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Carbohydrate Labeling and Analysis Kit

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**CAUTION**

Prior to using the system, refer to the *PA 800 Plus Pharmaceutical Analysis System Overview Guide* for detailed information on the safe use and operation of the system.

**Safety**

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

**CAUTION**

When installing or removing capillaries from the cartridge, wear safety goggles.

**Setup and Run Recommendations**

- Before using the instrument, turn the laser on and let it stabilize for 15 minutes. Turn it off when not in use for longer periods of time.
- If a capillary is being used for carbohydrate analysis, do not use the same capillary in another application.
- When the capillary is not in use or after finishing with all experiments, run a shutdown method to rinse the capillary and leave the ends immersed in water to avoid capillary blockage and to keep the capillary from drying out.
- Before using the capillary for the first time, SCIEX recommends rinsing the capillary for 10 minutes at 30 psi pressure with DDI water followed by a 10 minute rinse at 30 psi with the carbohydrate separation gel buffer.
Introduction

The SCIEX Carbohydrate Labeling & Analysis Kit uses capillary electrophoresis to separate and quantify oligosaccharides released from glycoproteins. This kit has been developed to work with the PA 800 Plus Pharmaceutical Analysis System, using the 488 nm laser and the 520 nm emission filter.

This kit contains the reagents, buffer, and coated capillaries required to label, separate, and quantify oligosaccharides. This kit also provides a glucose size marker for relative size determination and a maltose standard for quantitation and mobility characterization of the released oligosaccharides.


**NOTE** This application guide has been validated with the PA 800 Plus Pharmaceutical Analysis System.

Workflow

The carbohydrate analysis involves three tasks:

Enzymatic release of the N-linked oligosaccharides from the glycoproteins. Refer to Release the Oligosaccharides From the Glycoproteins.

Labeling of the mixture of released oligosaccharides. Refer to Prepare the Labeling Reagents and Perform the Labeling Reaction.

Analysis of the fluorophore-labeled oligosaccharides by capillary electrophoresis. Refer to Prepare the PA 800 Plus System and Evaluate the Results.

Intended Use

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

Equipment and Materials Required

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Reorder Part Number</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate Separation Buffer</td>
<td>56 mL</td>
<td>477623</td>
<td></td>
</tr>
<tr>
<td>N-CHO Coated Capillary</td>
<td>2</td>
<td>477601</td>
<td></td>
</tr>
<tr>
<td>Labeling Dye (APTS)</td>
<td>4 x 5 mg</td>
<td>501309</td>
<td>L6</td>
</tr>
<tr>
<td>Labeling Dye Solvent (15% Acetic acid)</td>
<td>1 mL</td>
<td></td>
<td>L3</td>
</tr>
<tr>
<td>Glucose Ladder Standard</td>
<td>50 mg</td>
<td></td>
<td>G20</td>
</tr>
<tr>
<td>Quantitation/Mobility Marker (Maltose)</td>
<td>0.18 mg</td>
<td></td>
<td>G22</td>
</tr>
<tr>
<td>APTS-M (Monosaccharide grade)</td>
<td>20 mg</td>
<td>725898</td>
<td></td>
</tr>
</tbody>
</table>
Customer-Supplied Equipment and Supplies

- Pipettors and appropriate tips
- Vortex mixer
- Microcentrifuge
- Double-deionized (DDI) (MS-grade water filtered through a 0.2 μm filter and with resistance greater than 18 MΩ)
- Water bath
- Analytical scale
- Centrifugal vacuum evaporator
- Powder-free gloves
- Safety glasses
- Laboratory coat
- Spatula
- Nonidet NP-40 non ionic detergent
- 1X phosphate buffered saline (PBS)
- 5% sodium dodecyl sulfate (SDS)
- Ethanol

Storage Conditions

The kit components are stable for one year when stored under the following conditions:

- Upon receipt, store the kit at 2 °C to 8 °C.
- Store the reconstituted quantitation control/mobility marker (G22) and labeled glucose ladder standard (G20) at −35 °C to −15 °C.
• Store the reconstituted labeling reagent (APTS) at −35 °C to −15 °C. It is stable for 2 weeks after reconstitution.

