SCIEX
The Power of Precision

RNA 9000 Purity & Integrity Kit

For the PA 800 Plus Pharmaceutical Analysis System

Application Guide

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EC Authorized Person

AB Sciex Netherlands B.V.
1e Tochtweg 11,
2913LN Nieuwerkerk aan den Ijssel
Netherlands

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

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RNA 9000 Purity & Integrity Kit

The RNA 9000 Purity & Integrity Kit contains reagents and supplies for sample preparation and methods to resolve RNA fragments by size and to quantify heterogeneity and impurities that might exist in an RNA preparation.

This document gives instructions for sample preparation with the RNA 9000 Purity & Integrity Kit. It also gives instructions for data acquisition and data analysis with the PA 800 Plus software.

Use the information in this application guide as a starting point. As required, change injection time, voltage, injection type, or other parameters to find the best conditions for your needs.

Note: For instructions for safe use of the system, refer to the document: *System Overview Guide*.

Safety

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

Intended Use

The RNA 9000 Purity & Integrity kit is for laboratory use only.

Introduction

The RNA 9000 Purity & Integrity kit is designed for biopharmaceutical scientists working on next generation RNA therapeutics. This kit provides high analytical resolution, helps alleviate method complexity, and simplifies transferability. The kit has been validated on both the PA 800 Plus and BioPhase 8800 systems.

The methodology involves heat denaturation of an RNA sample followed by immediate cooling in an ice water bath. This forces the nucleic acid into the structure that provides the most consistent mobility during the separation.

The RNA sample is separated by size in a bare-fused silica capillary containing a replaceable polymer gel that provides sieving selectivity while concurrently limiting counter electroosmotic flow (EOF). The fluorescent labeling dye SYBR™ Green II RNA Gel Stain[1] is added to the

[1] SYBR™ is a trademark of the Life Technologies Corporation. SYBR™ Green II RNA Gel Stain is not available for resale.

polymer gel matrix during reagent preparation. The dye preferentially binds to RNA molecules so that the RNA can be detected by laser-induced fluorescence (LIF) during the separation.

Workflow

Table 1 RNA 9000 Purity & Integrity Kit Workflow

Step	To Do This	Refer To
1	Prepare the RNA sample at a concentration between 50 ng/mL to 50 µg/mL.	The RNA preparation procedure for the laboratory.
2	(Optional) Dilute the ssRNA Ladder with Sample Loading Solution or nuclease-free water, denature at 70 °C for 5 minutes, and then cool immediately.	Prepare the ssRNA Ladder
3	Dilute each RNA sample with Sample Loading Solution or nuclease-free water, denature at 70 °C for 5 minutes, and then cool immediately.	Prepare the RNA Sample
4	Install the LIF detector.	Install the LIF Detector
5	Load the sample tray.	Load the Sample Tray
6	Install the cartridge.	Install the Cartridge and Calibrate the Detector
7	Prepare the gel buffer and load the buffer trays.	Load the Buffer Trays
8	For PA 800 Plus software users, create the RNA 9000 instrument and projects.	Create the Example Instrument and Create the Example Project
9	In the PA 800 Plus software, create a sequence and then start the run. Make sure that the sequence ends with the Shutdown Method - RNA 9000 method.	Create the Sequence and Start the Run
10	After the run, store the cartridge.	Store the Cartridge
11	Analyze the data.	Analyze the Data

Required Equipment and Materials

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

Note: The RNA 9000 Purity & Integrity Kit (PN C48231) is packaged as two parts: Nucleic Acid Extended Range Purity & Integrity Kit (PN 5087900) and ssRNA Ladder (0.05-9 kb) (PN 5088699). Neither part can be ordered separately.

Note: The ssRNA Ladder in the RNA 9000 Purity & Integrity Kit (PN C48231) is shipped separately from the other kit components.

Table 2 RNA 9000 Purity & Integrity Kit (PN C48231)

Component	Quantity	Reorder Part Number
Nucleic Acid Extended Range Purity & Integrity Kit (PN 5087900)		N/A
Acid Wash/Regenerating Solution (0.1 M HCl) (100 mL)	1	N/A
CE Grade Water (140 mL)	2	C48034
LIF Performance Test Mix (20 mL)	1	726022
Nucleic Acid Extended Range Gel (140 mL)	2	N/A
SYBR™ Green II RNA Gel Stain[2] (500×) (0.11 mL)	7	N/A
ssRNA Ladder (0.05-9 kb) (PN 5088699)		N/A
ssRNA Ladder (0.05 kb to 9 kb) (70 µL) (shipped separately)	2	N/A

Table 3 Additional Supplies from SCIEX

Component	Quantity	Part Number
(Optional) Capillary Performance Run Buffer A	1	338426
Capillary, bare fused-silica (50 µm i.d. × 67 cm long)	1	338451
Capillary cartridge, blank	1	144738
Capillary cartridge, pre-assembled	1	A55625
Filter, 520 nm emission filter	1	144940
LIF Cartridge Aperture Plug Assembly	1	721125
LIF Cartridge Probe Guide Assembly	1	721126
PCR microvials (200 µL)	100	144709
Sample Loading Solution	6 mL	608082

[2] SYBR™ is a trademark of the Life Technologies Corporation. SYBR™ Green II RNA Gel Stain is not available for resale.

Table 3 Additional Supplies from SCIEX (continued)

Component	Quantity	Part Number
Universal vial caps, blue	100	A62250
Universal vials	100	A62251

Table 4 Additional Required Reagents or Supplies

Description	Vendor	Part Number
Acrodisc 32 mm syringe filter with a 0.45 µm pore-size membrane	Pall	4654
Luer-Lok tip disposable syringe (10 mL)	BD	309604
(Optional) Nuclease-free water (10 × 2 mL)	Integrated DNA Technologies	11-04-02-01
(Optional) RNaseZap RNase Decontamination Solution (250 mL)	Thermo Fisher Scientific	AM9780

Storage Conditions

- Upon receipt, store the following at 2 °C to 8 °C:
  - Nucleic Acid Extended Range Gel
  - LIF Performance Test Mix
- Upon receipt, immediately wrap the SYBR™ Green II RNA Gel Stain[3] in aluminum foil to reduce photobleaching of the SYBR™ Green II RNA Gel Stain and then store at –35 °C to –15 °C.
- Upon receipt, store the ssRNA Ladder at –35 °C to –15 °C.
- Store the remainder of the kit contents at room temperature.

Customer-Supplied Equipment and Supplies

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

[3] SYBR™ is a trademark of the Life Technologies Corporation. SYBR™ Green II RNA Gel Stain is not available for resale.

- Table-top mini centrifuge
- Microcentrifuge, or equivalent, and nuclease-free microcentrifuge tubes
- Vortex mixer
- Pipettes and appropriate nuclease-free tips
- Water bath or heat block capable of 37 °C to 100 °C temperature
- Analytical balance
- Ice

Required Detector

A laser-induced fluorescence (LIF) detector with an excitation wavelength of 488 nm and a 520 nm emission filter and the supplies to calibrate it are required. Calibration supplies are:

- LIF Performance Test Mix, included with the kit
- CE Grade Water, included with the kit

Required Cartridge or Capillary

CAUTION: Potential Wrong Result. If a capillary is used with the RNA 9000 Purity & Integrity kit, then do not use the same capillary for another application. Mixing different buffers and sample types can cause sample carryover, nonspecific binding, and poor separation.

One of the following:

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

Condition the Capillary

CAUTION: Potential Wrong Result. Do not use a basic solution to clean the capillary because the solution might negatively ionize the capillary wall and cause nonspecific interactions with the sample. These interactions might cause poor separation and sample degradation.

- Before the capillary is used the first time, condition the capillary with the Conditioning Method-RNA 9000 method.

Methods and Sequences

Download the methods and sequences from sciex.com/products/methods. The methods and sequences can also be created manually with the 32 Karat software. Refer to the section: Methods.

Save the methods to the PA 800 Plus controller: `C:\32Karat\projects\RNA 9000\Method`.

Save the sequence to: `C:\32Karat\projects\RNA 9000\Sequence`.

At the time of publication, the following methods and sequence are available on the SCIEX website:

- Methods:
  - Conditioning Method-RNA 9000: Conditions the capillary.
  - Separation Method - Electrokinetic Injection - RNA 9000: Separates the sample with an electrokinetic injection of the sample.
  - Shutdown Method - RNA 9000: Cleans the capillary at the end of a sequence and turns off the light source.
  - Capillary Rinse - RNA 9000: Rinses the capillary. To save time after the capillary has been conditioned, replace Conditioning Method-RNA 9000 with this method in the sequence.
  - ANALYSIS - RNA 9000 - ssRNA Ladder: Contains the default integration parameters and named peaks to aid in the analysis of the ssRNA Ladder.
- RNA 9000 Test Sequence - Electrokinetic Sample Injection: A sequence with the separation method that uses electrokinetic sample injection.

Prepare the Samples

Best Practices for Working with RNA

Controlling RNase contamination is critical to the successful analysis of RNA. Precautions must be taken to ensure RNA integrity prior to ssRNA ladder or RNA sample separation. RNases are prevalent on human skin, perspiration, and saliva as well as bacteria and fungi spores and are therefore ubiquitous to the lab environment. Proper laboratory procedures will help control RNA degradation from RNases.

1. Wear gloves at all times and change gloves often when handling RNA samples.
2. Designate an RNase-free lab area and use RNase decontaminating reagents such as RNaseZap RNase Decontamination Solution or MP Bio RNase Erase Decontamination Solution for bench surfaces, lab racks, and micropipettors. Also, use a lab apparatus capable of UV-light disinfection to help control RNase contamination.

3. Dedicate pipettes for RNA use and use filtered pipette tips that are certified nuclease-free to decrease cross-contamination.
4. For anything that comes into contact with RNA, use nuclease-free plastic vials and labware. Before adding any RNA, shield plasticware from environmental contamination by covering and working in areas that limit airflow.
5. Use reagents such as nuclease-free water during RNA sample preparation. Use of Sample Loading Solution or deionized formamide during RNA sample preparation promotes an environment that stabilizes RNA and prevents RNA degradation from RNases.

Note: CE Grade Water is not certified as nuclease-free.

Prepare the ssRNA Ladder

Note: Use the ssRNA Ladder as a qualitative reference for estimating the size of an unknown RNA sample. It is not intended to be a quantitative standard.

1. Prepare the ssRNA Ladder.
   a. For the initial run, remove the vial of ssRNA Ladder from the freezer, and then let it thaw on ice.
   b. With a vortex mixer, mix briefly for a few seconds, and then use a centrifuge to spin the vial for a few seconds to bring the solution to the bottom of the vial.
   c. Measure out the solution in 8 µL aliquots into nuclease-free PCR vials.
   d. Reserve one aliquot, and then store the remaining aliquots at –35 °C to –15 °C.
2. Use one of the 8 µL aliquots of the ssRNA Ladder. If it is frozen, then thaw it on ice before use.
3. For every 50 µL of RNA sample, add 2 µL of the ssRNA Ladder to 48 µL of Sample Loading Solution or nuclease-free water.
4. Heat the sample at 70 °C for 5 min.
5. After 5 min, immediately put the mixture in an ice water bath to cool for a minimum of 2 min.

Note: Rapid cooling forces the RNA into a structure that gives the best separation results.

6. Put a microvial in a universal vial and then put the universal vial in the sample tray.
7. With a pipette, add between 50 µL and 200 µL of the cooled RNA solution to the microvial then cap the universal vial with a blue cap.
8. Put the sample tray in the system. Make sure that the sample compartment temperature is set to 10 °C.

Note: If the LIF laser is not warmed up or the detector has not been calibrated, then do not put the sample tray in the system. Instead, store the tray between 2 °C and 8 °C while the laser warms up or the calibration is performed and then put the sample tray in the system.

Prepare the RNA Sample

1. Thaw the RNA aliquot on ice.

   Keep the sample cool to help prevent the RNA from degrading.

2. Prepare the RNA sample in Sample Loading Solution or nuclease-free water between 50 ng/mL to 50 µg/mL.

   We recommend an RNA concentration between 1 µg/mL and 5 µg/mL.

