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-C

April 2020

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 SCIEX Fast Glycan Labeling and Analysis Kit provides reagents and supplies required to label, separate, and identify reducing carbohydrates for 100 samples using the PA 800 Plus Pharmaceutical Analysis System. This 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.

This document provides instructions for sample preparation using the Fast Glycan Labeling and Analysis Kit. It also provides instructions for data acquisition and data analysis using the PA 800 Plus Software and Waters Empower™ 3 (FR4) Software.

Note: Refer to the *System Overview Guide* for instructions for safe use of the system.

Note: For accurate results, we strongly recommend using 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 (SDS), available at sciex.com/tech-regulatory, for information about the proper handling of materials and reagents. Always follow standard laboratory safety guidelines. Refer to Hazardous Substance Information for hazardous substance information.

Intended Use

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

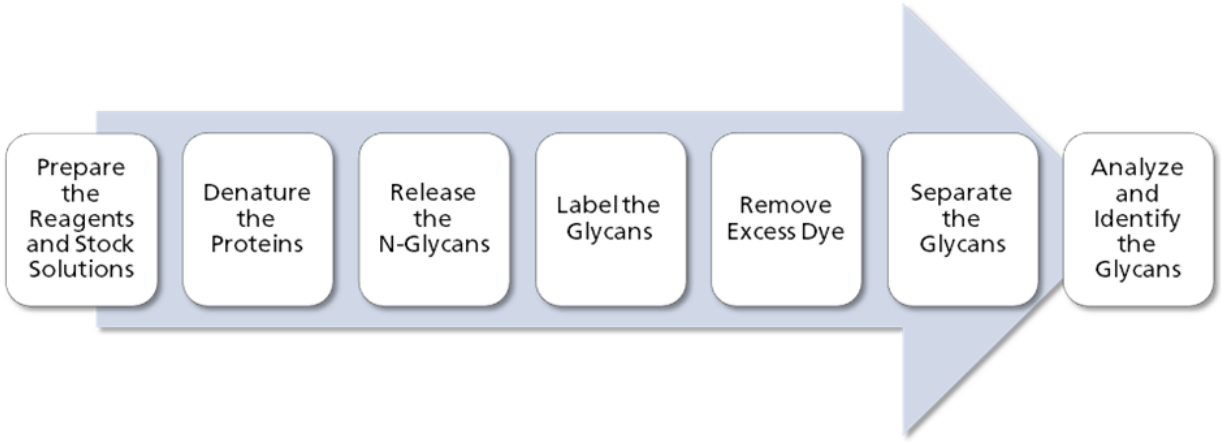
Introduction

The protocol consists of the following steps:

1. Enzymatic release of the N-glycans from glycoproteins.
2. Magnetic bead-mediated capture of the released glycans.

3. Labeling of the released glycans with a charged fluorophore.
4. Magnetic bead-mediated capture of the fluorophore-labeled glycans and dye removal, followed by release of the labeled glycans.
5. Separation of the labeled glycans by capillary electrophoresis with LIF detection.
6. Analysis of the capillary electrophoresis separation results to identify the type of glycans in the sample, using the GU Value Software.

Workflow



Equipment and Materials Required

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

Table 1 Fast Glycan Labeling and Analysis Kit (PN B94499PTO)

Component	Quantity	Reorder Part Number
Magnetic stand	1	N/A
M1 magnetic beads	1	N/A
D1 reagent	5	N/A
D2 reagent	5	N/A
D3 reagent	1	N/A

Table 1 Fast Glycan Labeling and Analysis Kit (PN B94499PTO) (continued)

Component	Quantity	Reorder Part Number
D4 reagent	2	N/A
L5 catalyst	1	N/A
L6 fluorophore	5	N/A
GU (glucose ladder); process control	1	N/A
IST (internal standard)	1	N/A
BST (bracketing standard)	1	N/A
HR-NCHO separation gel	1	N/A
Pre-assembled capillary cartridge with window (30 cm total length, 375 µm outer diameter (o.d.) x 50 µm inner diameter (i.d))	1	A55625

Table 2 Additional Supplies from SCIEX

Component	Quantity	Part Number
Micro vials, 200 µL	100	144709
Universal vial caps, blue	100	A62250
Universal vials	100	A62251

Table 3 Additional Required Reagents or Supplies

Description	Vendor	Part Number
Peptide-N-glycosidase F enzyme (PNGase F), 200 mU	Prozyme	GKE5006B
HPLC-grade acetonitrile	Other Lab Supplier	Various
1 M sodium cyanoborohydride in THF	Sigma-Aldrich	296813

Storage Conditions

- Upon receipt, store the Fast Glycan Labeling and Analysis kit at 2 °C to 8 °C.
- Store the cartridge at room temperature.

Customer-Supplied Equipment and Supplies

- Powder-free gloves, neoprene or nitrile recommended
- Safety glasses
- Laboratory coat
- 0.2 mL flat-cap PCR tubes (VWR USA PN 20170-012 or VWR EUR PN 732-0548)
- Table-top mini centrifuge
- Microcentrifuge, or equivalent, and microcentrifuge tubes
- Luer-Lok syringe, or equivalent, with a 22 gauge needle
- Heat block capable of maintaining 60 °C
- Vortex mixer
- Thermometer for measuring the heat block temperature
- Pipettes and appropriate tips
- Double-deionized (DDI) water (MS-grade water filtered through a 0.2 µm filter and with resistance above 18 MΩ)

Required Detector

An LIF detector and the supplies to calibrate it are required. Calibration supplies are:

- LIF Performance Test Mix (PN 726022)
- Capillary Performance Run Buffer A (PN 338426)

Required Cartridge or Capillary

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

One of the following:

- Pre-assembled cartridge (PN A55625)
- Capillary cartridge (PN 144738) and capillaries, bare-fused silica, 50 µm i.d. (PN 338451)

Required Software

The GU Value Software must be installed on the PA 800 Plus Pharmaceutical Analysis System controller.

For systems using 32 Karat™ Software version 10.3 or higher, the GU Value Software is included with the 32 Karat™ Software.

For systems using 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, go to sciex.com and then log in. Go to sciex.com/products/capillary-electrophoresis-instruments/pa-800-plus-pharmaceutical-analysis-system. Search for "Fast Glycan Software" and then download the installation file that contains the *Release Notes*, example data, and the GU Value Software. Follow the installation instructions in the *Release Notes*.

Methods and Sequences

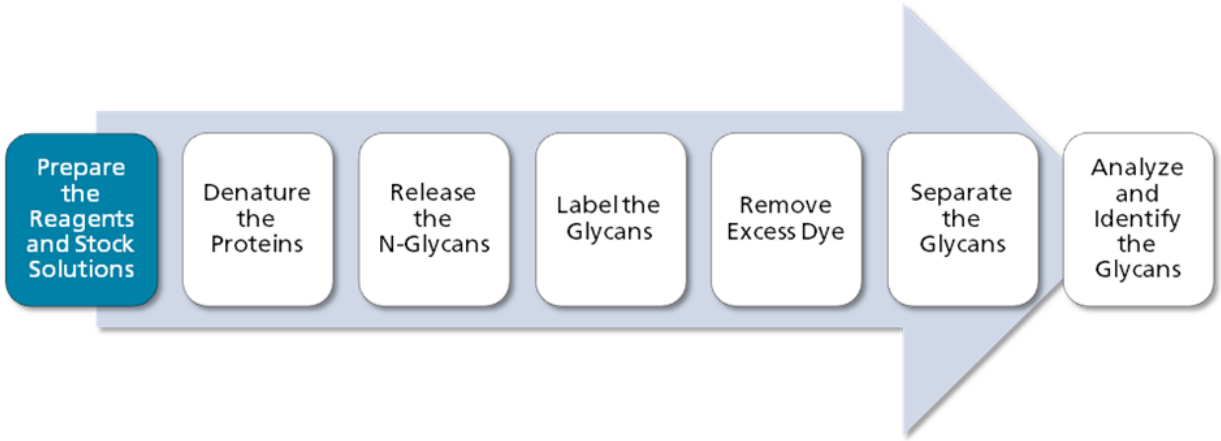
Note: The following information applies to users using the PA 800 Plus System with the PA 800 Plus and 32 Karat™ Software. If the system will be used with the Empower™ Software, then the methods will be different. Refer to Run the Samples with the Waters Empower™ Software.

For systems using 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 Methods.

- **HR-NCHO Fast Glycan.seq:** The sequence.
- **Fast Glycan Conditioning.met:** Conditions the capillary at the start of each day.
- **Fast Glycan Separation.met:** Performs a separation of the separated glycans.
- **Fast Glycan Shutdown.met:** Cleans the capillary at the end of a sequence, to rinse the capillary for storage, and to turn off the laser in the LIF detector.
- **Fast Glycan GU Ladder.met:** Performs a separation of the glucose ladder standard.

For systems using older versions of the 32 Karat™ Software (before 10.3), the methods and sequence are installed with the GU Value Software. Refer to Required Software.

Prepare the Reagents and Stock Solutions



Reconstitute the Bracketing Standard (BST)

1. Add 100 µL of DDI water to the BST vial and then vortex to mix.

   This makes a 50 nM solution.
2. Aliquot the solution into 20 µL portions.

   When not in use, store at –35 °C to –15 °C for up to six months.

Reconstitute the Internal Standard (IST)

1. Add 500 µL of DDI water to the IST vial and vortex to mix.

   This makes a 440 µM solution.
2. To limit repeated freezing and thawing, aliquot portions of the solution into 0.5 mL microfuge vials. Choose a volume appropriate for the experimental design.

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

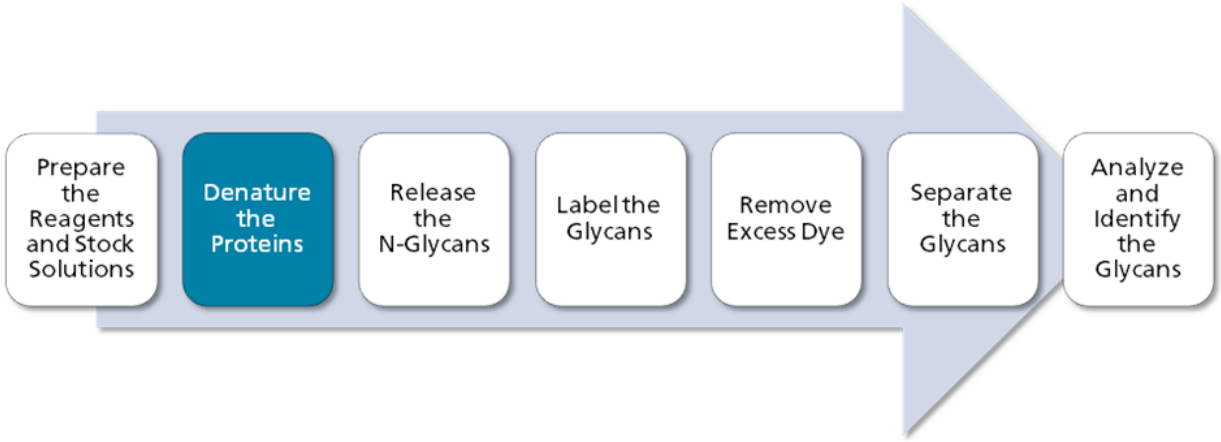
   When not in use, store at –35 °C to –15 °C for up to six months.

Reconstitute the Fluorophore Label (L6)

- Add 240 µL of L5 to the L6 vial and then vortex to mix.

When not in use, store at –35 °C to –15 °C for up to three months.

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 appropriate.

Prepare the Denaturation Solution

1. Reconstitute the D2 reagent.
   a. Add 50 µL of DDI water to the D2 vial.
   b. Vortex the vial to mix.

   The reconstituted D2 reagent can be stored 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 vortex the tube to mix. 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

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

Discard the unused portion after use.

Denature the Proteins

1. Pre-heat the heat block to 60 °C.
2. Prepare 100 µg of glycoprotein sample in 10 µL of DDI water.

Note: For highly-glycosylated samples, a smaller quantity of protein may be used.

3. Prepare the magnetic beads.
   a. Vortex the M1 vial at maximum speed until all beads are in solution.
   b. Using a pipette, 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. Vortex the sample tube for 10 sec at maximum speed. Make sure that the magnetic beads are mixed with the sample.
6. Add 5 µL of the previously-prepared denaturation solution to the sample tube, vortex briefly, and then incubate for 8 minutes at 60 °C in the heat block to denature the sample. Refer to 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: 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 appropriate.

Tips for Best Results

During the deglycosylation and labeling steps, keep the vials uncapped.

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 vortexing at high speed, some sample might be suspended from the micro vial cap. To prevent sample loss, spin the tube for 1 sec to 2 sec in a microcentrifuge to remove any solution that might be suspended from the cap.

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 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 vortex the tube for 5 sec at maximum speed. This procedure produces enough for 20 samples.

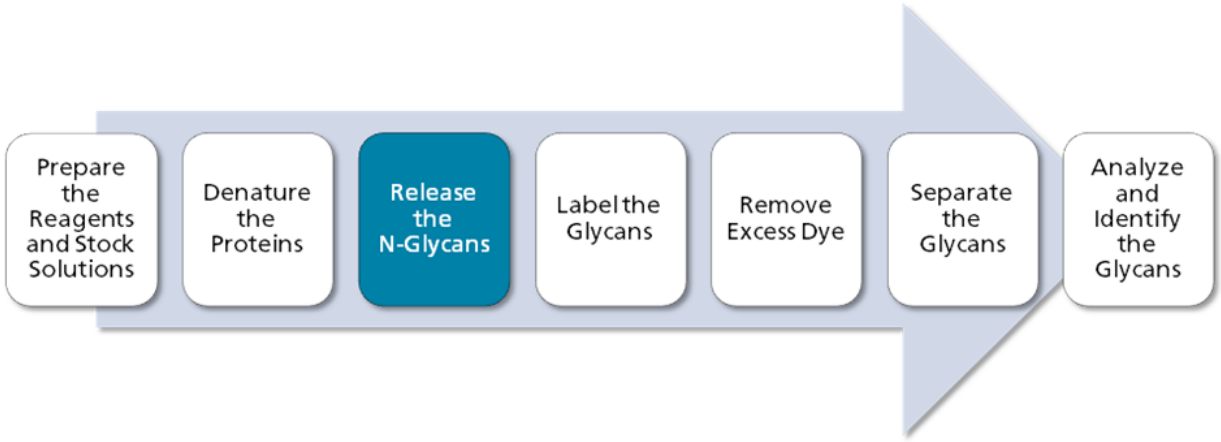
Reagent	For 1 Sample	For up to 20 Samples
D4	12 µL	240 µL
PNGase F enzyme	2 µL	40 µL

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 after use.

