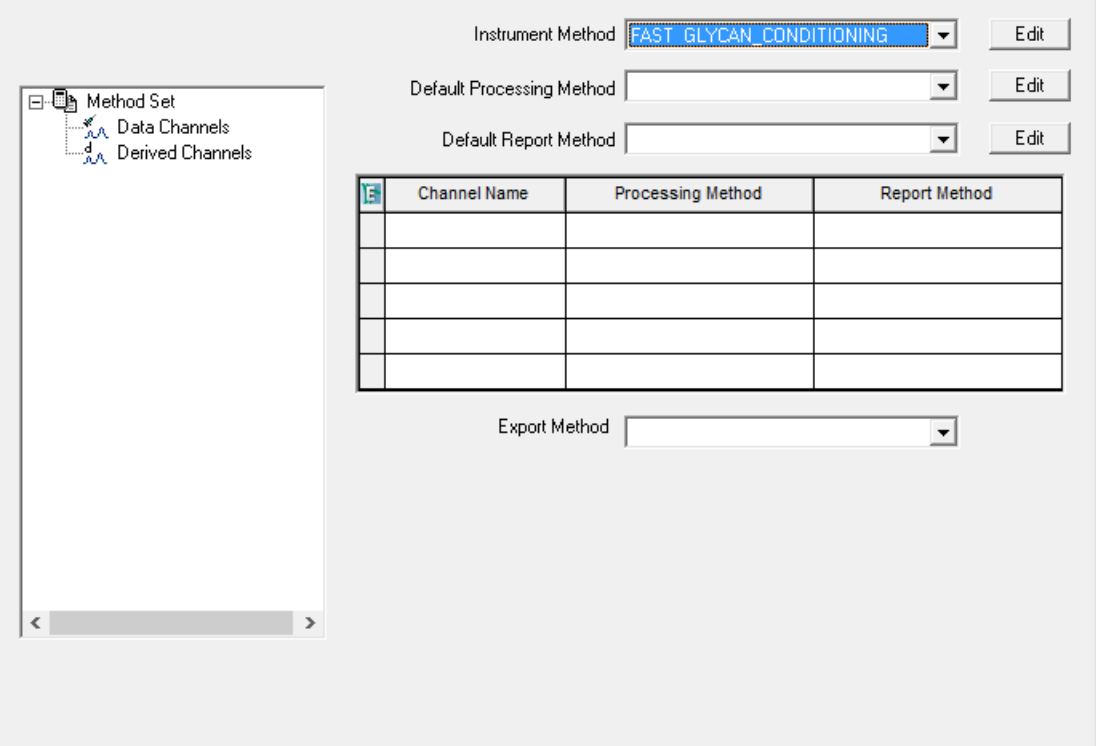
- Fast Glycan Conditioning
- Fast Glycan 96-Well Separation
- Fast Glycan 96-Well Shutdown

A fourth method set, Fast Glycan GU Ladder Method Set, is optional and is only needed if the glucose ladder is included as a sample.

Note: A method set can also include processing, report, and export methods. To create a processing or report method, refer to the documentation supplied with the Waters Empower™ software. To create an export method, refer to the section: Create an Export Method.

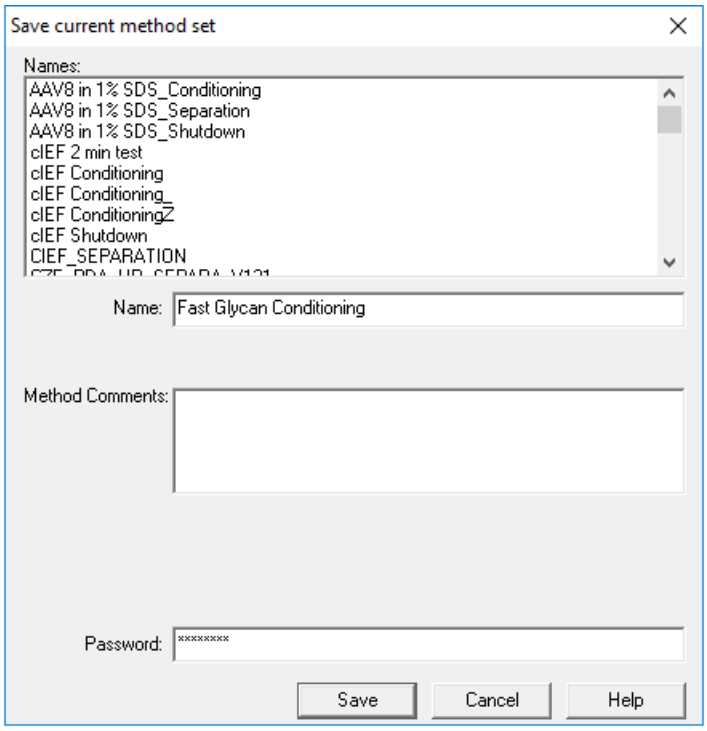
1. In the Waters Empower™ Software Project window, click **File** > **New Method** > **Method Set**.
2. Click **No** in the message.
   The Method Set Editor window opens.
3. In the **Instrument Method** list, click **FAST GLYCAN_CONDITIONING**. Do not make any other changes.