   Note: To use a higher sample concentration, increase the **Dynamic range** from 100 to 1,000 in the LIF Detector Initial Conditions tab in the separation method.

3. Heat the sample at 70 °C for 5 min.
4. After 5 min, immediately put the mixture in an ice water bath to cool for a minimum of 2 min.

   Note: Rapid cooling forces the RNA into a structure that gives the best separation results.

5. Put a microvial in a universal vial and then put the universal vial in the sample tray.
6. With a pipette, add between 50 µL and 200 µL of the cooled RNA solution to the microvial then cap the universal vial with a blue cap.
7. Put the sample tray in the system. Make sure that the sample compartment temperature is set to 10 °C.

   Note: If the LIF laser is not warmed up or the detector has not been calibrated, then do not put the sample tray in the system. Instead, store the tray between 2 °C and 8 °C while the laser warms up or the calibration is performed and then put the sample tray in the system.

Prepare the PA 800 Plus System

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

The procedures in this section assume that the system has already been correctly installed and initialized.

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

Install the LIF Detector

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

2. Turn on the system.
3. Turn on the laser and let it warm up for at least 30 minutes.

Clean the Interface Block

CAUTION: Potential System Damage. Do not let the gel collect on the electrodes, opening levers, capillary ends, and interface block. Gel accumulation might cause broken capillaries, bent electrodes, jammed vials, or missed injections.

Clean the electrodes, opening levers, capillary ends, and interface block after every use or when changing chemistries. For detailed instructions, refer to the document: *System Maintenance Guide*.

The gel buffer is very viscous and can collect in the system unless regular and thorough cleaning is performed.

Install the Cartridge and Calibrate the Detector

Note: To make sure that the analysis results are consistent over time, SCIEX recommends that the detector be calibrated each time it is installed in the PA 800 Plus system. Also, calibrate the detector after the capillary in the cartridge is replaced or a different cartridge is installed.

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

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

   Use the values in the following table in step two of the Calibration wizard.

   Note: The **Target RFU** is 40 so that the signal intensity from the PA 800 Plus system is almost the same as the signal from a BioPhase 8800 system. It it intended for transferability.

   Table 5 Calibration Parameters

Detector Channel	1
Target RFU	40
Capillary Dimensions	
Internal Diameter	50 µm

Table 5 Calibration Parameters (continued)

Total Length	30 cm or 50 cm, based on the length of the capillary

Load the Buffer Trays

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

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

Note: SYBR™ is a trademark of the Life Technologies Corporation. SYBR™ Green II RNA Gel Stain is not available for resale.

1. Add the reagents specified in the following table to a conical tube and then gently invert the tube a minimum of 20 times.

   While inverting the tube, make sure that no bubbles are formed.

   CAUTION: Potential Wrong Result. Do not prepare the gel buffer in advance. The SYBR™ Green II RNA Gel Stain in the gel buffer might degrade during storage, causing peaks with decreased intensity.

   Table 6 Gel Buffer (Nucleic Acid Extended Range Gel with SYBR™ Green II RNA Gel Stain)

Reagent	For 1 to 8 Samples	For 9 to 16 Samples	For 41 to 48 Samples
Nucleic Acid Extended Range Gel	5 mL	10 mL	30 mL
SYBR™ Green II RNA Gel Stain	10 µL	20 µL	60 µL

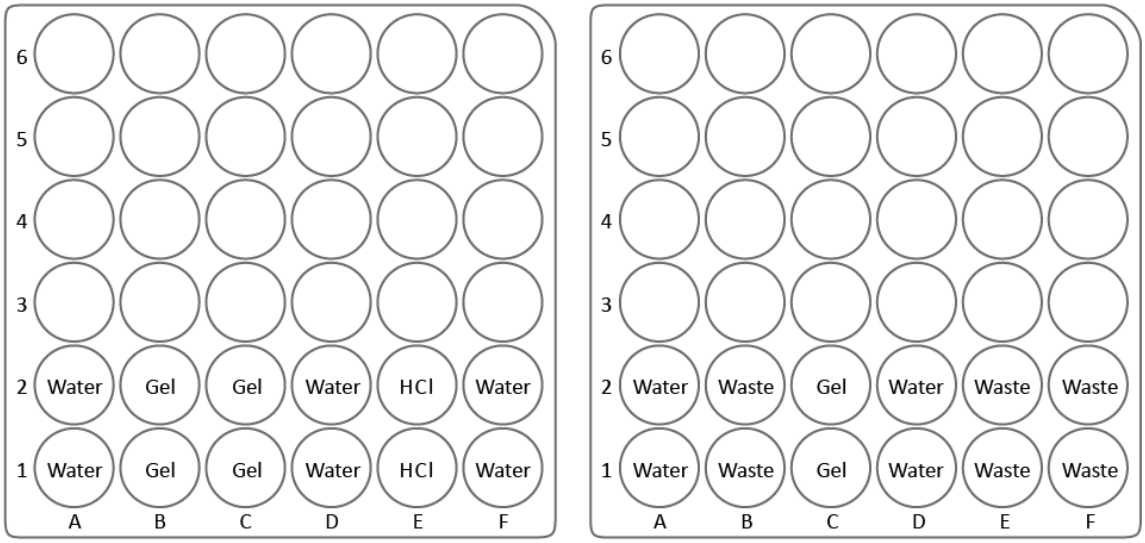
   Note: The gel buffer and dye mixture is referred to as "gel buffer" in the rest of the document.

   Tip! Wrap the vial that contains the prepared gel buffer in aluminum foil to reduce photobleaching of the SYBR™ Green II RNA Gel Stain.

2. With a 0.45 µm Acrodisc syringe filter and a Luer-Lok syringe, filter the gel buffer.
3. Put 16 universal vials in the buffer trays as shown in the following figure. Each row is sufficient for at least eight runs.

Note: The separation methods are programmed to increment after eight runs. Do not reuse the vials or caps because they might be contaminated with dried gel or other chemicals. The vials and caps are designed for a single use.

Figure 1 Buffer Tray Layout for 16 Samples



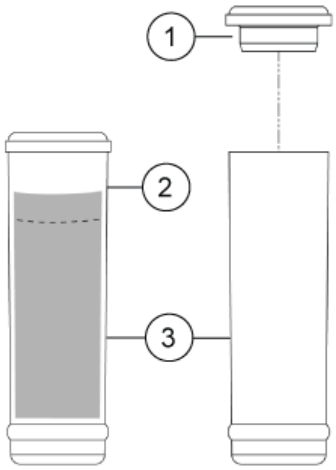
Note: For fewer than 8 samples, fill only row 1.

4. Fill the vials as shown in the following table. After filling each vial, cap it with a blue cap. Refer to the figure: Figure 2.

Table 7 Vials to Prepare

Label in Figure 1	No. of Vials	Vol./Vial (mL)	Reagent
Water	10	1.5	CE Grade Water
Gel	6	1.5	Gel buffer with SYBR™ Green II RNA Gel Stain
HCl	2	1.5	Acid Wash/Regenerating Solution
Waste	6	1.0	CE Grade Water

Figure 2 Universal Vial and Cap Setup



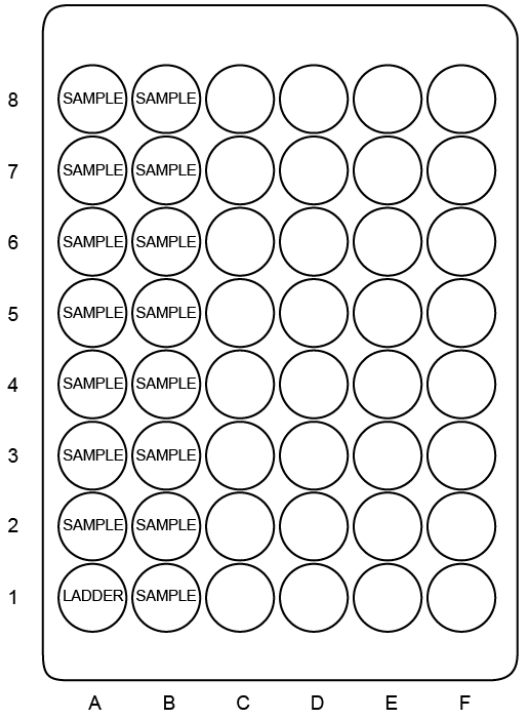
Item	Description
1	Universal vial cap
2	Maximum fill line
3	Universal vial

Load the Sample Tray

1. Put each microvial in a universal vial and then put the universal vial in the sample tray. Refer to the figure: Figure 3.

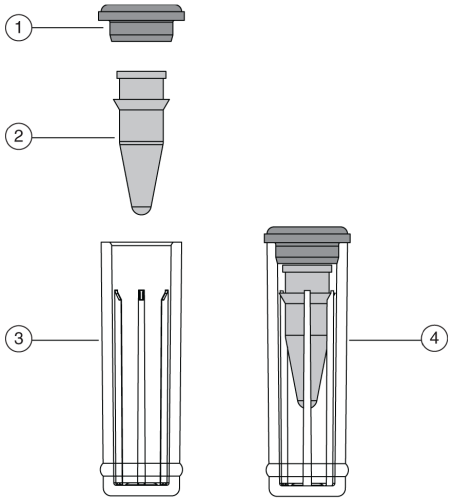
   Position A1 is for the ssRNA Ladder. Use the other positions for the other samples.

Figure 3 Sample Tray Layout



2. Add the samples to the microvials. For each sample:
   a. Transfer between 50 µL to 200 µL of the sample to a microvial.
   b. Put a blue cap on the universal vial. Refer to the figure: Figure 4.

Figure 4 Sample Vial Setup



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

Run the Samples

Tips for Best Results

SCIEX tested the separation performance of the ssRNA Ladder and other characteristics over a range of temperatures. 30 °C is used in the separation methods because it gave the best overall results.

Other temperatures can be used if a specific characteristic is to be maximized. Refer to the table: Table 8.

Table 8 Guidance for Setting the Capillary Temperature

Capillary Temperature (°C)	Capillary Run Life	9 kb Peak Migration Time (min)	Optimal Resolution by RNA Length	
			3 kb to 5 kb	≥ 5 kb
25	Highest	~21		
30	Higher	~20	✓	
35	Medium	~19		
40	Lower	~17		✓

Note: The capillary run life (the number of injections that can be performed) depends on the sample and the separation method. The preceding table illustrates how performance changes based on the capillary temperature. In general, a lower temperature increases the capillary run life.

Use the Rinse Method

A rinse method is supplied with the other methods for the kit.

To save time after the capillary has been conditioned, substitute the Capillary Rinse - RNA 9000 method for the Conditioning Method-RNA 9000 method in the sequence. The rinse method is approximately 30 minutes shorter than the conditioning method.

Create the Example Instrument

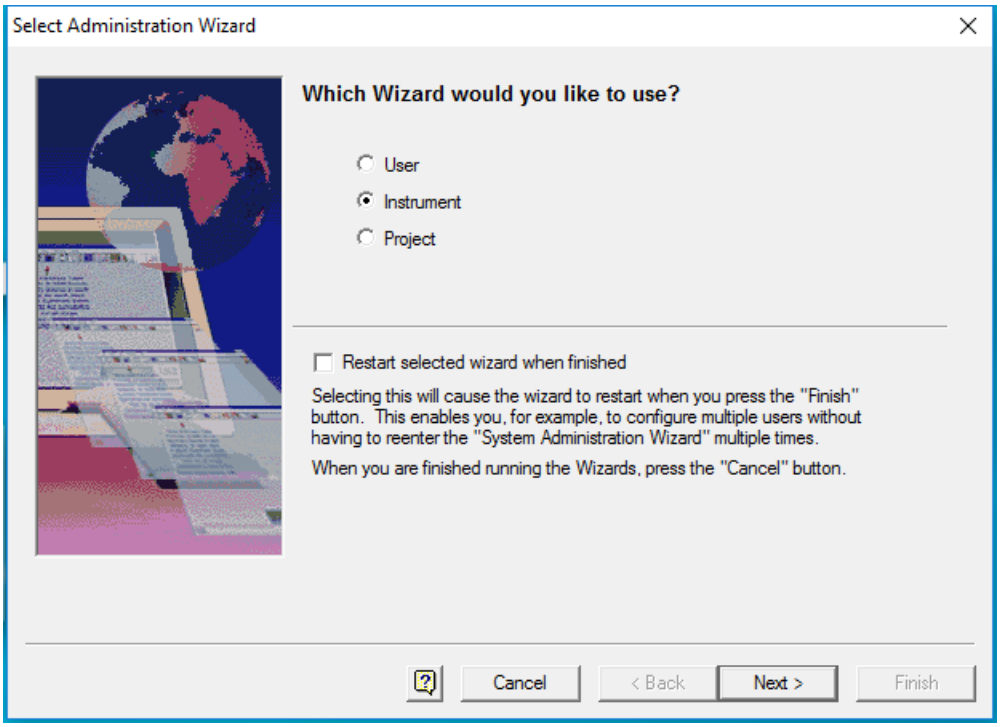
Note: The following procedure is required if the PA 800 Plus software will be used for data acquisition and analysis. If the 32 Karat software will be used instead, then this procedure is not required.

