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

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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 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 (SDSs), 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 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 has 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.

Two separation methods were developed for this application: one that uses an electrokinetic injection, and the other that uses a pressure injection. Both methods provide size-based separation for RNA molecules between 0.05 kb and 9 kb in approximately 40 minutes. For information about the injection types, refer to the section: Guidance for Selection of the Injection Type.

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	Install the cartridge.	Install the Cartridge
6	Load the sample tray.	Load the Sample Tray
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 `RNA 9000 Capillary Shutdown.met` 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 (100 mL)	1	5312349
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 Other Supplies from SCIEX

Component	Quantity	Part Number
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

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

Table 3 Other Supplies from SCIEX (continued)

Component	Quantity	Part Number
LIF Cartridge Probe Guide Assembly	1	721126
PCR microvials (200 µL)	100	144709
Sample Loading Solution	6 mL	608082
Universal vial caps, blue	100	A62250
Universal vials	100	A62251

Table 4 Other 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, to reduce photobleaching of the SYBR™ Green II RNA Gel Stain, remove the SYBR™ Green II RNA Gel Stain[3] from the box, immediately wrap it in aluminum foil, and then keep at –35 °C to –15 °C.
- Upon receipt, keep the following at 2 °C to 8 °C:
  - Nucleic Acid Extended Range Gel
  - LIF Performance Test Mix
- Upon receipt, keep the ssRNA Ladder at –35 °C to –15 °C.
- Keep the remaining contents of the kit at ambient temperature.

Customer-Supplied Equipment and Supplies

- Powder-free gloves, neoprene or nitrile recommended

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

- Protective eyewear
- Laboratory coat
- Analytical balance
- Microcentrifuge, or equivalent, and nuclease-free microcentrifuge tubes (USA Scientific PN 1615-5510)
- PCR tubes, 0.2 mL flat-cap nuclease-free (VWR USA PN 20170-012 or VWR EUR PN 732-0548)
- Ice
- Pipettes and applicable nuclease-free tips
- Table-top mini centrifuge
- 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

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), with a total length of 30.2 cm and 50 µm inner diameter (i.d.)
- Capillary cartridge (PN 144738) and bare-fused silica capillaries, 50 µm inner diameter (i.d.) × 67 cm (PN 338451)

Condition the Capillary

CAUTION: Potential Wrong Result. Do not use a basic solution to clean the capillary. The solution might negatively ionize the capillary wall and result in electro-osmotic flow detrimental to the separation of nucleic acids. These interactions might cause poor separation.

- Before a new capillary is used, condition the capillary with the `RNA 9000 Capillary Conditioning.met` 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:
  - `RNA 9000 Capillary Conditioning.met`: Conditions the capillary.
  - Separation methods:
    - `RNA 9000 Separation Method - Electrokinetic Injection.met`: Separates the sample with an electrokinetic injection of the sample.
    - `RNA 9000 Separation - Pressure Injection.met`: Separates the sample with a pressure injection of the sample.
  - `RNA 9000 Capillary Shutdown.met`: Cleans the capillary at the end of a sequence and turns off the light source.
  - `Capillary Rinse - RNA 9000.met`: Rinses the capillary. To save time after the capillary has been conditioned, replace `RNA 9000 Capillary Conditioning.met` with this method in the sequence.
  - `ANALYSIS - RNA 9000 - ssRNA Ladder.met`: Contains the default integration parameters and named peaks to aid in the analysis of the ssRNA Ladder.
- Sequences:
  - `RNA 9000 Test Sequence-EKI.seq`: A sequence with the separation method that uses electrokinetic sample injection.
  - `RNA 9000 Test Sequence-Pressure Injection.seq`: A sequence with the separation method that uses pressure 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.
4. Heat the sample at 70 °C for 5 minutes.
5. After 5 minutes, immediately put the mixture in an ice water bath for a minimum of 2 minutes.

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. Add between 50 µL and 200 µL of the cooled RNA solution to the microvial, and 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 warm, then do not put the sample tray in the system. Instead, keep the tray between 2 °C and 8 °C while the laser becomes warm, 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 minutes.
4. After 5 minutes, immediately put the mixture in an ice water bath for a minimum of 2 minutes.

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. Add between 50 µL and 200 µL of the cooled RNA solution to the microvial, and 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 warm, then do not put the sample tray in the system. Instead, keep the tray between 2 °C and 8 °C while the laser becomes warm, 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.

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

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

Clean the Electrodes, Insertion Levers, and Interface Block

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

Clean the electrodes, opening levers, capillary tips, and interface block after every use 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*.

The gel buffer is very viscous. To make sure that gel buffer does not collect in the system, do regular and thorough cleaning.

Install the Capillary

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

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

  The recommended capillary length is 20 cm to the window and 30.2 cm total length.

  Use the LIF aperture and probe guide.

Install the Cartridge

1. Remove the cartridge from the box.
2. For a pre-assembled cartridge, 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*.
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).

Load the Buffer Trays

Note: The gel buffer and dye mixture is referred to as *gel buffer* in this document.

CAUTION: Potential Wrong Result. Do not prepare the gel buffer before it is required. Degradation of the SYBR™ Green II RNA Gel Stain in the gel buffer might occur during storage and cause peaks with decreased intensity.

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 gel 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. Add the following reagents 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.