Figure D-8 Method Set Editor Window



4. Save the method set.
   a. Click **File** > **Save**.
   b. Type `Fast Glycan Conditioning` in the **Name** field.
   c. (Optional) Type information in the **Method Comments** field.
   d. If prompted, type the Waters Empower™ software password for the current user in the **Password** field, and then click **Save**.

Figure D-9 Save current method set Dialog



The method set is saved to the current project.

5. Repeat the previous steps to create the other method sets.

   a. To create the separation method set, select **FAST GLYCAN_SEPARATION** in the **Instrument Method** list. Save the method set as `Fast Glycan 96-Well Separation`.

      (Optional) If the data is to be exported, then click **Export Method** and select the export method before the separation method set is saved.

      Note: Only export the data to analyze it with the GU Value software.

   b. To create the shutdown method set, select **FAST GLYCAN_SHUTDOWN** in the **Instrument Method** list. Save the method set as `Fast Glycan 96-Well Shutdown`.

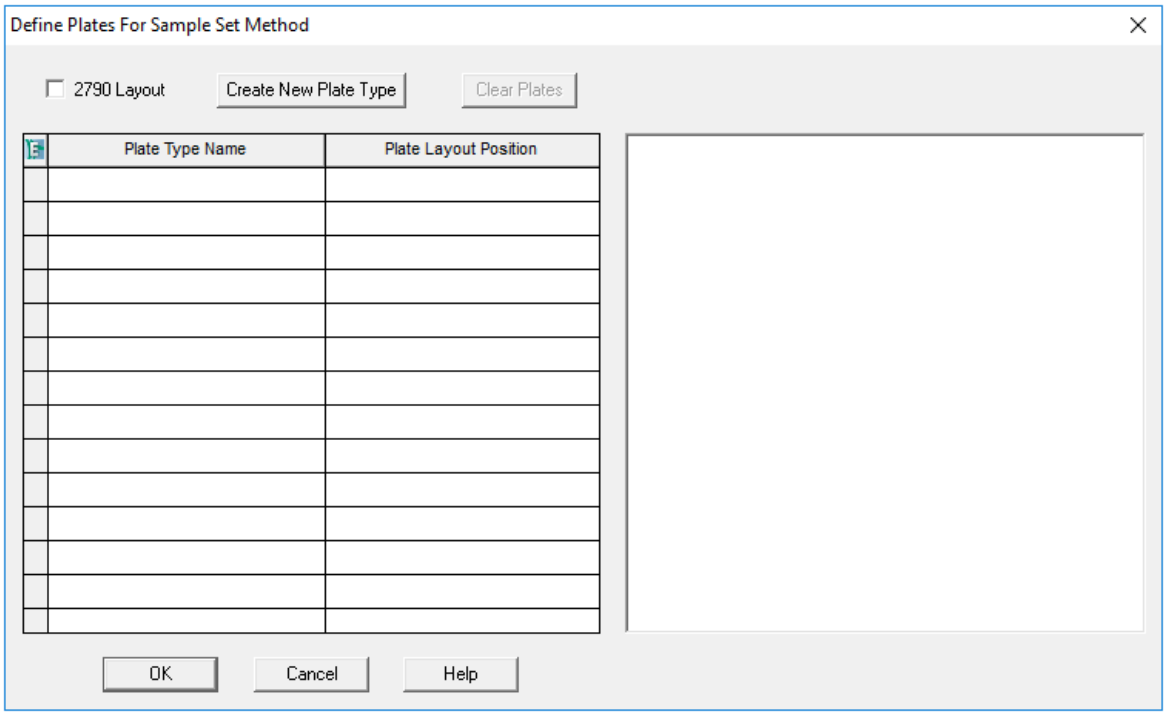
   c. (Optional) To create the GU ladder method set, select **GU LADDER_SEPARATION** in the **Instrument Method** list. Save the method set as `Fast Glycan GU Ladder`.

Configure the Software to Use Multiple Plates

The Waters Empower™ software is designed for chromatography systems that do not have buffer trays. To use the buffer trays, configure the Waters Empower™ software as follows.

1. In the Waters Empower™ software Run Samples window, click **Edit** > **Plates**.

Figure D-10 Define Plates for Sample Set Method Dialog



Note: If the dialog does not look like the preceding figure, then clear the **2790 Layout** check box.

2. In the first row, configure the buffer inlet tray.
   a. Click the **Plate Type Name** cell, and then select **PA 800 Plus Buffer Tray**.

