



Fast Glycan Labeling and Analysis Kit

Application Guide

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Fast Glycan Labeling and Analysis Kit

Note: Refer to the *PA 800 Plus Pharmaceutical Analysis System Overview Guide* for instructions about the 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 3*.

Safety

Refer to the Safety Data Sheets (SDS), available at [sciex.com/tech-regulatory](https://www.sciex.com/tech-regulatory), regarding the proper handling of materials and reagents. Always follow standard laboratory safety guidelines.

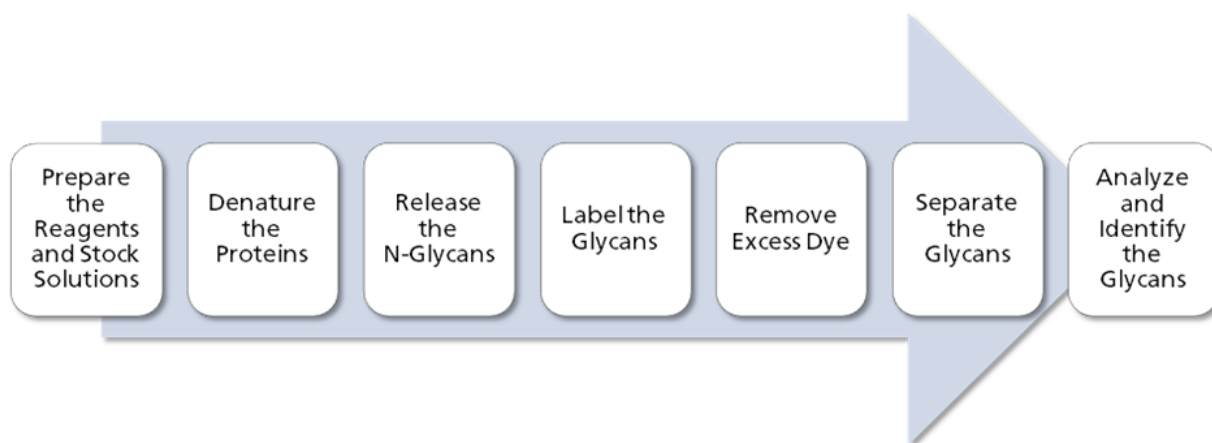
Overview

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 may lead to the discovery of new biomarkers for early diagnostics.

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 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 using 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



Intended Use

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

Equipment and Materials Required

Note: Unless a manufacturer name is specified, part numbers are for items available from SCIEX.

Required Software

In addition to the Fast Glycan Labeling and Analysis Kit and the equipment described in [Customer-Supplied Equipment and Supplies on page 6](#), the GU Value software must be installed on the PA 800 Plus Pharmaceutical Analysis System controller.

On a computer with internet access, go to sciex.com/products/capillary-electrophoresis-instruments/pa-800-plus-pharmaceutical-analysis-system. Search for "Fast Glycan Software" and download the installation file that contains the release notes and example data along with the GU Value software. Follow the installation instructions in the release notes.

Customer-Supplied Equipment and Supplies

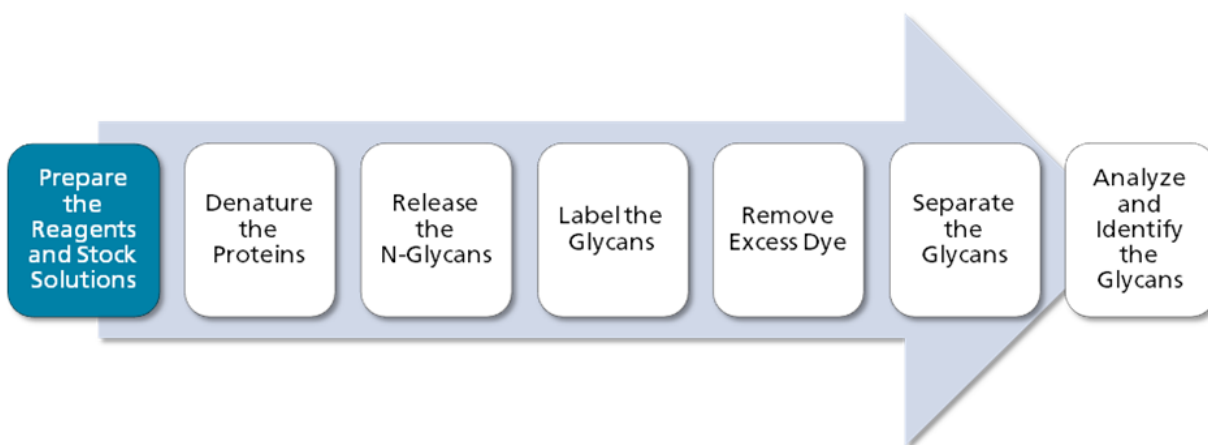
- Powder-free gloves (neoprene or nitrile recommended)
- Safety glasses
- Laboratory coat
- 0.2 mL flat-cap PCR tubes (VWR USA PN 20170-012 or VWR EUR PN 732-0548)

- Universal vials (PN A62251) and blue caps (PN A62250)
- Micro vials (PN 144709)
- Table-top mini centrifuge
- Microcentrifuge (or equivalent)
- Heat block capable of maintaining 60 °C
- Vortex mixer
- Thermometer for measuring the heat block temperature
- Pipettors (2 µL, 10 µL, 20 µL, 100 or 200 µL, and 1 mL) and appropriate tips
- Miscellaneous lab glassware for buffers and reagents

Customer-Supplied Reagents

- Peptide-N-glycosidase F enzyme (PNGase F) (Prozyme, 200 mU (PN GKE5006B))
- HPLC-grade acetonitrile
- 1 M sodium cyanoborohydride in THF (Sigma-Aldrich PN 296813)
- Double-deionized (DDI) water (MS grade water filtered through a 0.2 µm filter and with resistance above 18 MΩ)
- To calibrate the LIF detector:
 - LIF Performance Test Mix (Beckman Coulter PN 726022)
 - Capillary Performance Run Buffer A (PN 338426)

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 $-35\text{ }^{\circ}\text{C}$ to $-15\text{ }^{\circ}\text{C}$ 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.

