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Carbohydrate Labeling and Analysis Kit

For the PA 800 Plus Pharmaceutical Analysis System

Application Guide

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Carbohydrate Labeling and Analysis Kit

The Carbohydrate Labeling and Analysis kit uses capillary electrophoresis to separate and quantify carbohydrates that are released from glycoproteins.

This kit contains the reagents, buffer, and N-CHO capillaries that are required to label, separate, and quantify carbohydrates. This kit also supplies a glucose size marker for relative size determination and a maltose standard for quantitation and mobility characterization of the released carbohydrates.

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

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

Safety

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

Intended Use

The Carbohydrate Labeling and Analysis kit is for laboratory use only.

Introduction

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

The Carbohydrate Labeling and Analysis kit has been validated on the PA 800 Plus system.

Workflow

Table 1 Carbohydrate Labeling and Analysis Kit Workflow

Step	To Do This	Refer To
1	Release the N-Glycans.	Release the Carbohydrates from the Glycoproteins
2	Prepare the labelling reagents.	Prepare the Labeling Reagents
3	Label the glycans.	Label the Sample or G20-Glucose Ladder Standard
4	Install and condition the N-CHO capillary.	Install the Capillary
5	Install the LIF detector.	Install the LIF Detector
6	Prepare the gel buffer and load the buffer trays.	Load the Buffer Trays
7	Load the sample tray.	Load the Sample Tray
8	Create the sequence and then start the run.	Create the Sequence and Start the Run
9	Store the cartridge after use.	Stow the Cartridge
10	Analyze the data.	Analyze the Data for the Labeled G20-Glucose Ladder Standard

Required Equipment and Materials

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

Table 2 Carbohydrate Labeling and Analysis Kit (PN 477600)

Component	Quantity	Reorder Part Number
APTS-M (20 mg)	1	N/A
APTS Labeling Dye (5 mg)	4	501309
G20-Glucose Ladder Standard (50 mg)	1	N/A
G22-Quantitation Control (maltose) (0.18 mg)	1	N/A
L3-Labeling Dye Solvent (15% acetic acid) (1 mL)	1	N/A
N-CHO Capillary	2	477601

Table 2 Carbohydrate Labeling and Analysis Kit (PN 477600) (continued)

Component	Quantity	Reorder Part Number
N-Linked Carbohydrate Separation Buffer (56 mL)	1	477623

Table 3 Other Supplies from SCIEX

Component	Quantity	Part Number
Capillary cartridge, blank	1	144738
CE Grade water (140 mL)	1	C48034
LIF Performance Test Mix (20 mL)	1	726022
PCR microvials (200 µL)	100	144709
Universal vial caps, blue	100	A62250
Universal vials	100	A62251

Table 4 Other Required Reagents or Supplies

Description	Vendor	Part Number
1× phosphate buffered saline (PBS) (1 L)	Santa Cruz Biotechnology	sc-362182
1 M sodium cyanoborohydride in THF	MilliporeSigma	296813
2-mercaptoethanol	MilliporeSigma	M7154
NP-40 nonionic detergent	Other Lab Supplier	Various
Peptide-N-glycosidase F enzyme (PNGase F)	New England Biolabs	P0704S
5% sodium dodecyl sulfate (SDS)	Other Lab Supplier	Various

Storage Conditions

Note: For storage conditions for prepared reagents, refer to the preparation instructions.

- Upon receipt, keep the Carbohydrate Labeling and Analysis kit at 2 °C to 8 °C.

Customer-Supplied Equipment and Supplies

- Powder-free gloves, neoprene or nitrile recommended
- Protective eyewear
- Laboratory coat
- Analytical balance
- Applicable centrifuge
- Centrifugal vacuum evaporator
- Microfuge tubes, 1.5 mL
- Pipettes and applicable tips
- Parafilm
- Spatula
- Vortex mixer
- Water bath or heat block capable of 37 °C to 100 °C

Required Detector

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

Required Cartridge or Capillary

A capillary cartridge (PN 144738) and an N-CHO capillary, 50 µm inner diameter (i.d.), are required. The capillary must be trimmed to a total length of 50.2 cm and an effective length of 40 cm.

Methods and Sequences

The method files are installed on the PA 800 Plus controller in the `C:\32Karat\projects\CHO\method` folder. The sequence file is installed on the PA 800 Plus controller in the `C:\32Karat\projects\CHO\sequence` folder.

- Methods:
  - `CHO Conditioning - PA 800 plus.met`: Conditions the capillary.
  - `CHO Separation - PA 800 plus.met`: Separates the sample with a pressure injection of the sample.
  - `CHO Shutdown - PA 800 plus.met`: Cleans the capillary at the end of a sequence and turns off the light source.

- Sequence file:
  - `CHO - PA 800 plus.seq`: A sequence with the separation method that uses pressure sample injection.

Prepare the Reagents and Stock Solutions

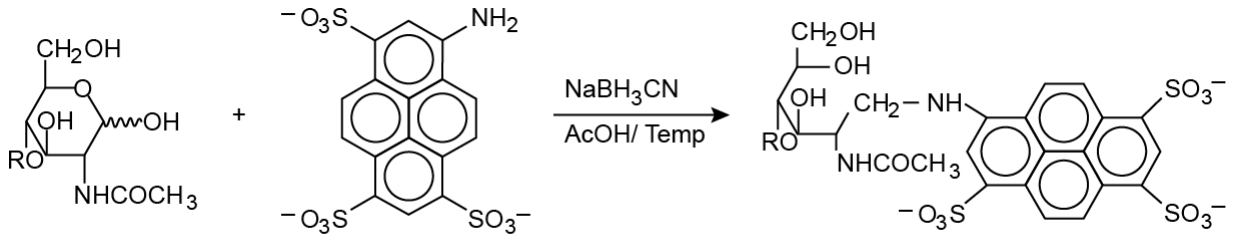
Note: The solutions in this section can be made ahead of time. Make sure that the solutions are stored correctly and are used before the solution aliquot expires.

Prepare the Labeling Reagents

Principle of the Labeling Method

After enzymatic or chemical release, the carbohydrates are labeled with the fluorophore 1-Aminopyrene-3,6,8-Trisulfonic Acid (APTS). The stoichiometry of the labeling reaction is one APTS molecule per molecule of carbohydrate. The following figure shows the labeling reaction of an N-linked carbohydrate with APTS.

Figure 1 Labeling Reaction of a Carbohydrate with APTS



Carbohydrate APTS Labeled Carbohydrate

Temperature and the amount of carbohydrates have an effect on the efficiency of the labeling reaction. This protocol was optimized for labeling 5 nmol or less of total carbohydrates.

Samples with amounts more than 5 nmol might give a lower reaction yield. Use G22-Quantitation Control as a control for the internal labeling reaction or as a marker for internal mobility.

Select the Fluorophore

- To label monosaccharides, oligosaccharides, or glycans, use APTS-M.
- To label glycans, use APTS-M or APTS Labeling Dye.
- For quantitation experiments, always use APTS-M.

APTS-M contains APTS dye lyophillized with citric acid as catalyst for the labeling reaction.

Prepare the APTS-M Labeling Dye

1. In the APTS-M vial, add 400 µL of CE Grade water.
2. Mix the solution for 5 seconds, or until all of the solid is dissolved.
3. Keep the prepared solution at –35 °C to –15 °C for a maximum of 2 weeks.

Prepare the APTS Labeling Dye

1. To a 5 mg vial of APTS Labeling Dye, add 48 µL of L3-Labeling Dye Solvent (15% acetic acid).
2. Mix the solution for 5 seconds, or until all of the solid is dissolved.
3. Keep the prepared solution at –35 °C to –15 °C for a maximum of 2 weeks.

Prepare the Standards

CAUTION: Potential Wrong Result. When the G22-Quantitation Control is used for quantitation, make sure to prepare a new solution and use it immediately. Bacterial contamination can cause errors in quantitation.

1. To prepare the G22-Quantitation Control, do this:
   a. To the G22-Quantitation Control, use a pipette to add 500 µL of CE Grade water. The reconstituted solution contains 500 nmol of maltose at a concentration of 1 nmol/µL.
   b. Use a pipette to add 5 µL of the reconstituted solution to the released oligosaccharides.
   c. Use centrifugal evaporator to dry the sample.
   d. Then do the procedure in the section: Label the Sample or G20-Glucose Ladder Standard.
   e. When it is not in use, keep the solution at –35 °C to –15 °C.
2. To prepare the G20-Glucose Ladder Standard, do this:
   a. Measure 5 mg of G20-Glucose Ladder Standard, and then put the G20-Glucose Ladder Standard into a 1.5 mL microfuge tube.
   b. In the 1.5 mL microfuge tube, add 80 µL CE Grade water.
   c. Use a vortex mixer to dissolve the G20-Glucose Ladder Standard fully.
   d. In ten 0.5 mL microfuge tubes, add 2 µL of the glucose ladder standard solution.
   e. Use a centrifugal vacuum evaporator to dry the aliquots.
      Keep the dried glucose standard tubes at ambient temperature.

Prepare the Samples

Release the Carbohydrates from the Glycoproteins

CAUTION: Potential Wrong Result. Use a deglycosylation procedure that does not cause damage to the reducing end of the released glycans. If the reducing ends of the glycans are damaged, then the released glycans cannot be labelled.

Multiple enzymatic and chemical procedures to deglycolsylate proteins that contain glycans are available. This procedure uses peptide-N-glycosidase F (PNGase F) to release glycans from proteins without damage to the reducing end.

Note: The Carbohydrate Labeling and Analysis kit does not contain the releasing enzyme, PNGase F. Refer to the section: Customer-Supplied Equipment and Supplies.