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 Phynexus. Refer to the manufacturer's instructions and to 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. When the denaturation step is complete, add 12 µL of the previously-prepared digestion solution to the sample tube and then incubate for 20 minutes at 60 ºC in the heat block. Refer to 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 vortex for 10 sec at maximum speed.
3. Add 200 µL of acetonitrile to the sample tube and then vortex for 10 sec at maximum speed.
4. Incubate for 1 minute at room temperature.
5. Using a microcentrifuge, spin the tube for 1 sec to 2 sec 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.

Prepare the Labeling Solution

DANGER! Toxic Chemical Hazard. Read the *Safety Data Sheet* for 1 M sodium cyanoborohydride (in THF) prior to use.

Note: The cyanoborohydride 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. Working in a fume hood, add the reagents specified in the following table to the Labeling Solution tube and then vortex the tube to mix. This procedure produces enough for 20 samples.

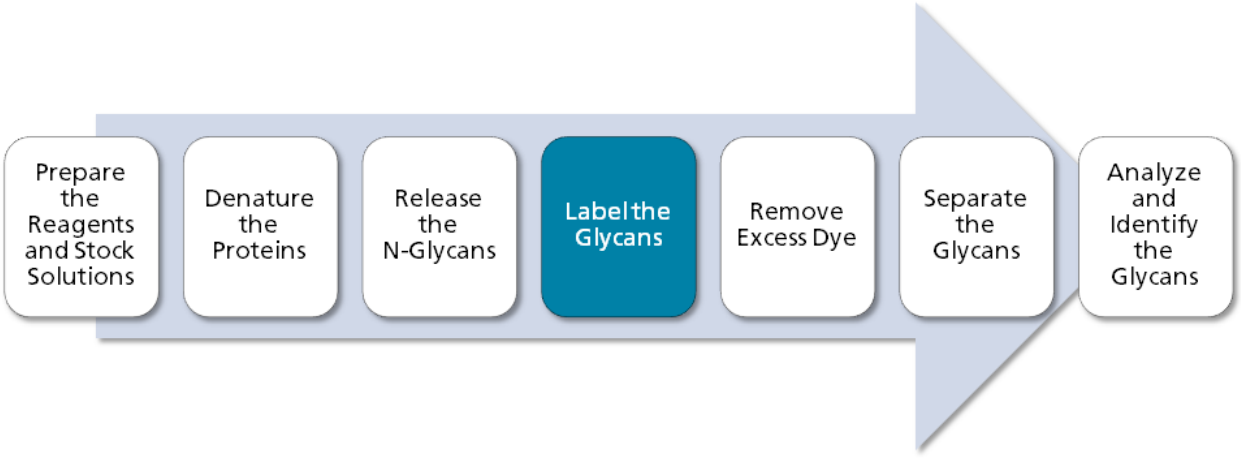
   Tip! Use the syringe to remove the cyanoborohydride from the vial.

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. Using a microcentrifuge, spin the tube for a few seconds to bring the solution to the bottom of the tube.

   Discard the unused portion after use.

Label the Released N-Glycans



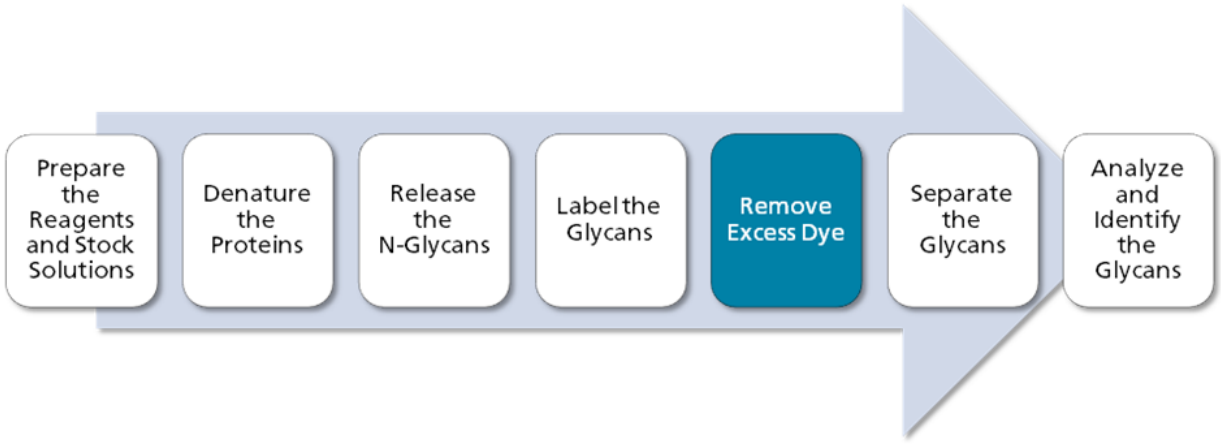
1. Working in a fume hood, add 11 µL of the previously-prepared labeling solution to each sample tube. Refer to Prepare the Labeling Solution.
2. Vortex the sample tube for 10 sec at maximum speed.

   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 vortex 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.

   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.

1. After the labeling reaction is complete, remove the sample tube from the heat block.
2. Rinse the labeled sample.
    a. Using a pipette, add 10 µL of D4 to the sample tube, close the lid, and then vortex for 10 sec at maximum speed.
    b. Using a pipette, add 160 µL of acetonitrile to the sample tube, close the lid, and then vortex for 10 sec at maximum speed.
    c. Allow the tube to incubate for 1.0 min at room temperature.
    d. If necessary, centrifuge the sample tube in a microcentrifuge for 1 sec to 2 sec to remove any solution suspended from the lid.
    e. 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.
    f. Remove the sample tube from the magnetic stand.
3. Wash the sample.
    a. Using a pipette, add 20 µL of DDI water to the sample tube, close the lid, and then vortex for 10 sec at maximum speed.
    b. Using a pipette, add 160 µL of acetonitrile to the sample tube , close the lid and then vortex for 10 sec at maximum speed.
    c. Allow the tube to incubate for 1.0 min at room temperature.

d. If necessary, centrifuge the sample tube in a microcentrifuge for 1 sec to 2 sec to remove any solution suspended from the lid.

e. 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. Repeat step 3 to wash the sample again.

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 DDI water to the sample tube and then vortex for 10 sec 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 200 µL micro vial.

   The supernatant contains the labeled and purified glycans.
9. Do one of the following:
   - Follow the instructions in Load the Sample Tray to load the samples and start the run.
   - Store the micro vials at –35 °C to –15 °C until analysis, for up to 1 month.

(Optional) Prepare and Label the Glucose Ladder Standard

DANGER! Toxic Chemical Hazard. Read the *Safety Data Sheet* for 1 M sodium cyanoborohydride (in THF) prior to use.

1. Add 5 mg of the GU standard to a 1.5 mL microfuge vial.
2. Add 80 µL of DDI water to the vial and then vortex the vial until the GU standard is completely dissolved.
3. Add 2 µL of the GU standard solution to a 0.5 mL microfuge vial.

   Discard the remainder of the GU standard solution.
4. Add 9 µL of reconstituted L6 to the GU standard.
5. Working in a fume hood, add 1 µL of 1 M sodium cyanoborohydride (in THF) to the GU vial and then cap the vial.

6. Vortex the vial for 10 sec at maximum speed.
7. Using a microcentrifuge, spin the vial for a few seconds to bring the solution to the bottom of the vial.
8. Open the vial cap, and then incubate the vial in the heat block for 40 min at 60 °C.

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 ladder standard is a dry yellow pellet.

9. Prepare the GU ladder standard stock solution.
   a. Add 100 µL of DDI water to the solid and then vortex until completely dissolved.
   b. Using a microcentrifuge, spin the vial for a few seconds to bring the solution to the bottom of the vial.

      This is the GU ladder standard stock solution. When not in use, store at –35 °C to –15 °C for up to six months.
10. Add 5 µL of the GU ladder standard stock solution to a 1.5 mL microfuge vial, add 195 µL of DDI water, and then vortex the vial well to mix.
11. Pipette 200 µL of the diluted stock solution in a PA 800 Plus micro vial and then put the vial in the sample tray. Refer to Figure 3.

Prepare the PA 800 Plus System

This section describes the steps to prepare the PA 800 Plus System to acquire data.

The procedures described in this section assume the system has already been properly installed and initialized.

Install the LIF Detector

1. Turn off the PA 800 Plus System and then install the LIF detector. Refer to the *System Maintenance Guide*.
2. Turn on the system.

Clean the Interface Block

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

Clean the electrodes, opening levers, capillary ends, and interface block weekly or when changing chemistries. Refer to the *System Maintenance Guide* for detailed instructions.

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

Install the Cartridge and Calibrate the Detector

Note: To make sure that analysis results are consistent over time, we strongly recommend calibrating the detector each time it is installed in the PA 800 Plus System. Also calibrate the detector after replacing the capillary in the cartridge or installing a different cartridge.

Note: For Empower™ Software users, calibration instructions are located in the *PA 800 Plus Empower™ Driver User Guide*.

1. Remove the cartridge from the box and, if necessary, install the capillary.
2. Remove the aperture from the cartridge and then install the LIF aperture and probe guide on the LIF detector. Refer to the *System Maintenance Guide*.
3. Install the cartridge in the PA 800 Plus System.
4. Calibrate the detector.

   Use the Calibration wizard, available from the Instrument Configuration dialog in the 32 Karat™ Software. Refer to the *System Maintenance Guide* for detailed instructions.

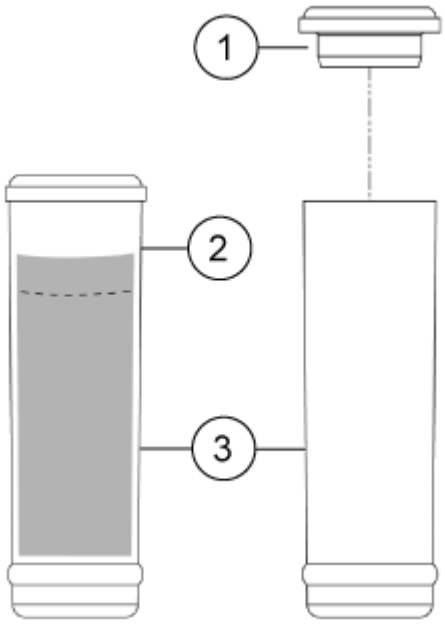
Load the Buffer Trays

CAUTION: Potential System Damage. Do not fill any vial with more than 1.8 mL of liquid. In addition, do not allow the waste vials to collect more than 1.8 mL. If a vial contains more than 1.8 mL, then the pressure system might be damaged.

1. Depending on the number of samples to be run, fill the appropriate number of vials and then cap them. Refer to Figure 1. For each set of 20 samples, prepare:
   - 5 universal vials, each containing 1.5 mL DDI water
   - 4 universal vials, each containing 1.5 mL HR-NCHO separation gel

- 1 universal vial containing 0.8 mL DDI water, for the Waste position in the outlet buffer tray

Figure 1 Universal Vial and Cap Setup



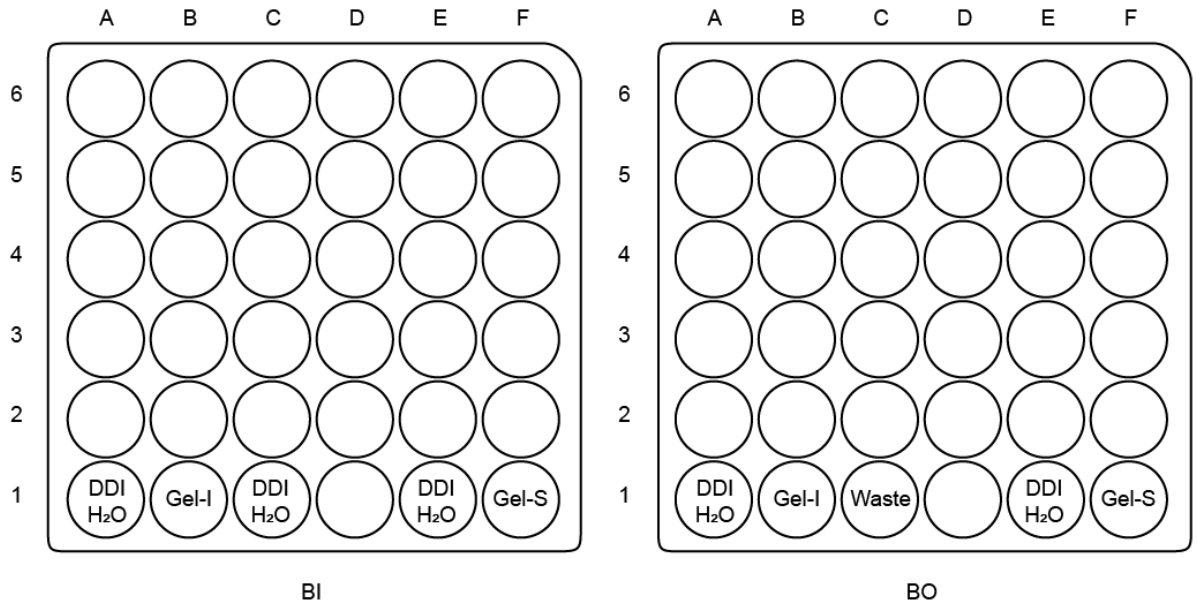
Item	Description
1	Universal vial cap
2	Maximum fill line
3	Universal vial

2. Put the vials in the buffer trays as shown in Figure 2. Each row is sufficient for at least 20 runs.

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

Note: For this application, all vials and caps are designed for a maximum of eight runs. Do not reuse the caps because they might be contaminated with dried gel and other chemicals.

Figure 2 Buffer Tray Layout

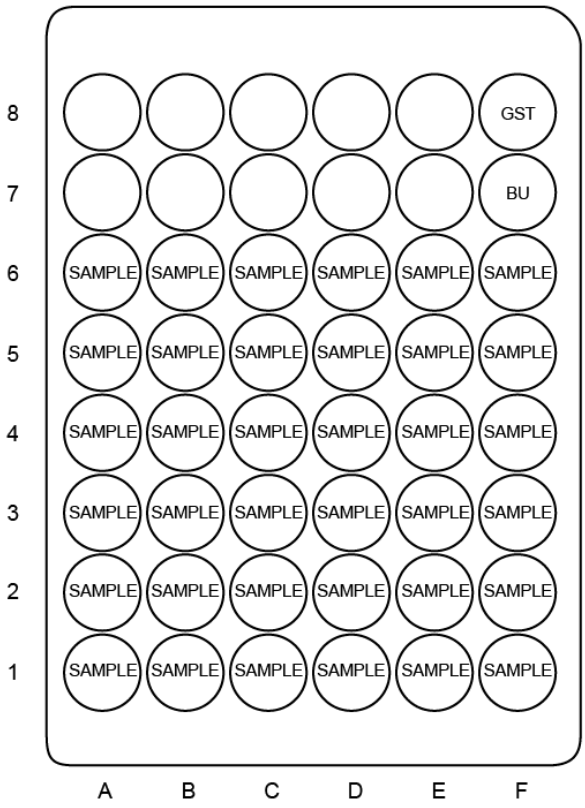


Note: During electrophoresis the ionic strength of the buffer will change. The separation method is programed to increment the buffer vials after 16 runs to avoid ionic depletion.