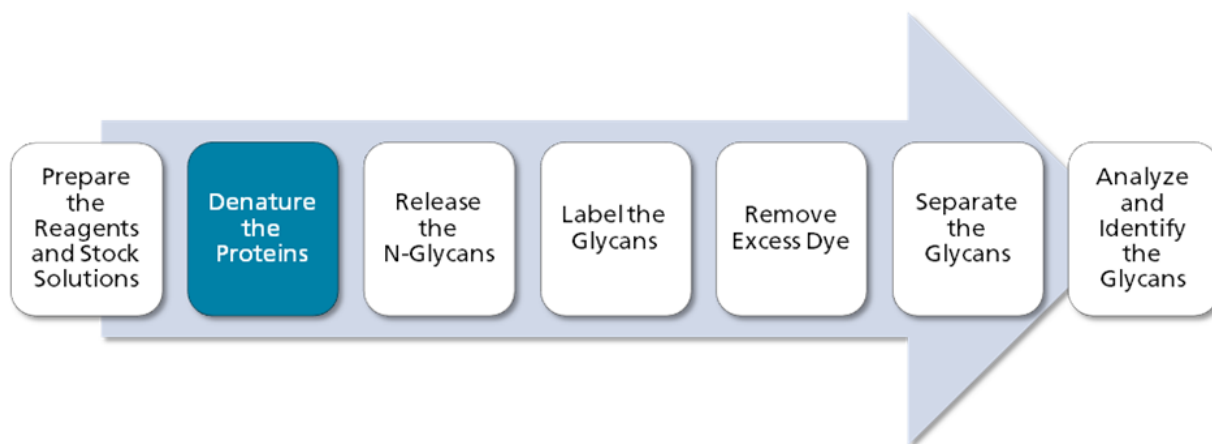
When not in use, store at $-35\text{ }^{\circ}\text{C}$ to $-15\text{ }^{\circ}\text{C}$ for up to six months.

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 $-35\text{ }^{\circ}\text{C}$ to $-15\text{ }^{\circ}\text{C}$ 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 can be stored at 4 $^{\circ}\text{C}$ 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
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 $^{\circ}\text{C}$.
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 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 tube from the magnetic stand.
4. Add 10 μ L of the glycoprotein sample solution (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 μ L of the previously-prepared denaturation solution to the sample tube, vortex briefly, and then incubate for 8 min at 60 °C in the heat block to denature the sample. Refer to [Prepare the Denaturation Solution on page 9](#).

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.

During the washing and eluting steps, to avoid aggregation of the beads, use the magnets (or stand) only for the time specified in the 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 vials.

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 μ L per sample.

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

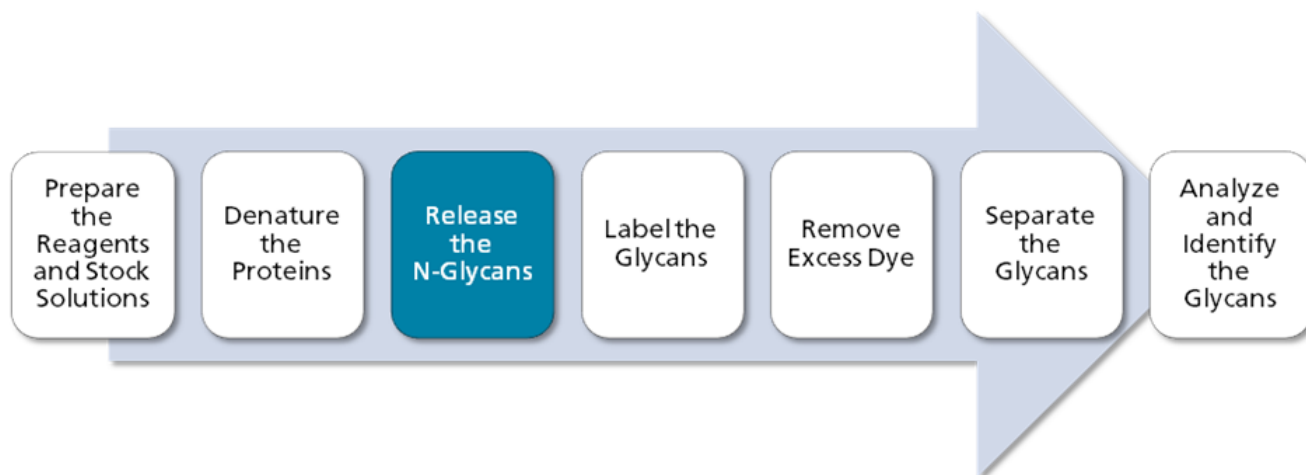
Reagent	For 1 Sample	For up to 20 Samples
D4	12 μ L	240 μ L
PNGase F enzyme	2 μ L	40 μ L

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 μ mol of denatured ribonuclease B per minute at 37 $^{\circ}$ 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 μ L of the previously-prepared digestion solution to the sample tube and then incubate for 20 minutes at 60 $^{\circ}$ C in the heat block. Refer to [Prepare the Digestion Solution on page 11](#).

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 μ L of acetonitrile to the sample tube and then vortex for 10 sec at maximum speed.
4. Allow the tube to incubate for 1 min 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 carefully remove the supernatant from the bottom of the sample tube without touching the beads with the pipette tip.
7. Remove the tube from the magnetic stand.

Prepare the Labeling Solution



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

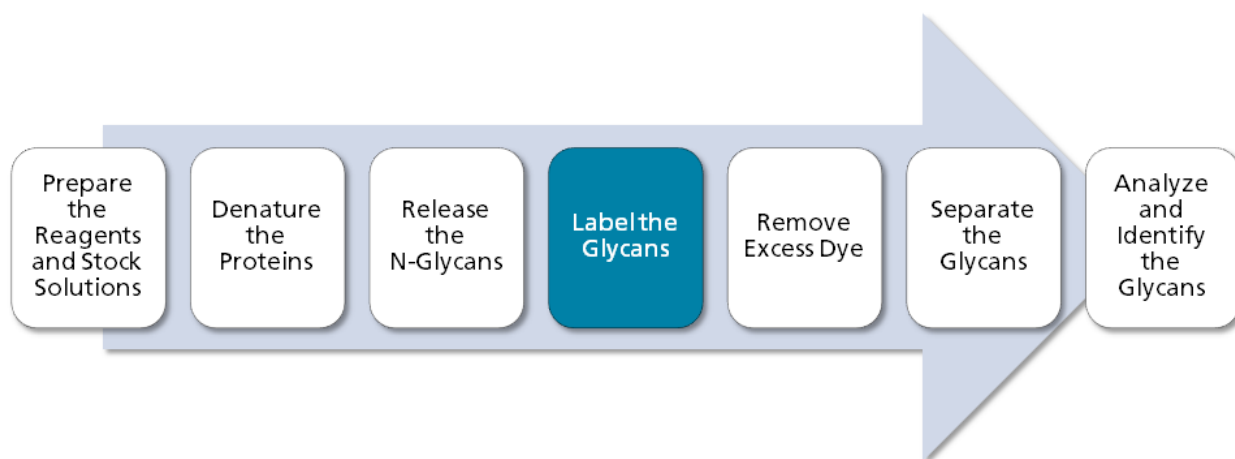
Note: This solution must be made fresh before use.