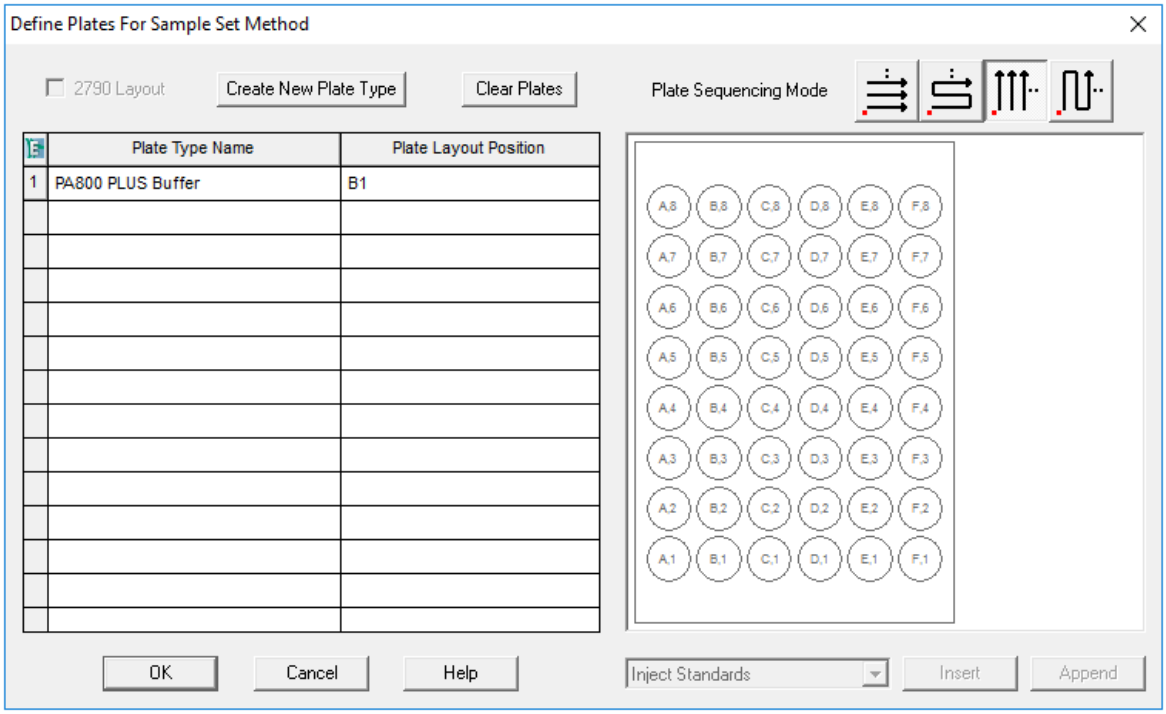
      Note: If **PA 800 Plus Buffer Tray** is missing, then the buffer and sample trays might not have been defined. Refer to the document: *PA 800 Plus Empower™ Driver User Guide*.

      The dialog updates with an image of the plate and buttons for the plate sequencing mode.

   b. Click the **Plate Layout Position** cell, and then type `BI`.

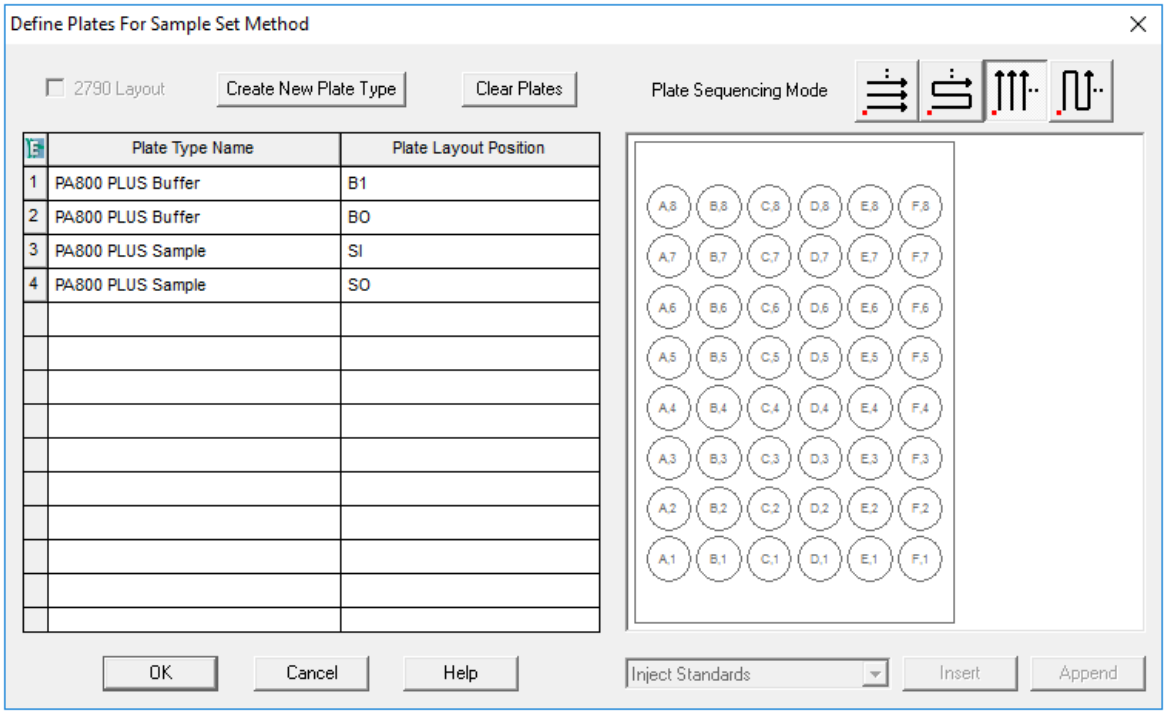
   c. Click  (**Vertical Discontinuous Plate Sequencing Mode**) to indicate the order in which the vials are accessed during the run.