**Prepare the Samples**

**Release the Oligosaccharides From the Glycoproteins**

**IMPORTANT** This labeling kit does not contain releasing enzymes.

There are multiple enzymatic and chemical procedures for releasing oligosaccharides from proteins. To successfully label the released oligosaccharide, it is essential that the reducing termini of the oligosaccharide is not destroyed by the deglycosylation method.

The following is a suggested protocol for N-deglycosylation, using N-glycosidase F (PNGase F):

**Enzymatic Release of the N-Linked Oligosaccharides**

1. Aliquot the sample to have between 25 μg to 300 μg of glycoprotein.

2. Dry the glycoprotein solution completely in a centrifugal vacuum evaporator.

3. Add 45 μL of 1X PBS buffer.

4. Add 1.0 μL of 5% SDS (or a volume that gives a final concentration of 0.1% SDS).

5. Add 1.5 μL of a 1:10 dilution of 2-mercaptoethanol in DDI water (or a volume that gives a final concentration of 50 mM 2-mercaptoethanol).

6. Heat the sample for 5 minutes at 100 °C.

   **IMPORTANT** If the denatured protein precipitates, discard the sample and restart this process, repeating steps 1 through 5. For step 6, denature the protein by incubating it for 10 minutes at 37 °C.

7. Cool the sample to room temperature.

8. Add 5 μL of Nonidet NP40 non-ionic detergent (or a sufficient volume that gives a final concentration of 0.75% Nonidet NP40 non-ionic detergent).
9  Add PNGase F enzyme, according to its activity.

10 Incubate for 15 hours at 37 °C.

11 Add approximately 150 μL of cold ethanol (or 3 times the actual reaction mixture volume.

12 Vortex the mixture and place it on ice for complete protein precipitation.

13 Centrifuge the samples for 5 minutes at 15 000 rpm.

14 Withdraw and save the supernatant.

   IMPORTANT  This solution contains the released N-linked oligosaccharides.

15 Discard the solid. This is the deglycosylated protein.

Working with the Supernatant

There are two options for working with the supernatant:

- For quantitative analysis, add an internal standard to the supernatant. Refer to Prepare the Standards beginning with step 1a.
- For qualitative analysis, bring the supernatant to dryness in the centrifugal vacuum evaporator. These oligosaccharides are ready to be labeled with APTS. Refer to Perform the Labeling Reaction.

Principle of the Labeling Method

After release (enzymatic or chemical), the oligosaccharides can be labeled with a fluorophore called 1-Aminopyrene-3,6,8-Trisulfonic Acid (APTS). The stoichiometry of the labeling reaction is one APTS molecule per molecule of oligosaccharide. The following figure illustrates the labeling reaction of an N-linked oligosaccharide with APTS. Refer to Figure 1.
Figure 1  Labeling Reaction of an Oligosaccharide with APTS

The efficiency of the labeling reaction is dependent on temperature and the amount of oligosaccharides. This protocol has been optimized for labeling 5 nmoles or less of total oligosaccharides. Samples with amounts greater than 5 nmoles may give a lower reaction yield. Use maltose (G22) as an internal labeling control or as an internal mobility marker.
Prepare the Labeling Reagents

Choose the Proper Fluorophore

- For labeling of monosaccharides, use APTS-M, a high-purity, monosaccharide-grade fluorophore. Refer to Prepare the APTS-M Labeling Dye.

  NOTE  APTS-M contains citric acid as a catalyst. Use APTS-M when the analysis goal is quantitation.

- For labeling glycans or oligosaccharides, either dye (APTS-M or APTS labeling dye – L6) can be used. Refer to Prepare the APTS-M Labeling Dye or Prepare the APTS Labeling Dye.


Prepare the APTS-M Labeling Dye

1. Add 400 μL of DDI water to the APTS-M vial.
2. Vortex the mixture for 5 seconds until all of the solid is dissolved.
3. Store the reconstituted APTS-M at −35 °C to −15 °C for up to two weeks.

Prepare the APTS Labeling Dye

1. Add 48 μL of labeling dye solvent (15% acetic acid – L3) to a vial of APTS labeling dye (L6).
2. Vortex for 5 seconds until complete dissolution.
3. Store at −35 °C to −15 °C for up to 2 weeks when not in use.