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.

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

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 Glycans



1. Working in a fume hood, add 11 μ L of the previously-prepared labeling solution to each sample tube. Refer to [Prepare the Labeling Solution on page 12](#).
2. Vortex the sample tube for 10 sec at maximum speed.

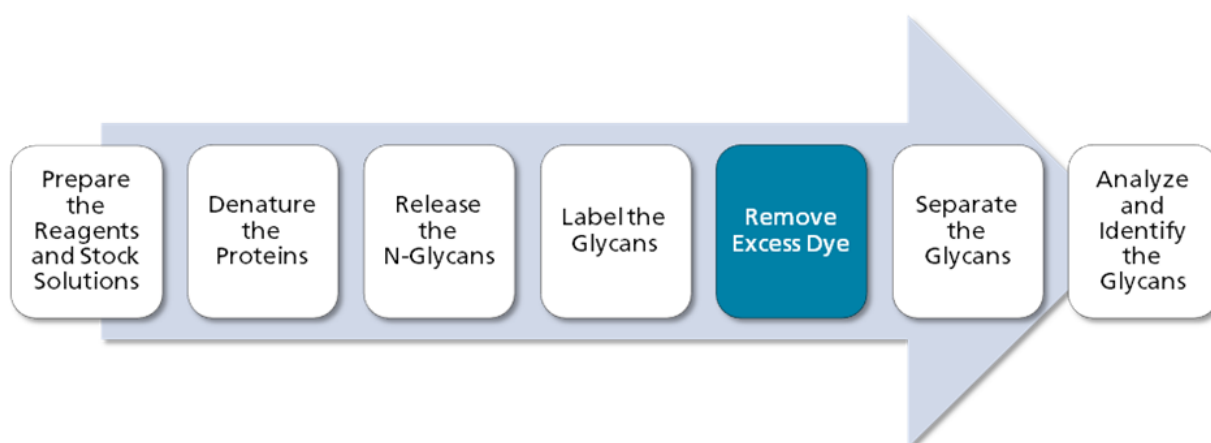
Make sure that the magnetic beads are well mixed with the labeling solution because the beads contain the glycans to be labeled. If the beads are not mixed, then vortex again.

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 μ L of D4 to the sample tube, close the lid, and then vortex for 10 sec at maximum speed.
 - b. Using a pipette, add 160 μ L 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 carefully remove the supernatant from the bottom of the sample tube without touching the beads with the pipette tip.
 - f. Remove the tube from the magnetic stand.
3. Wash the sample.
 - a. Using a pipette, add 20 μ L 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 μ L 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 carefully remove the supernatant from the bottom of the sample tube without touching the beads with the pipette tip.
4. 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 tube from the magnetic stand.
6. Add 100 μ L 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 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 μ L micro vial.

The supernatant contains the labeled and purified glycans.

9. Do one of the following:
 - Follow the instructions in [Load the Sample Tray on page 18](#) 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 1M 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 μ L of DDI water to the vial and then vortex the vial until the GU standard is completely dissolved.
3. Add 2 μ L of the GU standard solution to a 0.5 mL microfuge vial.

Discard the remainder of the GU standard solution.

Fast Glycan Labeling and Analysis Kit

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 $^{\circ}\text{C}$.

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.

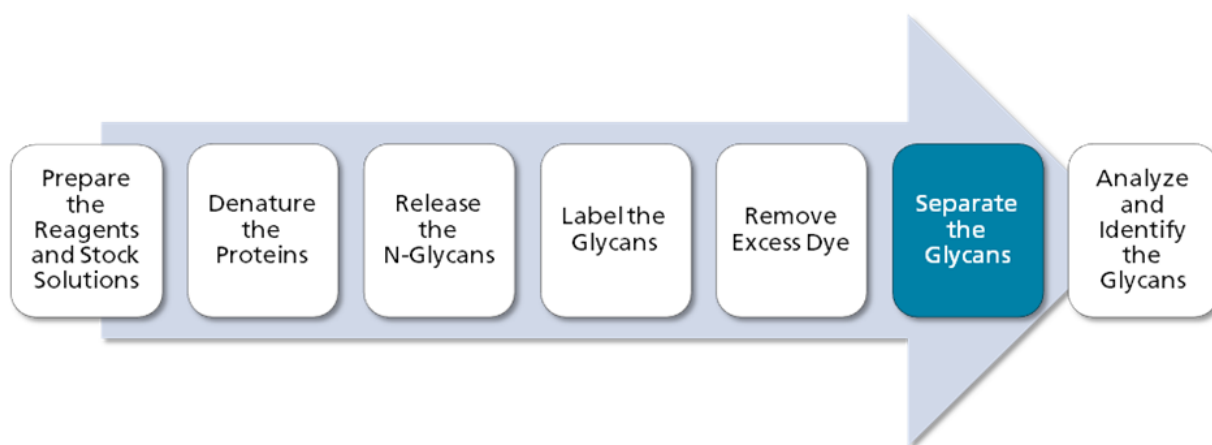
This is the GU ladder standard stock solution. When not in use, store at $-35\text{ }^{\circ}\text{C}$ to $-15\text{ }^{\circ}\text{C}$ for up to six months.
10. Add 5 μL of the GU ladder standard stock solution to a 1.5 mL microfuge vial, add 195 μL of DDI water, and then vortex the vial well to mix.
11. Pipette 200 μL of the diluted stock solution into a PA 800 Plus micro vial and then put the vial in the sample tray. Refer to [Figure 2 on page 19](#).

Waste Disposal



WARNING! Toxic Chemical Hazard. Follow local directives when disposing of chemicals, vials and caps, and the remains of the prepared samples, if applicable.

Separate the Glycans



Clean the Interface Block

Clean the electrodes, opening levers, capillary tips, and interface block weekly or when changing chemistries. Refer to the *PA 800 Plus Pharmaceutical Analysis System Maintenance Guide* for detailed instructions.

Prepare the Capillary Cartridge

Note: To ensure consistency of analysis results over time, we strongly recommend calibrating the LIF detector each time it is installed in the PA 800 Plus system or when the capillary cartridge is replaced. Use the Calibration wizard, available from the Instrument Configuration dialog in the 32 Karat software. Refer to the *PA 800 Plus Pharmaceutical Analysis System Maintenance Guide* for detailed instructions.