Load the Sample Tray

1. Prepare and load the bracketing standard.
   a. Add 30 µL of DDI water to one 20 µL aliquot of the previously-reconstituted bracketing standard and then vortex the vial to mix. Refer to Reconstitute the Bracketing Standard (BST).
   b. Put a 200 µL micro vial in a universal vial.
   c. Transfer all of the diluted bracketing standard to the micro vial.
   d. Put a blue cap on the universal vial.
   e. Put the universal vial in the sample tray in position SI:F8 as shown in Figure 3.
2. Prepare and load the samples. For each sample:
   a. If necessary, gently thaw the sample.
   b. Transfer at least 50 µL of the sample to a sample vial in the sample tray. Start at position SI:A1 and fill from A1 to F6.

Figure 3 Sample Tray Layout



3. (Optional) Load the GU ladder standard.

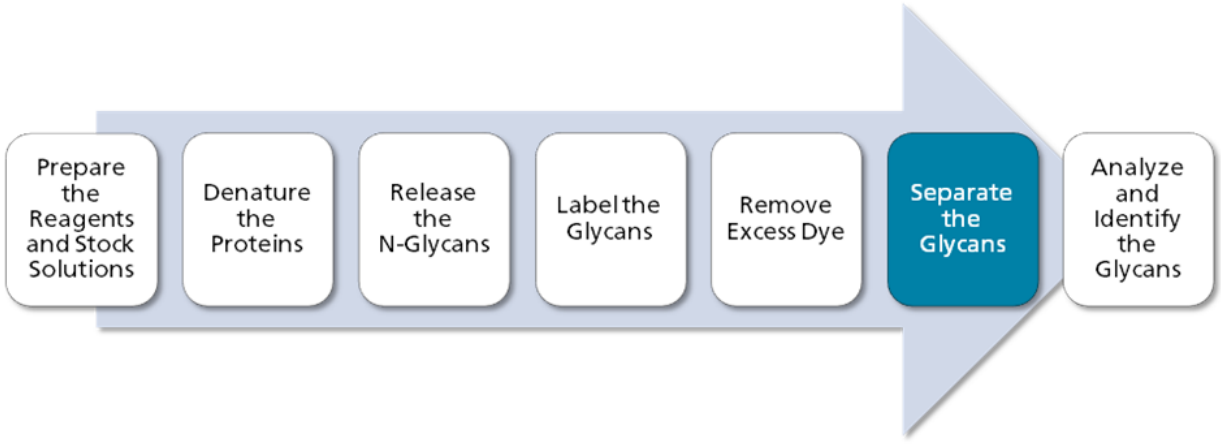
   The GU ladder standard serves as the process control.

   a. Put the micro vial containing the GU ladder standard in a universal vial.

   b. Put a blue cap on the universal vial.

   c. Put the universal vial in position SI:F7 in the sample tray as shown in Figure 3.

Separate the Glycans



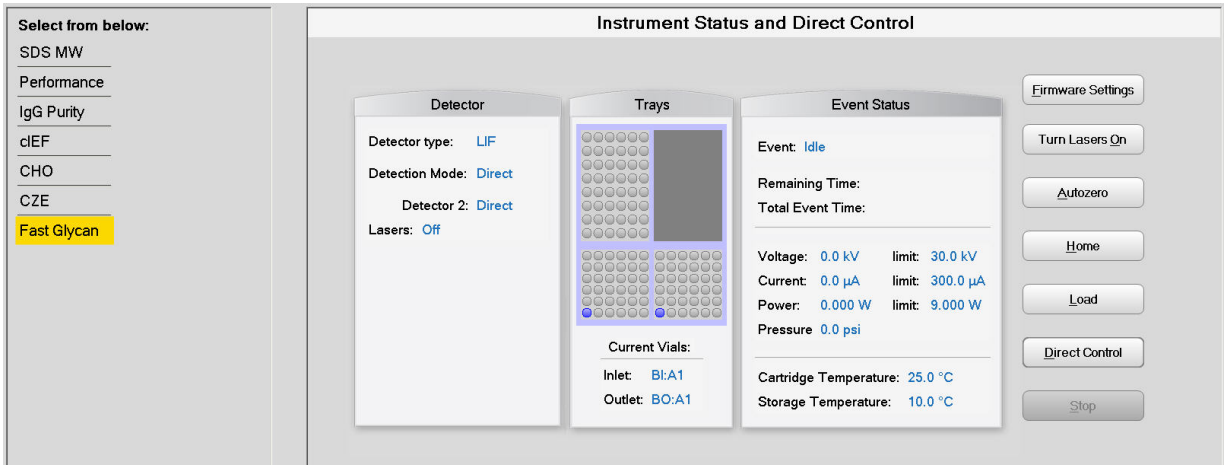
Create the Sequence and Start the Run

Note: For Empower™ Software users, refer to 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 of the window.
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 4 Instrument Status and Direct Control Page

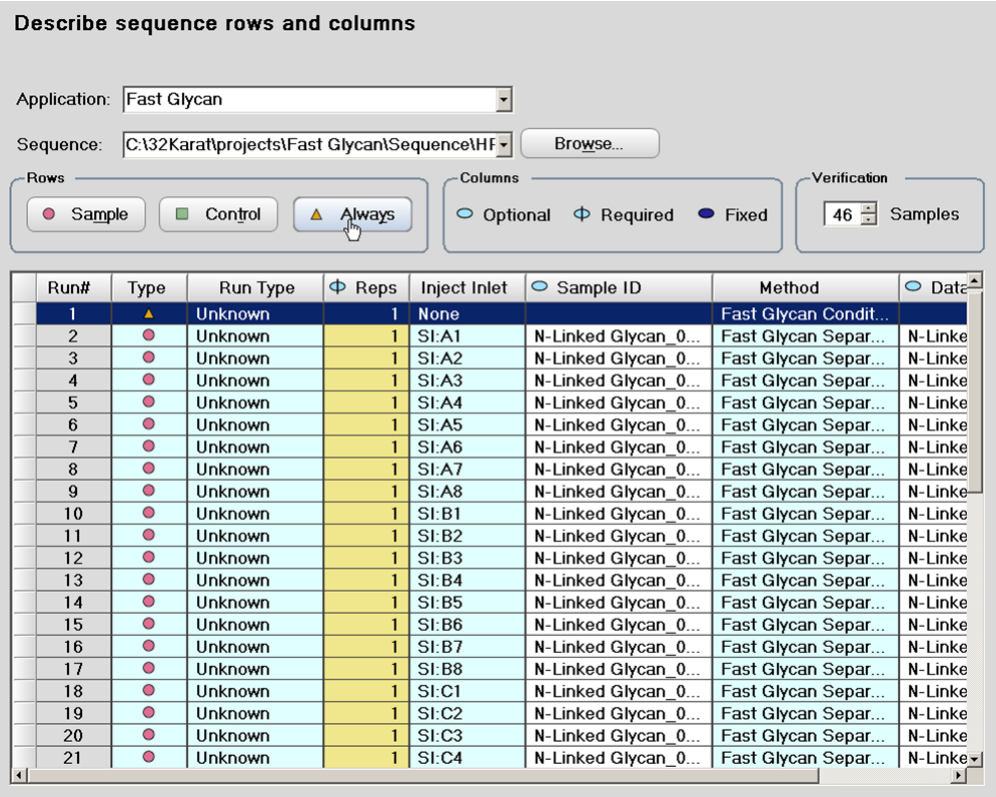


4. Click (**Describe**).
5. In the **Application** list, click **Fast Glycan**. In the **Sequence** list, click **Browse** and then browse to the **Fast Glycan** sequence. If prompted, type a user name and password.

   The page updates to show the selected sequence and all 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** area.
   b. Click the last row (with the Fast Glycan Shutdown method) to select it and then click Always (**Always**) in the **Rows** area.

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

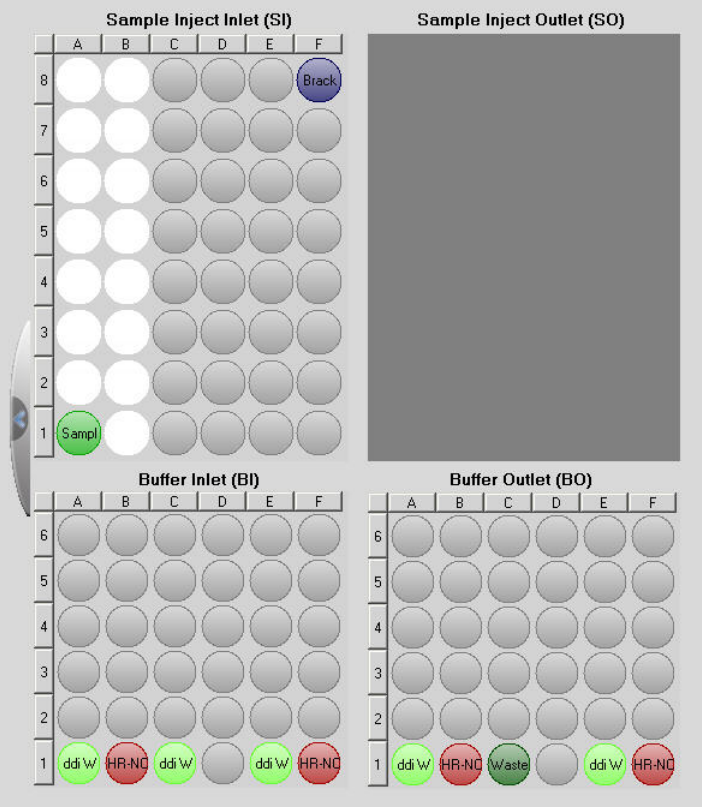
Figure 5 Describe sequence rows and columns Page—Conditioning Method Set to "Always"



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 6, one row of reagents is required for 16 samples.

Figure 6 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 7 Samples Loaded Prompt

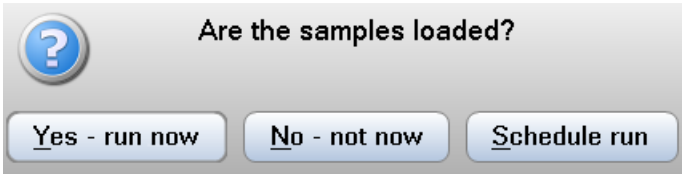
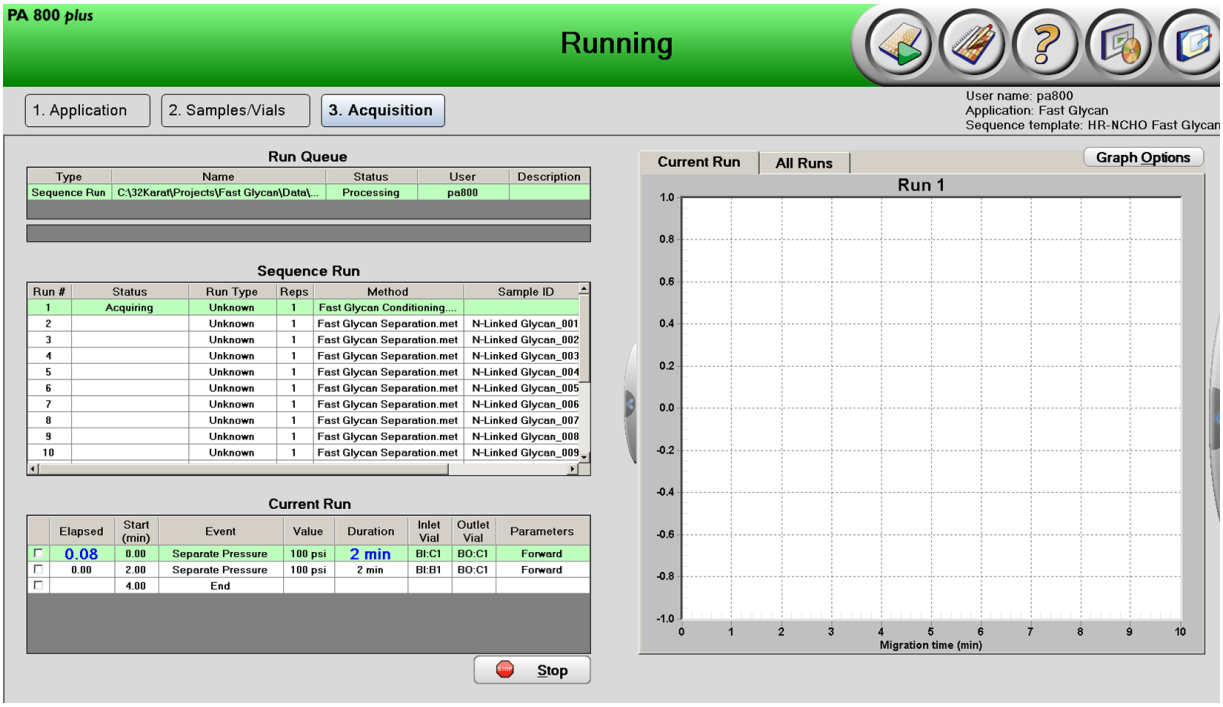


Figure 8 PA 800 Software During Data Acquisition



Waste Disposal

WARNING! Biohazard or Toxic Chemical Hazard. Follow local directives when disposing of chemicals, vials and caps, and the remains of the prepared samples, if applicable. They might contain regulated compounds and biohazardous agents.

Store the Cartridge

Store the Cartridge for Less Than 10 Days

1. Perform the shutdown method to clean the capillary.

   The shutdown method fills the capillaries with water.