   Table 5 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[4]	10 µL	20 µL	60 µL

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

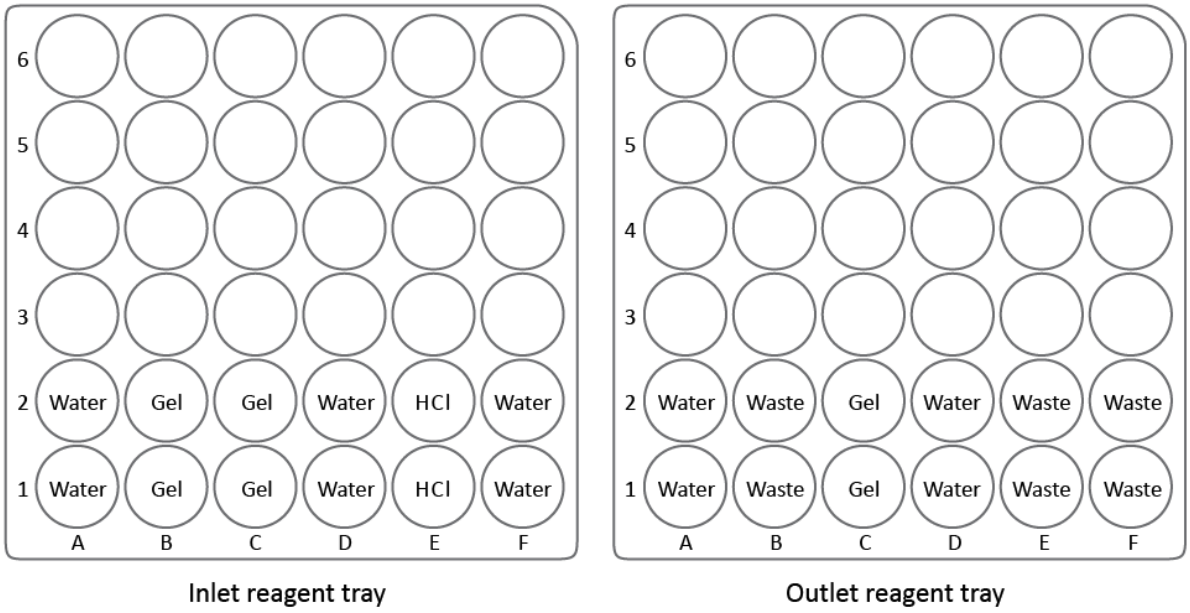
2. With a 0.45 µm Acrodisc syringe filter and a Luer-Lok syringe, filter the gel buffer.

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

3. Use the layout in the following figure to put the vials in the buffer trays. Each row is sufficient for at least eight runs. The following figure shows the layout for at least 16 runs.

Note: The separation methods are programmed to increment after eight runs.

Figure 1 Buffer Tray Layout



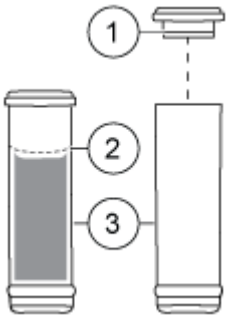
Note: For fewer than 8 samples, fill only row 1.

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

Table 6 Vials to Prepare

Label	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

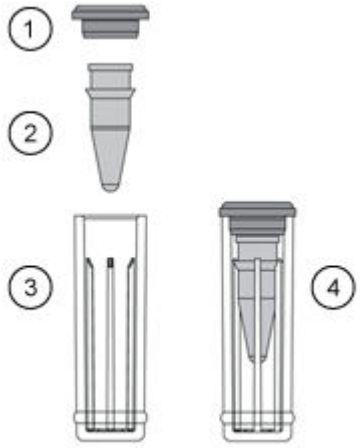
Load the Sample Tray

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 20 µL and 200 µL, with a standard volume of 100 µL, use microvials.

1. 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 3.

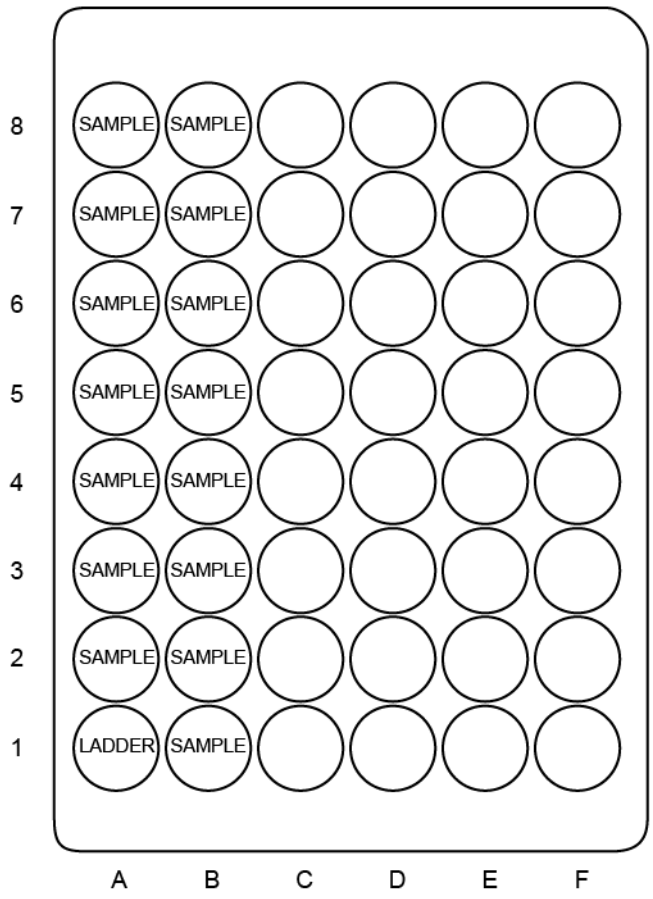
Figure 3 Microvial in a Universal Vial



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 4.
   Position A1 is for the ssRNA Ladder. Use the other positions for the other samples.