1. Remove the cartridge from the box.
2. Remove the aperture from the cartridge and install the probe retainer and probe guide on the LIF detector. Refer to the *System Maintenance Guide*.
3. Install the cartridge in the PA 800 Plus system.

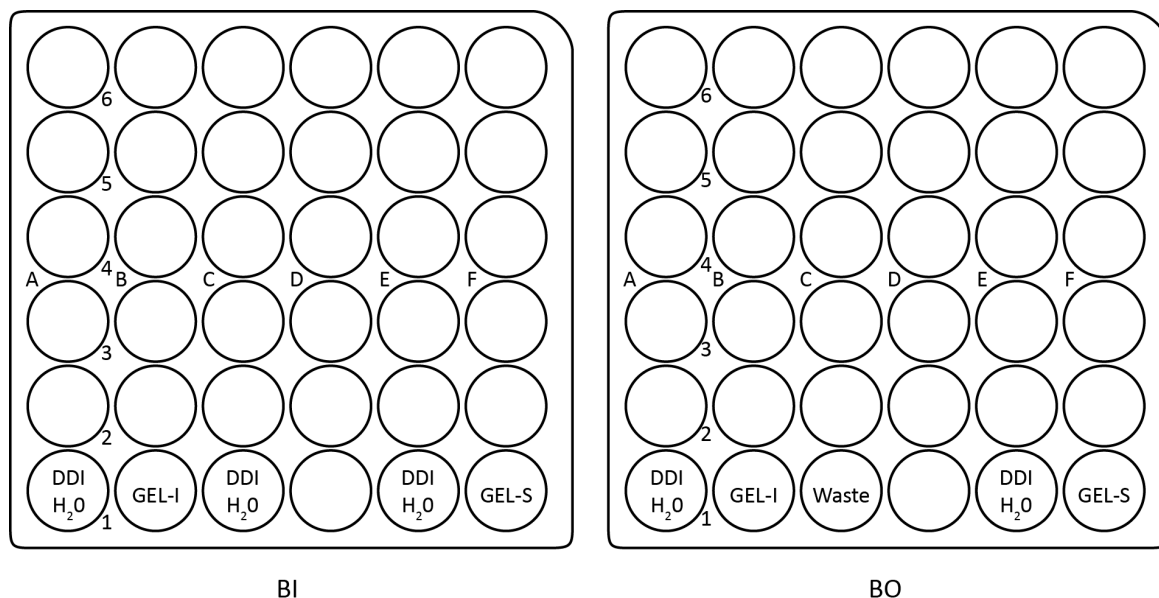
Load the Buffer Trays

1. Depending on the number of samples to be run, fill the appropriate number of vials and then cap them. 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
2. Put the vials in the buffer trays as shown in [Figure 1](#). 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.

Figure 1 Buffer Tray Layout



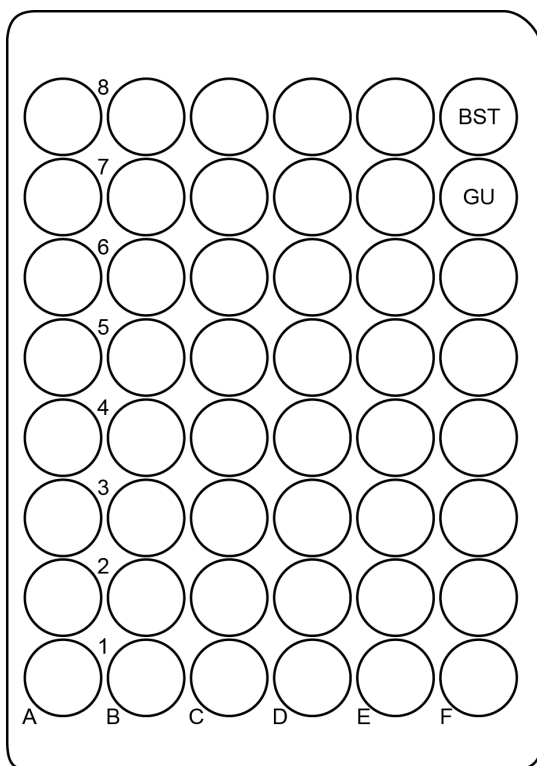
Note: During electrophoresis the ionic strength of the buffer will change. The separation method is pre-programmed to increment the buffer vials after 16 runs to avoid ionic depletion.

Load the Sample Tray

1. Prepare and load the bracketing standard.
 - a. Add 30 μ L of DDI water to one 20 μ L aliquot of the previously-reconstituted bracketing standard and then vortex the vial to mix. Refer to [Reconstitute the Bracketing Standard \(BST\) on page 8](#).
 - b. Put a 200 μ L micro vial into 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 position SI:F8 in the sample tray as shown in [Figure 2](#).
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 2 Sample Tray Layout




3. (Optional) Load the GU ladder standard.

The GU ladder standard serves as the process control.

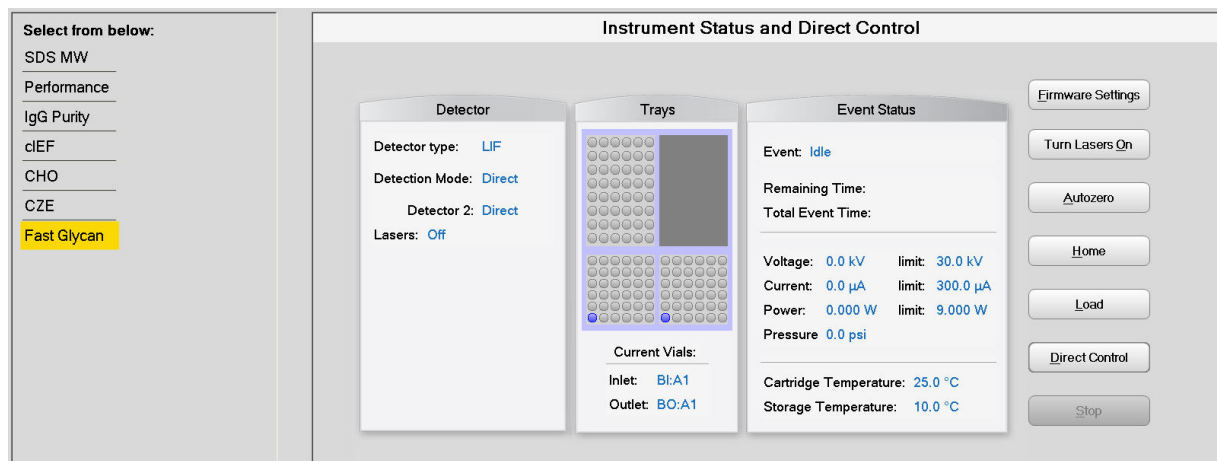
- a. Put the micro vial containing the GU ladder standard into a universal vial.
- b. Put a blue cap on the universal vial.
- c. Put the universal vial in position SI:F7 in the sample tray as shown in [Figure 2](#).