2. Store the cartridge for up to 10 days in the system with the capillary ends immersed in vials of DDI water.

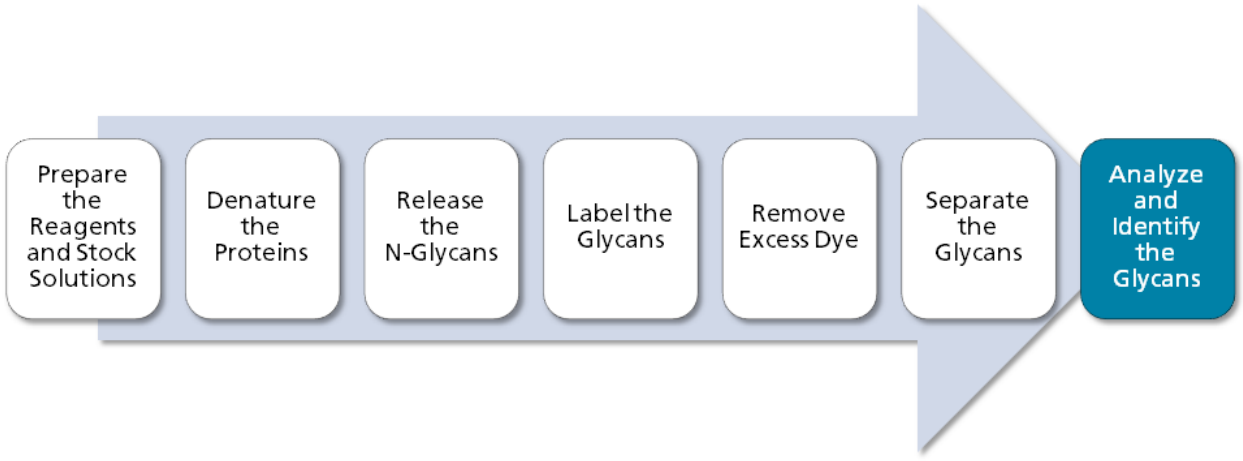
Store the Cartridge for More Than 10 Days

1. Perform the shutdown method to clean the capillary.
2. Rinse the capillary with DDI water for 10 min at 100 psi.
3. Remove the cartridge from the system.
4. Store the cartridge upright in the cartridge box at room temperature, with the capillary ends immersed in vials of DDI water.

Prepare the Cartridge After Storage

- If the cartridge has not been used for more than a day or it has been stored for an extended time, then condition the capillary using the Fast Glycan Conditioning method.

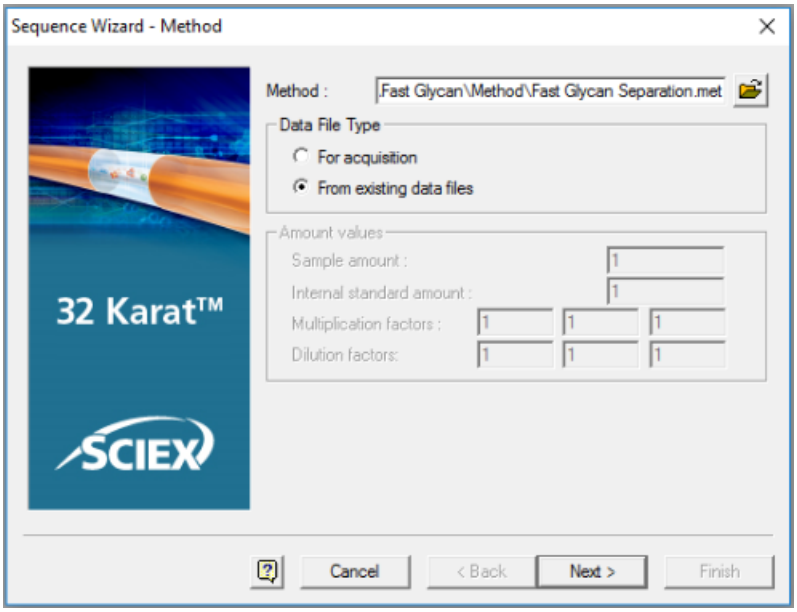
Analyze and Identify the Glycans



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 9 Method Page



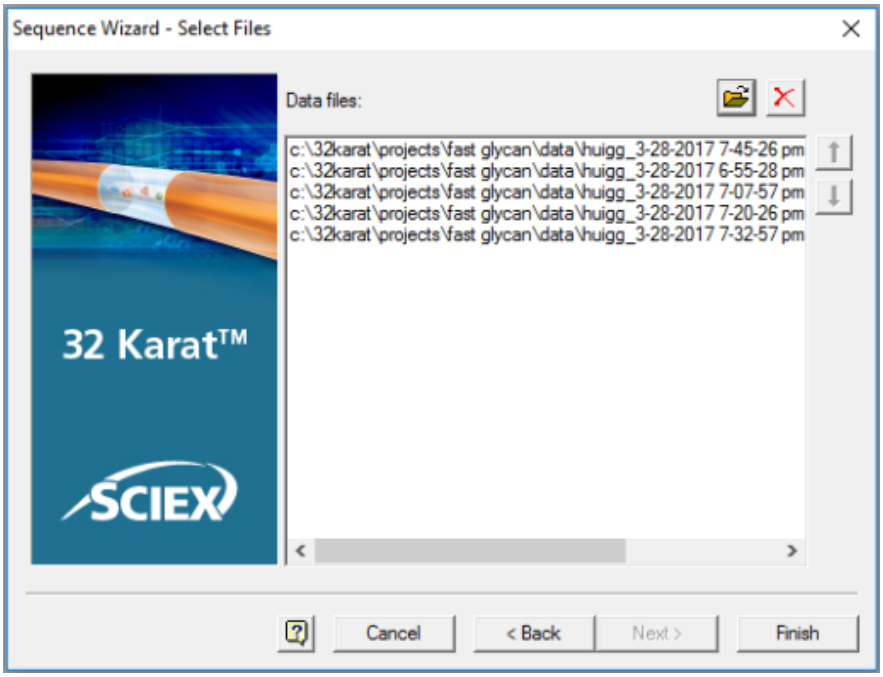
d. Click **Next**.

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 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 10 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.

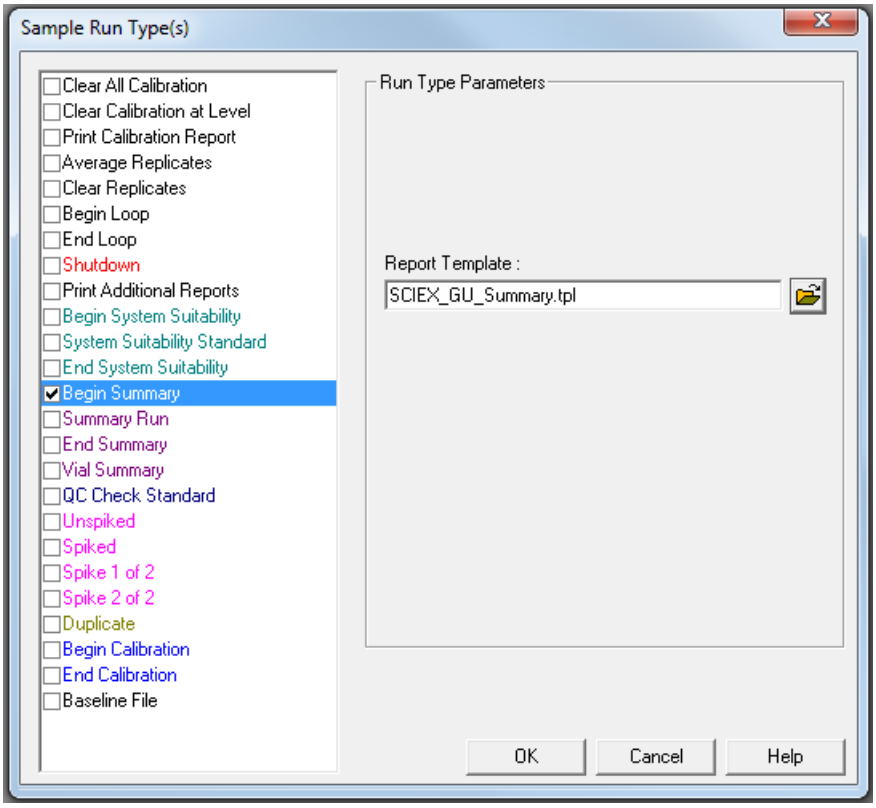
5. Click 

in the first row of the sequence.

The Sample Run Type(s) dialog opens.

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

Figure 11 Sample Run Type(s) Dialog

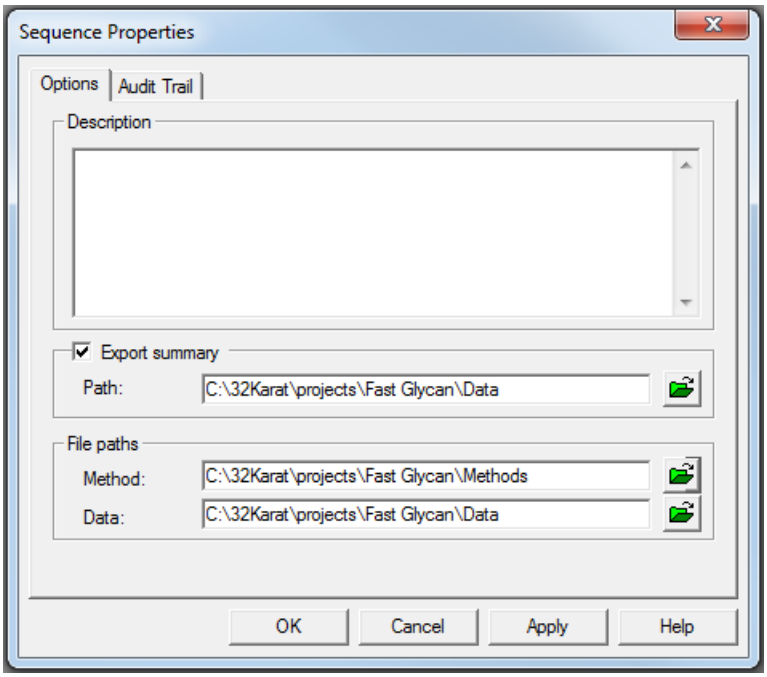


7. Click **Sequence > Properties** .

   The Sequence Properties dialog opens.

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

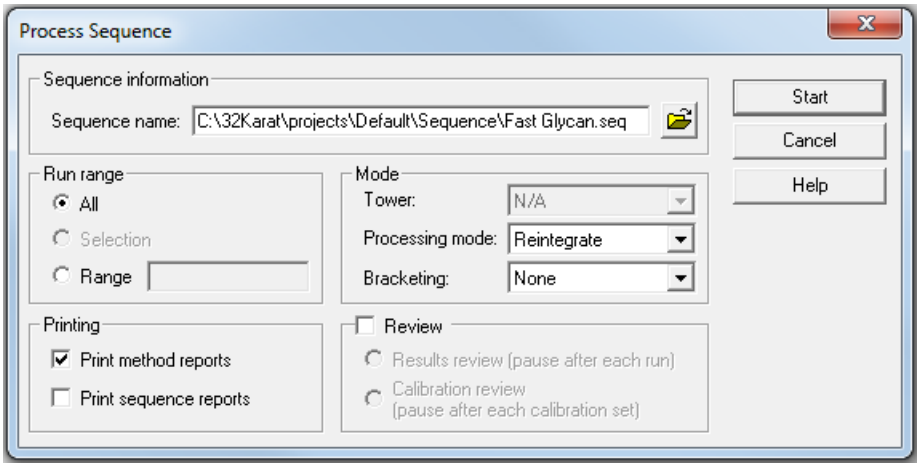
Figure 12 Sequence Properties Dialog



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 13 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 reports are created, in PDF format :

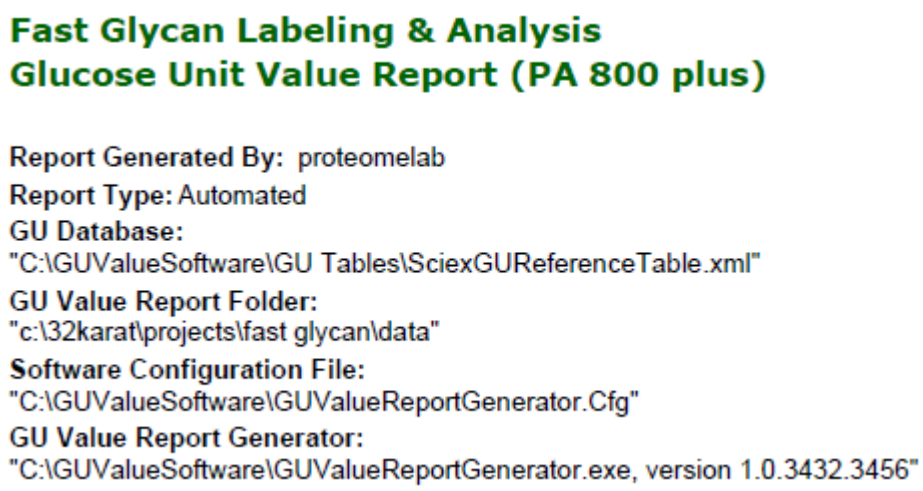
- The GU Value Report, which opens automatically at the end of the analysis. This report is saved to the folder specified in the **Data** folder in the Sequence Properties dialog and is named GU Value Report v1.0_MMDDYYYY_HHMMSS.pdf, where *MMDDYYYY* is the date and *HHMMSS* is the time the file was 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.

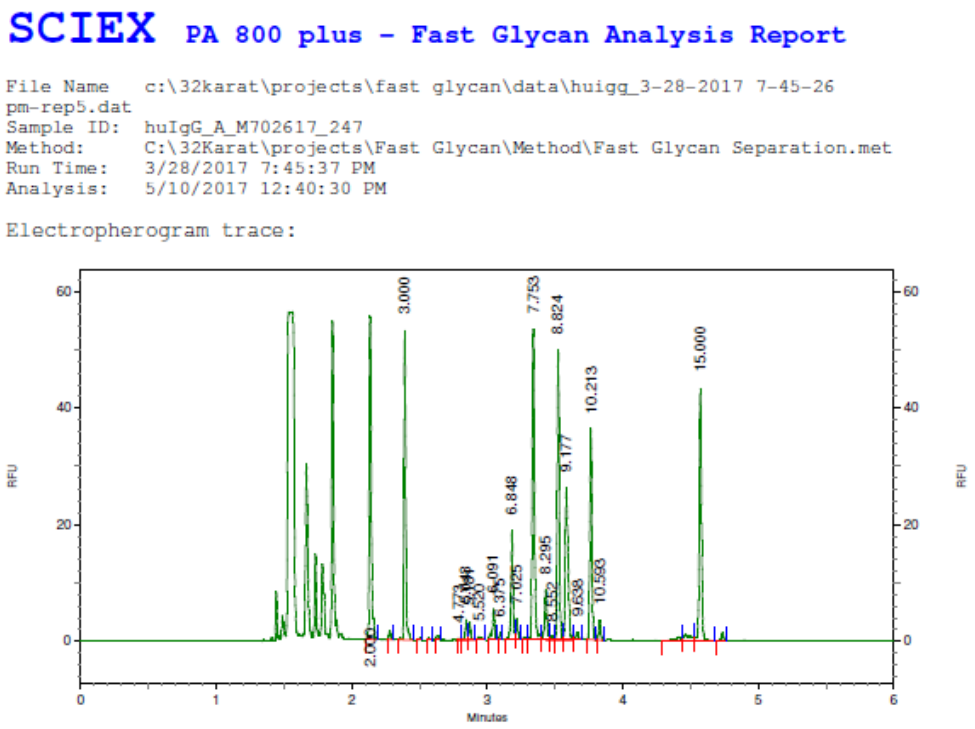
Figure 14 GU Value Report—Report Heading



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 (Figure 15) gives information including sample file name, sample ID, location of the file, and the location and name of the method.