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

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

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

Figure 5 Select Administration Wizard Window



4. Click **Instrument**, and then click **Next.**
5. Follow the instructions in the wizard to create the instrument. When prompted for the instrument name, type `RNA 9000`.
   The PA 800 plus System Configuration dialog opens.

6. Click **LIF Detector**,  , and then click **OK**.
7. Do one of the following:
   - If the system is connected to the controller and it is turned on, then click **Auto Configuration**.
   - If the system is not connected to the controller or it is turned off, then in the **Configured modules** list, right-click **LIF Detector** and select **Open**. Make sure that the trays are configured correctly, and then click **OK**.
8. Click **OK**.
   The PA 800 plus System Configuration dialog closes.

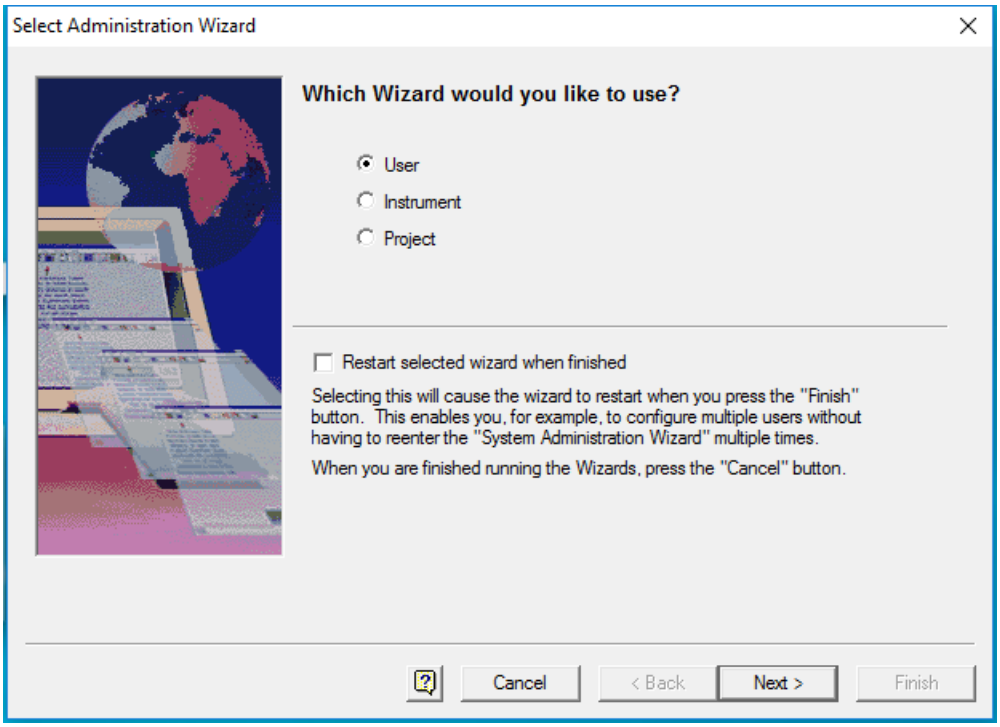
Create the Example Project

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

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

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

Figure 6 Select Administration Wizard Window



4. Click **Project** and then click **Next.**
5. Follow the instructions in the wizard to create the project. When prompted for the project name, type `RNA 9000`.

   Make sure to assign users to the project.

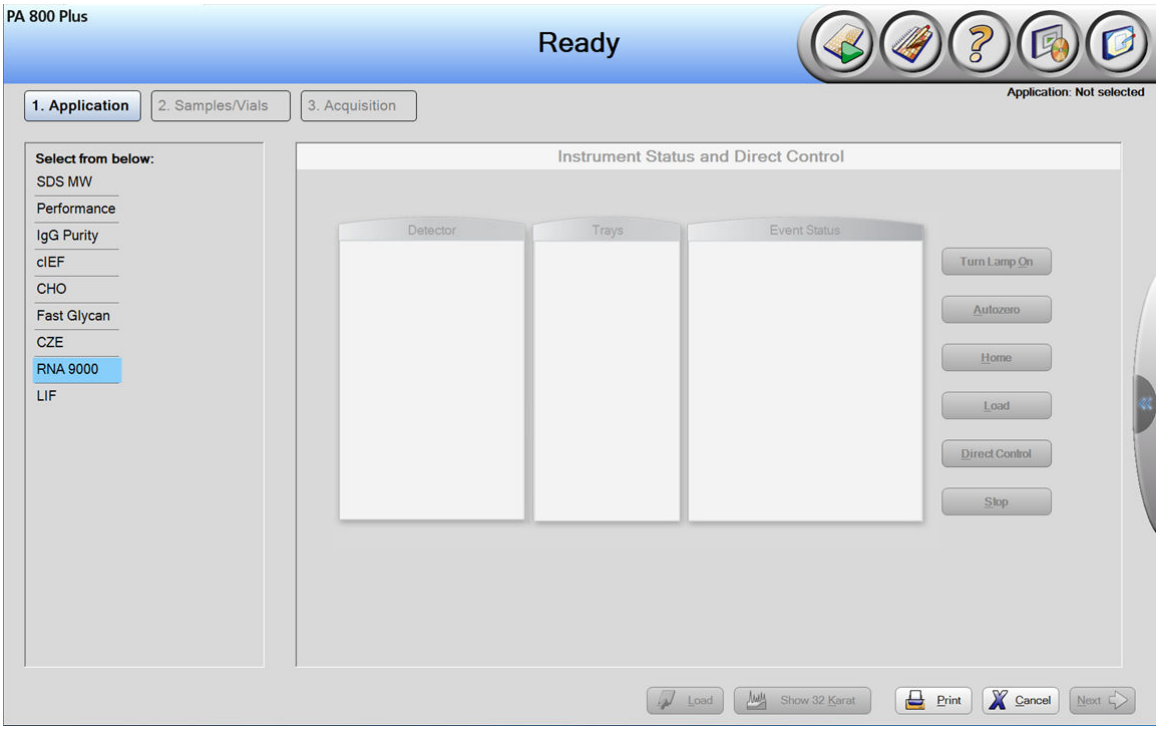
   For detailed instructions, refer to the documents: *32 Karat Software Help* or *System Administration Guide*.
6. If required, then download the method and sequence files from the SCIEX website. Refer to the section: Methods and Sequences.
7. Copy the methods to the methods folder for the project. By default, this is `C:\32Karat\projects\RNA 9000\Method`.
8. Copy the sequence to the sequence folder for the project. By default, this is `C:\32Karat\projects\RNA 9000\Sequence`.

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.
   The PA 800 plus window opens

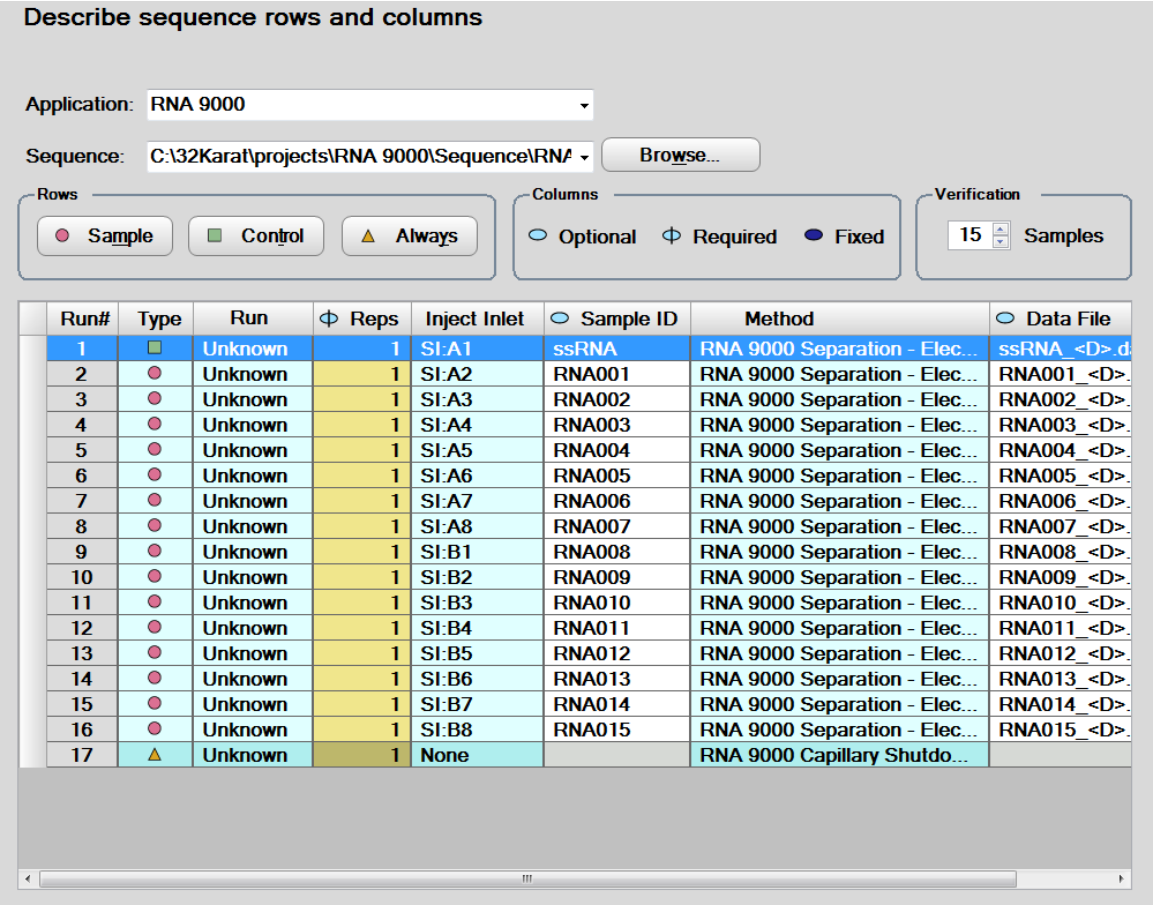
2. , click (**Run**) in the upper right corner of the window.
   The Instrument Status and Direct Control page opens.

Figure 7 Instrument Status and Direct Control Window



3. Click (**Describe**).
4. In the **Application** list, click **RNA 9000**. In the **Sequence** list, click **Browse**, and then browse to the **RNA 9000 Test Sequence - Electrokinetic Sample Injection** sequence. If prompted, type a user name and password.
   The page updates to show the selected sequence, and all of the rows in the sequence are designated as samples.
5. Click the first row to select it, and then click Control (**Control**) in the **Rows** area.
   The first row contains the ssRNA Ladder.
   The icon in the **Type** column in the first row in the sequence changes to a square.
6. Click the last row (with the **Shutdown Method - RNA 9000** method) to select it, and then click Always (**Always**) in the **Rows** area.
   The icon in the **Type** column in the sequence changes to a triangle.