Prepare the Standards

1. Prepare the maltose quantitative control/mobility marker (G22).
   a. Add 500 μL of DDI water to the maltose quantitative control (G22). This results in a solution containing 500 nmoles of maltose at a concentration of 1 nmol/μL.
   b. For quantitation purposes, add 5 μL of the reconstituted maltose control to your released oligosaccharides.
   c. Dry down the sample in a centrifugal evaporator and proceed to Perform the Labeling Reaction.
d. Store at −35 °C to −15 °C when not in use.

**NOTE** When using the maltose solution for quantitation purposes, make sure that the solution is prepared fresh and used right away. Bacterial contamination can digest the sugars, leading to errors in quantitation.

## 2 Prepare the glucose ladder standard (G20).

a. Weigh 5 mg of glucose ladder standard (G20) and then dissolve in 80 μL DDI water in a 1.5 mL microcentrifuge tube. Sonicate if necessary.

b. Aliquot ten 2 μL portions of the glucose ladder standard solution to 0.5 mL microcentrifuge vials and dry them in a centrifugal vacuum evaporator. The dried glucose ladder aliquots can be stored at room temperature or used immediately.

---

## Perform the Labeling Reaction

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

Refer to **APPENDIX A** for additional information.

## 1 Label the sample or glucose ladder standard (G20).

a. Add 2 μL of 1 M sodium cyanoborohydride (in THF) to the dried oligosaccharide sample.

b. Add 2 μL of APTS labeling reagent to the sample.

**IMPORTANT** Due to the reaction of sodium cyanoborohydride with water, avoid moisture and store this product under dry conditions. Use a dry needle to dispense this chemical. Pass dry argon into the chemical bottle through the septum while dispensing 2 μL from the bottle.

c. Incubate the reaction mixture:
   - For 4 hours at 37 °C
   - For 90 minutes at 60 °C
   - Overnight at room temperature (mildest reaction)

**NOTE** To prevent loss of sialic acid residues from the oligosaccharide chain, incubate the sample overnight at room temperature. When incubating the glucose ladder standard, use any of the incubation conditions.

*Figure 2* illustrates a summary of the overall labeling protocol.
2 Prepare the sample or glucose ladder standard (G20) for capillary electrophoresis.

a. To the 4 μL reaction mixture prepared above:
   - Add 96 μL of DDI water to the glucose ladder standard.
   - Add 46 μL of DDI water to all other samples.

   This solution can be stored at 2 °C to 8 °C.

b. Take an aliquot of 5 μL of the solution above and add 195 μL of DDI water.

c. Mix the contents well and transfer the solution into a micro vial for analysis in the PA 800 Plus system. Refer to Prepare the Sample Vials.

Figure 3 illustrates a summary of the sample preparation for injection.
Prepare the PA 800 Plus System

Before proceeding, review the following procedures in the PA 800 Plus System Maintenance Guide.

- Installation of the LIF Detector
- Calibration of the LIF Detector; also refer to APPENDIX B
- Capillary Cartridge Procedures
- Cleaning the Interface Block and the Opening Levers
Install and Pre-condition the N-CHO Capillary

Prior to using the PA 800 Plus system for carbohydrate analysis, the N-CHO capillary must be installed in a capillary cartridge. To properly install the capillary, refer to the capillary cartridge sections in the PA 800 Plus System Maintenance Guide.

**IMPORTANT** Be sure to pre-rinse a new capillary for 10 minutes at 30 psi pressure with DDI water followed by a 10 minute rinse at 30 psi with carbohydrate separation buffer before the first run.

This analysis uses a 50 μm I.D. N-CHO capillary. The length from the injection inlet to the detector should be 40 cm, with a total length of 50.2 cm. Measure the capillary dimensions accurately and record the dimensions in the Capillary/Performance tab of the Advanced Method Options dialog (Figure 4). This is required for accurate mobility determinations.

**Figure 4** Capillary/Performance Tab of the Advanced Method Options Dialog

![Capillary/Performance Tab](image)

**IMPORTANT** To get good reproducibility from capillary to capillary and accurate mobility assignments, it is important to follow the capillary pre-measurement procedure.

**IMPORTANT** Using magnification, carefully inspect the ends of the capillary. The cut must be clean (not jagged) and perpendicular to the capillary length (not angled). Poor cuts will result in poor resolution and poor sample loading.