Figure 4 Sample Tray Layout



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.

If a specific characteristic is to be maximized, other temperatures can be used. Refer to the table: Table 7.

Table 7 Guidance for Setting the Capillary Temperature

Capillary Temperature (°C)	Capillary Run Life	9 kb Peak Migration Time (minutes)	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 done) depends on the sample and the separation method. The preceding table shows 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, in the sequence, use the `Capillary Rinse - RNA 9000.met` method as an alternative to the `RNA 9000 Capillary Conditioning.met` method. The rinse method is approximately 30 minutes shorter than the conditioning method.

Guidance for Selection of the Injection Type

Two separation methods are supplied with the kit, one with an electrokinetic injection and one with a pressure injection. This section gives an explanation of the differences between the two types of injection. Select the separation method that is compatible with the goal of the experiment.

- Electrokinetic injection (EKI) uses an electric field to introduce the negatively charged RNA into the capillary.
  - Advantages: Assay sensitivity throughout the full separation range can be increased with a larger injection volume or more concentrated samples.
  - Disadvantages: Samples with higher ionic strength will have a lower signal intensity as a result of injection competition between nucleic acids and anions in solution. ssRNA ladder and sample peaks shorter than 300 bases might be more difficult to interpret and integrate as a result of decreased peak selectivity during electrokinetic sample introduction.
- Hydrodynamic (pressure) injection uses pressure-over-liquid to introduce the sample to the capillary.

- Advantages: Peak height is more consistent because the ionic strength in the sample does not change from injection to injection. Peaks below 300 bases keep peak selectivity which simplifies integration and analysis.
- Disadvantages: Assay sensitivity is decreased compared to electrokinetic injection.

The following figures show electropherograms of the separation of the ssRNA Ladder with the different sample injection methods. (Only the peaks from 50 bases to 300 bases are shown.) With electrokinetic injection the peak labeled *50* is much broader and thus has lower theoretical plates as compared to the same peak from a pressure injection. Refer to the figures: Figure 5 and Figure 6.

Figure 5 ssRNA Ladder Sample with Electrokinetic Injection, Annotated with Length and Theoretical Plates

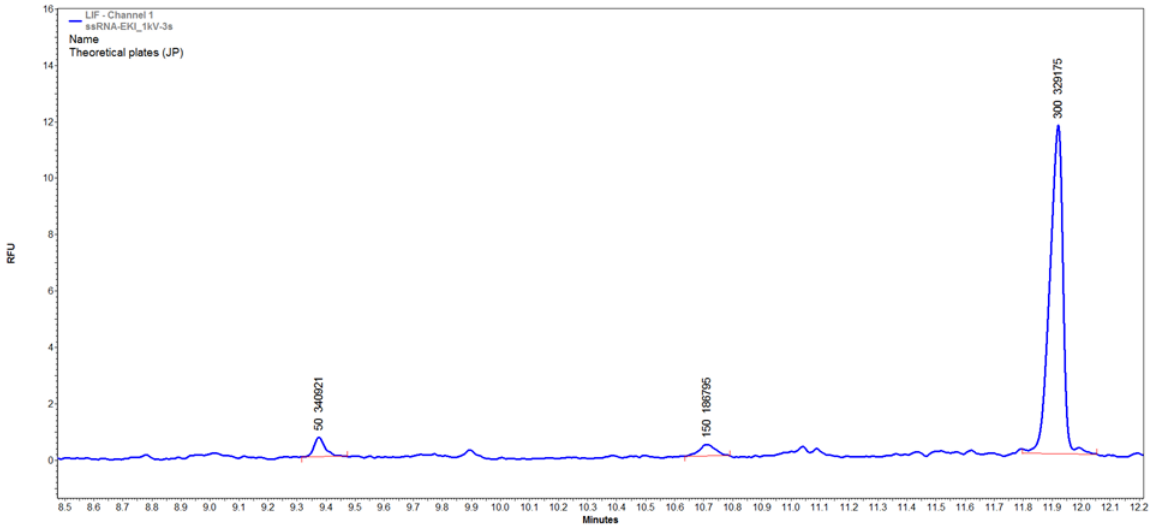
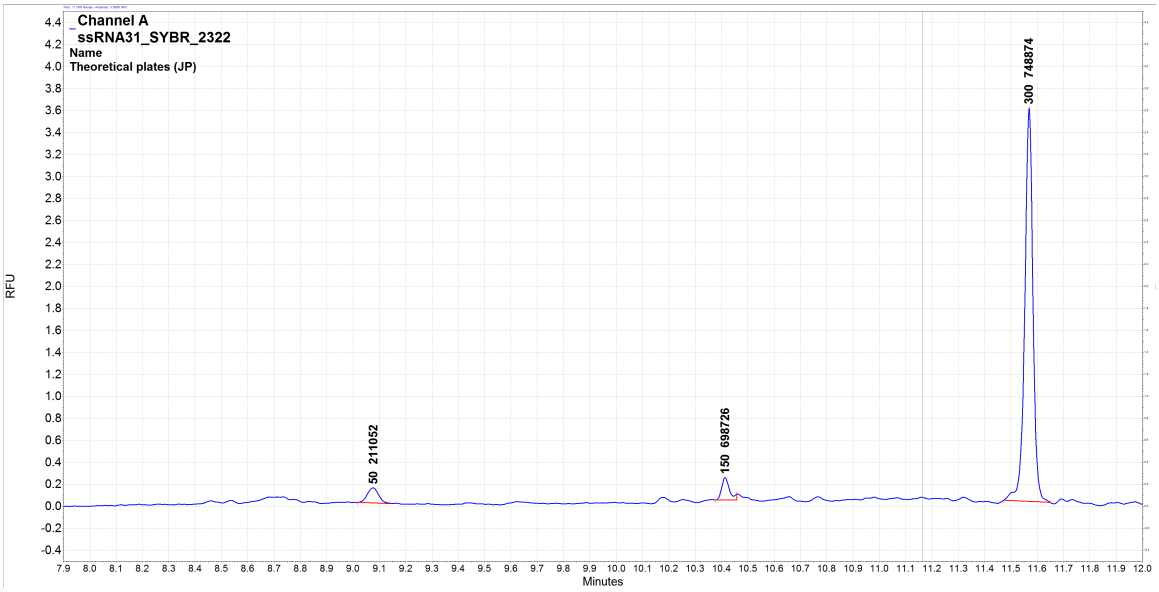