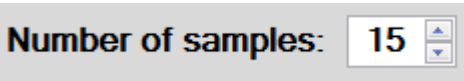
Figure 8 Describe sequence rows and columns Window



Run#	Type	Run	Reps	Inject Inlet	Sample ID	Method	Data File
1	■	Unknown	1	SI:A1	ssRNA	RNA 9000 Separation - Elec...	ssRNA_<D>.d
2	●	Unknown	1	SI:A2	RNA001	RNA 9000 Separation - Elec...	RNA001_<D>.
3	●	Unknown	1	SI:A3	RNA002	RNA 9000 Separation - Elec...	RNA002_<D>.
4	●	Unknown	1	SI:A4	RNA003	RNA 9000 Separation - Elec...	RNA003_<D>.
5	●	Unknown	1	SI:A5	RNA004	RNA 9000 Separation - Elec...	RNA004_<D>.
6	●	Unknown	1	SI:A6	RNA005	RNA 9000 Separation - Elec...	RNA005_<D>.
7	●	Unknown	1	SI:A7	RNA006	RNA 9000 Separation - Elec...	RNA006_<D>.
8	●	Unknown	1	SI:A8	RNA007	RNA 9000 Separation - Elec...	RNA007_<D>.
9	●	Unknown	1	SI:B1	RNA008	RNA 9000 Separation - Elec...	RNA008_<D>.
10	●	Unknown	1	SI:B2	RNA009	RNA 9000 Separation - Elec...	RNA009_<D>.
11	●	Unknown	1	SI:B3	RNA010	RNA 9000 Separation - Elec...	RNA010_<D>.
12	●	Unknown	1	SI:B4	RNA011	RNA 9000 Separation - Elec...	RNA011_<D>.
13	●	Unknown	1	SI:B5	RNA012	RNA 9000 Separation - Elec...	RNA012_<D>.
14	●	Unknown	1	SI:B6	RNA013	RNA 9000 Separation - Elec...	RNA013_<D>.
15	●	Unknown	1	SI:B7	RNA014	RNA 9000 Separation - Elec...	RNA014_<D>.
16	●	Unknown	1	SI:B8	RNA015	RNA 9000 Separation - Elec...	RNA015_<D>.
17	▲	Unknown	1	None		RNA 9000 Capillary Shutdo...	

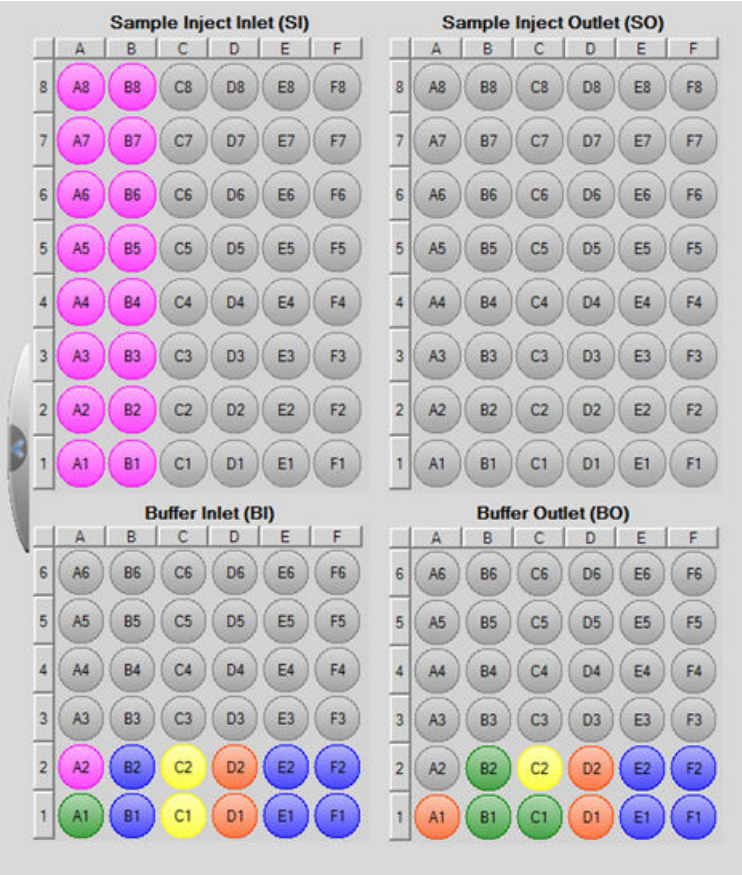
7. In the lower right corner of the window, click Save (**Save**), and then click Finish (**Finish**).
8. In the **Number of samples** field, click the arrow buttons to set the number of samples for the run.

Figure 9 Set the 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 1, one row of reagents is required for eight samples. Two rows of reagents are required for 16 samples.

Figure 10 Tray Map



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

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

Figure 11 Samples Loaded Prompt

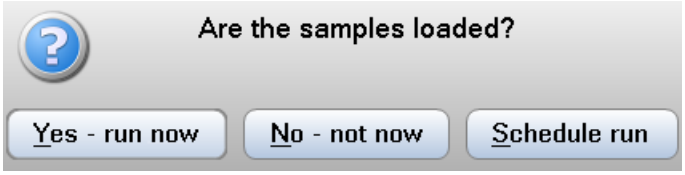
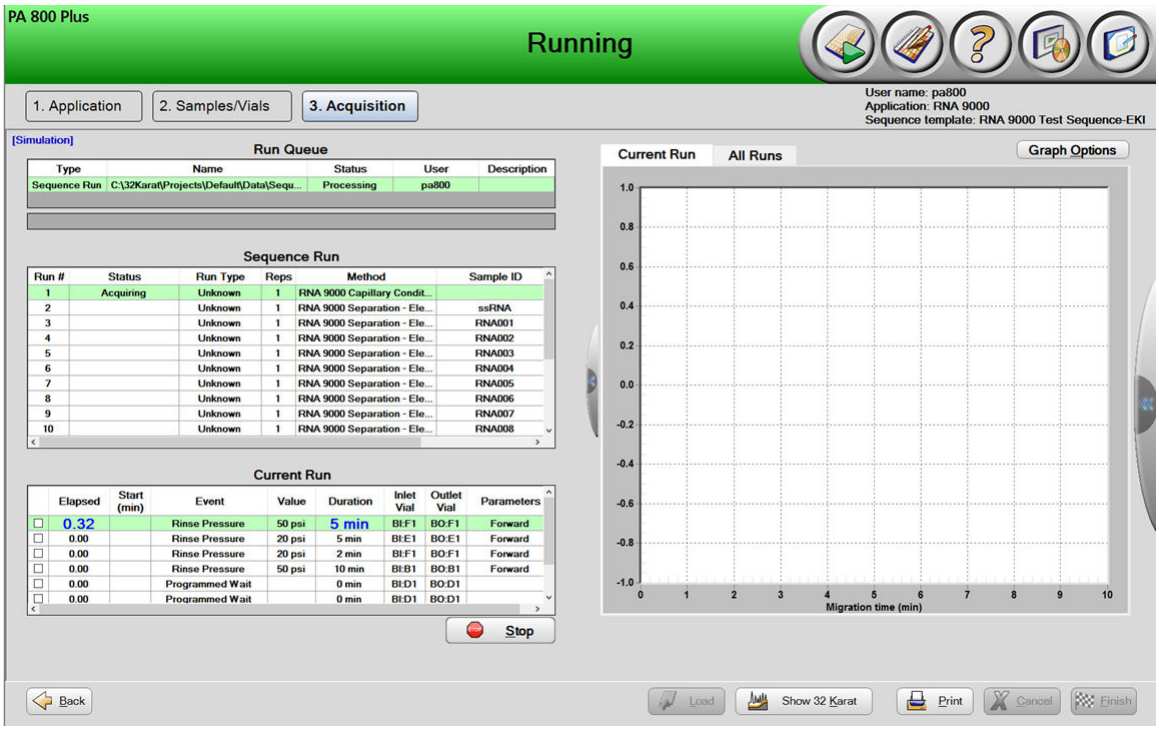


Figure 12 PA 800 Software During Data Acquisition



Waste Disposal

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

Store the Cartridge

Store the Cartridge for Less Than 24 Hours

1. Use the shutdown method to clean the capillary.

   The shutdown method fills the capillaries with CE Grade Water and decreases the cartridge temperature to 15 °C.

2. Store the cartridge for up to 24 hours in the system, with the capillary ends immersed in vials of CE Grade Water.

Store the Cartridge for More Than 24 Hours

1. Use the shutdown method to clean the capillary.

2. Remove the cartridge from the system.
3. Put the cartridge in the cartridge storage box with the capillary ends immersed in vials of CE Grade Water.
4. Store 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 or if it has been stored for an extended time, then condition the capillary by rinsing it with CE Grade Water for 5 min at 50 psi.

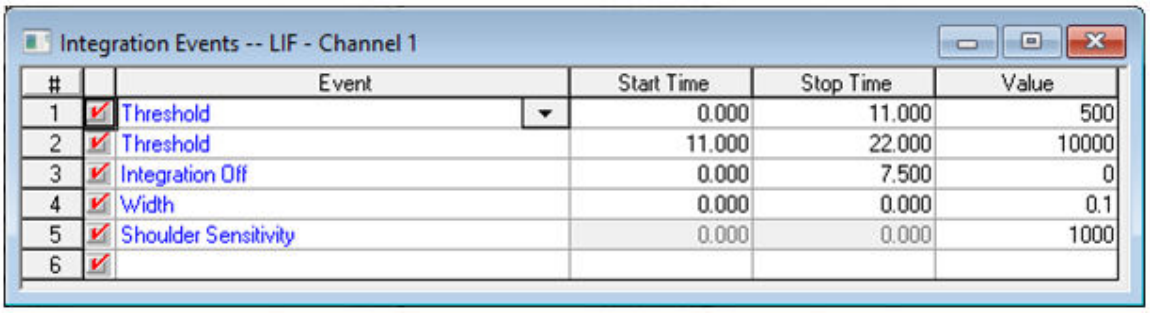
Analyze the Data

Analyze the Data for the ssRNA Ladder

1. In the 32 Karat software, open the sequence from the run.
2. Open the data file for the first run.
3. Click **File** > **Open** > **Method**, select **ANALYSIS - RNA 9000 - ssRNA Ladder**, and then click **OK**.

   For reference, the integration parameters and named peaks table are shown in the following figures.

Figure 13 Integration Parameters



#	Event	Start Time	Stop Time	Value
1	Threshold	0.000	11.000	500
2	Threshold	11.000	22.000	10000
3	Integration Off	0.000	7.500	0
4	Width	0.000	0.000	0.1
5	Shoulder Sensitivity	0.000	0.000	1000
6				

Note: The peak names correspond to the length of the RNA in the ssRNA Ladder.

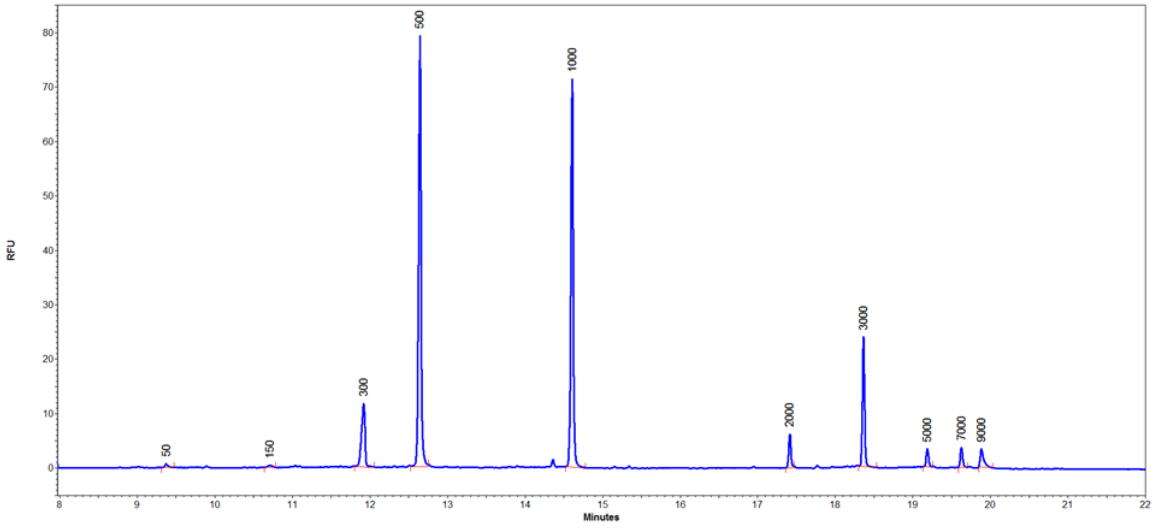
Figure 14 Named Peaks Table

Named Peaks | Groups

#		Name	ID	Mig. Time	MT Window
1	✓	50	1	9.15208	0.457604
2	✓	150	2	10.4875	0.524376
3	✓	300	3	11.6667	0.583334
4	✓	500	4	12.4479	0.622396
5	✓	1000	5	14.6083	0.723646
6	✓	2000	6	17.3937	0.863854
7	✓	3000	7	18.1292	0.906458
8	✓	5000	8	18.8937	0.944688
9	✓	7000	9	19.3104	0.96552
10	✓	9000	10	19.5896	0.97948
11	✓				

4. Adjust the integration events until all of the peaks in the test sample are integrated correctly.
   Refer to the chapter: "Integration" in the document: *Methods Development Guide*.
5. Click **File** > **Save**, to save the method and then apply it to the rows in the sequence that contain the ssRNA Ladder samples.
   The electropherogram is labeled with the peak names for the components of the ssRNA Ladder.