Create the Sequence and Start the Run

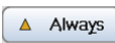

1. Double-click the PA 800 Plus software icon on the desktop to open the PA 800 Plus software.
2. In the **PA 800 plus** window, click  (Run) in the upper right corner of the window.
3. Click the **Fast Glycan** instrument, and then click **HR-NCHO Fast Glycan**. If system administration is enabled,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

The Instrument Status and Direct Control page opens.

Figure 3 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 **HR-NCHO 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) in the **Rows** area.
 - b. Click the last row (with the Fast Glycan Shutdown method) to select it and then click  (Always) in the **Rows** area.

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

Figure 4 Describe sequence rows and columns Page—Conditioning Method Set to "Always"

Describe sequence rows and columns

Application:

Sequence:

Rows: ☒ Sample ☐ Control ☒ Always

Columns: ☐ Optional ☒ Required ☐ Fixed

Verification: Samples

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) If there are rows containing system suitability samples, click each row to select it and then click

☒ Control (Control).

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

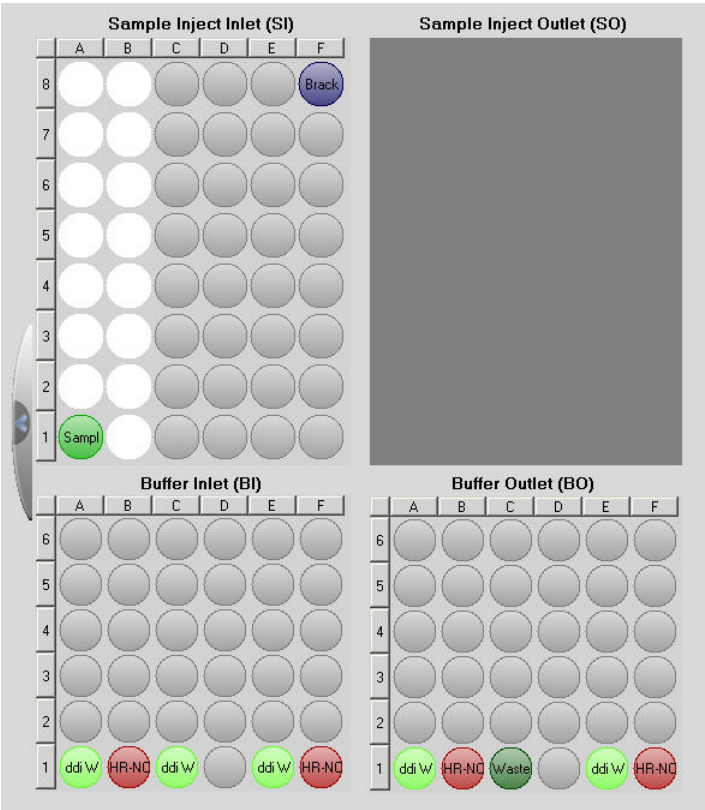
9. In the **Number of samples** field, click the arrow buttons to set the number of samples for the run.

Number of samples:

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 5](#), one row of reagents is required for 16 samples.

Figure 5 Tray Map for Fast Glycan





10. If the buffer and sample trays have not been loaded, click  (Load), load the buffer and sample trays in the PA 800 Plus system, and then close the door.
11. Click  (Next) and then click **Yes - run now**.

Figure 6 Samples Loaded Prompt

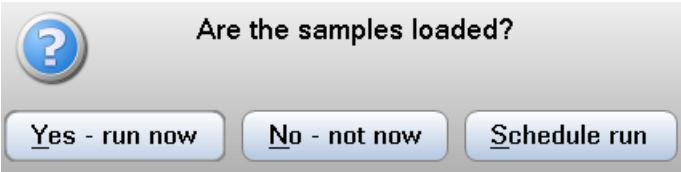
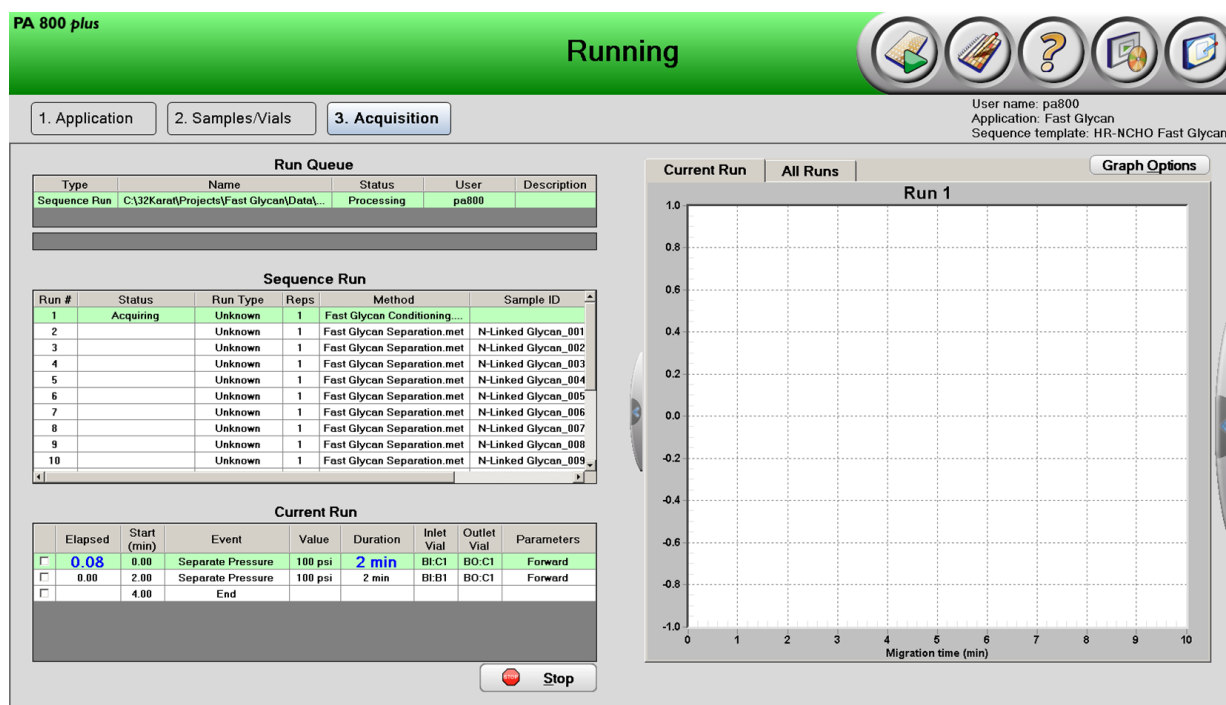