**NOTE** When not in use, submerge the capillary ends in DDI water to prevent the capillary coating from drying out.

- Turn off the PA 800 Plus system and install the LIF detector.
- Turn on the system and permit the laser to warm up for at least 30 minutes prior to calibration.
Clean the Interface

Carefully clean the system electrodes, opening levers, capillary tips, and interface block following the cleaning procedure as described in the PA 800 Plus System Maintenance Guide. Do this on a weekly basis or when changing chemistries on the PA 800 Plus system. Accumulation of reagent deposits on the interface block can lead to current leakage errors.

Insert the Cartridge and Calibrate the LIF Detector

Insert the cartridge into the system. Close the cartridge and the sample covers. Calibrate the LIF detector. Refer to APPENDIX B.

Prepare and Load the Buffer Vials

Fill the vials with the appropriate volumes of each reagent according to the buffer tray maps (Figure 6 and Figure 7).

1. Fill the four water (H₂O) vials with 1.5 mL of DDI water.

2. Fill the 1 gel rinse (Gel-R) vial with 1.5 mL of carbohydrate separation buffer.

3. Fill the 2 gel separation (Gel-S) vials with 1.3 mL of carbohydrate separation buffer.

4. Fill the 1 waste vial with 0.8 mL of DDI water.

WARNING

Do not fill the waste vial with more than 0.8 mL. If the vial has more than 0.8 mL, the pressure system might be damaged.
**NOTES**

Carefully fill the buffer vials without producing bubbles and use the recommended volume.

5 Load the reagent vials into the inlet (Figure 6) and outlet (Figure 7) buffer trays.

**NOTES**

- During the separation, salts migrate from one vial of gel buffer to the other, changing the composition of the buffer. Therefore, SCIEX recommends that the vials of buffer be replaced with fresh buffer after 20 runs. For unattended operation of more than 20 runs, simply program the increment function to increment the water and buffer vials after 20 runs. Vials will increment in a forward direction. Make sure that the tray is filled with the appropriate number of vials to manage the number of runs to be performed.

- The H2O vials clean the capillary tips after the sample introduction step to prevent sample carryover. Replace the vials with fresh water if the rinse water becomes contaminated.
Figure 6  Inlet Buffer Tray Map

Figure 7  Outlet Buffer Tray Map
Prepare the Sample Vials

Before putting the 200 μL sample vials into the universal vials, make sure that no bubbles are at the bottom of the sample vials. If bubbles exist, centrifuge the sample vials for 2 minutes at 1000 g and repeat if necessary. Put a blue cap on the universal vial and make sure it is secure. Refer to Figure 8.

Put the universal vials into the 48-position inlet sample tray from positions A1 through C8.

![Figure 8 Sample Vial Setup](image)

1. Universal Cap
2. Micro Vial
3. Universal Vial
4. Micro Vial inside Universal Vial

Create the Methods

Initial Conditions

<table>
<thead>
<tr>
<th>Table 4  Test Run: Initial Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameter</strong></td>
</tr>
<tr>
<td>Capillary Temperature</td>
</tr>
<tr>
<td>Sample Storage Temperature</td>
</tr>
<tr>
<td>Auxiliary Data Channel</td>
</tr>
</tbody>
</table>
LIF Detector Initial Conditions

Table 5 Test Run: LIF Detector Initial Conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Settings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection</td>
<td>Laser Induced Fluorescence</td>
</tr>
<tr>
<td>Wavelength</td>
<td>Excitation - 488 nm</td>
</tr>
<tr>
<td></td>
<td>Emission - 520 nm</td>
</tr>
<tr>
<td>Data Rate</td>
<td>4 Hz</td>
</tr>
</tbody>
</table>
NOTE Before use, calibrate the LIF detector using the procedure in APPENDIX B. For comparable results, the detection system should be calibrated at system start-up or whenever a capillary is replaced.

**Time Program**

1. Rinse the capillary with carbohydrate separation buffer for 3 minutes at 30 psi from BI:B1 to waste at vial BO:B1.

2. Inject the sample at 0.5 psi for 3 seconds from the sample vial to the buffer vial BO:C1.
   
   **NOTE** A 3 second to 20 second, 0.5 psi pressure injection is recommended. Longer injection times might cause peak shape and migration times to vary.