Figure 15 Example Electropherogram for the ssRNA Ladder



Guidance for Developing Acceptance Criteria

Acceptance criteria that are created for use with this kit for SOPs or other purposes should be based on parameters inherent to the quality of the separation and attributes that reflect critical

sample qualities. Differences between gel and capillary lots and different systems might lead to variation in absolute migration times.

For the ssRNA ladder, the ratio between the migration time of the 9 kb and 0.5 kb peaks more accurately reflects the apparent size of the nucleic acids in the gel and can be used to identify the resolving power of the separation gel and consistency of the separation. SCIEX strongly discourages the use of absolute migration time as an acceptance criterion.

Troubleshooting

Note: SYBR™ is a trademark of the Life Technologies Corporation. SYBR™ Green II RNA Gel Stain is not available for resale.

Symptom	Possible Cause	Corrective Action
Broad peaks, poor resolution	1. The capillary end is damaged. 2. The sample concentration is too high. 3. The capillary is blocked. 4. The internal surface of the capillary is contaminated. 5. The lifetime of the capillary has been exceeded. 6. The SYBR™ Green II RNA Gel Stain concentration in the gel buffer is too high.	1. Inspect the capillary ends under magnification. If a cut is jagged, then cut the end again or replace the capillary. 2. Do one or all of the following: • Dilute the sample again with the sample diluent. • Decrease the **Duration** in the **Inject** event in the separation method to inject less sample. If the results are not satisfactory, then decrease the **Pressure** or **Voltage**. 3. Replace the capillary or capillary cartridge. 4. Replace the capillary or capillary cartridge. 5. Perform a test separation of the ssRNA Ladder. If the peak widths are consistently wider than previous runs then replace the capillary or capillary cartridge. 6. Make sure that the SYBR™ Green II RNA Gel Stain is diluted between 100× and 1000× in the gel buffer. Refer to the section: Load the Buffer Trays.

Symptom	Possible Cause	Corrective Action
Broad peaks, poor resolution (continued)	1. The Nucleic Acid Extended Range Gel was left at room temperature too long. 2. The capillary cartridge was left at room temperature for more than a week.	1. Prepare fresh Nucleic Acid Extended Range Gel, making sure to store it between 2 °C and 8 °C. Minimize the time that the gel is at room temperature. 2. Replace the capillary or capillary cartridge. For the new capillary, make sure to run the shutdown method at the end of the day.
Carryover	1. The sample concentration is too high. 2. The vials or caps are contaminated.	1. Do one or all of the following: • Decrease the **Duration** in the **Inject** event in the separation method to inject less sample. If the results are not satisfactory, then decrease the **Pressure** or **Voltage**. • Dilute the sample again with the sample diluent. 2. Replace the vials and caps or modify the method: • Fill clean vials with freshly prepared reagents, cover the vials with clean caps, and then replace the vials in the tray. Do not reuse vials or caps. • Make sure that the waste vials contain 1.0 mL of water and are present in the outlet buffer tray. • In the separation method, add one or more water dip steps after sample injection.

Symptom	Possible Cause	Corrective Action
Extra peaks	1. Non-nucleic acid components of the sample interacted with the SYBR™ Green II RNA Gel Stain. 2. The plasticware used during sample preparation or the sample vials are contaminated with materials that interact with SYBR™ Green II RNA Gel Stain. 3. Light scattering due to particulates larger than 1 µm in the gel buffer.	1. Prepare the sample again, making sure it is pure. 2. Prepare the sample again, using clean plasticware and clean sample vials. Do not reuse vials or caps. 3. Filter the gel buffer with a syringe filter before adding it to the buffer trays.
High current	1. The gel buffer is contaminated. 2. The buffer trays are not set up correctly. 3. The Nucleic Acid Extended Range Gel was left at room temperature too long.	1. Replace the vials and caps with clean ones. Do not reuse vials or caps. 2. Make sure that the vials in the buffer tray contain the correct reagents and are in the correct location. Refer to the section: Load the Buffer Trays. 3. Prepare fresh Nucleic Acid Extended Range Gel, making sure to store it between 2 °C and 8 °C. Minimize the time that the gel is at room temperature.

Symptom	Possible Cause	Corrective Action
Low signal	1. The end of the capillary tip is dirty or plugged. 2. The sample concentration is too low. 3. The salt concentration in the sample is too high. 4. The initial nucleic acid concentration is too low.	1. Replace the capillary or capillary cartridge. 2. Do one or all of the following: • Increase the **Duration** up to 15 s in the **Inject** event in the separation method to inject more sample. If the results are not satisfactory, then increase the **Pressure** or **Voltage**. • Prepare the sample again, with the recommended concentration. The recommended concentration is 50 ng/mL to 50 µg/mL. 3. Do one or all of the following: • If the separation method uses an electrokinetic injection, then use a pressure injection instead. • Prepare the sample at a lower ionic strength. 4. Do one or all of the following: • Increase the **Duration** up to 15 s in the **Inject** event in the separation method to inject more sample. If the results are not satisfactory, then increase the **Pressure** or **Voltage**. • Prepare the sample again, with the recommended concentration. The recommended concentration is 50 ng/mL to 50 µg/mL.

Symptom	Possible Cause	Corrective Action
Low signal (continued)	1. The nucleic acids in the sample have degraded due to the presence of RNAse or other nucleases.	1. Prepare the sample again, making sure to limit exposure to RNase. Refer to the section: Best Practices for Working with RNA.
Low current	1. The end of the capillary tip is dirty or plugged. 2. The capillary window or end is broken. 3. There are dried reagents on the electrodes, opening levers, capillary ends, or interface block. 4. The buffer trays are not set up correctly.	1. Replace the capillary or capillary cartridge. 2. Inspect the capillary window and tip. If either is broken, then replace the capillary or the capillary cartridge. 3. Clean the interface block daily or as needed. Refer to the section: Clean the Interface Block. 4. Make sure that the vials in the buffer tray contain the correct reagents and are in the correct locations. Refer to the section: Load the Buffer Trays.
Low or unsteady current	1. The end of the capillary tip is dirty or plugged. 2. The gel buffer has air bubbles.	1. Replace the capillary or capillary cartridge. 2. Sonicate the buffer from 10 s to 20 s to remove air bubbles.

Symptom	Possible Cause	Corrective Action
No electrical current during separation	1. The capillary is damaged. 2. An electrode is broken or bent. 3. The end of the capillary tip is dirty or blocked. 4. The buffer trays are not set up correctly. 5. The capillary is filled with air bubbles. 6. There are dried reagents on the electrodes, opening levers, capillary ends, or interface block.	1. Replace the capillary or capillary cartridge. 2. Replace the electrode. 3. Replace the capillary or capillary cartridge. 4. Make sure that the vials in the buffer tray contain the correct reagents and are in the correct locations. Refer to the section: Load the Buffer Trays. 5. Do one or all of the following: • Make sure that there is 100 µL of sample in the microvial. • Make sure that the vials in the buffer and sample trays are in the correct locations. Refer to the sections: Load the Buffer Trays and Load the Sample Tray. • Sonicate the buffer from 10 s to 20 s to remove air bubbles. 6. Clean the interface block daily or as needed. Refer to the section: Clean the Interface Block.

Symptom	Possible Cause	Corrective Action
No peaks	1. The lifetime of the LIF detector laser has been exceeded. 2. The method parameters are incorrect. 3. There is an air bubble at the bottom of the sample vial. 4. The capillary window or end is broken. 5. The sample volume is too low. 6. The sample is missing or not in the correct position in the sample tray. 7. The capillary was cleaned with a basic solution such as 0.1 N NaOH. 8. An end of the capillary extends beyond the electrode.	1. Contact SCIEX Technical Support at sciex.com/request-support. 2. Do the following: a. Open the separation method in the software and then make sure that the method is correct. Refer to the section: Separation Method. b. Make sure that the positions of the samples and reagents in the trays agree with the tray layouts. 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 capillary or the capillary cartridge. 5. Make sure that there is 100 µL of sample in the microvial. 6. Make sure that the samples are in the correct locations in the sample tray. Refer to the section: Load the Sample Tray. 7. Replace the capillary or capillary cartridge. 8. Inspect the capillary ends under magnification. If an end extends beyond the electrode, then cut the end again or replace the capillary.

Symptom	Possible Cause	Corrective Action
No peaks (continued)	1. A pipetting error occurred during preparation of the sample. 2. The salt concentration in the sample is too high.	1. Do one or all of the following: • If the separation method uses an electrokinetic injection, then use a pressure injection instead. • Prepare the sample at a lower ionic strength. 2. Prepare a new sample.
Slower migration time with or without concurrent low current	1. The end of the capillary tip is dirty or plugged.	1. Replace the capillary or capillary cartridge.
Dramatic shift in migration time between runs on the same day	1. The capillary has not been conditioned sufficiently. 2. The gel buffer has evaporated.	1. Condition the capillary. Refer to the section: Condition the Capillary. Do a blank separation run to equilibrate the capillary surface. 2. Fill clean vials with freshly prepared reagents, cover the vials with clean caps, and then replace the vials in the tray. Do not reuse vials or caps.
Spikes in electropherogram	1. The gel buffer has air bubbles.	1. Sonicate the buffer from 10 s to 20 s to remove air bubbles. If air bubbles are still present, then prepare fresh gel buffer. Do not mix the buffer with a vortex mixer, instead invert the tube gently a minimum of 20 times.

Symptom	Possible Cause	Corrective Action
Broad or split peaks	1. After the denaturation step, the nucleic acid sample still has secondary structure. 2. The cooling step after denaturation was too slow. 3. The SYBR™ Green II RNA Gel Stain concentration in the gel buffer is too high. 4. The lifetime of the capillary has been exceeded.	1. Dilute the samples with Sample Loading Solution instead of nuclease-free water. 2. Cool the samples immediately to prevent the formation of secondary structure. 3. Make sure that the SYBR™ Green II RNA Gel Stain is diluted between 100× and 1000× in the gel buffer. Refer to the section: Load the Buffer Trays. 4. Do a test separation of the ssRNA Ladder. If the peak widths are consistently wider than previous runs, then replace the capillary or capillary cartridge.
Saturated peaks	1. In the **LIF Detector Initial Conditions** for the separation method, the **Dynamic range** is too small. 2. The sample concentration is too high.	1. Increase the value for the **Dynamic range**. 2. Do one or all of the following: a. Dilute the sample again with the sample diluent. b. Decrease the **Duration** in the **Inject** event in the separation method to inject less sample. If the results are not satisfactory, then decrease the **Pressure** or **Voltage**.
Unstable baseline	1. The concentration of dye in the inlet buffer tray is not the same as the concentration in the outlet buffer tray.	1. Make enough gel buffer for both the inlet and outlet buffer trays.

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. The safety data sheets are available upon request or can be downloaded from our website, at sciex.com/tech-regulatory.