Figure 7 PA 800 Software During Data Acquisition



Store the Cartridge

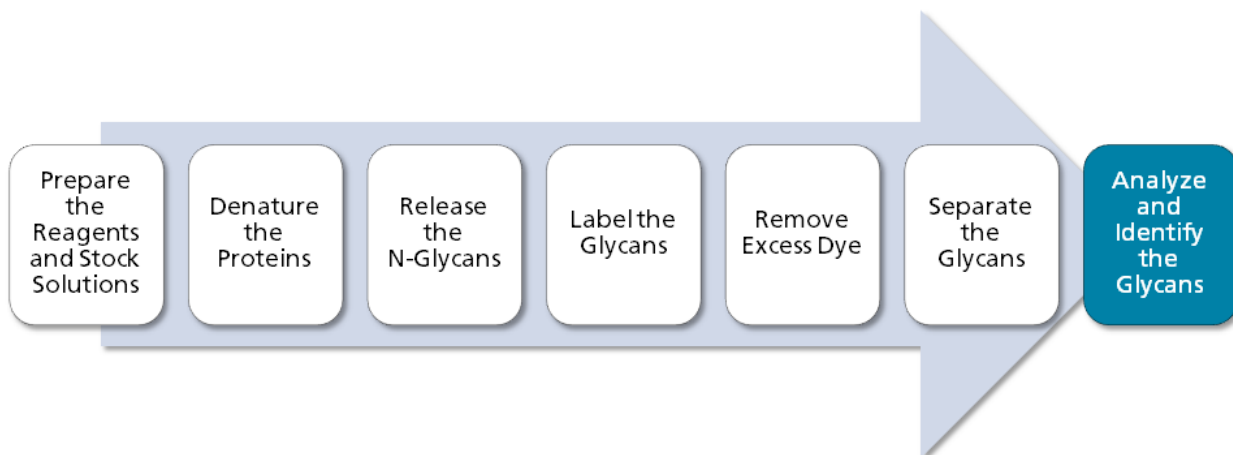
1. Make sure that the Fast Glycan Shutdown method (downloaded from the SCIEX website) was performed. The shutdown method fills the capillaries with water.
2. Store the cartridge in the system with the capillary ends immersed in vials of DDI water.


If the cartridge is removed from the system, then store 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 three or more hours, then condition the capillary using the Fast Glycan Conditioning method (downloaded from the SCIEX website).

Analyze and Identify the Glycans



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

- c. Click the **From existing data files** radio button.

Figure 8 Method Page

Sequence Wizard - Method

PA 800 plus

Method : Fast Glycan\Method\Fast Glycan Separation.met

Data File Type

☐ For acquisition

☒ From existing data files

Amount values


Sample amount : 1

Internal standard amount : 1

Multiplication factors : 1 1 1

Dilution factors : 1 1 1

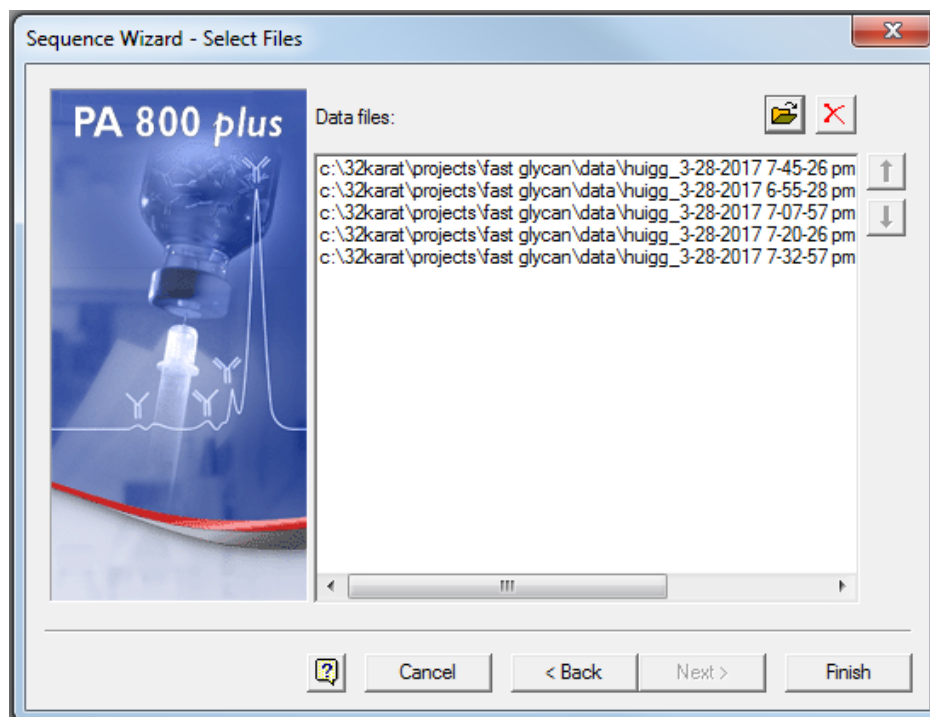
< Back Next > Cancel Finish

- 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**. When 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 9 Select Files Page



- g. Click **Finish**.

The wizard closes and a window with the sequence opens.