Figure 15 Method Report—Heading and Electropherogram



The second part of the report (Figure 16) 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.

Figure 16 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 (Figure 17, Figure 18, and Figure 19) which shows structures for all of the glycans that the GU Value can identify.

Figure 17 Translation Table

Oxford and Composition Letter Nomenclature Translation Table

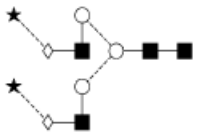
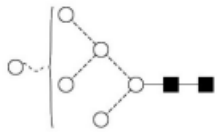
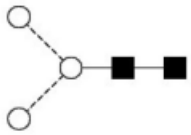
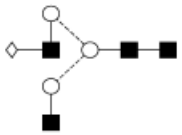
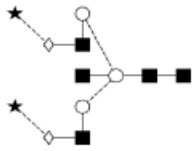
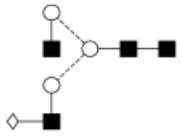
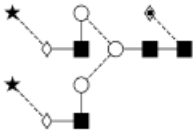
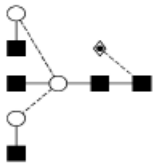
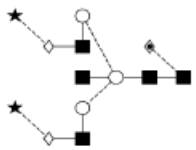
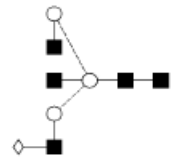
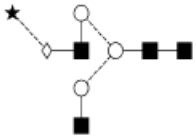
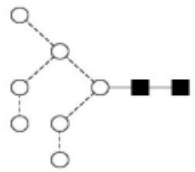
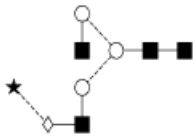
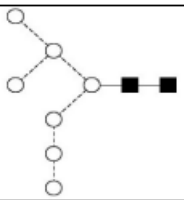
Oxford ID* (composition**)	Glycan Name	Glycan Structure	Oxford ID* (composition**)	Glycan Name	Glycan Structure
A2G2S2 (H5N4A2)	G2S2		M6 (H6N2)	Man-6	
M3 (H3N2)	Man-3		A2(6)G1 (H4N4)	G1	
A2BG2S2 (H5N5A2)	G2BS2		A2(3)G1 (H4N4)	G1’	
FA2G2S2 (H5N4F1A2)	G2FS2		FA2B (H3N5F1)	G0FB	
FA2BG2S2 (H5N5F1A2)	G2FBS2		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 18 Translation Table (continued)

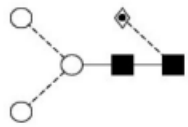
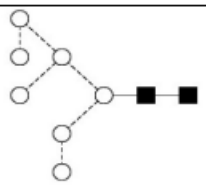
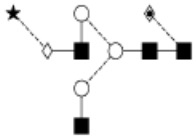
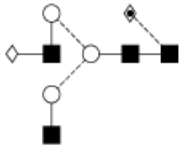
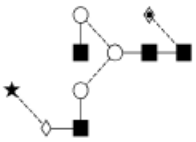
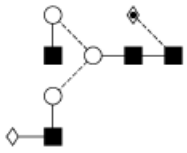
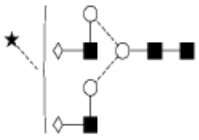
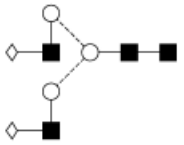
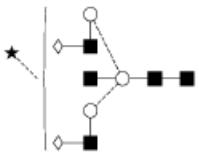
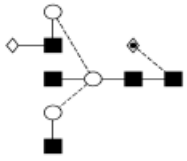
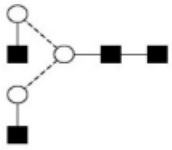
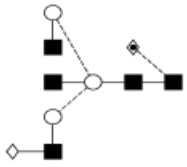
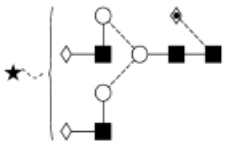
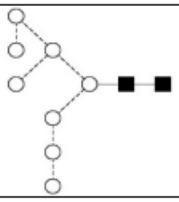
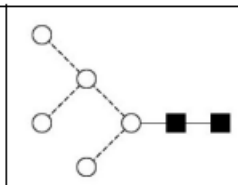
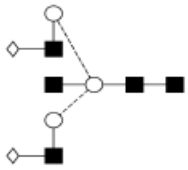
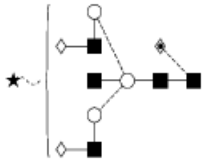
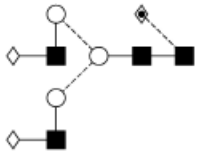
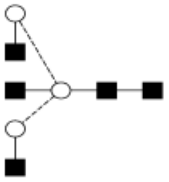
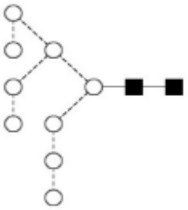
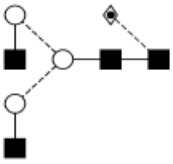
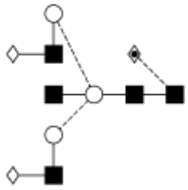
FM3 (H3N2F1)	Man-3F		M7[D1] (H7N2)	Man-7[D1]	
FA2(6)G1S1 (H4N4F1A1)	G1FS1		FA2(6)G1 (H4N4F1)	G1F	
FA2(3)G1S1 (H4N4F1A1)	G1'FS1		FA2(3)G1 (H4N4F1)	G1'F	
A2G2S1 (H5N4A1)	G2S1		A2G2 (H5N4)	G2	
A2BG2S1 (H5N5A1)	G2BS1		FA2B(6)G1 (H4N5F1)	G1FB	
A2 (H3N4)	G0		FA2B(3)G1 (H4N5F1)	G1'FB	
FA2G2S1 (H5N4F1A1)	G2FS1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

Figure 19 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.	1. Make sure that the probe is properly connected to the clamp bar on the LIF detector. Refer to the *System Maintenance Guide*. 2. Open the separation method in the software and then make sure that: • The voltage is correct. • The **Reverse** polarity check box 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. Centrifuge 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 DDI water. • Clean the aperture in a water bath. After cleaning, assemble the cartridge and then run the samples again.

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 starting at 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 min and that the temperature of the heat block is 60 °C. 2. Inspect the peak intensity for the IST peak. If it is greater than 50 RFU, then digest the sample again, adding more PNGase F enzyme or using 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 using 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	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.	1. Open the separation method in the software and then make sure that the capillary temperature is correct. 2. Inspect 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 plugged. • Make sure that aperture plug is seated in the cartridge and that the probe guide is securely attached to the plug. Refer to the *System Maintenance Guide*. 4. Fill clean vials with freshly prepared reagents, cover with clean caps, and then replace the vials in the tray.
Shifts in migration time between runs on the same day	The capillary was not properly equilibrated.	Perform 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 for 2 minutes at 75 psi with DDI water.
Carryover	The vials or caps are contaminated.	Replace the buffer vials with clean vials filled with buffer, cover with clean caps, and then increment as necessary. Replace the water vials with clean vials filled with DDI water, cover with clean caps, and then increment as necessary. Make sure that the waste vials contain 1.0 mL of water and are present 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.	Sonicate the buffer for 10 sec to 20 sec to remove air bubbles.

Symptom	Possible Cause	Corrective Action
Extra peaks	The L6 reagent reacted with contaminants in the vials.	Use new micro vials, especially for steps related to labeling.
Fewer small glycans (DP < 5) than expected	Small glycans were washed away during dye removal.	Perform fewer wash steps during the dye removal step.
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 *GU Value Software Release Notes*.
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 *GU Value Software Release Notes*.

Hazardous Substance Information A

The following information must be noted and the relevant safety measures taken. Refer to the respective safety data sheets for more information. These are available upon request or can be downloaded from our website sciex.com/tech-regulatory.

Hazard classification according to HCS 2012.

BST Bracketing Standard

WARNING! Causes serious eye irritation. Causes skin irritation. May cause respiratory irritation.

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
- GU glucose ladder
- IST internal standard
- L6 fluorophore
- M1 magnetic 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 using the PA 800 Plus System with the PA 800 Plus and 32 Karat™ Software. If the system is used with the Empower™ Software, then the methods are different. Refer to Run the Samples with the Waters Empower™ Software.

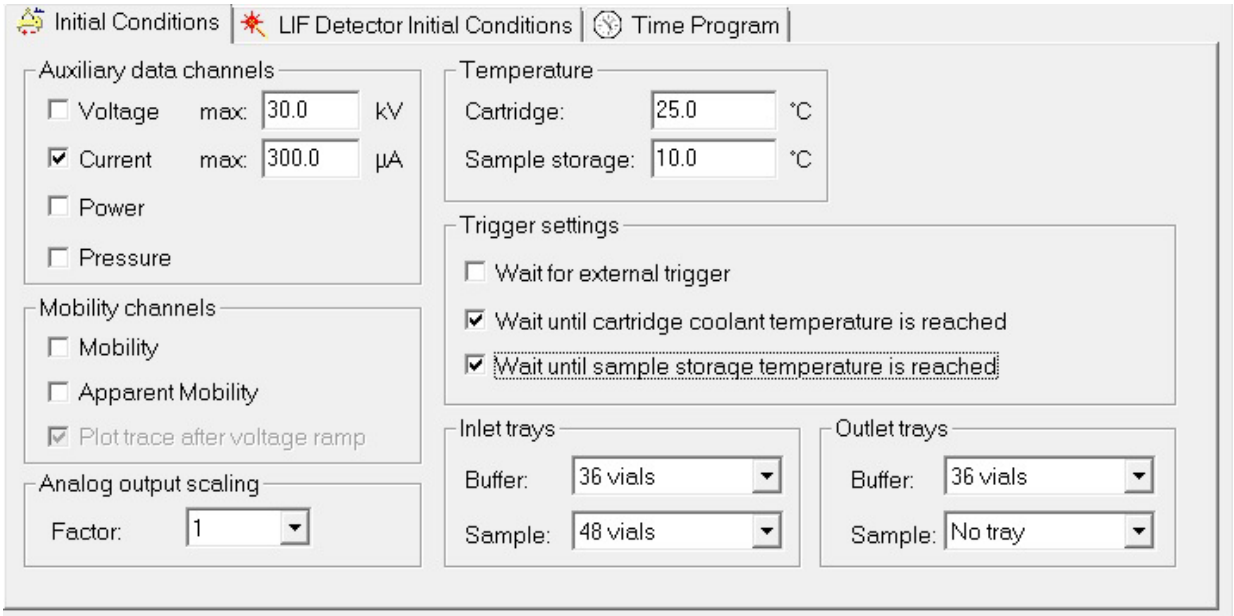
The Fast Glycan Labeling and Analysis application requires three methods.

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.

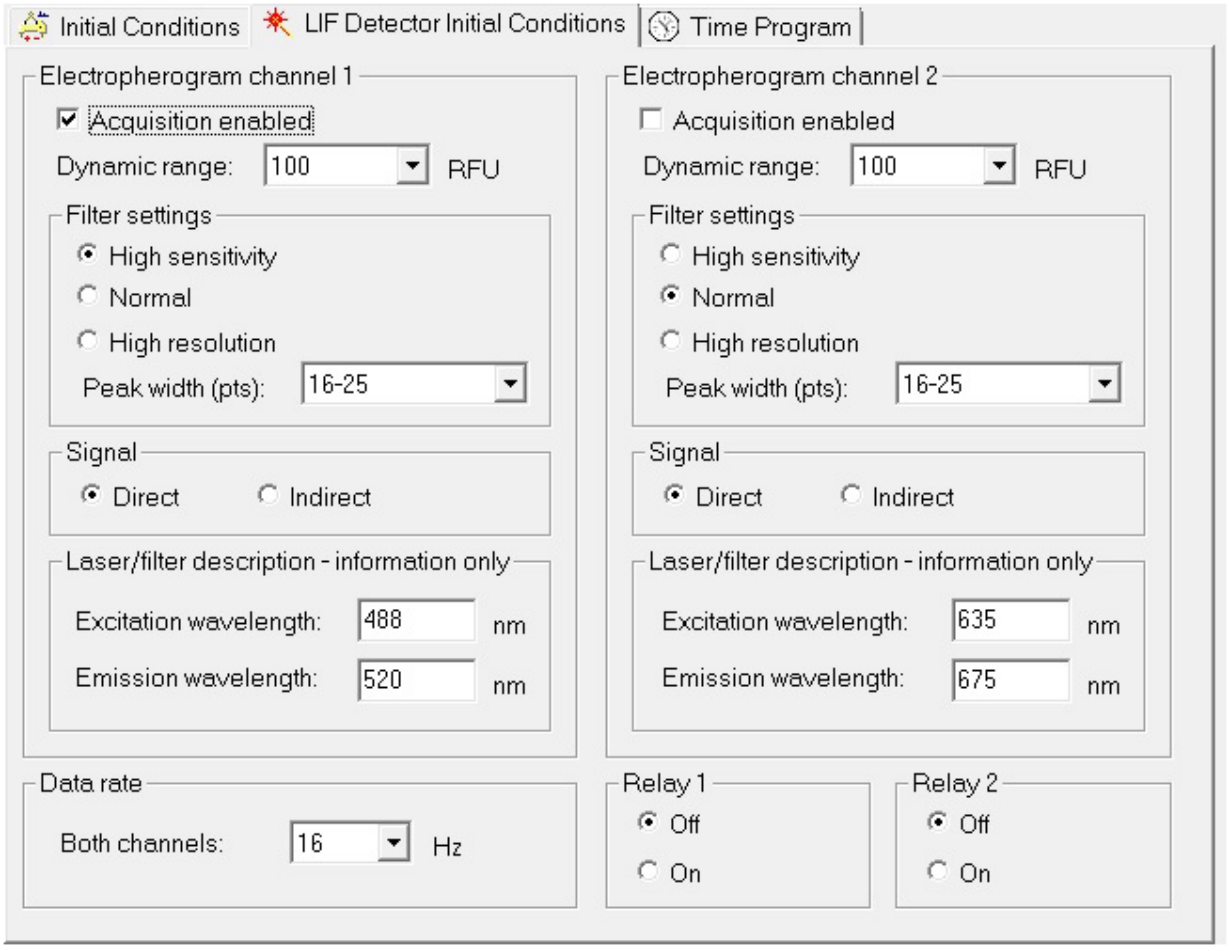
Initial Conditions

Figure B-1 Initial Conditions Tab for All Methods



Detector Initial Conditions

Figure B-2 LIF Detector Initial Conditions Tab for All Methods



Time Programs

The time programs are different for each method.