1. Use a centrifugal vacuum evaporator to fully dry 25 µg to 300 µg of glycoprotein solution.
2. Add 45 µL of 1× PBS buffer.
3. Add 1.0 µL of 5% SDS, or a volume that gives a final concentration of 0.1% SDS.
4. Add 1.5 µL of a 1:10 dilution of 2-mercaptoethanol in CE Grade water, or a volume that gives a final concentration of 50 mM 2-mercaptoethanol.
5. To denature the protein, incubate the sample at 100 °C for 5 minutes.
6. If the denatured protein precipitates, then discard the sample and do these steps:
   a. Do step 1 to step 4.
   b. To denature the protein, incubate the sample at 37 °C for 10 minutes.
7. Let the sample temperature decrease to the ambient temperature.
8. Add 5 µL of Nonidet NP-40 detergent, or a volume that gives a final concentration of 0.75% Nonidet NP-40 detergent.
9. Add the correct amount of PNGase F enzyme.

   Tip! To calculate the correct amount of PNGase F enzyme to add, refer to specified enzymatic activity from the product insert of the PNGase F enzyme and the amount of glycoprotein solution used in step 1.
10. Incubate the sample at 37 °C for 15 hours.
11. Add 150 µL of cold ethanol, or three times the actual reaction mixture volume.
12. Mix the sample.
13. To precipitate the proteins, put the sample on ice for two hours.
14. Use a centrifuge to spin the samples between 18,000 × *g* and 20,000 × *g* for 5 minutes.

Note: The supernatant contains the released N-linked glycans.

15. Collect the supernatant, and then do one of these actions:
    - For quantitative analysis, add an internal standard to the supernatant. Refer to the section: Prepare the Standards.
    - For qualitative analysis, use a centrifugal vacuum evaporator to dry the supernatant, and then label the oligosaccharides with APTS. Refer to the section: Prepare the APTS Labeling Dye.
16. Discard the pellet.

Note: Samples with more than 5 nmol might give a lower reaction yield. Use G22-Quantitation Control as an internal labeling control or as an internal mobility marker.

Tips for Best Results

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

Label the Sample or G20-Glucose Ladder Standard

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

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

Use this procedure to label either each of the glycan samples or the G20-Glucose Ladder Standard, or both.

1. Add 2 µL of 1 M sodium cyanoborohydride/THF solution to each vial of dried oligosaccharide sample or G20-Glucose Ladder Standard.
2. Add 2 µL of APTS labeling reagent to each vial of sample or G20-Glucose Ladder Standard.
3. Use any of these conditions to incubate the mixture.

Note: To select an incubation option, think about the available time and the type of oligosaccharide to be labeled. If the oligosaccharides in the sample are sensitive to heat, then select an incubation option that uses a lower temperature. For example, since sialic acid species are sensitive to heat, use the mildest incubation conditions for samples that contain sialic acid.

- For 90 minutes at 60 °C

- For 4 hours at 37 °C
- Overnight at ambient temperature (mildest reaction)

Prepare the Labeled Samples for Analysis

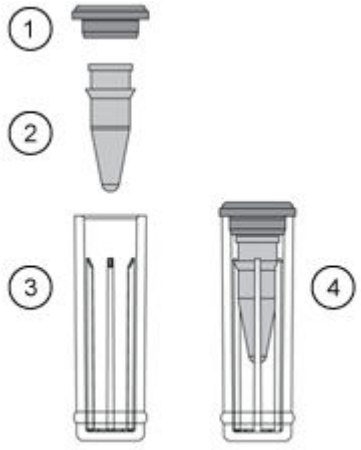
Use the solutions that were prepared in the section: Label the Sample or G20-Glucose Ladder Standard.

1. In the vial of labeled G20-Glucose Ladder Standard, add 96 µL of CE Grade water.
2. In the vial of labeled sample, add 46 µL of CE Grade water.
3. Use a vortex mixer to dissolve the solids fully.

Tip! Make sure that all solids are dissolved fully and that the solution is homogeneous.

4. Keep the labeled solutions at 2 °C to 8 °C.
5. Dilute the solutions from step 1 and step 2.
   a. Use a pipette to transfer 5 µL of each of the solutions into new 1.5 mL microfuge tubes.
   b. To each tube, add 195 µL of CE Grade water.
6. Use a vortex mixer to mix the sample fully.
7. Transfer the samples to new microvials. Put each microvial in a universal vial, and then attach a cap. Refer to the following figure.

Figure 2 Microvial in a Universal Vial



Item	Description
1	Universal vial cap
2	Microvial

Item	Description
3	Universal vial
4	Microvial inside a universal vial

The samples are now ready to be loaded in the sample tray. Refer to the section: Load the Sample Tray.

Prepare the PA 800 Plus System

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

Install the LIF Detector

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

Clean the Electrodes, Insertion Levers, and Interface Block

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

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

Install the Capillary

CAUTION: Potential System Damage. Do not let the capillary become dehydrated. The coating inside the capillary starts to dehydrate within 5 minutes to 10 minutes after the tip of the capillary is trimmed.

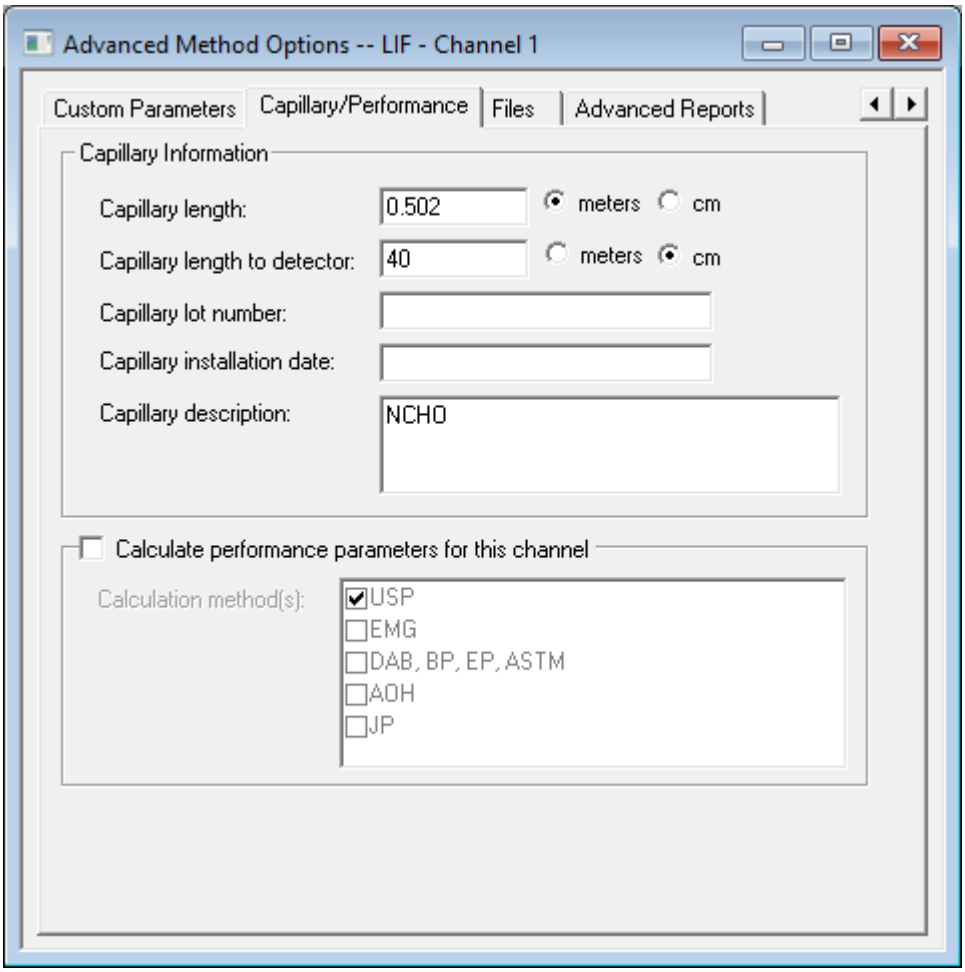
CAUTION: Potential System Damage. Do not cut the capillary to its final length before it is installed in the cartridge.

1. Install the capillary into a capillary cartridge. Refer to the document: *Capillary Cartridge Rebuild Instructions*.

   The recommended capillary length is 40 cm to the window and 50.2 cm total length. The inner diameter (i.d.) is 50 µm.

2. Measure the capillary dimensions accurately. Record the dimensions on the Capillary Performance tab of the Advanced Method Options dialog. Refer to the following figure.

Figure 3 Advanced Method Options Dialog: Capillary/Performance Tab



3. To minimize damage to the capillary coating, do this:
   a. Cut off the end-cap on the inlet side of the capillary, and then install the capillary in the cartridge. After the capillary is in the cartridge, cut the end-cap from the outlet side, and then complete the cartridge assembly.
   b. Trim the capillary tips to the recommended length, and then submerge both tips in vials filled with CE Grade water.

Install the Cartridge

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

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

4. (Optional) Calibrate the LIF detector.

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

Condition the Capillary

- Before a new capillary is used, condition the capillary with the `CHO Conditioning - PA 800 plus.met` method.