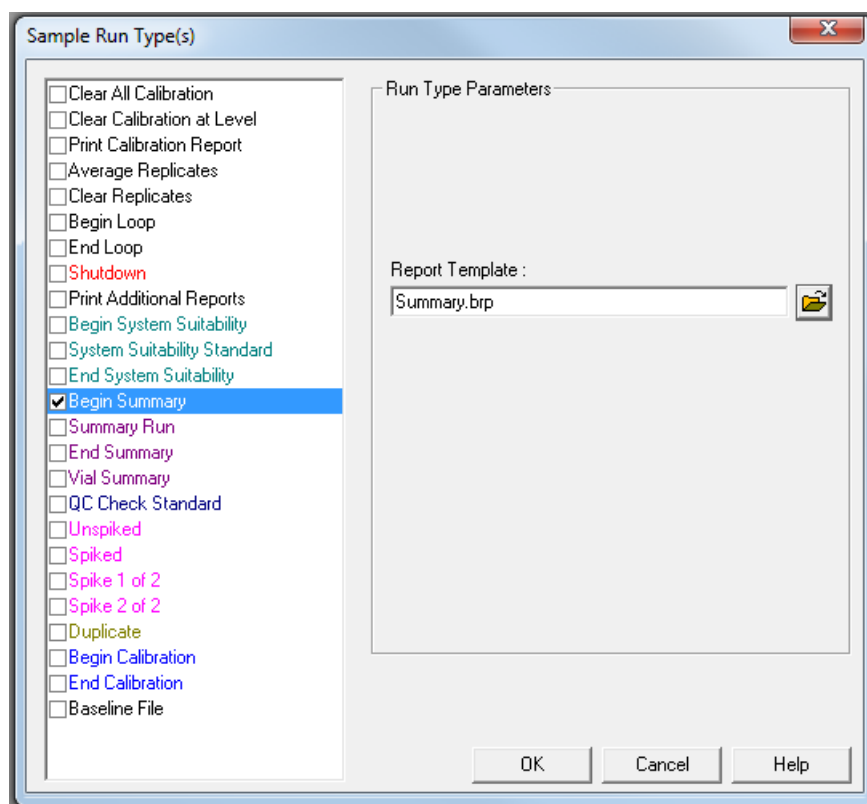
4. Click the **Run Type** column heading to select the column and then right-click and select **Set Run Types > Summary**

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 **Summary B...** in the first row of the sequence to open the Sample Run Type(s) dialog.
6. Make sure that the **Begin Summary** check box is selected and then click **OK** to close the dialog.

Figure 10 Sample Run Type(s) Dialog




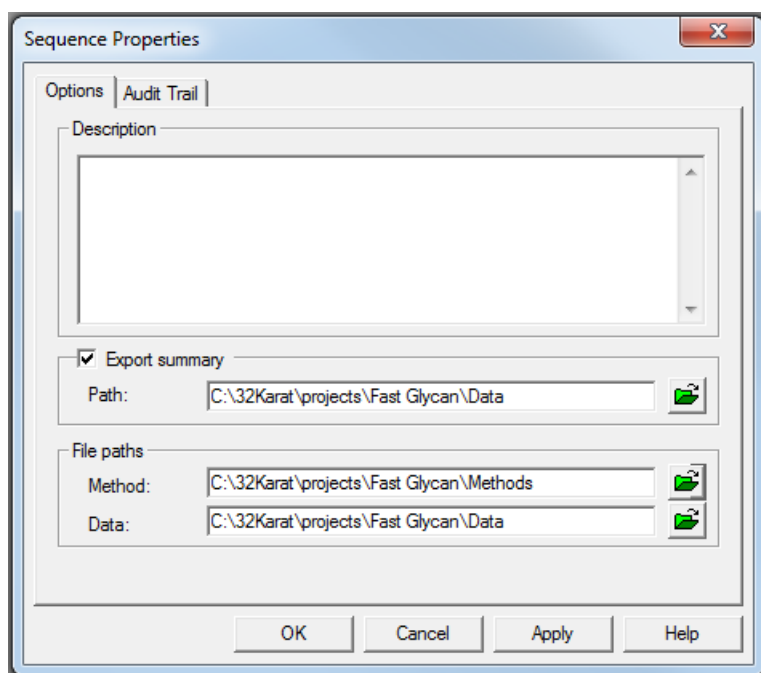
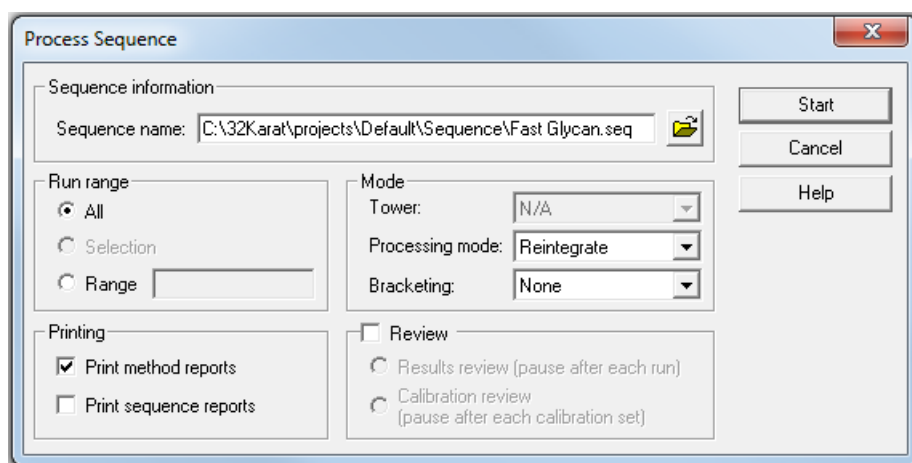
7. Click **Sequence > Properties** to open the Sequence Properties dialog.
8. Select **Export summary** and then click  (Browse) and select the folder to save the results.

Figure 11 Sequence Properties Dialog



9. Click **File > Sequence > Save As** and then type a name for the sequence.
10. Click **Sequence > Process** to open the Process Sequence dialog.
11. Select the **Print method reports** check box and then click **Start**.

Figure 12 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. When the analysis is finished, several reports in PDF format are created:

- The GU Value Report, which opens automatically at the end of the analysis. This report is saved to the Data folder in the Sequence Properties dialog box 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 software 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 13 GU Value Report—Report Heading

Fast Glycan Labeling & Analysis **Glucose Unit Value Report (PA 800 plus)**

Report Generated By: proteomelab
Report Type: Automated
GU Database:
"C:\GUValueSoftware\GU Tables\SciexGUReferenceTable.xml"
GU Value Report Folder:
"c:\32karat\projects\fast glycan\data"
Software Configuration File:
"C:\GUValueSoftware\GUValueReportGenerator.Cfg"
GU Value Report Generator:
"C:\GUValueSoftware\GUValueReportGenerator.exe, version 1.0.3432.3456"