Hazard classification according to HCS 2012.

Acid Wash/Regenerating Solution (0.1 M HCl)

DANGER! Causes severe skin burns and eye damage.

SYBR™ Green II RNA Gel Stain[4]

WARNING! Combustible liquid. Causes skin irritation.

Other Reagents

These components are not classified as hazardous:

- CE Grade Water
- LIF Performance Test Mix
- Nucleic Acid Extended Range Gel

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

[4] SYBR™ is a trademark of the Life Technologies Corporation. SYBR™ Green II RNA Gel Stain is not available for resale.

Methods B

Capillary Conditioning Method

Figure B-1 Initial Conditions Tab

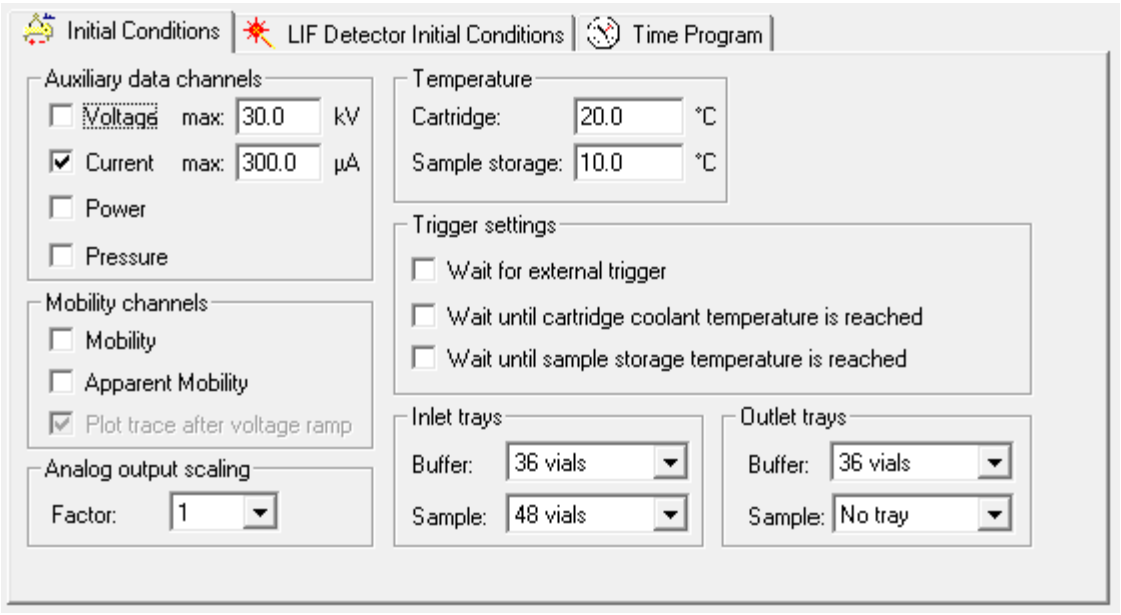


Figure B-2 LIF Detector Initial Conditions Tab

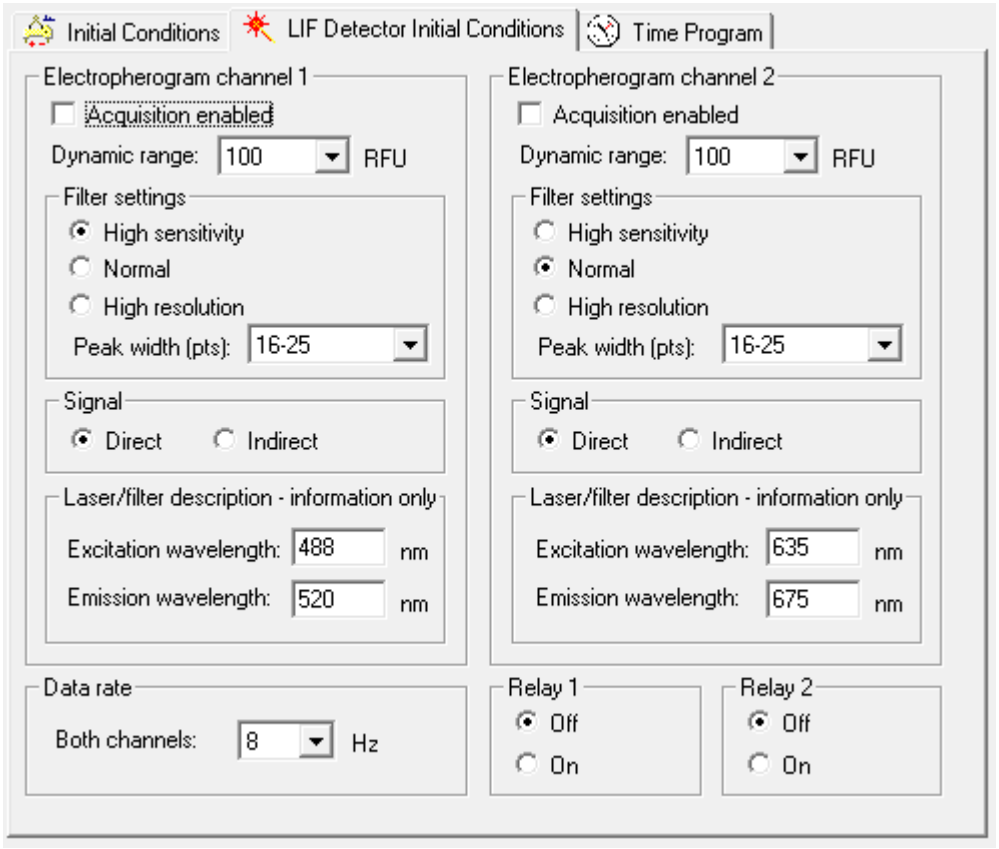


Figure B-3 Capillary Conditioning Method Time Program Tab

Initial Conditions | LIF Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	50.0 psi	5.00 min	BI:F1	BO:F1	forward	Water rinse
2		Rinse - Pressure	20.0 psi	5.00 min	BI:E1	BO:E1	forward	HCl rinse
3		Rinse - Pressure	20.0 psi	2.00 min	BI:F1	BO:F1	forward	Water rinse
4		Rinse - Pressure	50.0 psi	10.00 min	BI:B1	BO:B1	forward	Gel Rinse
5		Wait		0.00 min	BI:D1	BO:D1		water dip
6		Wait		0.00 min	BI:D1	BO:D1		water dip
7	0.00	Separate - Voltage	6.0 KV	20.00 min	BI:C1	BO:C1	2.00 Min ramp, reverse polarity	Separation
8	20.00	Wait		0.00 min	BI:D1	BO:D1		water dip
9	20.01	End						
10								

Separation Method

Figure B-4 Initial Conditions Tab

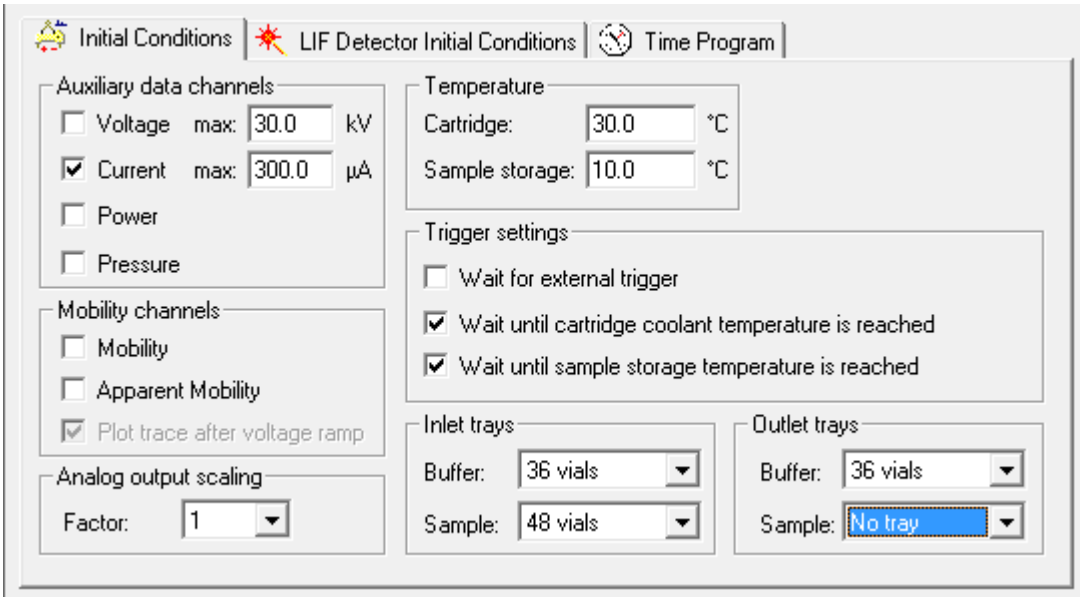


Figure B-5 LIF Detector Initial Conditions

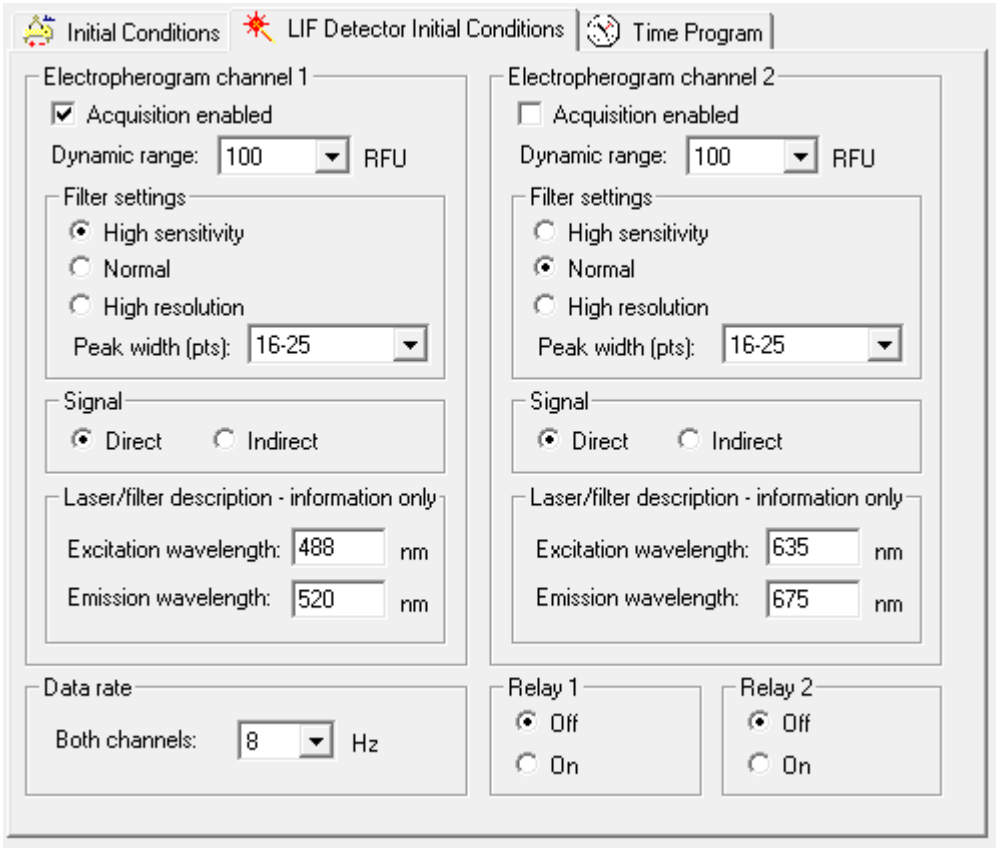


Figure B-6 Separation Method Time Program Tab for Electrokinetic Injection

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	70.0 psi	1.00 min	BI:E1	BO:E1	forward, In / Out vial inc 8	HCl Rinse
2		Rinse - Pressure	70.0 psi	1.00 min	BI:F1	BO:F1	forward, In / Out vial inc 8	Water Rinse
3		Rinse - Pressure	50.0 psi	5.00 min	BI:B1	BO:B1	forward, In / Out vial inc 8	Gel Rinse
4		Separate - Voltage	30.0 KV	2.00 min	BI:C1	BO:C1	0.17 Min ramp, reverse polarity, In / Out vial inc 8	pre-voltage
5		Wait		0.00 min	BI:D1	BO:D1	In / Out vial inc 8	water dip
6		Inject - Voltage	1.0 KV	3.0 sec	SI:A1	BO:C1	Override, reverse polarity	Electrokinetic Injection
7		Wait		0.00 min	BI:D1	BO:D1	In / Out vial inc 8	water dip
8	0.00	Separate - Voltage	6.0 KV	22.00 min	BI:C1	BO:C1	2.00 Min ramp, reverse polarity, In / Out vial inc 8	Separation
9	8.00	Autozero						
10	22.00	End						
11								