Load the Buffer Trays

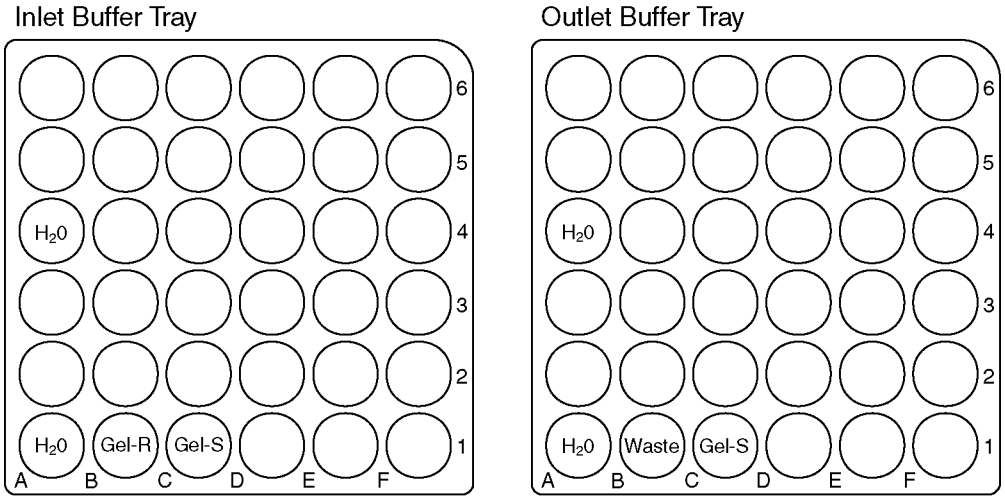
CAUTION: Potential System Damage. Do not fill any vial with more than 1.5 mL of liquid. Fill waste vials with 1.0 mL of liquid. Do not let more than 1.5 mL of liquid collect in waste vials. If a vial is filled with more than 1.5 mL of liquid, then the pressure system can be damaged.

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

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

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

Figure 4 Buffer Tray Layout



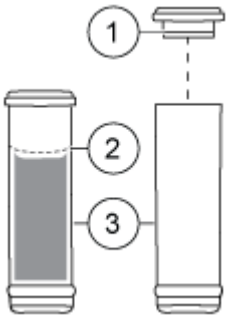
Note: During electrophoresis, the ionic strength of the buffer changes. The separation method is programed to increment the buffer vials after 20 runs to prevent ionic depletion.

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

Table 5 Vials to Prepare

Label	No. of Vials	Vol./Vial (mL)	Reagent
Water	4	1.5	CE Grade water
Gel-R	1	1.5	N-Linked Carbohydrate Separation Buffer
Gel-S	2	1.3	N-Linked Carbohydrate Separation Buffer
Waste	1	1.0	CE Grade water

Figure 5 Universal Vial and Cap Setup



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

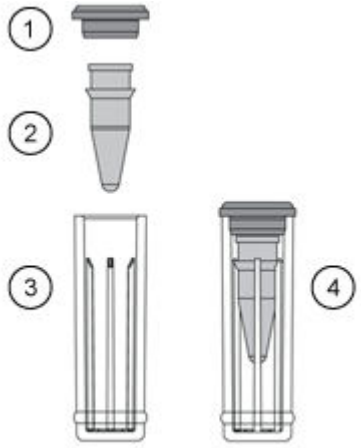
Load the Sample Tray

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

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

1. Prepare the samples. Refer to the section: Prepare the Samples.
   If required, put the microvial in a universal vial, and then attach a cap. Refer to the following figure.

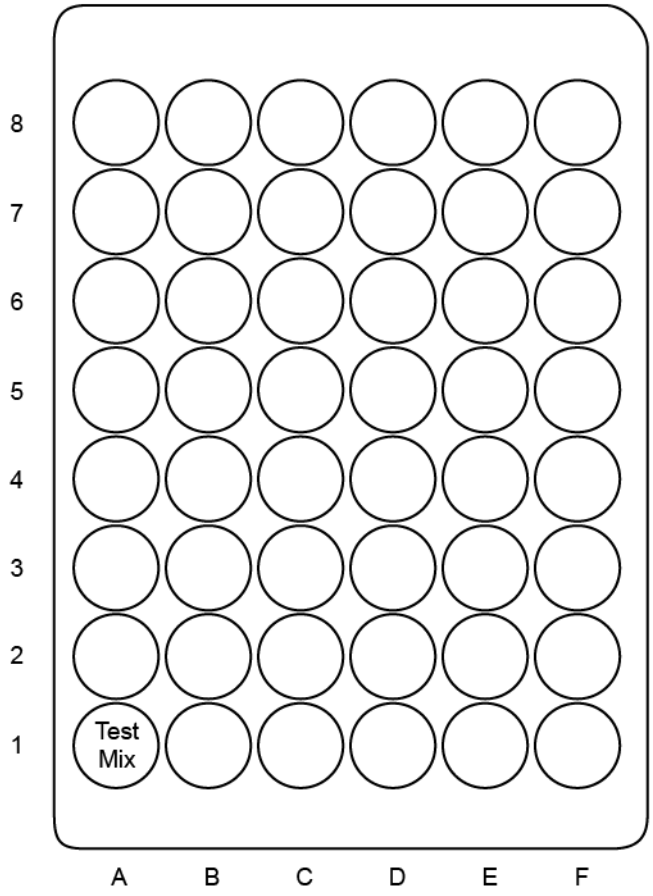
   Figure 6 Microvial in a Universal Vial

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

2. Put each universal vial in the sample tray. Refer to the figure: Figure 7.
   Position A1 is for the APTS-labeled G20-Glucose Ladder Standard (Test Mix). Use the other positions for the other samples.

Figure 7 Sample Tray Layout



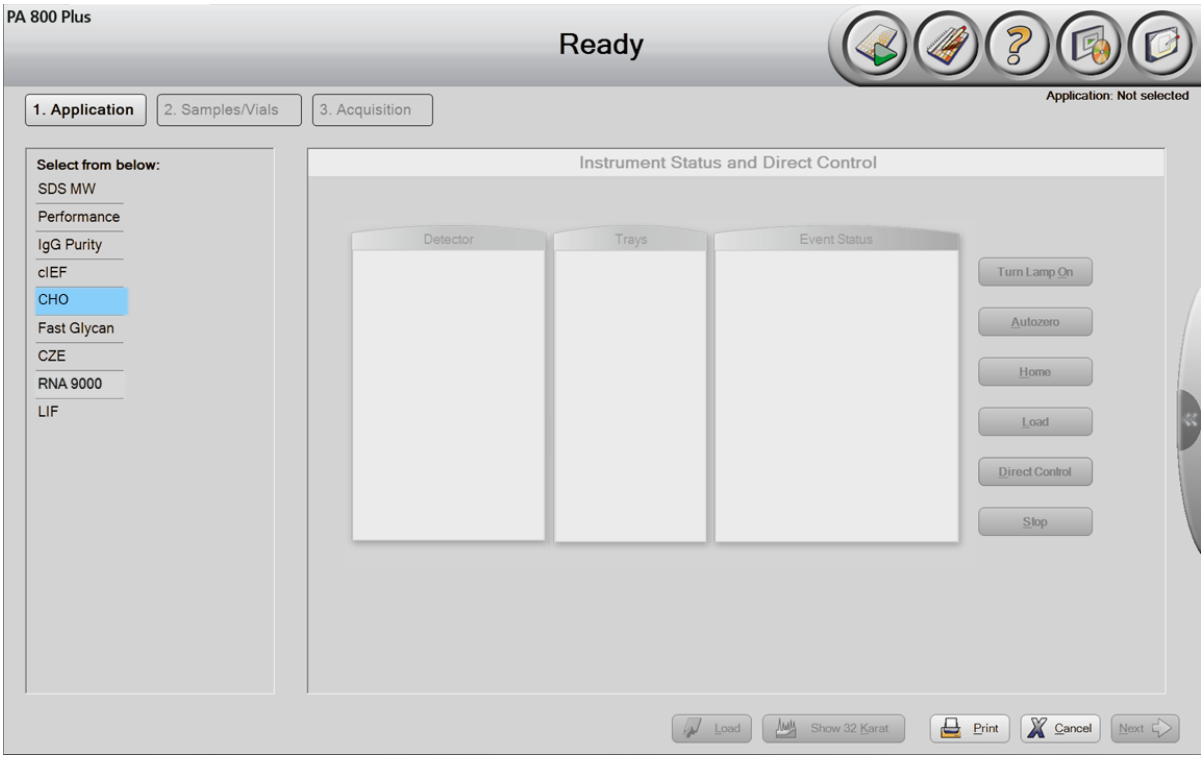
Run the Samples

Create the Sequence and Start the Run

1. To open the PA 800 Plus software, double-click the PA 800 Plus software icon on the desktop.
   The PA 800 plus window opens.

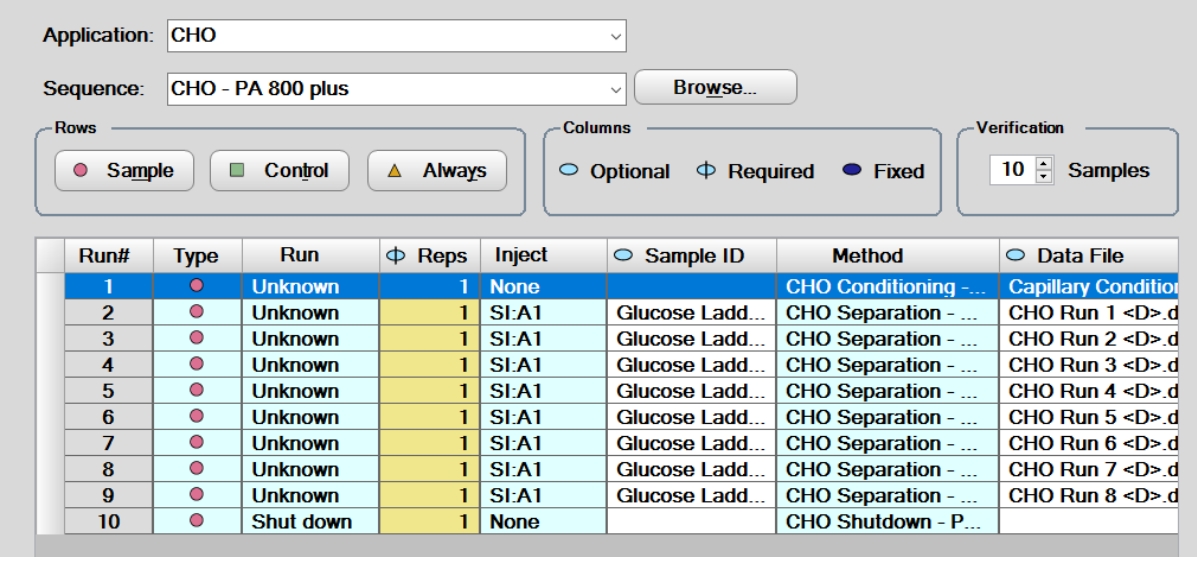
2. In the top right corner of the PA 800 plus window, click  (**Run**).