Figure 6 ssRNA Ladder Sample with Pressure Injection, Annotated with Length and Theoretical Plates



Create the Example Instrument

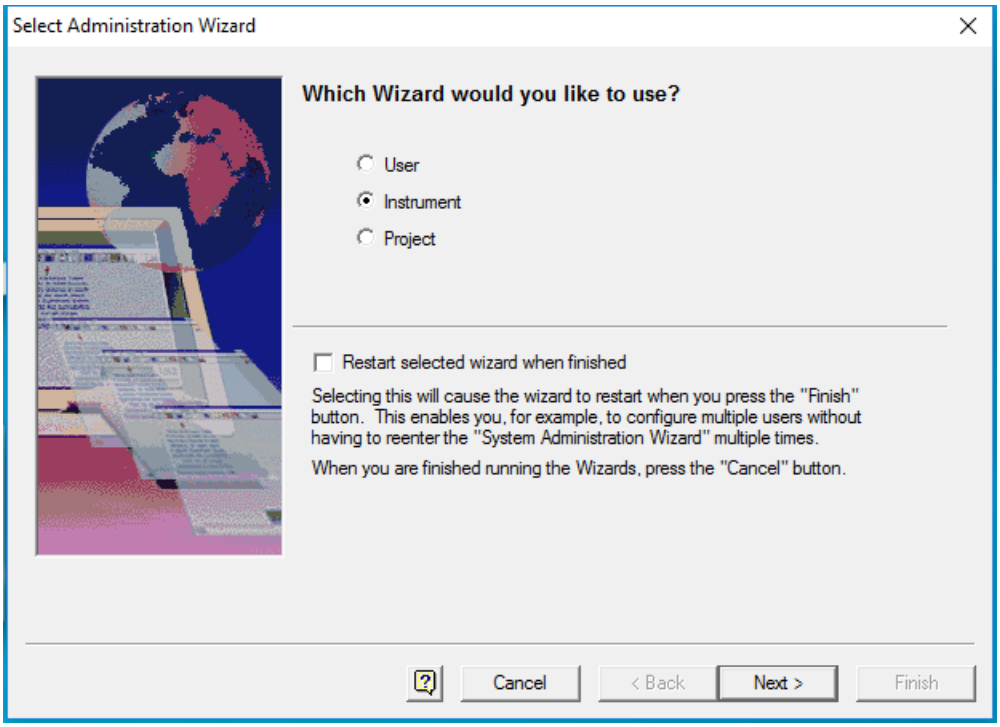
Note: The following procedure is required if the PA 800 Plus software will be used for data acquisition and analysis. If the 32 Karat software will be used instead, then this procedure is not required.

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 7 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.

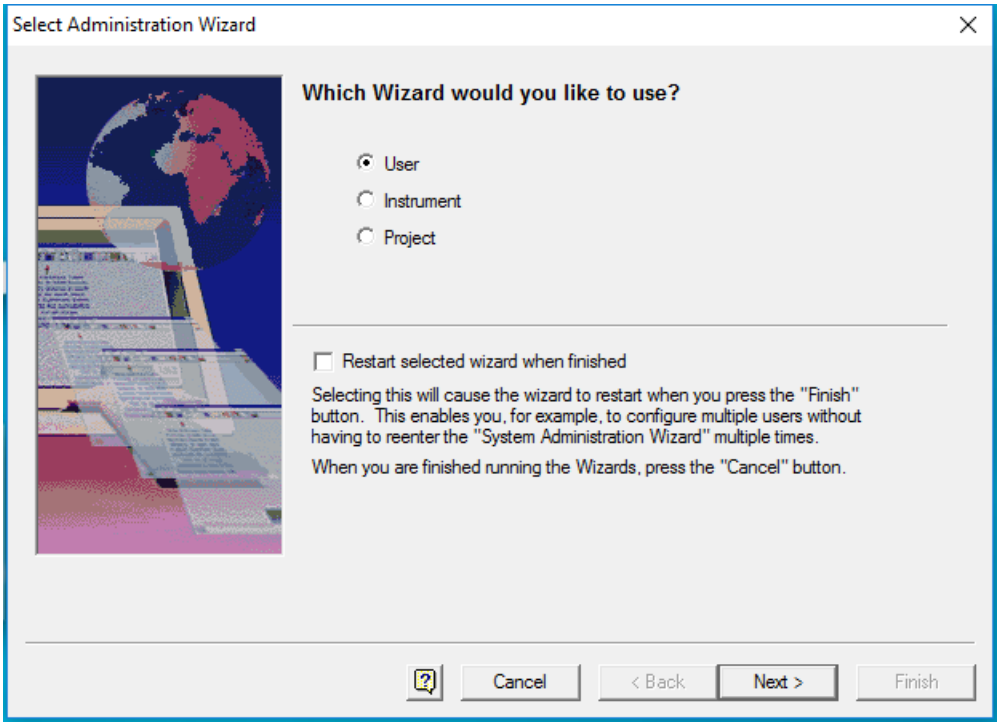
Create the Example Project

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 8 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.