Figure D-11 After Defining the Buffer Inlet Plate



3. Repeat step 2 to configure the buffer outlet tray in the second row. Type `BO` for the **Plate Layout Position**.
4. In the third row, configure the sample inlet tray.
   a. Click the **Plate Type Name** cell, and then select the correct plate type: either **PA 800 Plus Sample Tray** or **PA 800 Plus 96 Well Sample Tray**.
   b. Click the **Plate Layout Position** cell, and then type `SI`.
   c. Click  (**Vertical Discontinuous Plate Sequencing Mode**) to indicate the order in which the vials are accessed during the run.
5. Repeat step 4 to configure the sample outlet tray in the fourth row. Type `SO` for the **Plate Layout Position**.

Figure D-12 After Defining All Plate Types

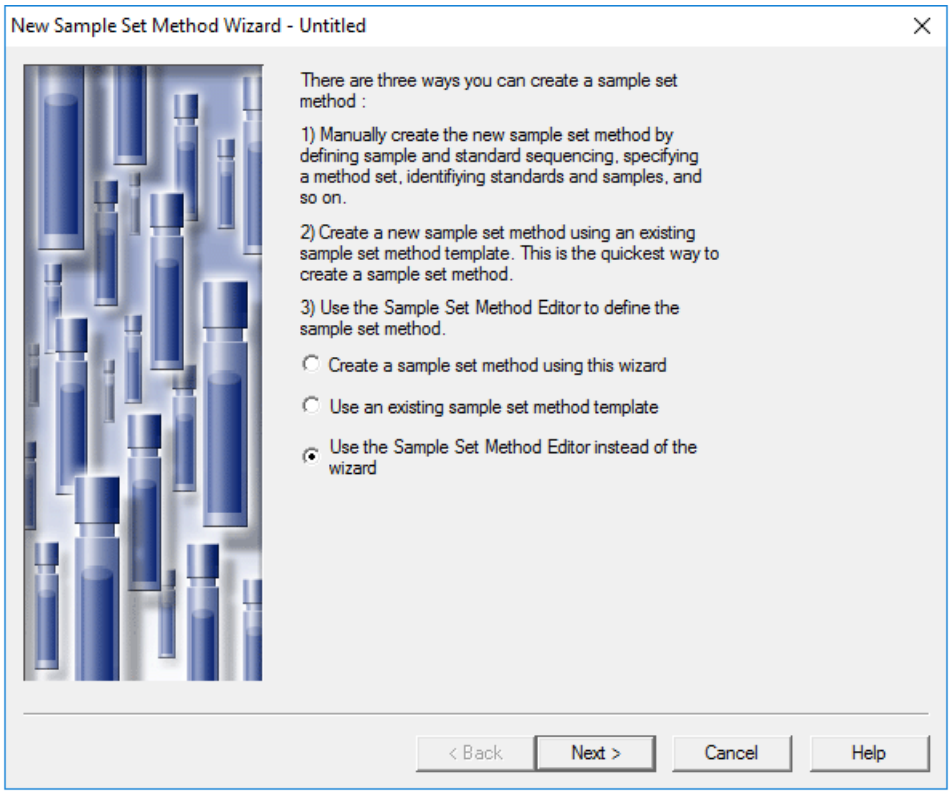


6. Click **OK** to save the changes and close the dialog.

Create the Sample Set Method and Run the Samples

1. In the Waters Empower™ Software Project window, click **File** > **New Method** > **Sample Set Method**.
   The New Sample Set Method Wizard opens.
2. Click **Use the Sample Set Method Editor instead of the wizard**, and then click **Next**.

Figure D-13 New Sample Set Method Wizard



The Sample Set Method Editor opens.