3. Wait 0.2 minutes with vials BI:A4 and BO:A4. Refer to Figure 11. This step dips the capillary in water to protect against sample carry over. Change the rinse water vials if they are contaminated.
4 Separate Step - 20 minutes from vial Bl:C1 to vial BO:C1 (buffer). Refer to Figure 11. The constant voltage should be set at 30 kV, with reverse polarity, and a 0.17 ramp time.

5 Autozero at 1.0 minute.

6 End at 20.0 minutes.

**Figure 11** Time Program Tab of the Instrument Setup Dialog

---

**Evaluate the Results**

The test mixture contains the APTS-labeled glucose oligomers consisting of at least 20 individual oligomers. An example of this test mix is highlighted in Figure 12.

**Figure 12** Typical Current Profile
Table 6  Typical Current Profile

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Settings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field Strength Generated</td>
<td>598 V/cm</td>
</tr>
<tr>
<td>Typical Current</td>
<td>under 20 μA</td>
</tr>
</tbody>
</table>

**Figure 13  Electropherogram - Glucose Ladder**

**System Shutdown and Capillary Storage**

**Short Term Storage (< 24 hours) of Capillary**

Perform a 3 minute, 30 psi rinse with water. The capillary may be stored on the instrument with the capillary ends immersed in water.

If the capillary has not been used for three hours or longer, rinse the capillary by performing a 3 minute, 30 psi rinse with water and then a 10 minute rinse with carbohydrate separation buffer before performing a separation.

**Long Term Storage (> 24 hours) of Capillary**

1. Perform a 3 minute, 30 psi rinse with water. Repeat the rinse with carbohydrate separation buffer for 3 minutes.

2. Remove the capillary from the instrument and place in a cassette box with the capillary ends placed in vials of DDI water.
3 Store the cartridge box at 2 °C to 8 °C in an upright position.

Troubleshooting

Table 7 Troubleshooting

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Possible Cause</th>
<th>Corrective Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>No peaks</td>
<td>LIF detector was not calibrated.</td>
<td>Calibrate the LIF detector.</td>
</tr>
<tr>
<td>Laser is off.</td>
<td></td>
<td>Turn on laser.</td>
</tr>
<tr>
<td>Fiber optic connector was not pushed all the way inside the LIF cartridge probe stabilizer.</td>
<td>Make sure that the lock down bar is pushed all the way down and that the fiber optic is pushed all the way inside the LIF probe stabilizer.</td>
<td></td>
</tr>
<tr>
<td>Wrong polarity is set in method.</td>
<td>Make sure that reverse polarity is used in the separation method; during separation orange LED will be on.</td>
<td></td>
</tr>
<tr>
<td>No sample was injected due to air bubble present in sample vial.</td>
<td>Spin down micro vial for a few seconds to remove air bubbles.</td>
<td></td>
</tr>
<tr>
<td>Capillary window is broken.</td>
<td>Replace capillary.</td>
<td></td>
</tr>
<tr>
<td>Capillary window and/or LIF probe aligner is dirty.</td>
<td>Clean capillary window and/or probe aligner.</td>
<td></td>
</tr>
<tr>
<td>Low intensity peaks</td>
<td>Low labeling yield is due to Tris presence.</td>
<td>Buffer exchange protein sample with a non-Tris buffer.</td>
</tr>
<tr>
<td>Low labeling yield is due to water.</td>
<td>Make sure that the carbohydrate pellet is fully dry before performing the labeling reaction.</td>
<td></td>
</tr>
<tr>
<td>Low deglycosylation yield.</td>
<td>Make sure that the amount of enzyme is enough to deglycosylate the protein sample completely.</td>
<td></td>
</tr>
<tr>
<td>Low electrical current during the separation</td>
<td>Capillary is longer than 50 cm.</td>
<td>Make sure that capillary length is 50 cm.</td>
</tr>
<tr>
<td>Separation temperature is lower than 20 °C.</td>
<td>Make sure that the method uses a capillary temperature of 20 °C.</td>
<td></td>
</tr>
<tr>
<td>Buffer vials set incorrectly in buffer trays.</td>
<td>Make sure that separation method uses a vial filled with Carbohydrate Gel Buffer in the voltage separation step.</td>
<td></td>
</tr>
<tr>
<td>Shift in migration time between runs, within the same day</td>
<td>Injection volumes are changing between separations.</td>
<td>Make sure that the sequence uses the same injection parameters between different samples.</td>
</tr>
<tr>
<td>Carryover</td>
<td>Water dip and/or buffer vials are contaminated with sample.</td>
<td>Change water dip and/or buffer vials or increment them more often in the separation method.</td>
</tr>
<tr>
<td>Spikes in electropherogram</td>
<td>Air is dissolved in the gel buffer.</td>
<td>Sonicate the gel for 5 minutes before use.</td>
</tr>
<tr>
<td>Extra peaks</td>
<td>Tube used in labeling reaction has contaminants that react with APTS during the labeling step.</td>
<td>Use extra clean 0.5 mL micro-centrifuge vials, especially during the labeling step.</td>
</tr>
</tbody>
</table>
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.