Figure B-3 Time Program for the Fast Glycan Conditioning Method

Initial Conditions | LIF Detector Initial Conditions | Time Program

	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								

Figure B-4 Time Program Tab Tab for the Fast Glycan Separation Method

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								

Figure B-5 Time Program Tab for the Fast Glycan Shutdown Method

Initial Conditions | LIF Detector Initial Conditions | Time Program

	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								

Figure B-6 Time Program Tab for the Fast Glycan GU Ladder Separation Method

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:B6	BO:C6	forward	Rinse with HR-CHO capillary
2		Wait		0.10 min	BI:A6	BO:A6		Capillary Tip Wash
3		Inject - Pressure	1.0 psi	5.0 sec	BI:C6	BO:C6	No override, forward	Water Plug Injection
4		Inject - Voltage	1.0 KV	5.0 sec	SI:F7	BO:B6	No override, reverse polarity	Sample Injection
5		Wait		0.10 min	BI:A6	BO:A6		Capillary Tip Wash
6		Inject - Pressure	1.0 psi	5.0 sec	BI:B6	BO:B6	No override, forward	HR-Separation Gel Push
7	0.00	Separate - Voltage	30.0 KV	6.00 min	BI:F6	BO:F6	0.10 Min ramp, reverse polarity, both	Separation Reversed Polarity with 0.5 psi pressure applied on both sides
8	0.50	Autozero						
9	6.00	Stop data						
10	6.10	Wait		0.10 min	BI:A6	BO:A6		Capillary Tip Wash
11	6.20	End						
12								

Run the Samples with the Waters Empower™ Software C

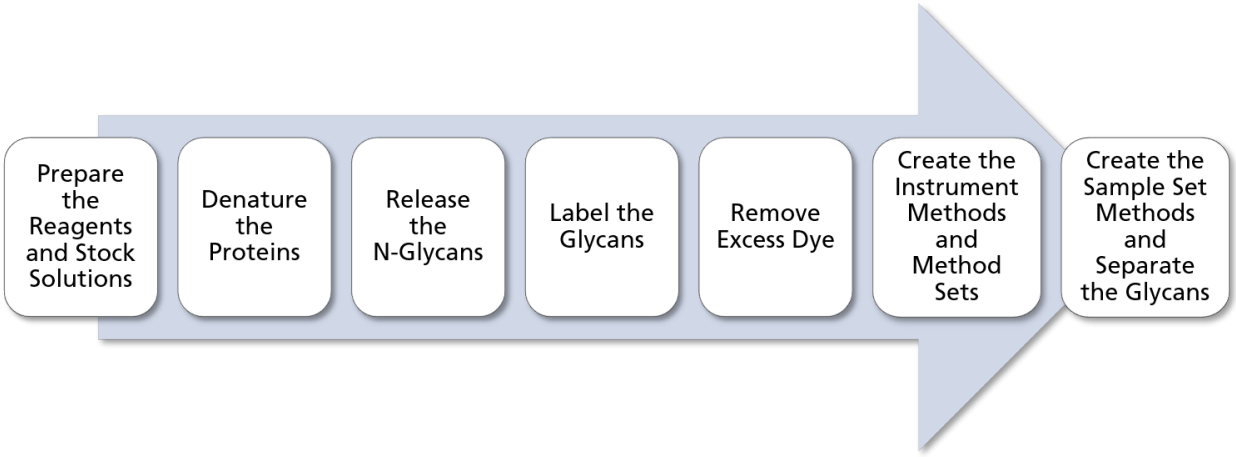
This section gives instructions on data acquisition using the Empower™ Software.

Data analysis to determine the glucose unit (GU) values is not available in 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 Empower™ Software.
- In the Empower™ Software, create an export method and then export the data to the AIA format. In the 32 Karat™ and the GU Value Software, open and then analyze the exported data. Refer to Create an Export Method.

Tip! Add the export method to the method set for automatic export after data acquisition. Refer to the documentation supplied with the Empower™ Software.

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

Step	To do this...	Refer to...
4	Label the glycans	Label the Released N-Glycans
5	Remove excess dye	Remove the Excess Dye from the Samples
6	Create the instrument methods and method sets in the Empower™ Software	Create the Instrument Methods and then Create the Method Sets
7	Separate the glycans	Create the Sample Set Method and Run the Samples

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 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.

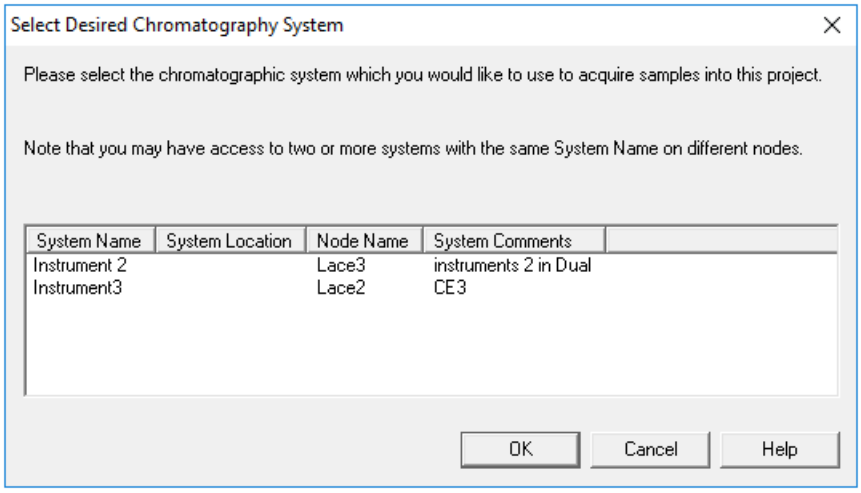
Note: The values on the General and Detector tabs are the same for all of the methods.

Note: Pressure values can be shown in millibar (mbar) or pounds per square inch (psi), depending on a registry setting for the Empower™ Software. The default unit is millibar. To change the units, refer to the *PA 800 Plus Empower™ Driver Release Notes.*

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

   The Select Desired Chromatography System dialog opens.

Figure C-1 Select Desired Chromatography System Dialog



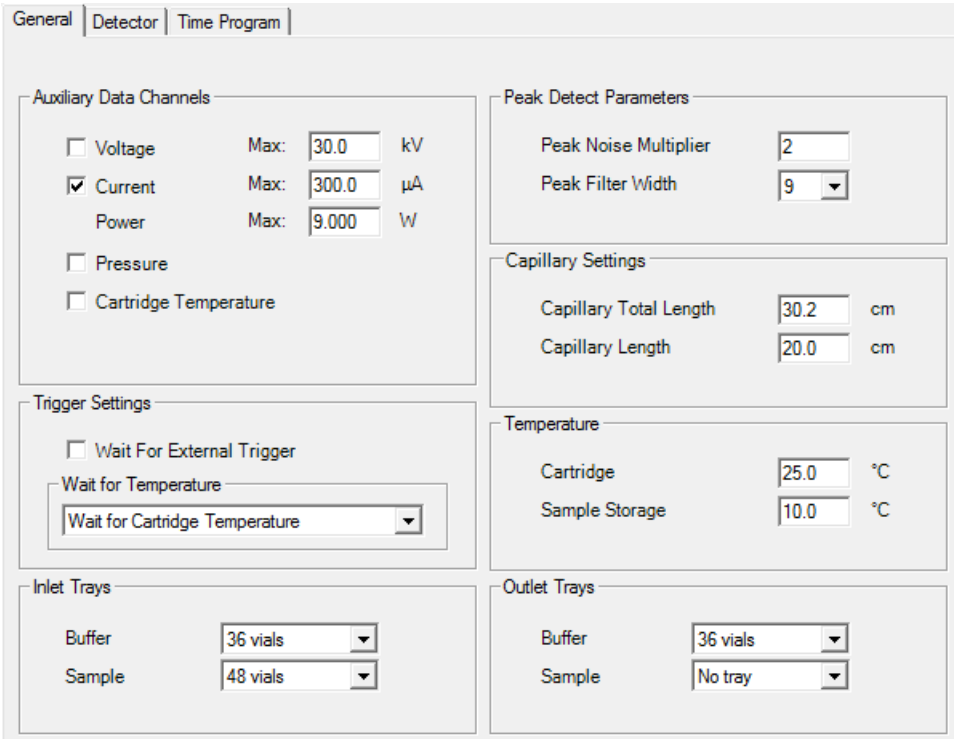
2. Click the system to be used and then click **OK**.

   Make sure that the instrument is configured with an LIF detector.

   The Instrument Method Editor opens.

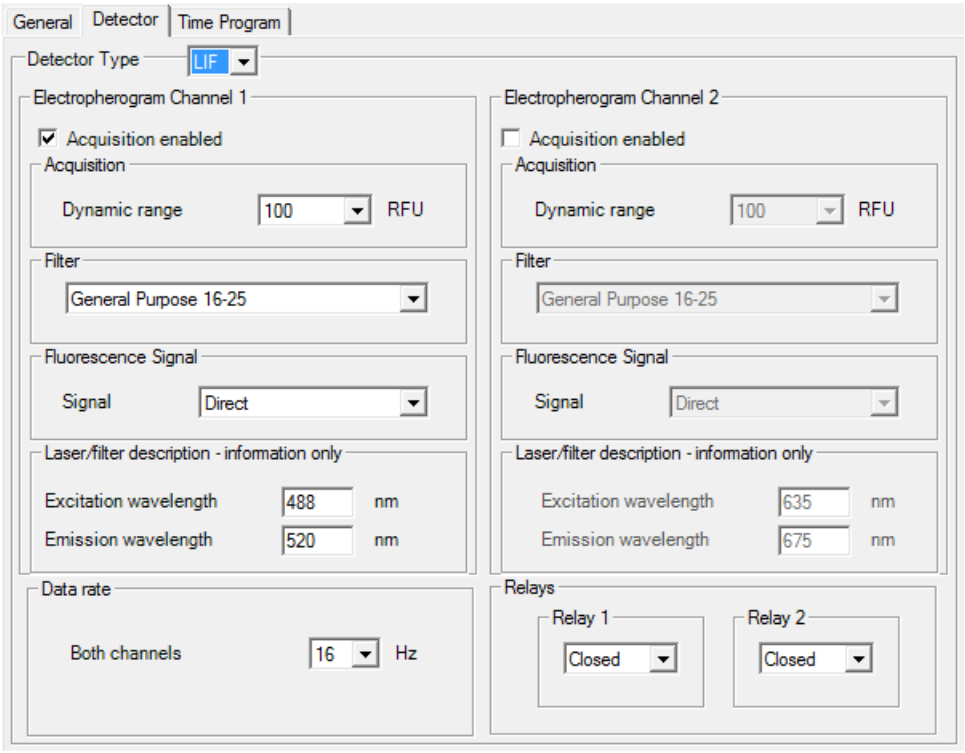
3. Set the parameters on the **General** tab.

Figure C-2 General Parameters for FAST GLYCAN_CONDITIONING Instrument Method



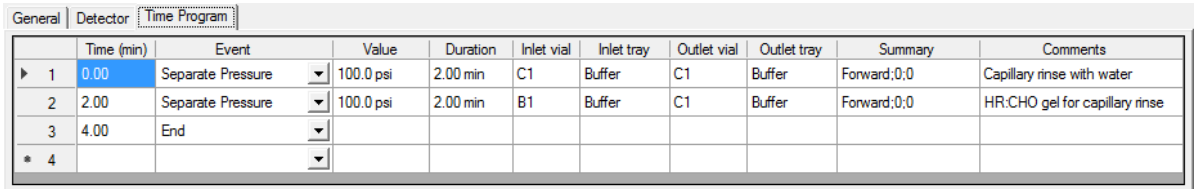
4. Click the **Detector** tab, click **LIF** in the **Detector Type** list, and then set the parameters.

Figure C-3 Detector Parameters for FAST GLYCAN_CONDITIONING Instrument Method



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

Figure C-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 is using 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 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 Figure C-2.

b. Set the parameters on the Detector tab. Refer to Figure C-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 C-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 is using 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.
- 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 Figure C-2.

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

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

Figure C-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 is using 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.

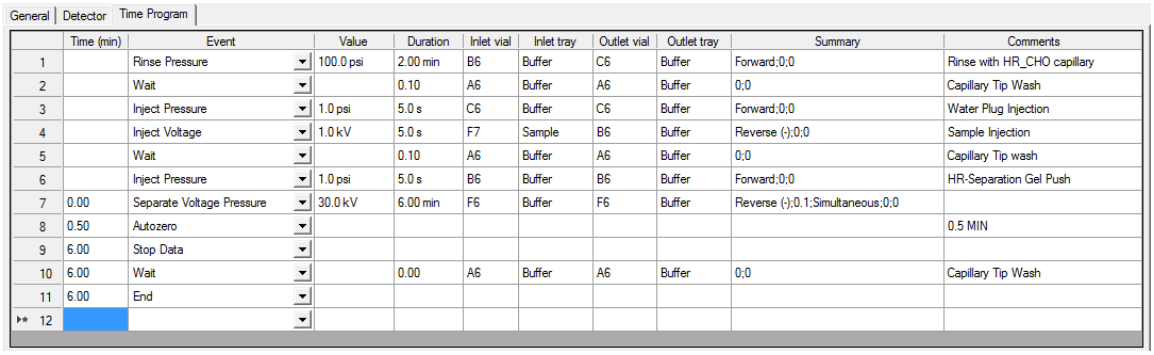
a. Set the parameters on the General tab. Refer to Figure C-2.

b. Set the parameters on the Detector tab. Refer to Figure C-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 C-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 is using 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 Method Set
- Fast Glycan Separation Method Set
- Fast Glycan Shutdown Method Set

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 Empower™ Software. To create an export method, refer to Create an Export Method.