3. Set up the sample set method.
   a. In the first row, select **FAST GLYCAN_CONDITIONING** in the **Method Set/Report or Export Method** cell.
   b. For rows 2 through 17, select **FAST GLYCAN_SEPARATION** in the **Method Set/ Report or Export Method** cell.
   c. (Optional) If the glucose ladder standard is included, then select **Fast Glycan GU Ladder** in the **Method Set/Report or Export Method** cell that corresponds to the location of the ladder standard in the sample tray.
   d. For row 18, select **FAST GLYCAN_SHUTDOWN** in the **Method Set/Report or Export Method** cell.
   e. Add the required information for the samples. Refer to the table: Table D-1.

      Use the default values for the other fields. Refer to the section: Create an Export Method

Table D-1 Required Fields for a Sample Set Method

Name	Description
Plate/Well	The position of the sample in the sample tray.
# of Injs	The number of times the sample is to be injected.
SampleName	The name of the sample.
Run Time (Minutes)	The duration of the run. **CAUTION: Potential Wrong Result. Make sure that the Run Time is higher than or equal to the duration of the time program in the instrument method. If the Run Time is shorter, then the system stops the run before the time program is complete.**

The completed sample set is shown in the following figure.

Note: The **Level** and **Label Reference** columns are hidden in the following figure.

Figure D-14 Sample Set Method

	Plate/Well	Inj Vol (uL)	# of Injs	Label	SampleName	Function	Method Set / Report or Export Method	Processing	Run Time (Minutes)
1						Clear Calibration	FAST GLYCAN_SEPARATION	Normal	
2	BI:A,1	1.0	1		Conditioning	Inject Samples	FAST GLYCAN_CONDITIONING	Normal	6.00
3	SI:A,1	1.0	4	S0101	GU STD	Inject Standards	FAST GLYCAN_SEPARATION	Normal	6.00
4	SI:A,2	1.0	1	U0101	1Glycan	Inject Samples	FAST GLYCAN_SEPARATION	Don't Process or Report	6.00
5	SI:A,3	1.0	1	U0102	2Glycan	Inject Samples	FAST GLYCAN_SEPARATION	Don't Process or Report	6.00
6	SI:A,4	1.0	1	U0103	3Glycan	Inject Samples	FAST GLYCAN_SEPARATION	Don't Process or Report	6.00
7	SI:A,5	1.0	1	U0104	4Glycan	Inject Samples	FAST GLYCAN_SEPARATION	Don't Process or Report	6.00
8	SI:A,6	1.0	1	U0105	5Glycan	Inject Samples	FAST GLYCAN_SEPARATION	Don't Process or Report	6.00
9	SI:A,7	1.0	1	U0106	6Glycan	Inject Samples	FAST GLYCAN_SEPARATION	Don't Process or Report	6.00
10	SI:A,1	1.0	1	S0201	GU STD	Inject Standards	FAST GLYCAN_SEPARATION	Normal	6.00
11	SI:A,3	1.0	1	U0201	7Glycan	Inject Samples	FAST GLYCAN_SEPARATION	Don't Process or Report	6.00
12	SI:A,3	1.0	1	U0202	8Glycan	Inject Samples	FAST GLYCAN_SEPARATION	Don't Process or Report	6.00
13	SI:A,3	1.0	1	U0203	9Glycan	Inject Samples	FAST GLYCAN_SEPARATION	Don't Process or Report	6.00
14	SI:A,3	1.0	1	U0204	10Glycan	Inject Samples	FAST GLYCAN_SEPARATION	Don't Process or Report	6.00
15	SI:A,3	1.0	1	U0205	11Glycan	Inject Samples	FAST GLYCAN_SEPARATION	Don't Process or Report	6.00
16	SI:A,3	1.0	1	U0206	12Glycan	Inject Samples	FAST GLYCAN_SEPARATION	Don't Process or Report	6.00
17	SI:A,1	1.0	1	S0301	GU STD	Inject Standards	FAST GLYCAN_SEPARATION	Normal	6.00
18	BI:A,3	1.0	1		Shutdown	Inject Samples	FAST GLYCAN_SHUTDOWN	Normal	6.00
19						Calibrate	FAST GLYCAN_SEPARATION	Normal	
20						Quantitate	FAST GLYCAN_SEPARATION	Normal	

4. (Optional) Add instructions to export the data.

Note: Only export the data to analyze it with the GU Value software.

Note: If the separation method set already includes the export method, then skip this step and go to step 5.

a. Create an export method. Refer to the section: Create an Export Method.

b. In row 19, select **Export** in the **Function** cell.

c. In the **Method Set/Report or Export Method** cell, click the name of the export method.

d. If required, type information in the **Comment** cell.

5. Save the sample set method.

   a. Click **File** > **Save**.
   The Save current sample set method dialog opens.

   b. Type `Fast Glycan Sample Set Method` in the **Name** field.