Hazardous Substances

APTS-M (Monosaccharide grade)

WARNING! Causes serious eye irritation.

Carbohydrate Separation Buffer

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

Labeling Dye Solvent (15% Acetic Acid)

DANGER! Causes severe skin burns and eye damage.
**LIF Detector Calibration Procedure**

This procedure is used to calibrate the detection system for carbohydrate analysis. This procedure makes sure that consistent results are obtained from day to day and from capillary to capillary, as detection is measured in relative fluorescence units (RFU). This calibration procedure allows normalization of the signal generated from the analyte, relative to a known concentration of sodium fluorescein. Perform the calibration procedure when installing a new capillary or after detector and cartridge changes.

**IMPORTANT** The following calibration procedure is different from previous versions, resulting in different RFU values. The calibration correction factor (CCF) is a scalar multiple of all data points, so there is no impact on signal to noise performance. This change was made to reduce the risk of saturating the detector from excessively high fluorescence signals.

**Table B.1 Required Reagents**

<table>
<thead>
<tr>
<th>Materials</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIF Performance Test Mix</td>
<td>726022</td>
</tr>
<tr>
<td>DDI water</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Calibrate the LIF Detector

Setting the Calibration Corrector Factors (CCFs)

IMPORTANT The user must have instrument administration privileges.

1 Launch the 32 Karat software.

2 Go to Tools > Enterprise Login.

3 Enter the user name PA 800 and password plus and select Log in.

4 Select the CHO instrument icon and then Configure Instrument.

5 Select Configure on the instrument configuration dialog.

6 Select the LIF Detector icon in the right pane of the PA 800 plus System Configuration dialog.

7 The PA800 System Instrument Configuration dialog opens.

Figure B.1 PA 800 Plus System Configuration Dialog
8 Click LIF Calibration Wizard in the Instrument Configuration dialog to display the Calibration Wizard.

Automatic Calibration

1 Choose Auto as the calibration option and click Next.

Figure B.2 Calibration Wizard Dialog - Step 1
2 Set the target RFU value. The target value for the N-CHO-coated capillary is 7. Click Next.

Figure B.3 Calibration Wizard Dialog - Step 2

3 Fill one buffer vial with 1.5 mL DDI water and another with 200 μL of diluted LIF Performance Test Mix (diluted 1:2 with DDI water) in a micro vial. Place vials in the buffer vial positions as indicated in Figure B.4.

4 Place an empty vial in the waste position.
**IMPORTANT** The system performance test kit (PN 713360) contains instructions for performing detector noise tests on a bare-fused silica capillary, which uses borate buffer. Do not use the borate buffer for calibration of the N-CHO capillary. Use a 50% LIF Performance Test Mix solution (100 μL of LIF Test Mix with 100 μL of DDI water) in a micro vial.

5 Click **Next** to perform the automatic calibration.

![Figure B.4 Calibration Wizard Dialog - Step 3](image)

6 After the calibration is completed, a dialog opens. Refer to **Figure B.5**.

A number will appear in the Calibration Correction Factor field.

- If the number is below 10, click **Accept** to complete the calibration procedure.
- If the number is above 10, refer to the CCF Troubleshooting Procedures.