Shutdown Method

Figure B-7 Initial Conditions Tab

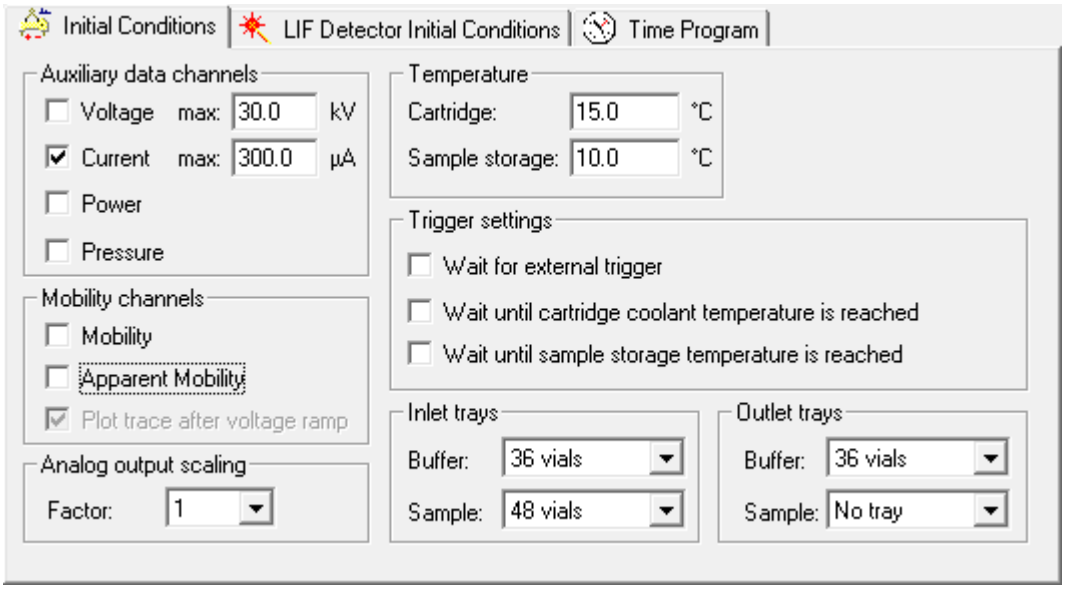


Figure B-8 LIF Detector Initial Conditions Tab

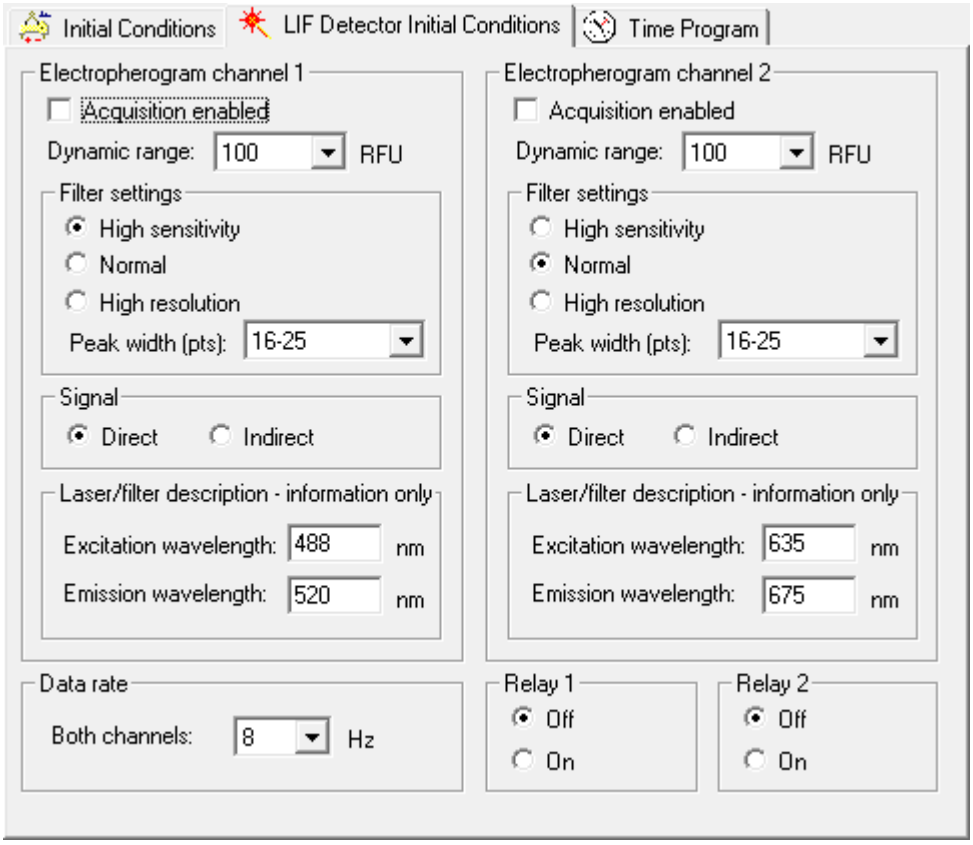
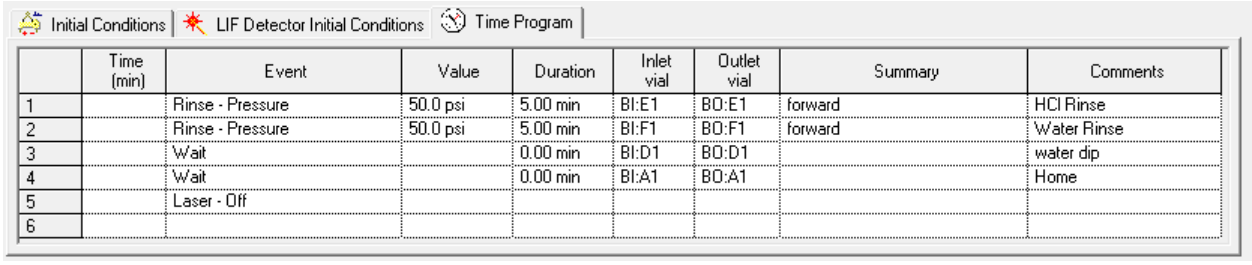


Figure B-9 Shutdown Method Time Program Tab



	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	50.0 psi	5.00 min	BI:E1	BO:E1	forward	HCl Rinse
2		Rinse - Pressure	50.0 psi	5.00 min	BI:F1	BO:F1	forward	Water Rinse
3		Wait		0.00 min	BI:D1	BO:D1		water dip
4		Wait		0.00 min	BI:A1	BO:A1		Home
5		Laser - Off						
6								

Capillary Rinse Method

Figure B-10 Initial Conditions Tab

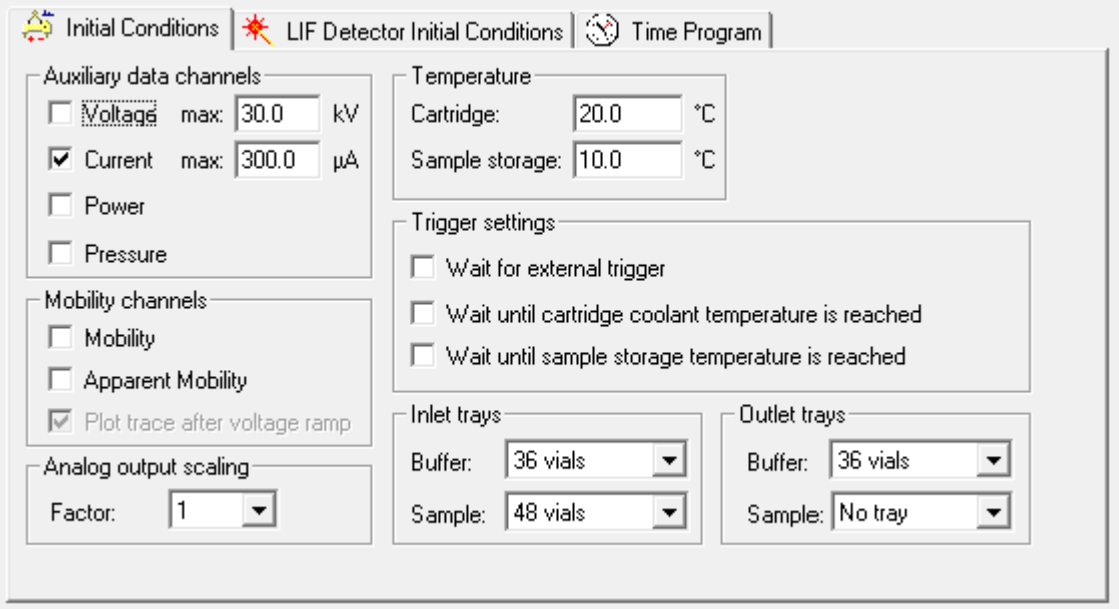


Figure B-11 LIF Detector Initial Conditions Tab

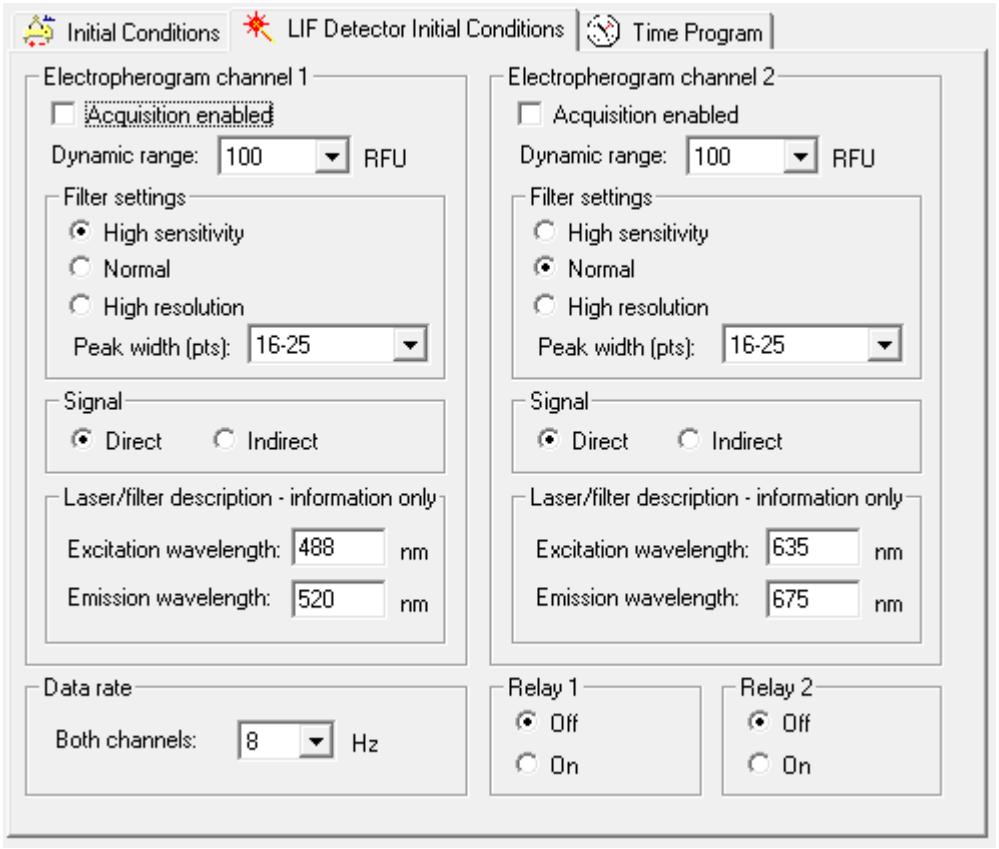
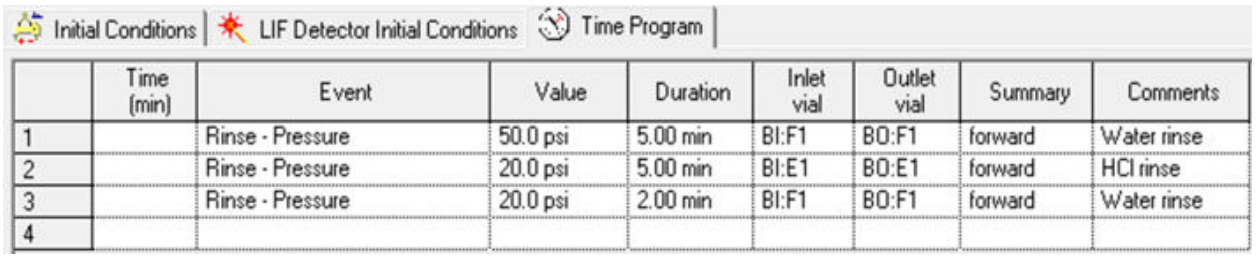