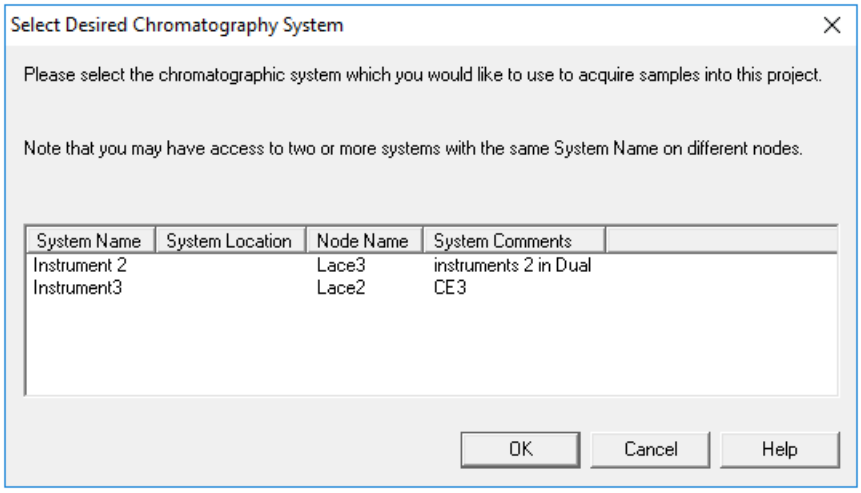
   c. (Optional) Type information in the **Method Comments** field.

   d. If required, type the Waters Empower™ software password for the current user in the **Password** field, and then click **Save**.

   The method set is saved to the current project.

6. Click **Tools** > **Run Samples**.

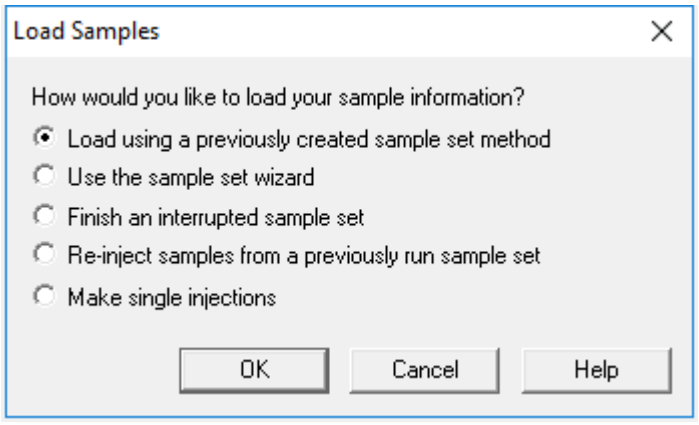
Figure D-15 Select Desired Chromatography System Dialog



7. Click the system to be used, and then click **OK**.
   Make sure that the instrument is configured with an LIF detector.
   The Run Samples window opens.

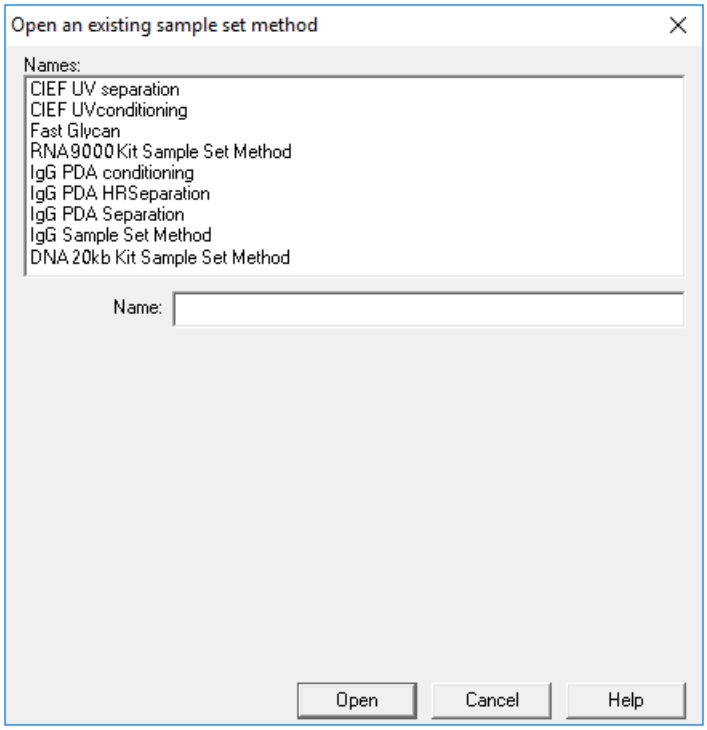
8. Click (**Load Sample Set**).