Figure 8 Instrument Status and Direct Control Window



3. Click (**Describe**).
4. In the **Application** list, click **CHO**.
5. To the right of the **Sequence** list, click **Browse**, and then select `CHO - PA 800 plus.seq`. If a prompt is shown, then type a user name and password.
   The page changes to show the selected sequence. All of the rows in the sequence are identified as samples.
6. Click the first row to select it, and then click Control (**Control**) in the Rows section.
   The first row contains the G20-Glucose Ladder Standard.
   The icon in the **Type** column in the first row changes to a square.
7. Click the last row (with the `CHO Shutdown - PA 800 plus.met` method) to select it, and then click Always (**Always**) in the Rows section. The last row contains the shutdown method.
   The icon in the **Type** column changes to a triangle.

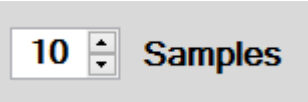
Figure 9 Describe sequence rows and columns Window



Run#	Type	Run	Reps	Inject	Sample ID	Method	Data File
1		Unknown	1	None		CHO Conditioning -...	Capillary Conditio
2		Unknown	1	SI:A1	Glucose Ladd...	CHO Separation - ...	CHO Run 1 <D>.d
3		Unknown	1	SI:A1	Glucose Ladd...	CHO Separation - ...	CHO Run 2 <D>.d
4		Unknown	1	SI:A1	Glucose Ladd...	CHO Separation - ...	CHO Run 3 <D>.d
5		Unknown	1	SI:A1	Glucose Ladd...	CHO Separation - ...	CHO Run 4 <D>.d
6		Unknown	1	SI:A1	Glucose Ladd...	CHO Separation - ...	CHO Run 5 <D>.d
7		Unknown	1	SI:A1	Glucose Ladd...	CHO Separation - ...	CHO Run 6 <D>.d
8		Unknown	1	SI:A1	Glucose Ladd...	CHO Separation - ...	CHO Run 7 <D>.d
9		Unknown	1	SI:A1	Glucose Ladd...	CHO Separation - ...	CHO Run 8 <D>.d
10		Shut down	1	None		CHO Shutdown - P...	

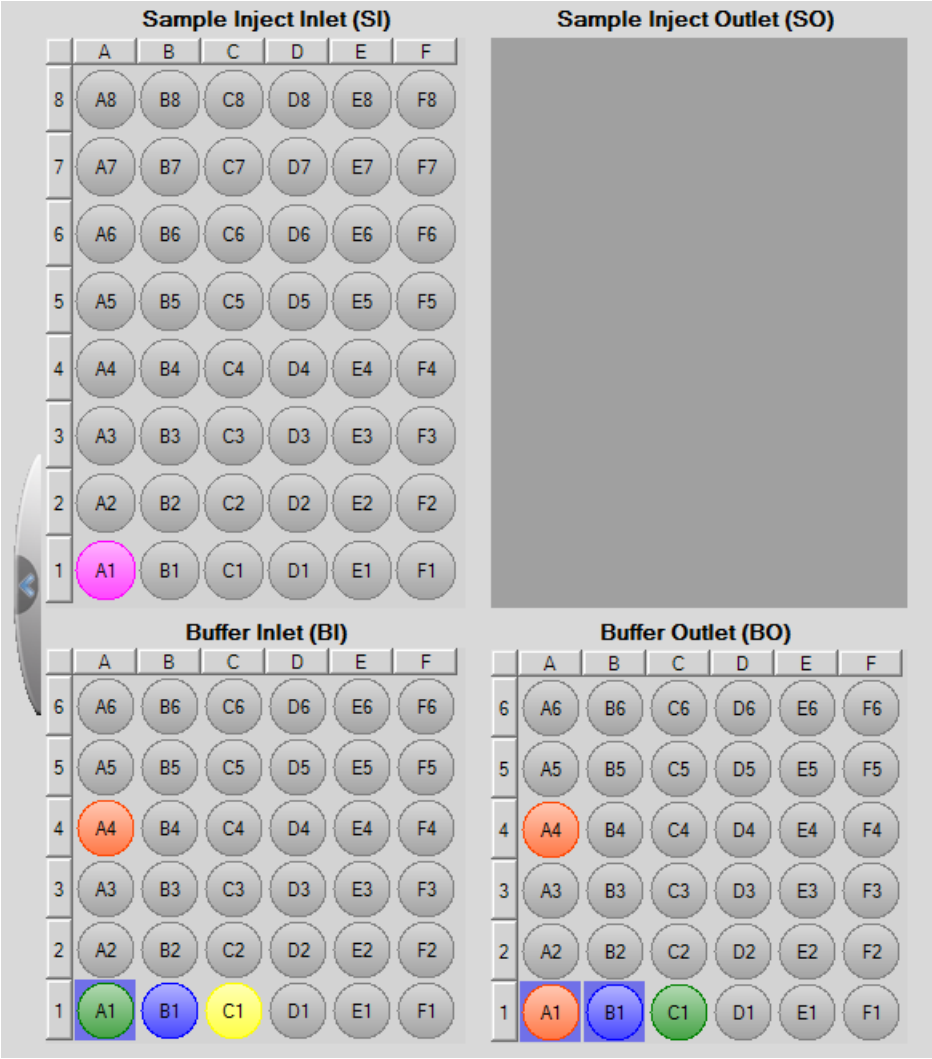
8. In the bottom right corner of the window, click Save (**Save**), and then click Finish (**Finish**).
9. In the **Number of samples** field, click the arrow buttons to set the number of samples for the run.

Figure 10 Set the Number of Samples



As the number of samples changes, the images of the buffer and sample trays on the right change to show the correct number of vials and their locations for the run.

Figure 11 Tray Map



10. If the buffer and sample trays have not been loaded, then do this:
    a. Click Load (**Load**).
    b. Load the buffer and sample trays in the PA 800 Plus system.
    c. Close the door.
11. Click Next (**Next**), and then click **Yes - run now**.

Figure 12 Samples Loaded Prompt

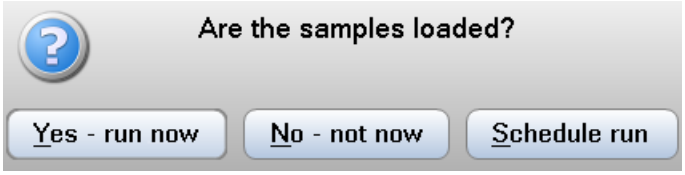
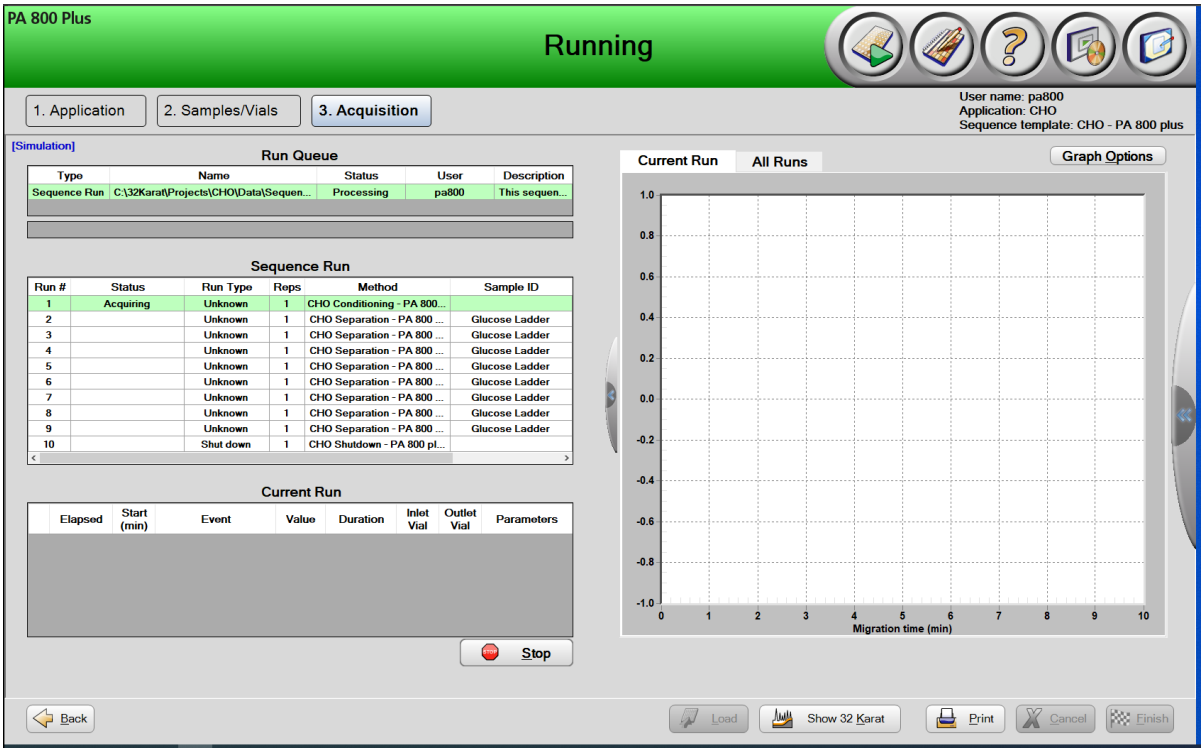


Figure 13 PA 800 Software During Data Acquisition



Waste Disposal

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

Stow the Cartridge

Stow the Cartridge Less Than 24 Hours

1. Use the shutdown method to clean the capillary.

The shutdown method fills the capillary with CE Grade water.