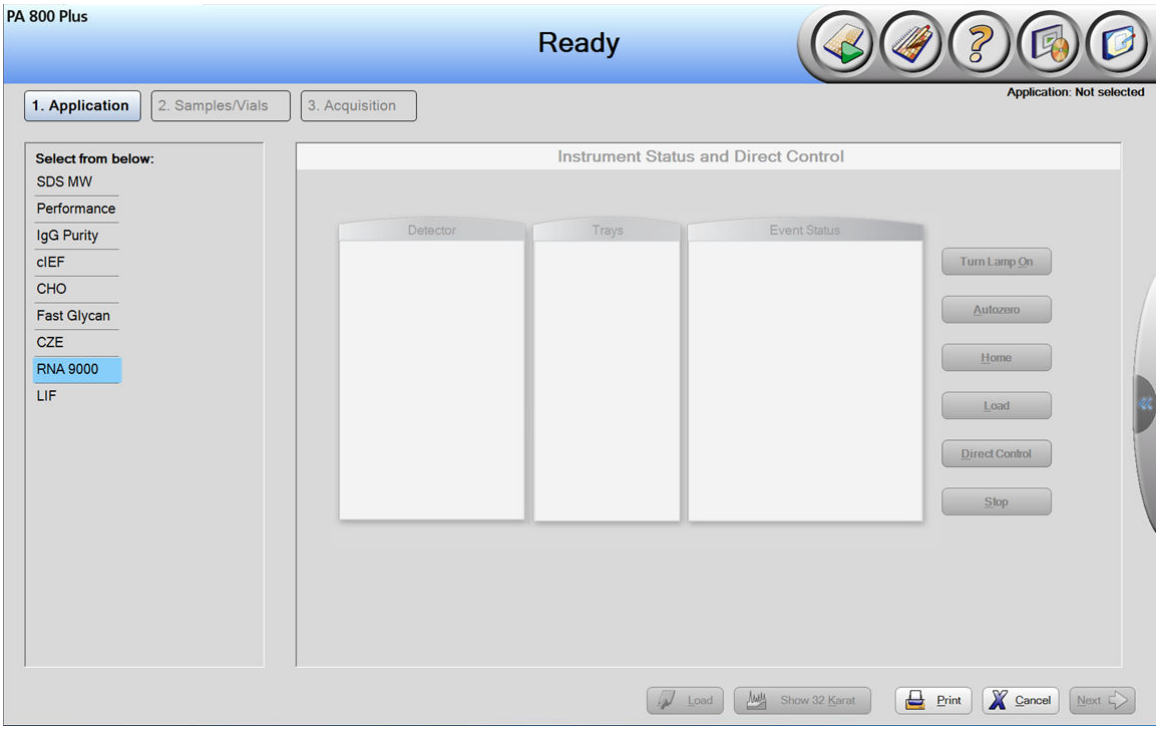
   For detailed instructions, refer to the documents: *32 Karat Software Help* or *System Administration Guide*.

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.
   The PA 800 plus window opens
2. In the upper right corner of the PA 800 plus window, click (**Run**).
   The Instrument Status and Direct Control page opens.

Figure 9 Instrument Status and Direct Control Window



3. Click (**Describe**).
4. In the **Application** list, click **RNA 9000**.
5. In the **Sequence** list, click **Browse**, and then browse to the sequence. Choose either of the following:

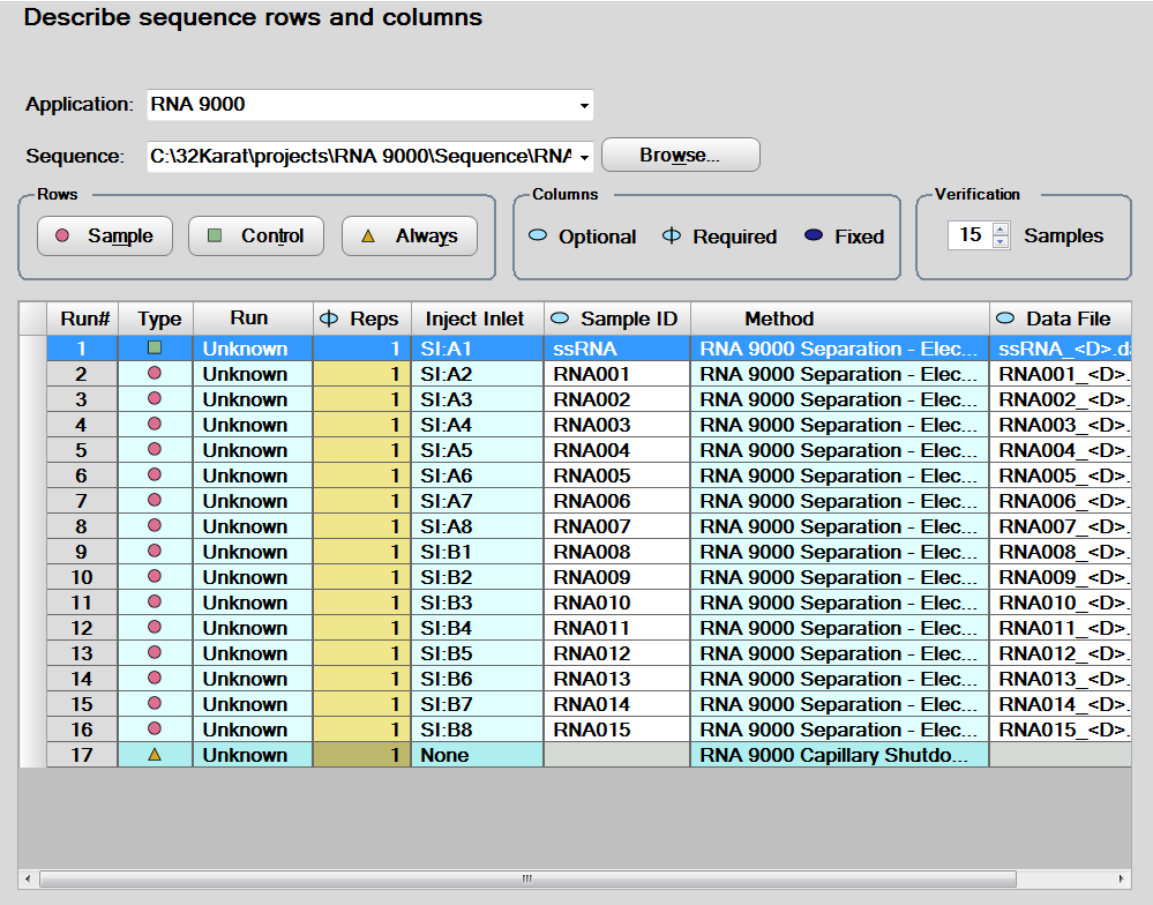
- **RNA 9000 Test Sequence-EKI.seq**
- **RNA 9000 Test Sequence-Pressure Injection.seq**

For information about the injection types, refer to the section: Guidance for Selection of the Injection Type.

6. If a prompt is shown, then type a user name and password.
   The page changes to show the selected sequence, and all of the rows in the sequence are designated as samples.
7. 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.
8. Click the last row (with the **RNA 9000 Capillary Shutdown.met** method), and then click Always (**Always**) in the **Rows** area.
   The icon in the **Type** column in the sequence changes to a triangle.

Note: If the RNA 9000 Test Sequence-Pressure Injection.seq sequence was selected in step 5, then the information in the **Method** column is different in the following figure.

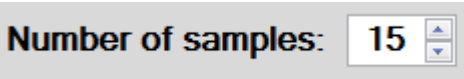
Figure 10 Describe sequence rows and columns Window



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...	

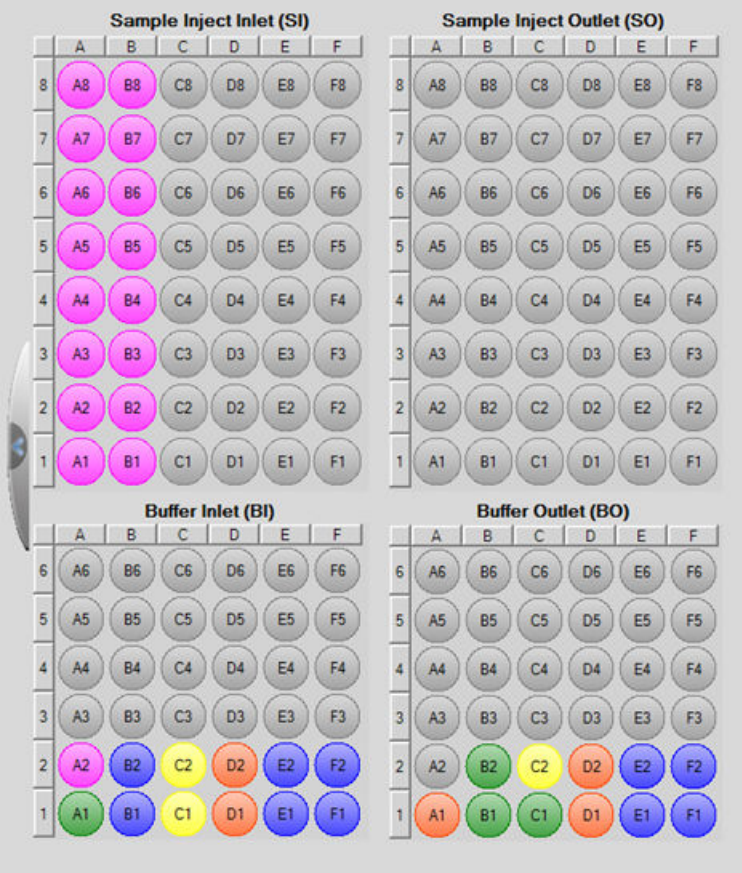
9. In the lower right corner of the window, click Save (**Save**), and then click Finish (**Finish**).
10. To set the number of samples, in the **Number of samples** field, click the arrow buttons.

Figure 11 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. For example, in Figure 1, one row of reagents is required for eight samples. Two rows of reagents are required for 16 samples.

Figure 12 Tray Map



11. 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.