Figure B-12 Capilllary Rinse Method Time Program Tab



	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	50.0 psi	5.00 min	BI:F1	BO:F1	forward	Water rinse
2		Rinse - Pressure	20.0 psi	5.00 min	BI:E1	BO:E1	forward	HCl rinse
3		Rinse - Pressure	20.0 psi	2.00 min	BI:F1	BO:F1	forward	Water rinse
4								

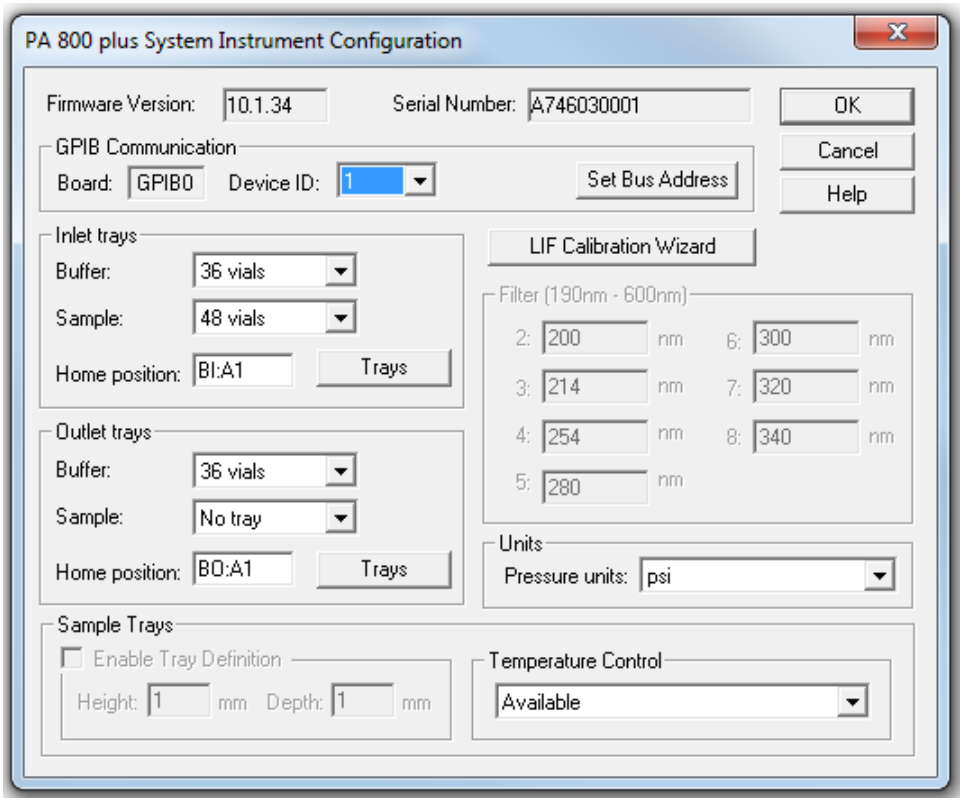
Calibrate the LIF Detector C

Calibrate the LIF detector after installation of a new capillary or a different cartridge, or after installation of the LIF detector.

Reagent
• LIF Performance Test Mix • Capillary Performance Run Buffer A • CE Grade Water

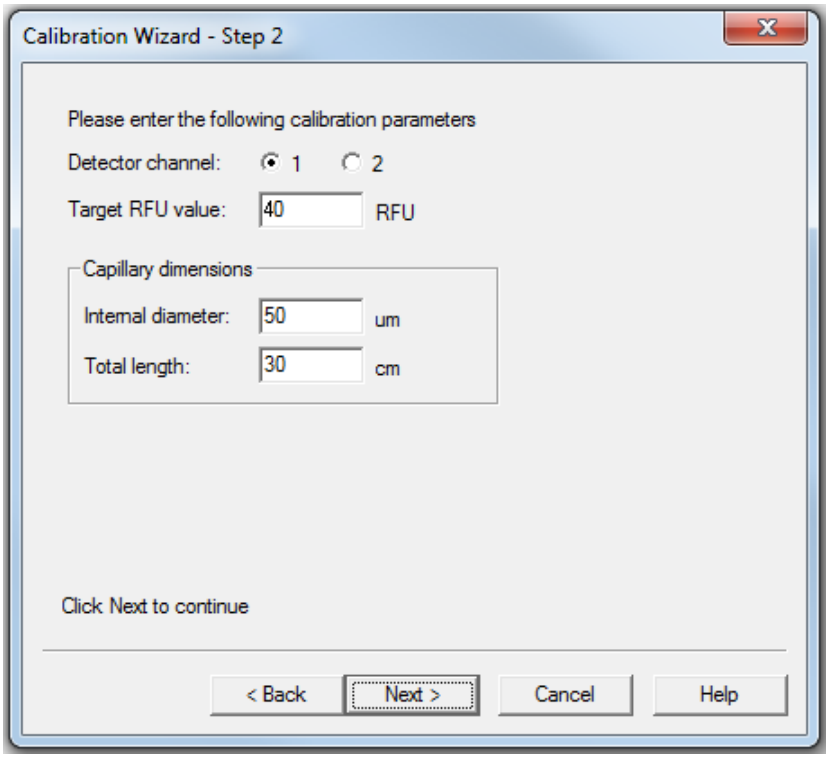
1. Turn off the PA 800 Plus system, and then install the LIF detector.
2. Turn on the PA 800 Plus system, and then let the laser warm up for at least 30 minutes.
3. Open the 32 Karat software.
4. Click **Tools** > **Enterprise Login**, and then log in as a user with Administrative privileges.
5. Click the **RNA 9000** instrument icon, and then right-click **Configure** > **Instrument**.
   The Instrument Configuration dialog opens.
6. Click **Configure**.
   The PA 800 Plus System Configuration dialog opens.
7. In the right pane, click the **LIF Detector** icon, and then right-click and select **Open**.
   The PA 800 plus System Instrument Configuration dialog opens.

Figure C-1 PA 800 plus System Instrument Configuration Dialog



8. Click **LIF Calibration Wizard**.
9. Do the calibration.
    a. Click **Auto**, and then click **Next**.
    b. Type `40` in the **Target RFU** field.
    c. Make sure that the values in the **Capillary dimensions** area are correct, and then click **Next**.

Figure C-2 Calibration Wizard - Step 2



10. Fill the vials and start the calibration.

    For vial positions, refer to the figure: Figure C-3.

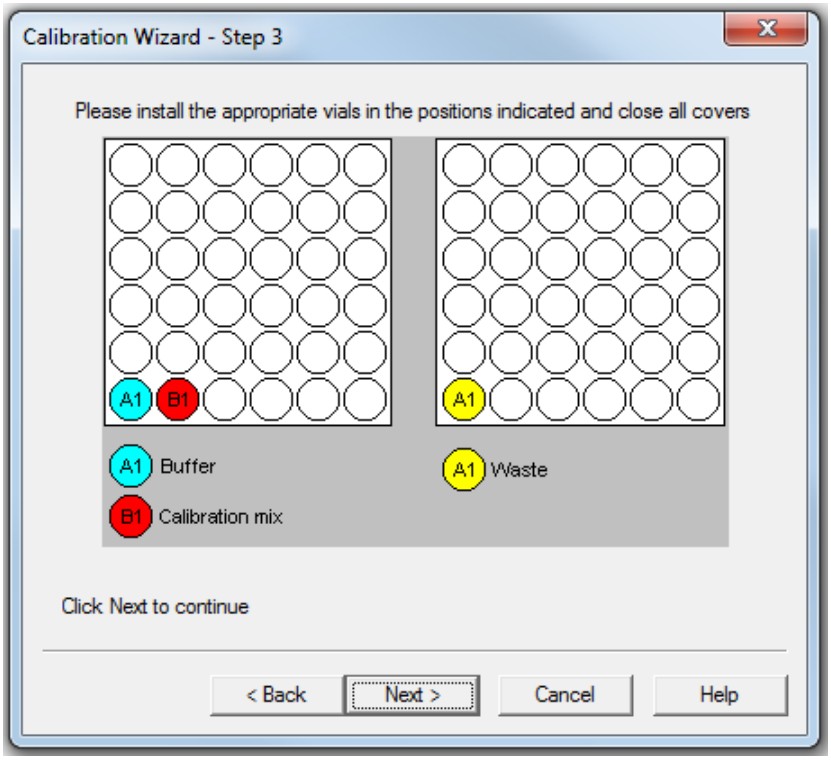
    a. Put a universal vial in the Buffer position, fill it with 1.5 mL Capillary Performance Run Buffer A, and then put a cap on the vial.

    b. Put a universal vial in the Calibration mix position, add 1.5 mL of LIF Performance Test Mix diluted 1:1 with CE Grade Water, and then put a cap on the vial.

    c. Put a universal vial in the Waste position, fill it with 1.0 mL CE Grade Water, and then put a cap on the vial.

    d. Click **Next**.

Figure C-3 Calibration Wizard - Step 3

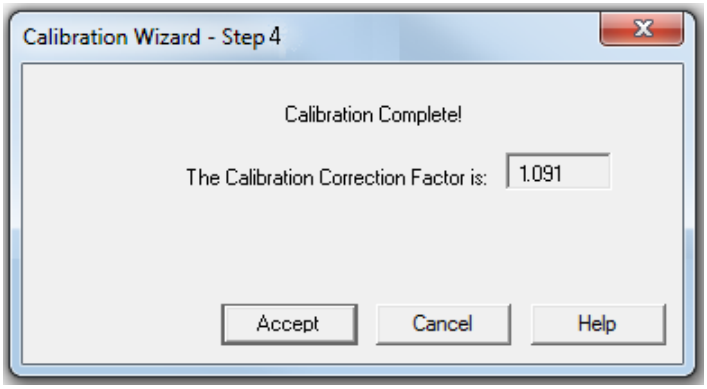


When the calibration is finished the Calibration Wizard - Step 4 dialog opens.

11. Examine the value for **Calibration Correction Factor** to evaluate the results.

    - If the number is less than 10, then the calibration is successful. Click **Accept** to save the results and close the Calibration wizard.
    - If the number is greater than 10, then click **Cancel**, and then refer to the section: CCF Values for LIF Detector Calibration.

Figure C-4 Calibration Wizard - Step 4



12. Use Direct Control to set the sample storage temperature to 10 °C.

CCF Values for LIF Detector Calibration

Reported CCF Value	Action

Less than 0.1
or
System performance is not acceptable
	• Make sure that the correct capillary was used, and that it is not broken.
	• Make sure the laser output for the laser in use on the PA 800 Plus system.
	• 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 repeat the calibration. If the problem persists, then contact SCIEX Technical Support at sciex.com/request-support.

Between 0.1 and 10.0	There is no problem with the system. Run a standard and make sure that the system performance is satisfactory.

Greater than 10
or
System performance is not acceptable
	• Make sure that the laser output for the laser in use on the PA 800 Plus 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 repeat the calibration. If the problem continues, then contact SCIEX Technical Support at sciex.com/request-support.

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