2. Keep the cartridge up to 24 hours in the system, with the capillary tips immersed in vials of CE Grade water.

Stow the Cartridge More Than 24 Hours

1. Use the shutdown method to clean the capillary.

   The shutdown method fills the capillary with CE Grade water.

2. Remove the cartridge from the system.
3. Put the cartridge in the cartridge storage box with the capillary tips immersed in vials of CE Grade water.
4. Keep the cartridge storage box upright in the refrigerator between 2 °C and 8 °C.

Prepare the Cartridge After Storage

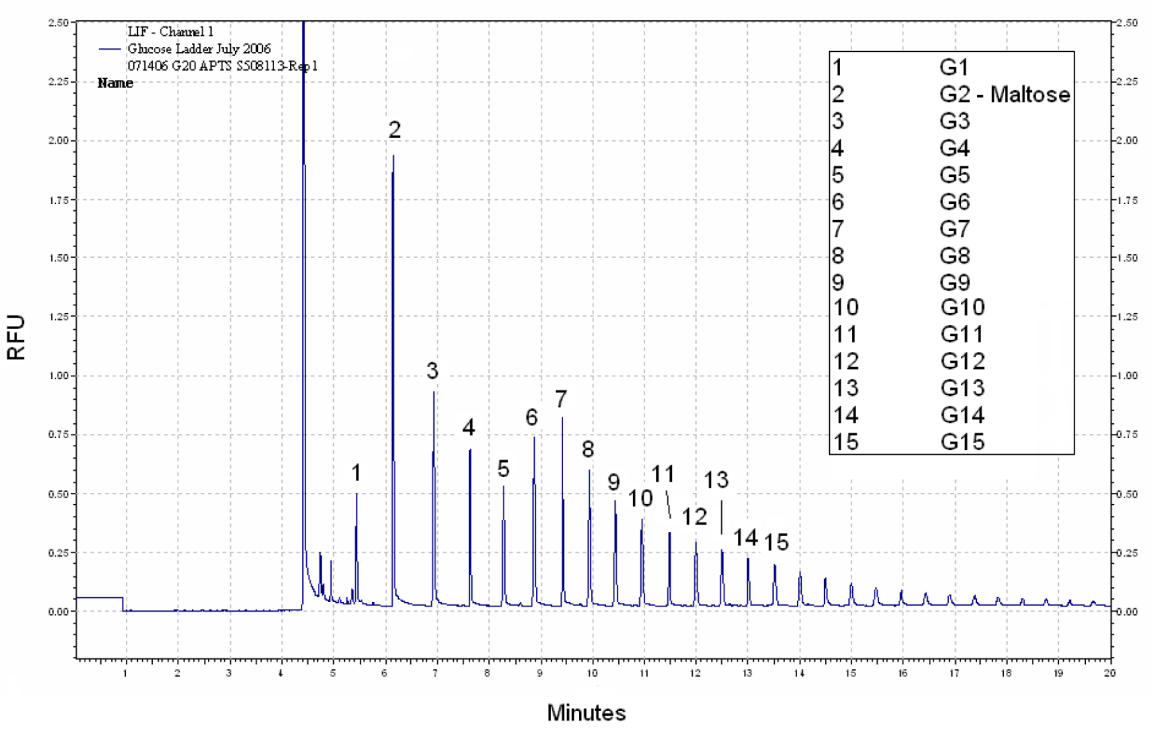
- If the cartridge has not been used for more than a day, then use the `CHO Conditioning - PA 800 plus.met` method to condition the capillary.

Analyze the Data

Analyze the Data for the Labeled G20-Glucose Ladder Standard

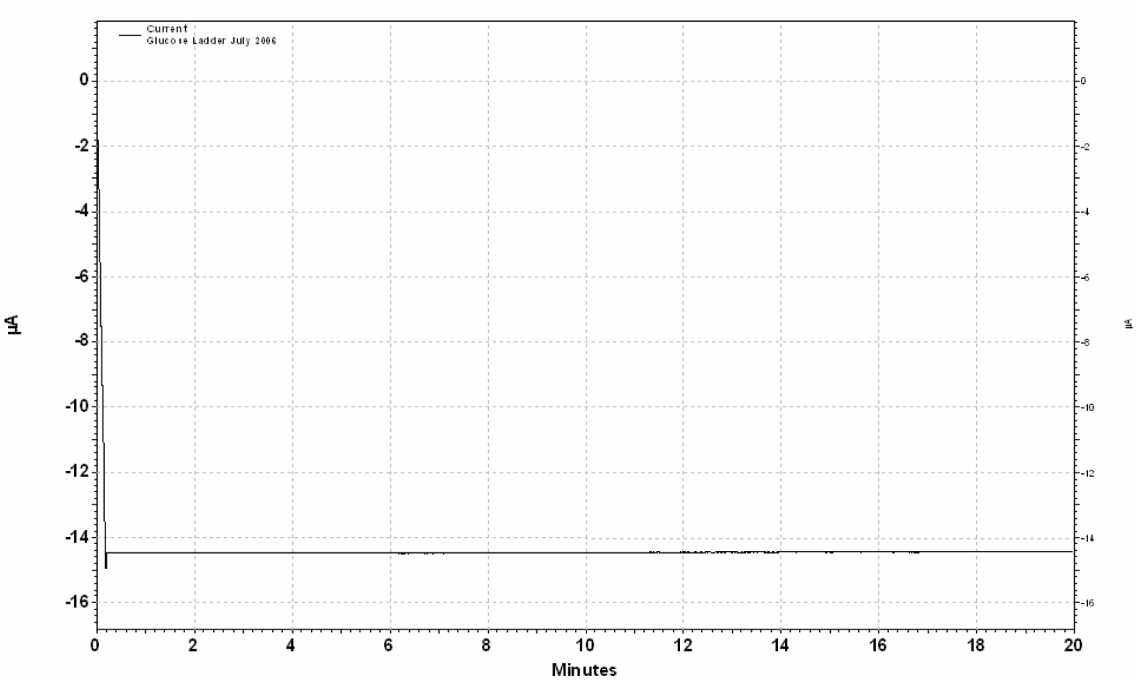
The labeled G20-Glucose Ladder Standard contains at least 20 individual glucose oligomers bonded to APTS Labeling Dye. For an example, refer to the following figure.

Figure 14 Electropherogram: Glucose Ladder



9019300L.png

Figure 15 Typical Current Profile



9019299L.png

Table 6 Typical Current Profile Parameters

Parameter	Settings
Field Strength Generated	598 V/cm
Typical Current	Less than 20 µA

Troubleshooting

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

Symptom	Possible Cause	Corrective Action
Low intensity peaks	1. The sample concentration is too low. 2. The deglycosylation was not complete. 3. Tris in the labeling reaction causes low yield.	1. Make sure that the amount of protein is between 25 µg and 300 µg. If the sample concentration is significantly lower, then concentrate the sample in a spin filter with a 10 kDa molecular weight cutoff (MWCO). 2. Make sure the that amount of protein is between 25 µg and 300 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. 3. Use a buffer that does not contain Tris. If needed, do a buffer exchange on the protein sample with a buffer that does not contain Tris.
Saturated peak intensity	The sample concentration is too high.	Dilute the sample with water, and then run the sample again.

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

Symptom	Possible Cause	Corrective Action
Carryover	The vials or caps are contaminated.	Do not use vials or caps more than once. Replace the buffer vials with clean vials filled with buffer, attach clean caps to the vials, and then increment as required. Replace the water vials with clean vials filled with CE Grade water, attach clean caps to the vials, and then increment as required. Make sure that the waste vials contain 1.0 mL of water and have been put in the outlet buffer tray. Add one or more water dip steps to the time program after the sample injection step.
Spikes in electropherogram	Air is dissolved in the separation gel.	To remove bubbles from the gel buffer, do one or all of these: • Sonicate the buffer vials for 10 seconds to 20 seconds to remove air bubbles. • Use a centrifuge to spin the vial at 30 × *g* for 5 minutes to remove air bubbles. If air bubbles are still present, then prepare new gel buffer vials. Do not mix the buffer with a vortex mixer.
Extra peaks	The plasticware used during sample preparation or the sample vials are contaminated with materials that interact with APTS-M.	Use new microvials, especially for steps related to labeling.

Hazardous Substance Information A

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

Hazard classification according to HCS 2012.

APTS-M

WARNING! Causes serious eye irritation.

L3-Labeling Dye Solvent

DANGER! Causes severe skin burns and eye damage.

N-Linked Carbohydrate Separation Buffer

WARNING! Causes mild skin irritation. May be harmful if swallowed.

Other Reagents

These components are not classified as hazardous:

- APTS Labeling Dye
- G20-Glucose Ladder Standard
- G22-Quantitation Control

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

References B

1. Fu Tai, A.Chen, Thomas S. Dobashi and Ramon A. Evangelista, *Glycobiology*, volume 8, pp 1045-1052, 1998. "Quantitative Analysis of Sugar Constituents of Glycoproteins by Capillary Electrophoresis."