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

Figure 13 Samples Loaded Prompt

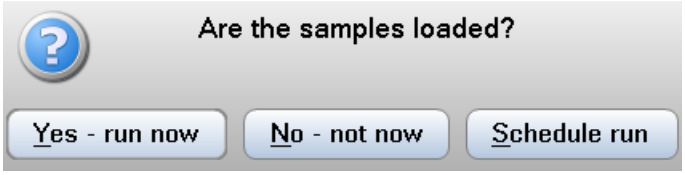
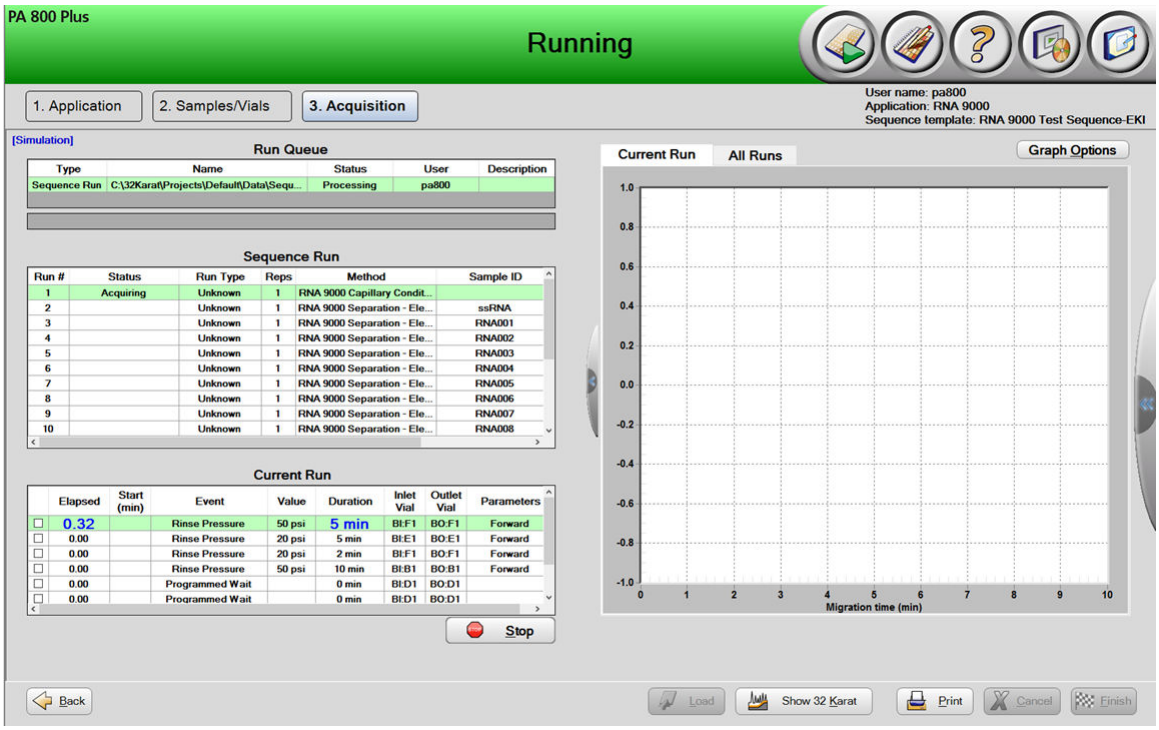


Figure 14 PA 800 Software During Data Acquisition



Waste Disposal

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

Store the Cartridge

Store 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 and decreases the cartridge temperature to 15 °C.

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

Store 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 and decreases the cartridge temperature to 15 °C.

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 `Capillary Rinse - RNA 9000.met` method to rinse the capillary.

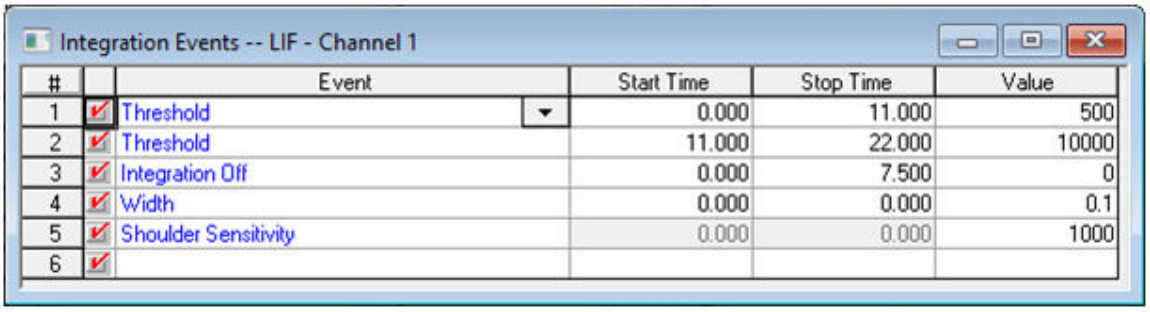
Analyze the Data

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.met`**, and then click **OK**.