Figure D-16 Load Samples Dialog



9. Click **Load using a previously created sample set method**, and then click **OK**.

Figure D-17 Open an existing sample set method Dialog



10. Click **`Fast Glycan Sample Set Method`** in the list, and then click **Open**.
    The sample set method opens in the Samples tab.

11. In the Waters Empower™ Software Project window, click  (**Start**).

Data acquisition starts. During the run, the text in the row in the Sample Set Method window for the sample being acquired is red.

12. Do the following:

    - (Optional) To halt data acquisition, click  (**Stop**).
    - See the voltage and current data.

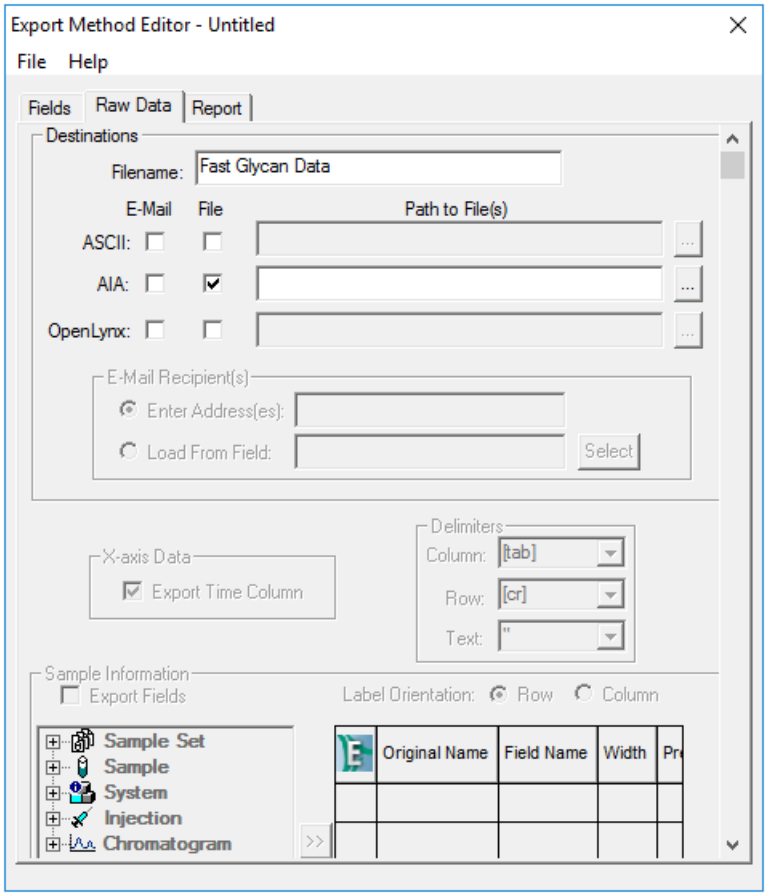
    When the run ends, the text in all rows in the Sample Set Method window is red.

Create an Export Method

Note: Only export the data to analyze it with the GU Value software.

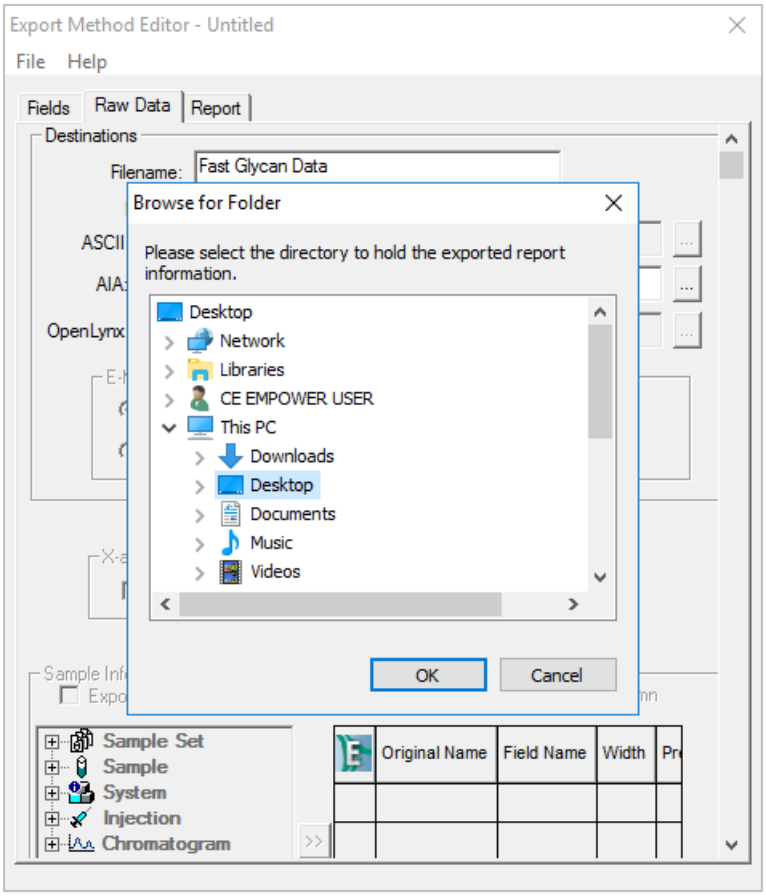
1. In the Waters Empower™ Software Project window, click **File** > **New Method** > **Export Method**.
2. Click the **Raw Data** tab, and then type a name for the export file in the **Filename** field, up to 32 characters.
3. In the **AIA** row, select **File**.