Methods C

The Carbohydrate Labeling and Analysis application requires three methods.

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

Conditioning Method

Figure C-1 Initial Conditions Tab

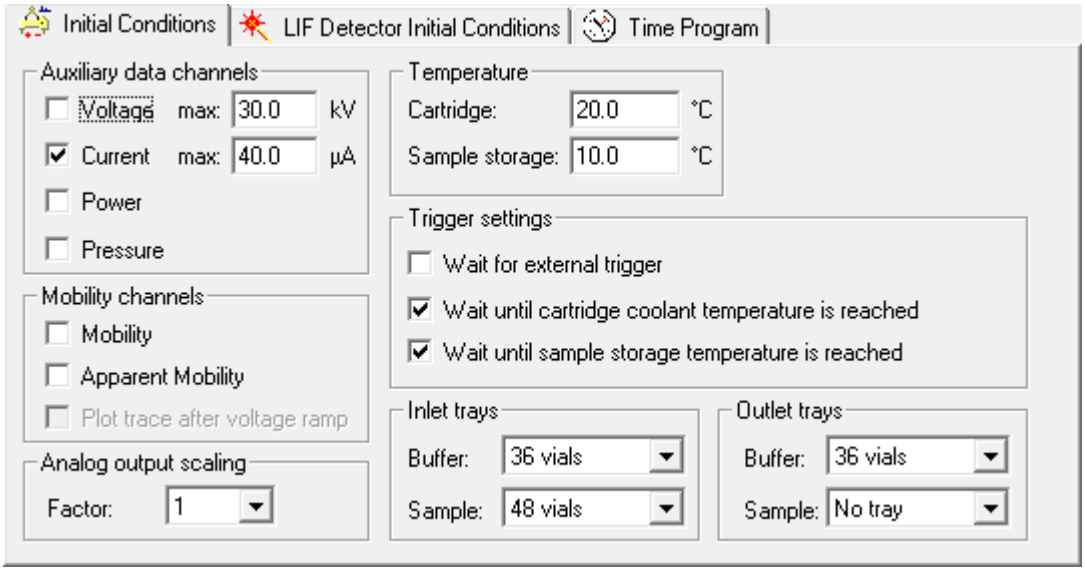


Figure C-2 LIF Detector Initial Conditions Tab

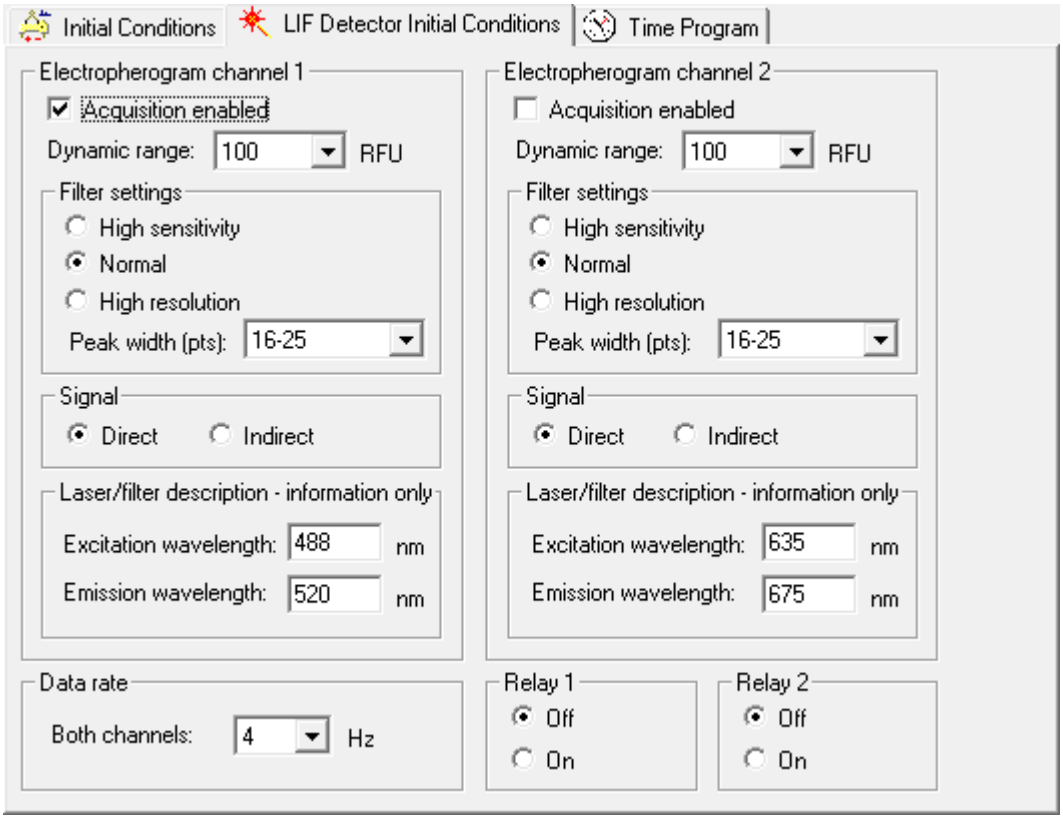
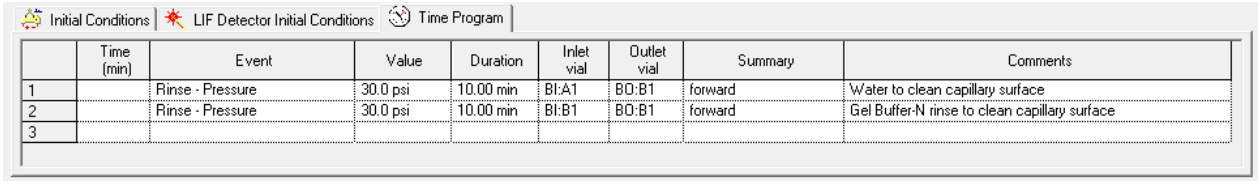


Figure C-3 Time Program Tab



	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	30.0 psi	10.00 min	BI:A1	BO:B1	forward	Water to clean capillary surface
2		Rinse - Pressure	30.0 psi	10.00 min	BI:B1	BO:B1	forward	Gel Buffer-N rinse to clean capillary surface
3								