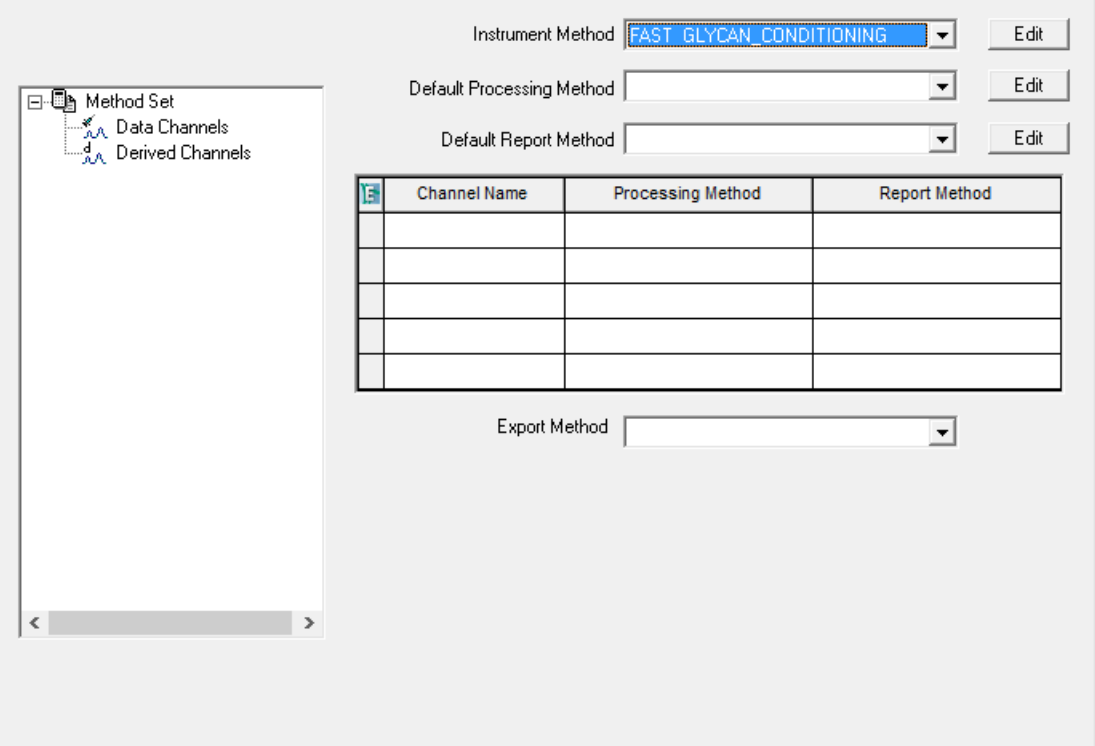
1. In the Empower™ Software Project window, click **File > New Method > Method Set**.

2. Click **No** in the message.

   The Method Set Editor window opens.

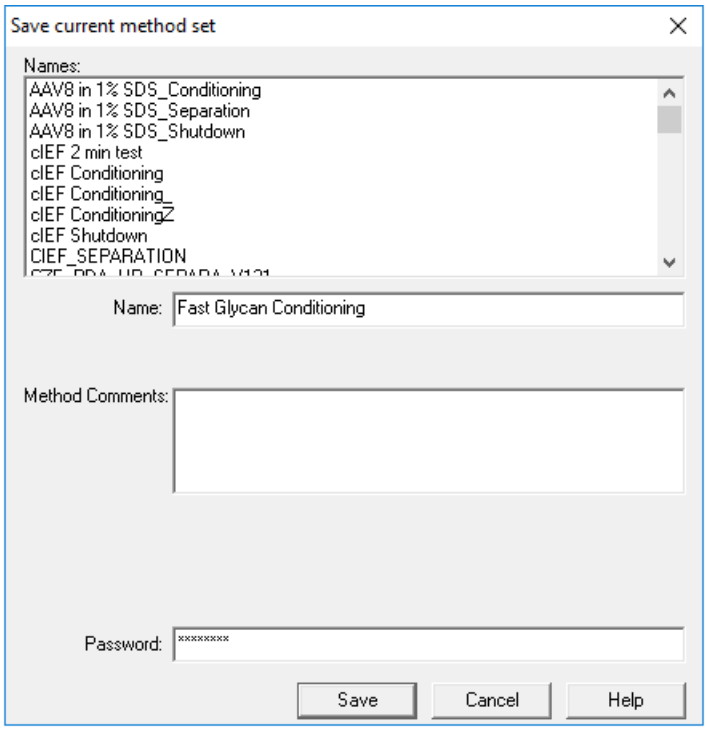
3. In the **Instrument Method** list, click **FAST GLYCAN_CONDITIONING**. Do not make any other changes.

Figure C-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 Empower™ Software password for the current user in the **Password** field and then click **Save**.

Figure C-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. Create the separation method set by selecting **FAST GLYCAN_SEPARATION** in the **Instrument Method** list. Save the method set as "Fast Glycan Separation".

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

      Note: Only export the data to analyze it using the GU Value Software.

   b. Create the shutdown method set by selecting **FAST GLYCAN_SHUTDOWN** in the **Instrument Method** list. Save the method set as "Fast Glycan Shutdown".
   c. (Optional) Create the GU ladder method set by selecting **GU LADDER_SEPARATION** in the **Instrument Method** list. Save the method set as "Fast Glycan GU Ladder".

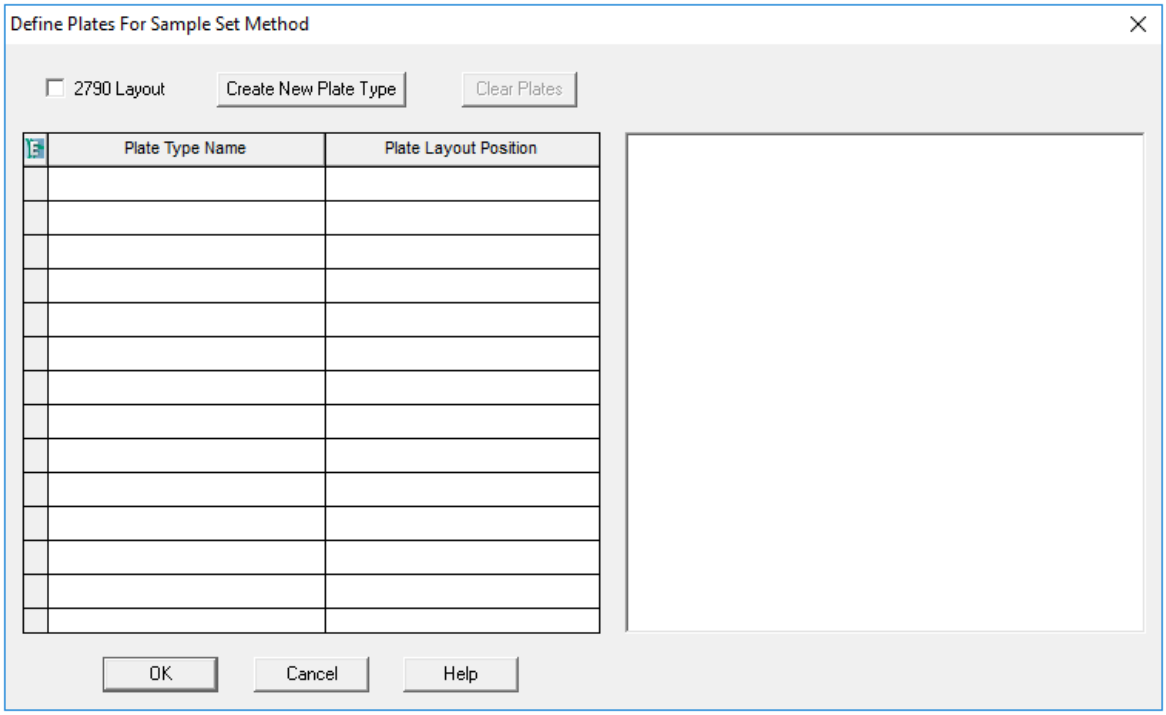
Configure the Software to Use Multiple Plates

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

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

   The Define Plates for Sample Set Method dialog opens.

Figure C-10 Define Plates for Sample Set Method Dialog



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

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

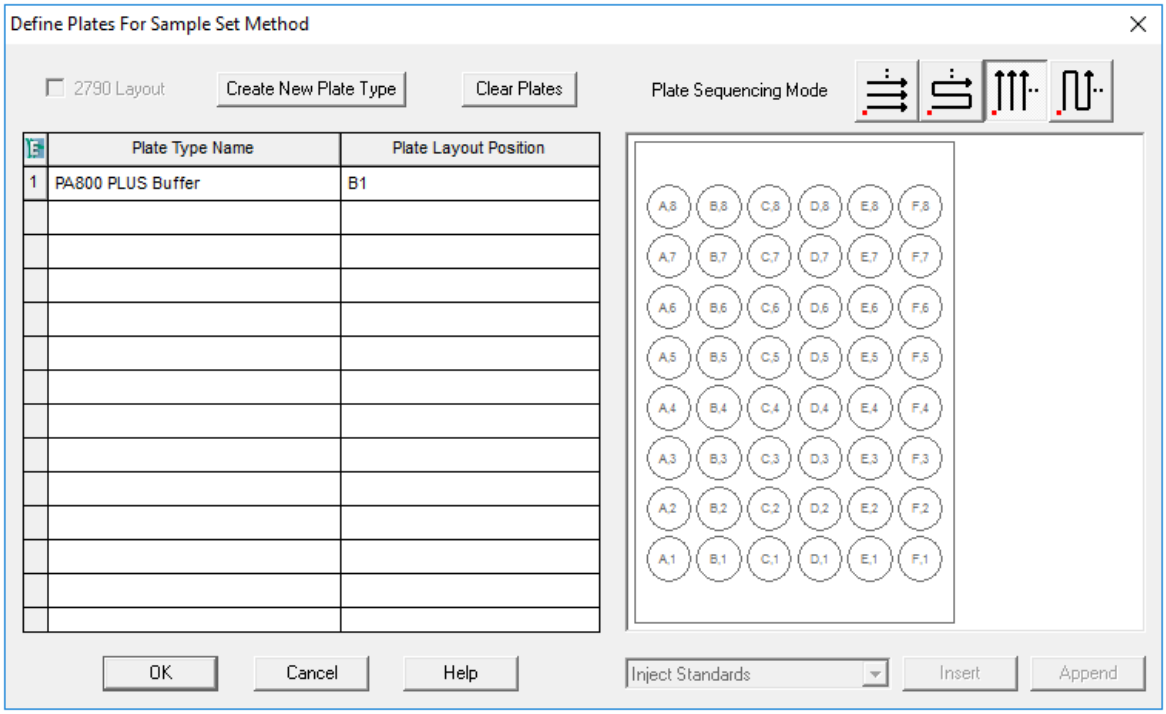
      Note: If **PA 800 Plus Buffer Tray** is missing, then the buffer and sample trays might not have been defined. Refer to the *PA 800 Plus Empower™ Driver User Guide*.

      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**.

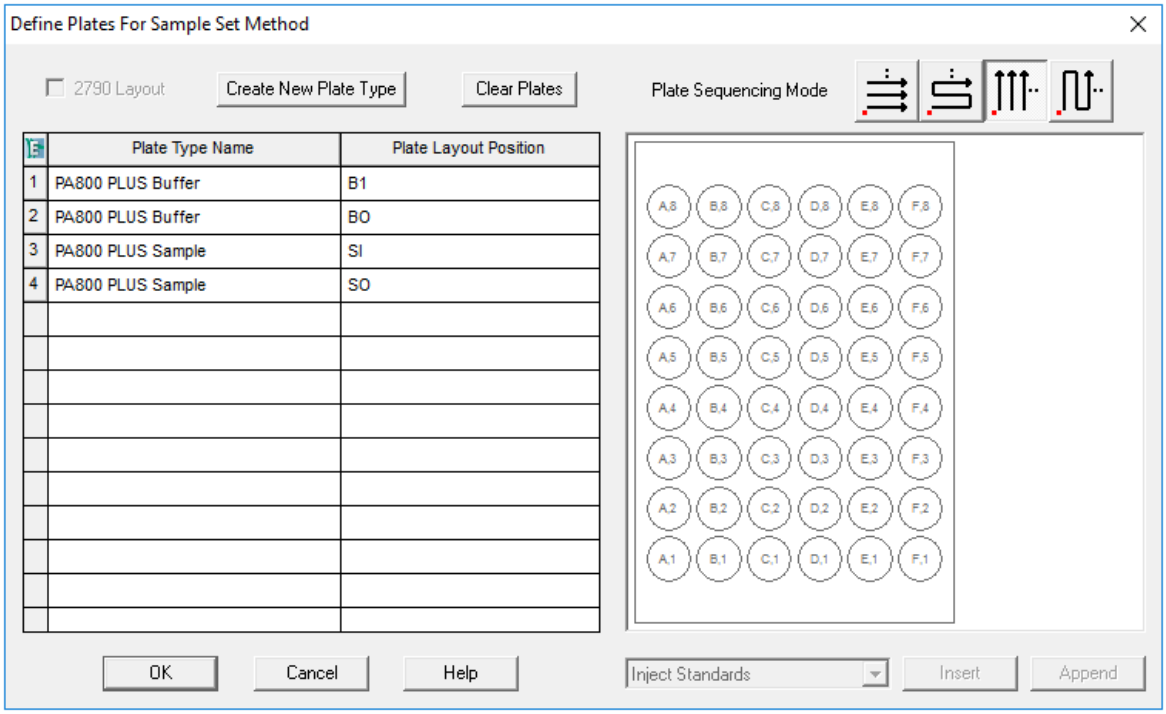
   c. Click  (**Vertical Discontinuous Plate Sequencing Mode**) to indicate the order in which the vials are accessed during the run.

Figure C-11 After Defining the Buffer Inlet Plate



3. Repeat step 2 to set up the buffer outlet tray in the second row. Type **BO** for the **Plate Layout Position**.
4. In the third row, set up 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 set up the sample outlet tray in the fourth row. Type **SO** for the **Plate Layout Position**.

Figure C-12 After Defining All Plate Types

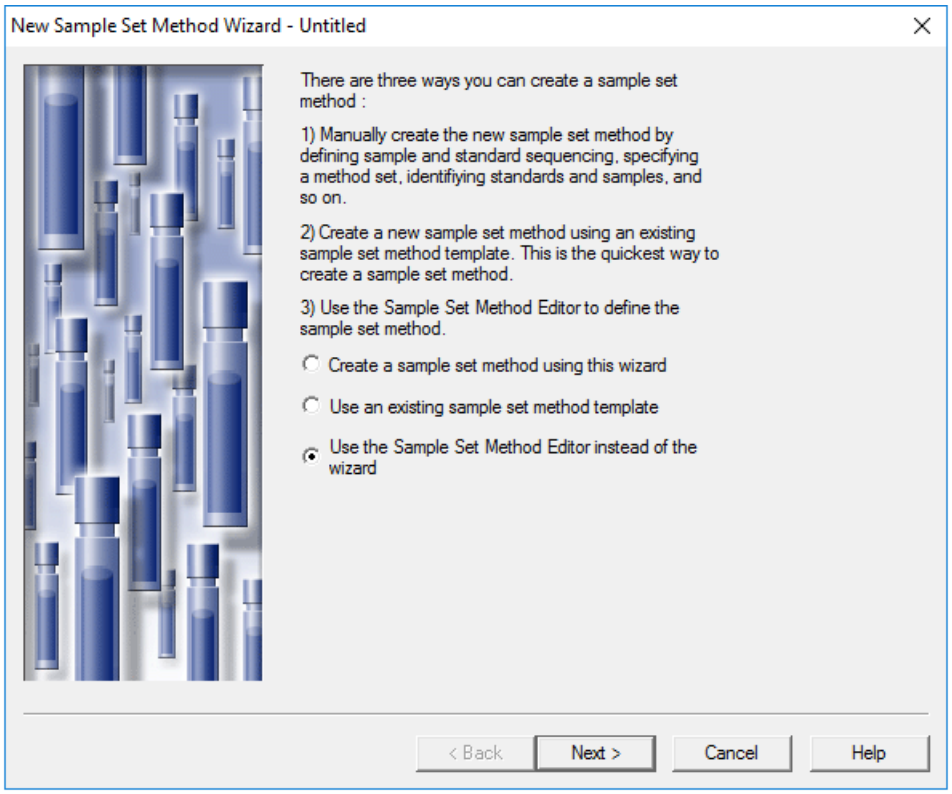


6. Click **OK** to save the changes and close the dialog.

Create the Sample Set Method and Run the Samples

1. In the 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 C-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 corresponding 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 Table C-1.