![Figure B.5 Calibration Wizard Dialog - Step 4](image)
Calibrate the LIF Detector

## CCF Troubleshooting Procedures

### Table B.2  CCF Troubleshooting

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Possible Cause</th>
<th>Corrective Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCF is between 0.1 and 1</td>
<td>No problem with the system</td>
<td>Run a standard and verify acceptable performance of the system.</td>
</tr>
<tr>
<td>CCF is less than 0.1 however system performance is not acceptable</td>
<td>Wrong or broken capillary used</td>
<td>Verify that the capillary for the carbohydrate analysis was used. Verify capillary is not broken.</td>
</tr>
<tr>
<td></td>
<td>Laser output faulty</td>
<td>Verify laser output for the laser in use on the PA 800 Plus system.</td>
</tr>
<tr>
<td></td>
<td>Incorrect filters installed in the LIF detector</td>
<td>Verify that the correct filters are installed in the LIF detector</td>
</tr>
<tr>
<td>CCF is between 1.0 and 10</td>
<td>No problem with synthesis.</td>
<td>Run a standard to determine if the system performance is still acceptable.</td>
</tr>
<tr>
<td>CCF is greater than 10 or the system performance is not acceptable</td>
<td>Laser output faulty</td>
<td>Verify laser output for the laser in use on the PA 800 Plus system.</td>
</tr>
<tr>
<td></td>
<td>Incorrect filters installed in the LIF detector</td>
<td>Verify that the correct filters are installed in the LIF detector</td>
</tr>
<tr>
<td></td>
<td>LIF Performance Test Mix prepared incorrectly.</td>
<td>Prepare LIF Performance Test Mix as described in this guide.</td>
</tr>
<tr>
<td></td>
<td>Capillary internal diameter is larger than 50 microns.</td>
<td>Make sure that the NCHO capillary is installed in cartridge.</td>
</tr>
<tr>
<td></td>
<td>Instrument failure.</td>
<td>Contact sciex.com/request-support.</td>
</tr>
</tbody>
</table>

### No Step Change Detected

The LIF Calibration method compares the detector signal from a non-fluorescent solution to the signal from a fluorescent solution. When rinsed with the non-fluorescent solution followed by a fluorescent solution rinse, you should see the first part of the detector signal near zero and the second part of the detector signal near the target fluorescent value. This detector output is step shaped in appearance.

If no step change is observed, the appropriate solutions are not passing the detector or the detector cannot detect them.

1. Verify that the solution is rinsing through the capillary by performing a direct control 5 minute, 20 psi pressure rinse of water, from buffer inlet A1 to an empty buffer vial in outlet B1.

2. Once the rinse has begun, lift the sample cover.

3. Observe the outlet end of the capillary in B1. Droplets should be formed on the outlet end of the capillary.
If no droplets are formed, either the capillary is plugged or the instrument has a pressure failure. Replace the capillary and repeat the rinse. If there is still no droplet observed, contact SCIEX Technical Support at sciex.com/request-support. Once flow through the capillary is verified, the detection system is the only other possible cause, when no step is detected.

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Verify that the correct filters are installed in the LIF detector.</td>
</tr>
<tr>
<td>5</td>
<td>Verify that the laser provided with the PA 800 Plus system is connected and on.</td>
</tr>
<tr>
<td>6</td>
<td>Repeat the calibration with a new capillary and fresh reagents. If there is still no step change, contact SCIEX Technical Support at sciex.com/request-support.</td>
</tr>
</tbody>
</table>
Revision History

Initial Issue, A51969AA, April 2009
32 Karat Software version 9.1
PA 800 plus Software version 1.1
PA 800 plus Firmware version 9.0

First Revision, A51969AB, December 2009
Revised corporate address

Second Revision, A51969AC, February 2011
32 Karat Software version 9.1 patch
PA 800 plus Software version 1.1 patch
PA 800 plus Firmware version 9.2
Numerous syntax and grammatical edits

Third Revision, A51969AD, January 2014
PA 800 plus Software version 10.1 (includes 32 Karat software)
PA 800 plus Firmware version 10.1 (incompatible with 9.x)
Formatting updates, Aminopyrene Trisulfonic Acid naming standardization

Fourth Revision, A51969AE, April 2018

This guide applies to the latest software and firmware listed above, and any higher subsequent versions. When a subsequent software or firmware version affects the information in this guide, a new issue will be released to the SCIEX website. For updates, go to www.sciex.com and download the latest version of the guide.