Separation Method

Figure C-4 Initial Conditions Tab

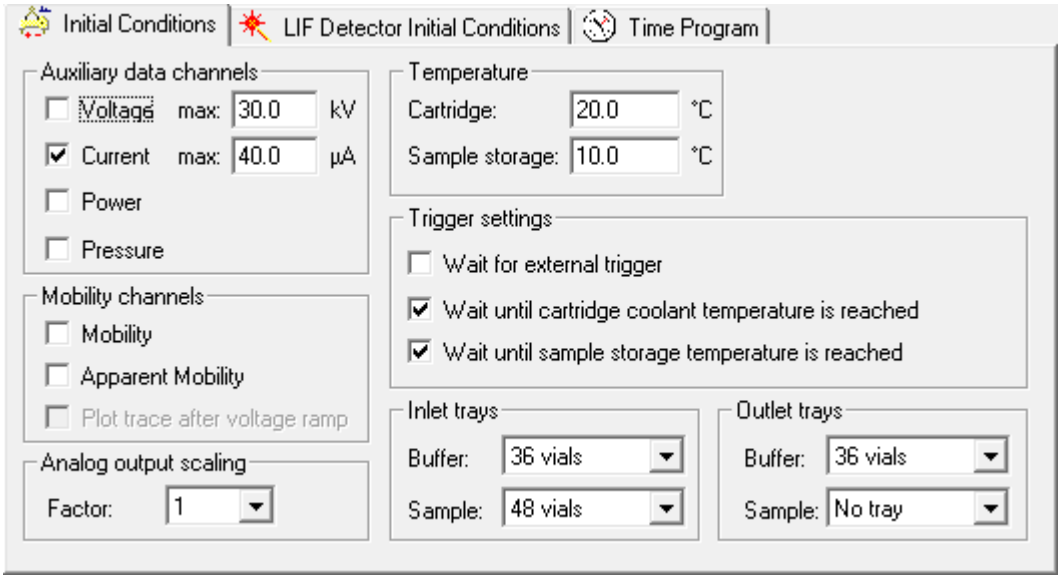


Figure C-5 LIF Detector Initial Conditions

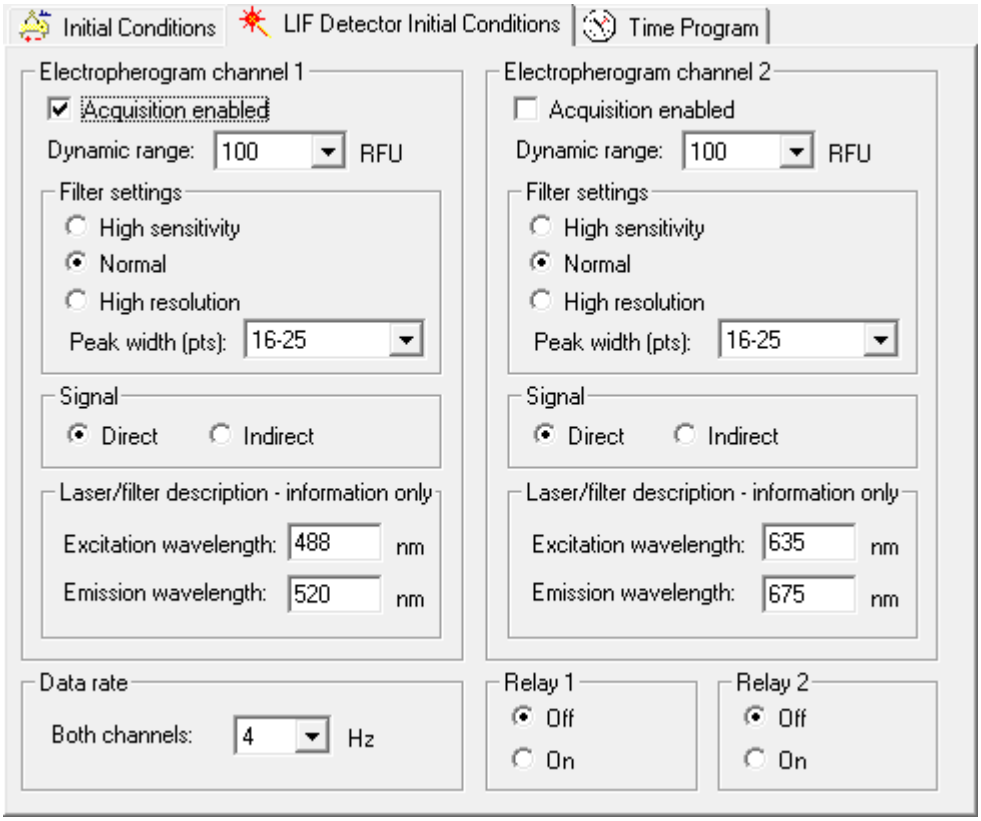
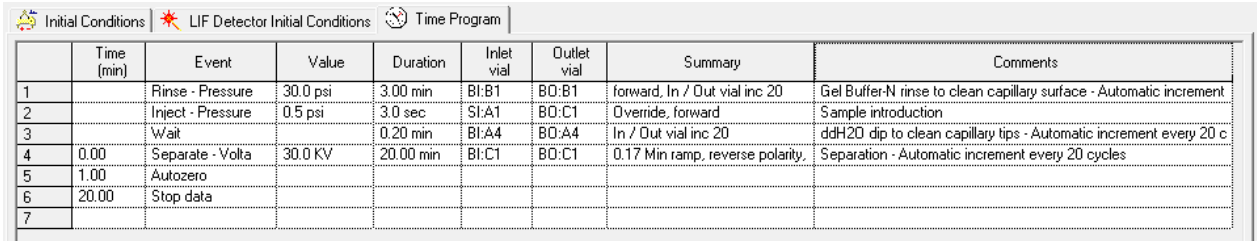


Figure C-6 Separation Method Time Program Tab



	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	30.0 psi	3.00 min	BI:B1	BO:B1	forward, In / Out vial inc 20	Gel Buffer-N rinse to clean capillary surface - Automatic increment
2		Inject - Pressure	0.5 psi	3.0 sec	SI:A1	BO:C1	Override, forward	Sample introduction
3		Wait		0.20 min	BI:A4	BO:A4	In / Out vial inc 20	ddH2O dip to clean capillary tips - Automatic increment every 20 c
4	0.00	Separate - Volta	30.0 KV	20.00 min	BI:C1	BO:C1	0.17 Min ramp, reverse polarity,	Separation - Automatic increment every 20 cycles
5	1.00	Autozero						
6	20.00	Stop data						
7								

Shutdown Method

Figure C-7 Initial Conditions Tab

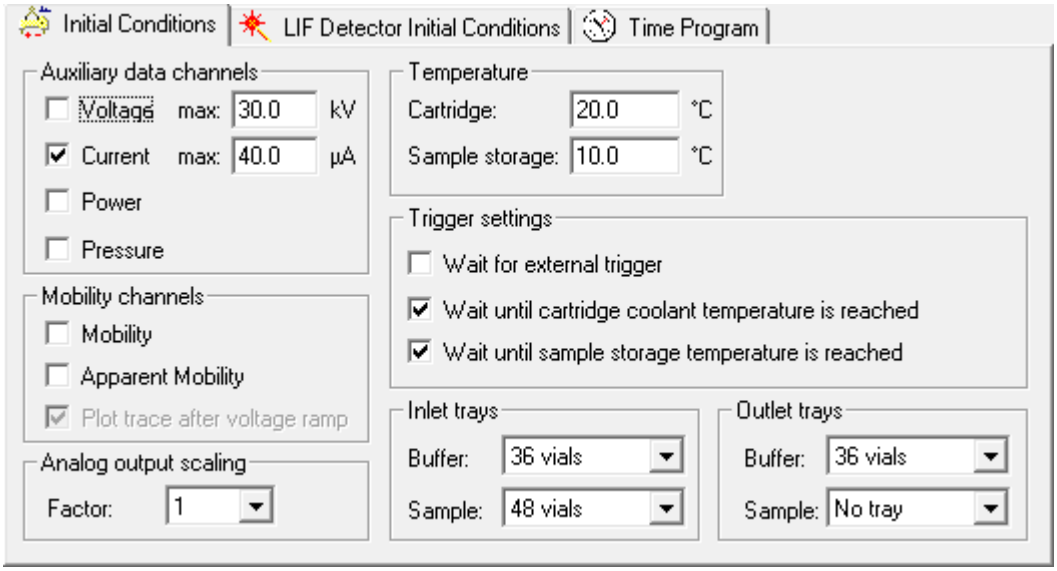


Figure C-8 LIF Detector Initial Conditions Tab

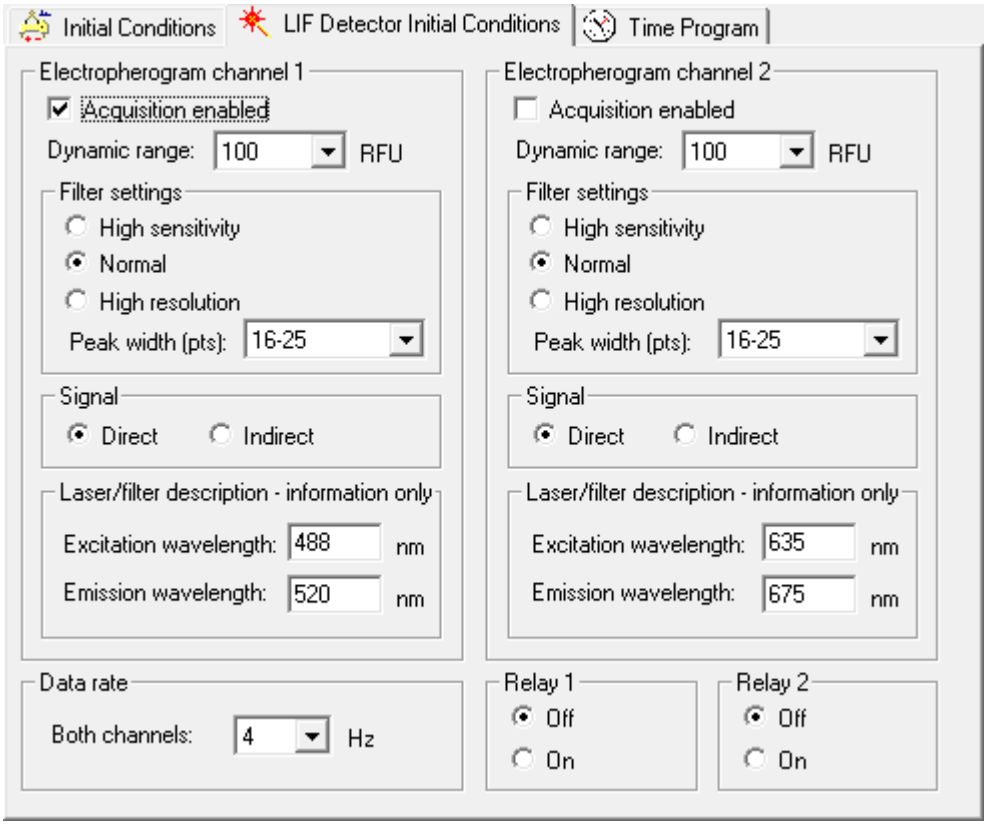
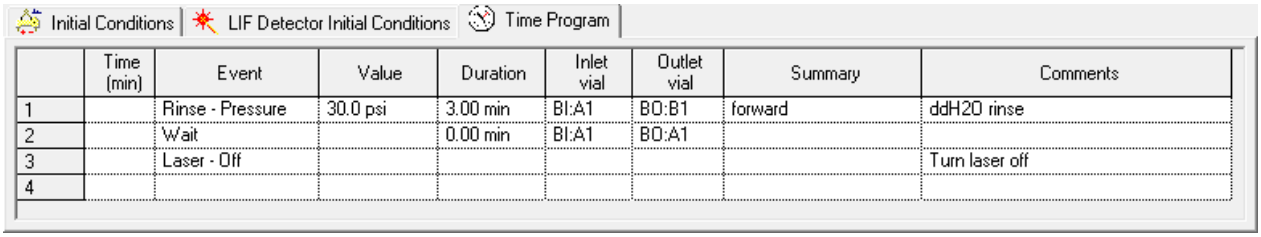


Figure C-9 Shutdown Method Time Program Tab



	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	30.0 psi	3.00 min	BI:A1	BO:B1	forward	ddH20 rinse
2		Wait		0.00 min	BI:A1	BO:A1		
3		Laser - Off						Turn laser off
4								