Use the default values for the other fields. Create an Export Method

Table C-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: Possible Wrong Result. Make sure that the Run Time is greater than or equal to the duration of the time program in the instrument method. If the Run Time is shorter, 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 C-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 using the GU Value Software.

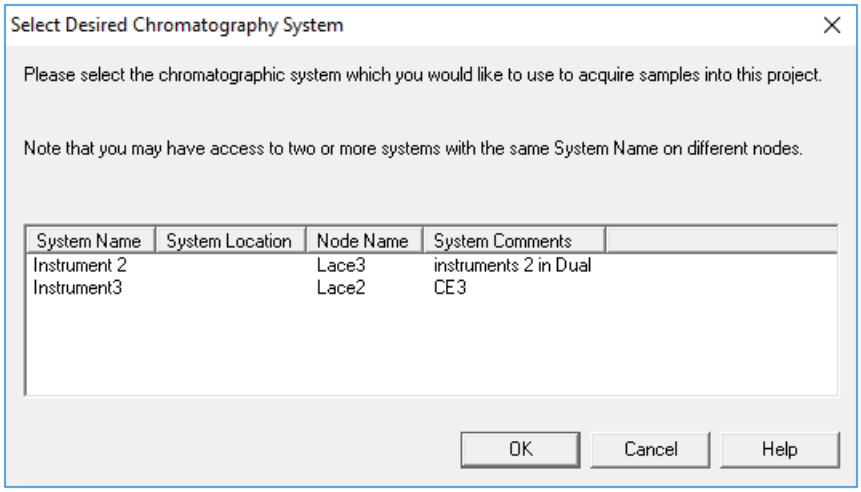
   Note: If the separation method set already includes the export method, skip this step and go to step 5.

   a. Create an export method. Refer to 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 needed, 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 prompted, type the 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 C-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**).

   The Load Samples dialog opens.

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

Figure C-16 Load Samples Dialog

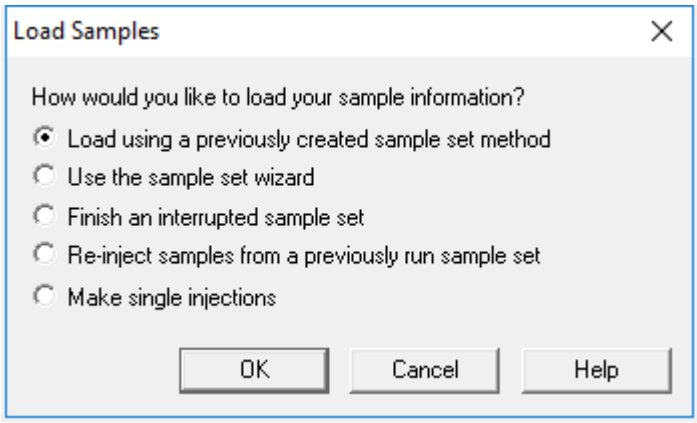
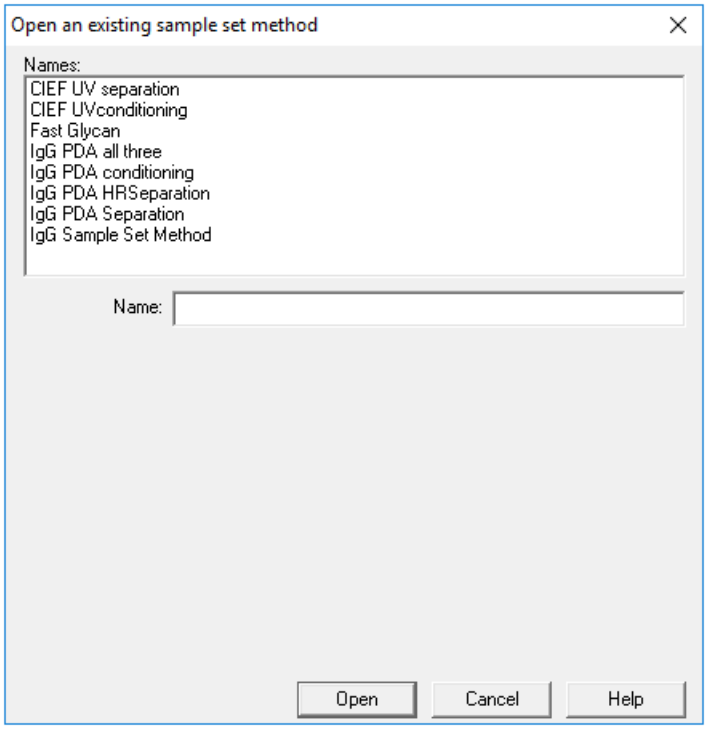


Figure C-17 Open an existing sample set method Dialog



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 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. During the run, the following actions are available:

    - (Optional) Click  (**Stop**) to halt data acquisition.
    - View the voltage and current data.

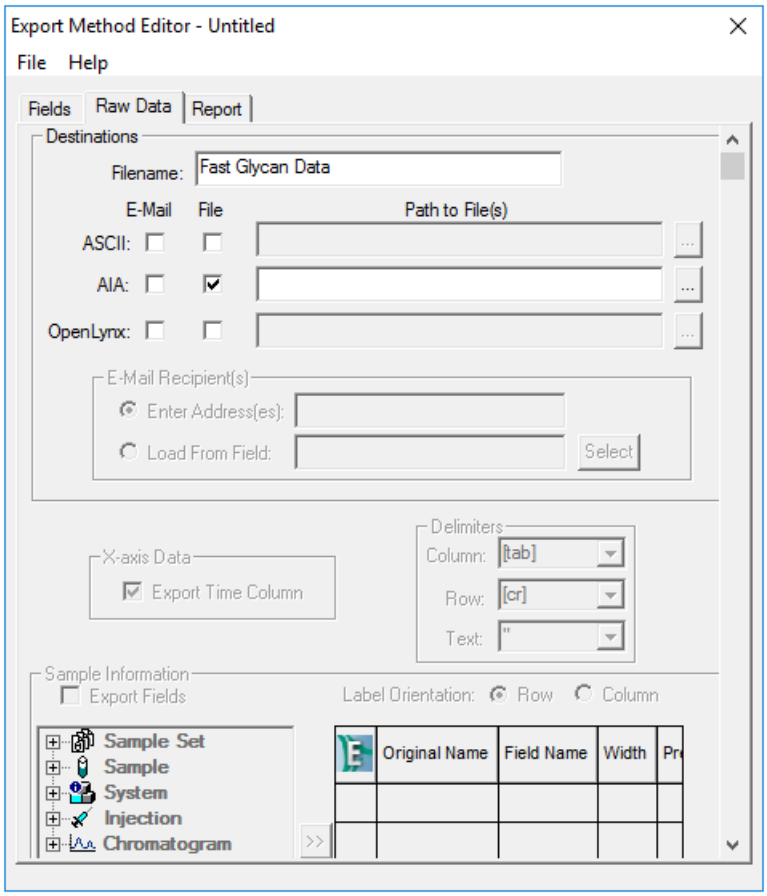
    When the run ends, the text in all rows in the Sample Set Method window is red.

Create an Export Method

Note: Only export the data to analyze it using the GU Value Software.

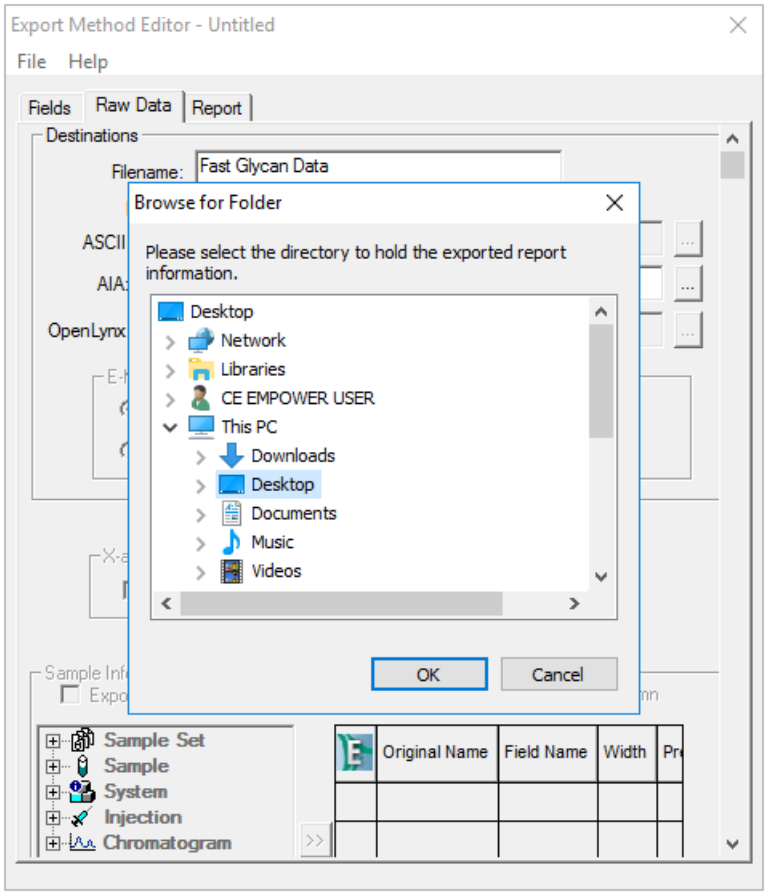
1. In the Empower™ Software Project window, click **File > New Method > Export Method**.
2. Click the **Raw Data** tab and then type a name for the export file in the **Filename** field, up to 32 characters.
3. In the **AIA** row, click **File**.

Figure C-18 Export Method Editor Window



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

Figure C-19 Browse for Folder Dialog

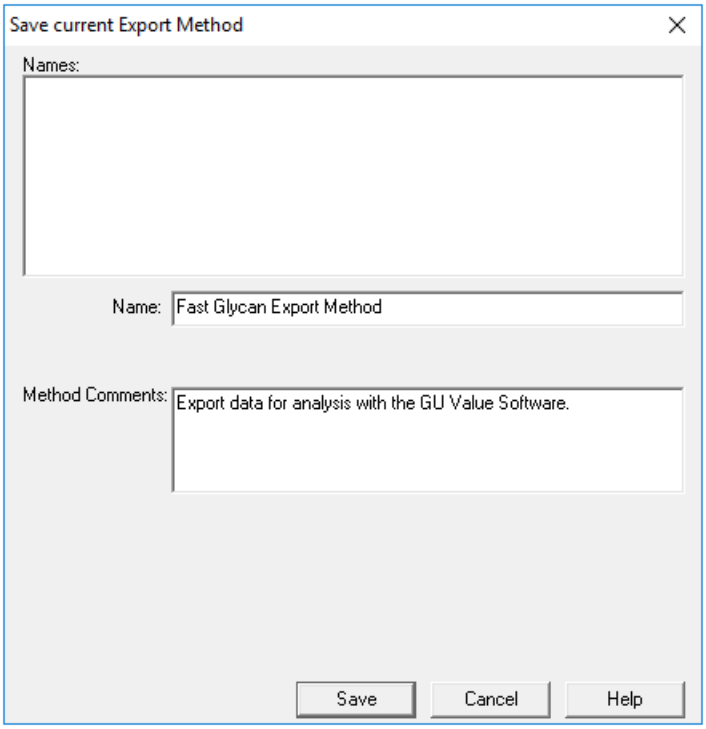


5. Save the export method.
   a. Click **File > Save As**.
   b. In the **Name** field, type a name for the export method.

      The **Names** field at the top of the dialog shows the existing export methods.

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

Figure C-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 Empower™ Software Pro Interface window, click **Browse Projects**, click the project of interest and then click **OK**.

Figure C-21 Empower™ Software Pro Interface Window

The Project window opens.

3. Click 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.

Revision History

Revision	Reason for Change	Date
A	First release of document.	May 2017
B	Changes for ECR 1614: • Changed wording of tip to recommend the PA 800 Plus System have a current *Operational Qualification 3*. • Corrected kit part number and bill of materials, moved "Ordering Information" to an appendix • Updated the download location of the GU Value • Moved steps for preparing the PA 800 Plus System to the "Separate the Glycans" section • Added storage conditions to "Prepare the Denaturation Solution" • Updated washing instructions in "Remove the Excess Dye from the Samples" • Added figures showing the entire "Translation Table in GU Value Report" • Changed wording in "Troubleshooting" • Deleted incorrect hazard images in Appendix A, "Hazardous Substance Information" • Deleted "Download the Methods from the SCIEX Website", "Condition the Capillary", Appendix B, "Calibrate the LIF Detector" and Appendix C, "Create the Methods"	July 2017

Revision History

Revision	Reason for Change	Date
C	Updated template. Deleted "Ordering Information" and moved content to "Equipment and Materials Required". Reorganized "Equipment and Materials Required" section. Added new section "Prepare the PA 800 Plus System" with relevant topics from "Separate the Glycans". Moved "Waste Disposal" topic after "Separate the Glycans". Reorganized "Troubleshooting" section. Added Appendix B, "Methods" with PA 800 Plus Software methods. Added Appendix C, "Run the Samples with Empower Software". Added "Contact Us" chapter.	March 2020

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 University™

Purchase Consumables

Reorder SCIEX consumables online at store.sciex.com. To set up an order, use the account number, found on the quote, order confirmation, or shipping documents. The SCIEX online store is currently limited to the US, UK, and Germany but will be expanding to other countries in the future. For customers in other countries, contact the local SCIEX representative.

SCIEX Support

SCIEX and its representatives maintain a staff of fully-trained service and technical specialists located throughout the world. They can answer questions about the system or any technical issues that might arise. For more information, visit the SCIEX website at sciex.com or contact us in one of the following ways:

- 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 supercedes all previous versions of this document.

To view this document electronically, Adobe Acrobat Reader is required. To download the latest version, go to https://get.adobe.com/reader.

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

To find hardware product documentation, refer to the *Customer Reference* DVD 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.