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

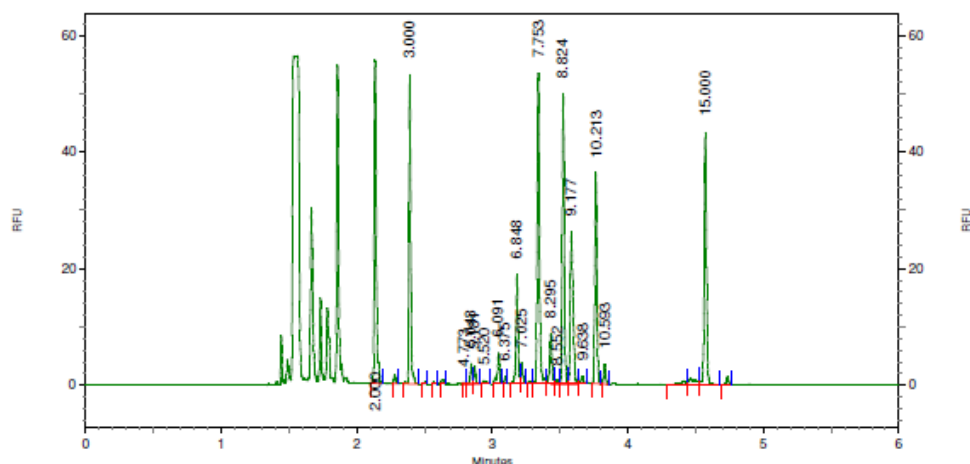
After the sample index is a series of method reports, one for each row in the sequence. The first part of the report (Figure 14) gives information including sample file name, sample ID, location of the file, and the location and name of the method.

Figure 14 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 [Figure 15](#) 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 15 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 16, Figure 17, and Figure 18) that shows structures for all of the glycans that the GU Value software can identify.

Figure 16 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 17 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 18 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: trimannosyl 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	LIF detector issues	<p>Make sure that the LIF detector is on and has been calibrated (refer to the <i>System Maintenance Guide</i>).</p> <p>Make sure that the probe stabilizer is properly connected to the lock down bar on the LIF detector (refer to the <i>System Maintenance Guide</i>).</p>
	Incorrect separation method	<p>Open the separation method in the 32 Karat software and then make sure that:</p> <ul style="list-style-type: none"> The voltage is correct. The Reverse polarity check box is selected. <p>During the run, observe the amber LED on the PA 800 Plus system. It should be lit during the time that the reverse polarity voltage is applied.</p>
	Air bubble at the bottom of the sample vial	Centrifuge the sample tube to make sure that there are no bubbles at the bottom.
	Capillary window or tip is broken	<p>Inspect the capillary window and tip. If either is broken, then replace the cartridge.</p> <p>Clean the probe aligner with a cotton swab moistened with DDI water.</p>
Low intensity peaks	Labeling reaction not performed properly	<p>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:</p> <ul style="list-style-type: none"> Prepare fresh L6. Make sure to add L5 to the L6 vial. Inspect 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.

Symptom	Possible Cause	Corrective Action
	Enzyme activity low	Inspect the peak intensity for the IST peak. If it is above 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.
	Sample concentration low	Make sure that the amount of protein is 100 µg (concentration 10 mg/mL). If the sample concentration is significantly lower, concentrate the sample using a spin filter with a pore size of 10 kDa MWCO.
	Incomplete deglycosylation	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	Sample concentration too high	Dilute the sample with water and then run the sample again.
Low current	Problems with the capillary	Make sure that the capillary temperature is correct. Make sure that the capillary is not blocked. Inspect the capillary window and tip. If either is broken, then replace the cartridge. Make sure that the probe stabilizer is properly connected to the lock down bar on the LIF detector (refer to the <i>System Maintenance Guide</i>).
	Contaminated reagents	Replace vials and vial caps with clean ones.
Shifts in migration time between runs on the same day	Problems with the capillary	Make sure that the capillary is properly equilibrated.
Shifts in migration time over an extended period of time with low current	Capillary partially blocked or the surface is contaminated	Treat the capillary as follows: 1. Rinse for 2 minutes at 90 psi with DDI water. 2. Rinse for 2 minutes at 90 psi with 0.1 M NaOH. 3. Rinse for 2 minutes at 90 psi with 0.1 M HCl. 4. Rinse for 2 minutes at 90 psi with DDI water.

Symptom	Possible Cause	Corrective Action
Carryover	Contaminated vials or caps	Replace the dip vials with clean vials filled with DDI water, cover with clean caps, and then increment as necessary. Replace the buffer vials with clean vials and caps, cover with clean caps, and then increment as necessary. Make sure the waste vials contain 0.8 mL of water and are present in the buffer outlet tray.
Spikes in electropherogram	Air dissolved in the separation gel	Sonicate the separation gel for 10 sec to 20 sec before use.
Extra peaks	Reaction of L6 with contaminants in the vials	Use new micro vials, especially for steps related to labeling.
Fewer small glycans (DP < 5) than expected	Small glycans washed away during dye removal	Perform fewer wash steps during the dye removal step.
Multiple Save file as dialogs appear during data analysis	Sciex PDF Writer software folder location incorrectly configured	Make sure that the folder location in the Preferences - PDF Writer dialog exists and the other settings are correct. Refer to <i>GU Value Software Release Notes</i> .
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 <i>GU Value Software Release Notes</i> .

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.

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 according the European Union Legislation:

- D4 reagent
- GU glucose ladder
- IST internal standard
- L6 fluorophore
- M1 magnetic beads

Ordering Information

B

Fast Glycan Labeling and Analysis Kit (Part Number B94499PTO)

This kit contains enough reagents to analyze 100 samples.

Component	Quantity
Magnetic stand	1
M1 magnetic beads	1
D1 reagent	5
D2 reagent	5
D3 reagent	1
D4 reagent	2
L5 catalyst	1
L6 fluorophore	5
GU (glucose ladder); process control	1
IST (internal standard)	1
BST (bracketing standard)	1
HR-NCHO separation gel	1
Pre-assembled capillary cartridge with window (30 cm total length, 375 µm outer diameter (o.d.) x 50 µm inner diameter (i.d)) (PN A55625)	1

Components Available Separately

Part Number	Component	Quantity
A55625	Pre-assembled capillary cartridge with window (30 cm total length, 375 µm outer diameter (o.d.) x 50 µm inner diameter (i.d))	1

Revision History

Revision	Reason for Change	Date
A	First release of document.	May 2017
B	<p>Changes for ECR 1614:</p> <ul style="list-style-type: none">• Changed wording of tip to recommend the PA 800 Plus system have a current <i>Operational Qualification 3</i>.• Corrected kit part number and bill of materials, moved "Ordering Information" to an appendix• Updated the download location of the GU Value software• 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