Figure D-18 Export Method Editor Window



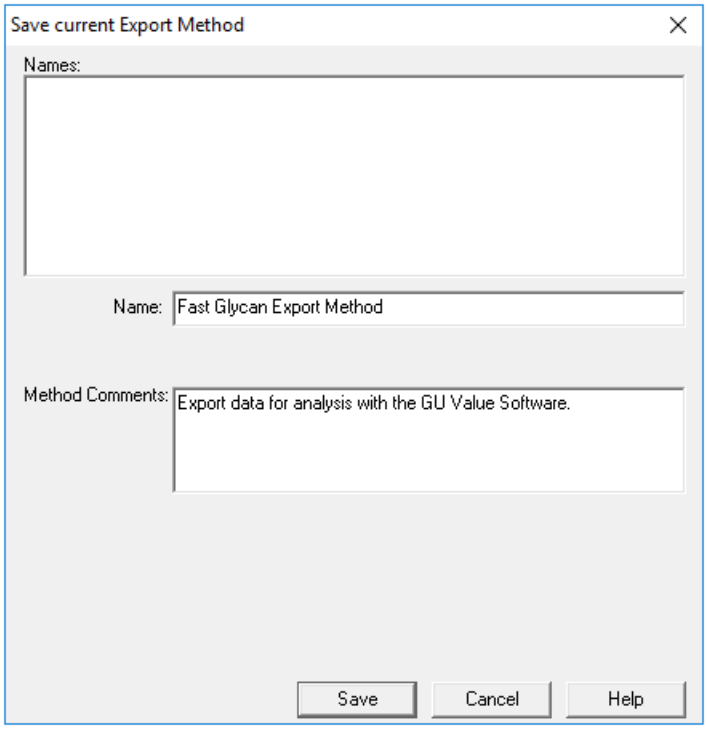
4. Click **...** (**Browse**), navigate to the location for the exported data, and then click **OK**.

Figure D-19 Browse for Folder Dialog



5. Save the export method.
   a. Click **File** > **Save As**.
   b. In the **Name** field, type a name for the export method.
      The **Names** field at the top of the dialog shows the existing export methods.
   c. (Optional) Type information in the **Method Comments** field.

Figure D-20 Save current Export Method Dialog



d. Click **Save**.

The export method is saved to the current project.

6. Click **File** > **Exit**.
   The Export Method Editor window closes.

Import the Instrument Methods

1. Open the **Methods** folder on the PA 800 Plus Empower™ Driver DVD.
2. In the Waters Empower™ Software Pro Interface window, click **Browse Projects**, click the project of interest, and then click **OK**.

Figure D-21 Waters Empower™ Software Pro Interface Window

The Project window opens.

3. Open the Methods tab.
4. On the Windows desktop, click each `min` file in the `Methods` folder, and then drag it to the Project window.
   The instrument method is added to the project, and can be edited and added to a method set like any other method.

Contact Us

Customer Training

- In North America: NA.CustomerTraining@sciex.com
- In Europe: Europe.CustomerTraining@sciex.com
- Outside the EU and North America, visit sciex.com/education for contact information.

Online Learning Center

- SCIEX Now Learning Hub

Purchase Supplies and Reagents

Reorder SCIEX supplies and reagents online at store.sciex.com. To set up an order, use the account number, found on the quote, order confirmation, or shipping documents. Currently, customers in the United States, Canada, United Kingdom, Belgium, Netherlands, France, Germany, and Switzerland have access to the online store, but access will be extended to other countries in the future. For customers in other countries, contact a local SCIEX representative.

SCIEX Support

SCIEX and its representatives have a global staff of fully-trained service and technical specialists. They can supply answers to questions about the system or any technical issues that might occur. For more information, go to the SCIEX website at sciex.com or use one of the following links to contact us.

- sciex.com/contact-us
- sciex.com/request-support

Cybersecurity

For the latest guidance on cybersecurity for SCIEX products, visit sciex.com/productsecurity.

Documentation

This version of the document supersedes all previous versions of this document.

To find software product documentation, refer to the release notes or software installation guide that comes with the software.

Contact Us

To find hardware product documentation, refer to the documentation that comes with the system or component.

The latest versions of the documentation are available on the SCIEX website, at sciex.com/customer-documents.

Note: To request a free, printed version of this document, contact sciex.com/contact-us.