Calibrate the LIF Detector (Optional) D

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

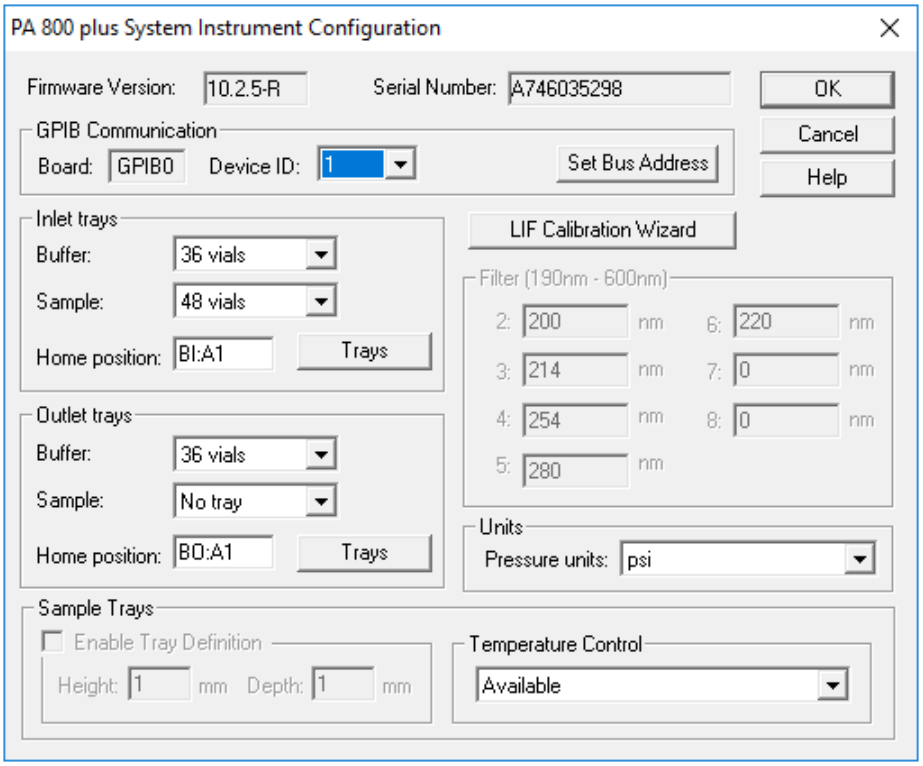
Calibrate the LIF detector after the LIF detector is installed, after a different cartridge is installed, or after a new capillary is installed in the cartridge.

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

Required Materials
• LIF Performance Test Mix • CE Grade water

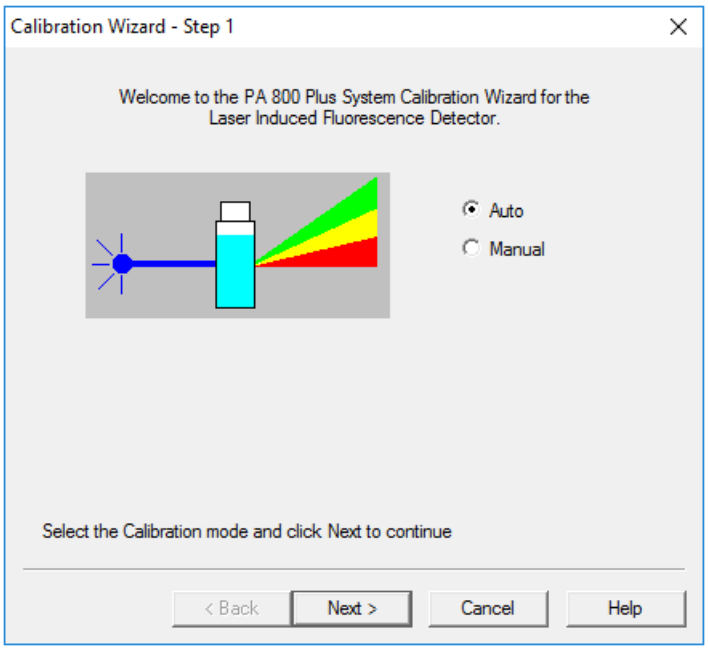
1. Turn on the PA 800 Plus system.
2. Open the 32 Karat software.
   The 32 Karat Software Enterprise window opens.
3. Open the LIF instrument, open the Direct Control window, and then turn on the laser.
4. In the 32 Karat Software Enterprise window, click **Tools** > **Enterprise Login**, and then log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

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



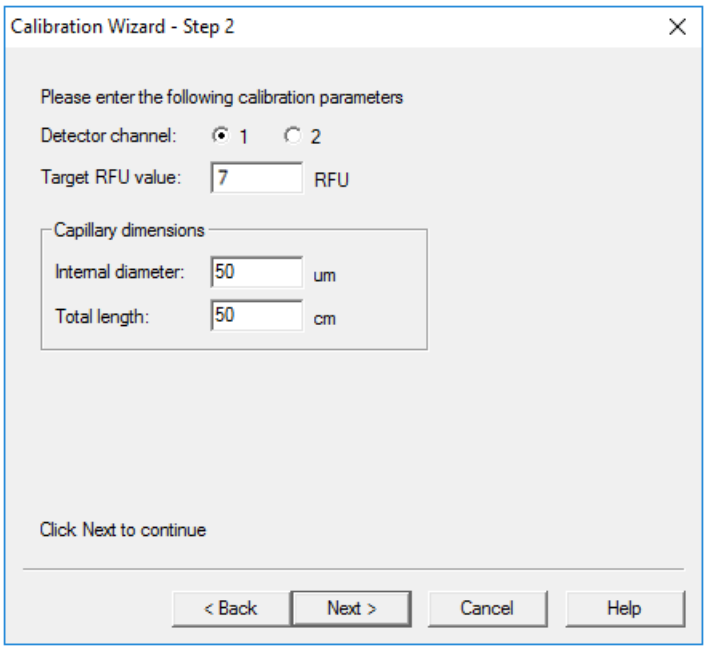
8. Click **LIF Calibration Wizard**.
9. Do the calibration:
    a. Click **Auto**, and then click **Next**.

Figure D-2 Calibration Wizard - Step 1



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

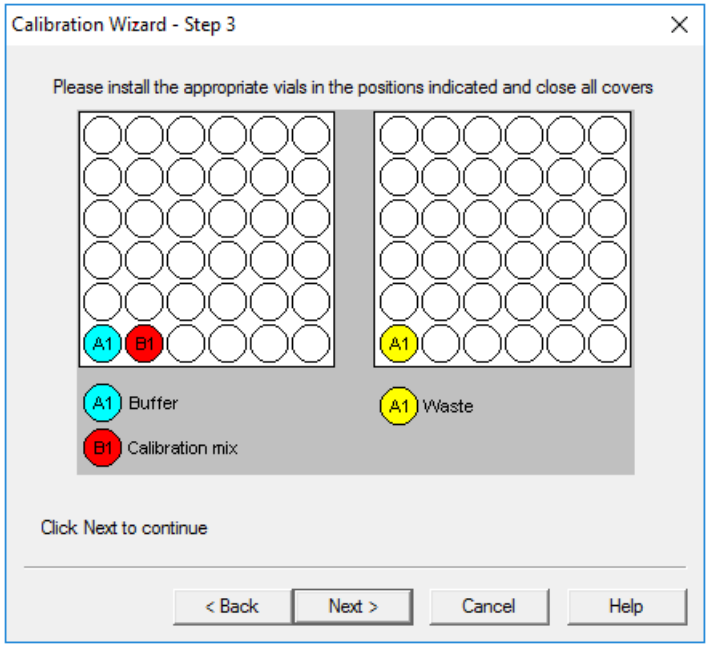
Figure D-3 Calibration Wizard - Step 2



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

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

Figure D-4 Calibration Wizard - Step 3



12. Fill the vials, and then put caps on the vials:
    - Inlet buffer tray position A1 (labeled `Buffer`): 1.5 mL of CE Grade water
    - Inlet buffer tray position B1 (labeled `Calibration mix`): 1.5 mL of LIF Performance Test Mix

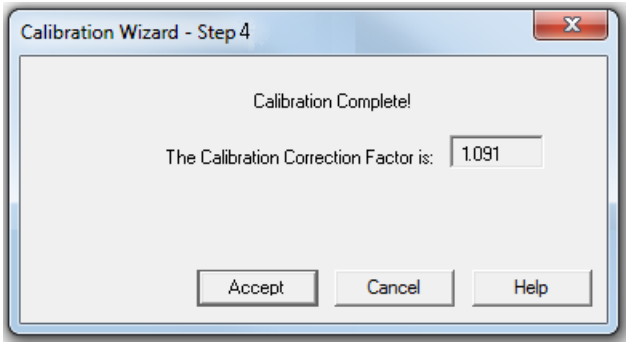
      Note: For the N-CHO capillary, dilute the LIF Performance Test Mix 1:1 with CE Grade water.

    - Outlet buffer tray position A1 (labeled `Waste`): 1.0 mL of CE Grade water
13. Click **Next**.
    The 32 Karat software does the calibration. When the calibration is complete, the Calibration Wizard - Step 4 window opens.

    If the message `No step change detected` is shown, then the detector cannot detect the solution. For troubleshooting procedures, refer to the section: No Step Change Detected.
14. Examine the value in the **Calibration Correction Factor** field:

- If the CCF value is less than 0.1, then click **Cancel**. Refer to the section: CCF Values for LIF Detector Calibration.
- If the CCF value is between 0.1 and 10, then the calibration was successful. Click **Accept** to save the results.
- If the CCF value is more than 10, then click **Cancel**. Refer to the section: CCF Values for LIF Detector Calibration.

Figure D-5 Calibration Wizard - Step 4



15. In the Direct Control window, set the sample storage temperature to 10 °C.
16. Close all of the dialogs and windows.

Troubleshoot the LIF Detector Calibration

CCF Values for LIF Detector Calibration

Issue	Action

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

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

Issue	Action

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

No Step Change Detected

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

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

7. If no step change is detected, then do the calibration procedure again. Refer to the section: Calibrate the LIF Detector (Optional).

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

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

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- 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.

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- SCIEX Now Learning Hub

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SCIEX Support

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

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

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

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