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

Figure 15 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 16 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 17 ssRNA Ladder: Data Collected with Electrokinetic Injection

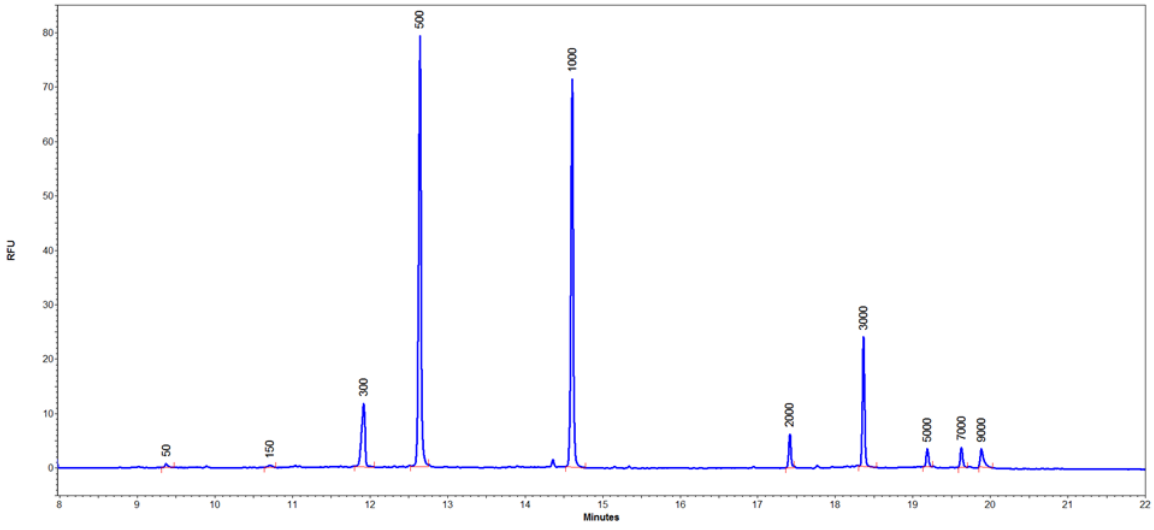
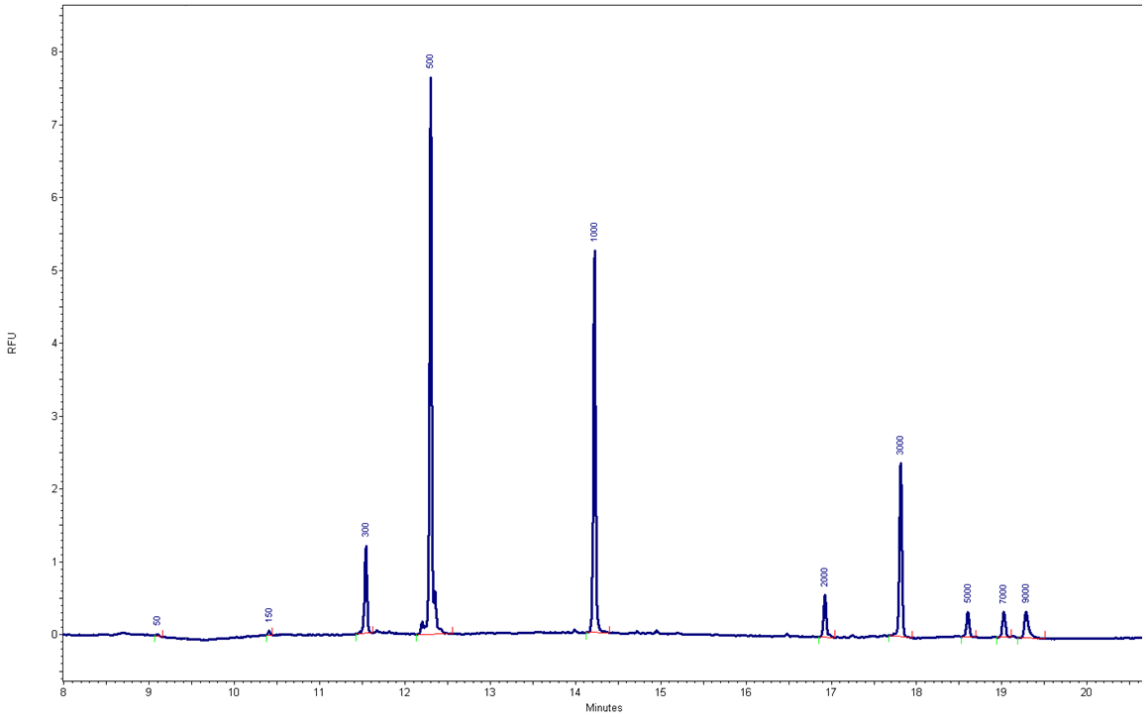


Figure 18 ssRNA Ladder: Data Collected with Pressure Injection



Guidance for Acceptance Criteria

To create acceptance criteria for use with this kit for SOPs or other purposes, use parameters that are inherent to the quality of the separation and attributes that show critical sample qualities. Differences between gel and capillary lots and different systems might cause variations 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 tip 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 tips under magnification. If a cut is jagged, then cut the tip 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 new Nucleic Acid Extended Range Gel. Make sure to keep it between 2 °C and 8 °C. Minimize the time that the gel is at ambient 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 change the method: • Fill clean vials with newly prepared reagents, cover the vials with clean caps, and then replace the vials in the tray. Do not use vials or caps more than once. • Make sure that the waste vials contain 1.0 mL of water and have been put 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. Make sure it is pure. 2. Prepare the sample again, with clean plasticware and clean sample vials. Do not use vials or caps more than once. 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 use vials or caps more than once. 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 new Nucleic Acid Extended Range Gel. Make sure to keep it between 2 °C and 8 °C. Minimize the time that the gel is at ambient temperature.

Symptom	Possible Cause	Corrective Action
Low signal	1. 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 seconds 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. • Prepare the sample at a lower ionic strength. 4. Do one or all of the following: • Increase the **Duration** up to 15 seconds 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. Make sure to limit exposure to RNase. Refer to the section: Best Practices for Working with RNA.
Low current	1. The capillary tip is dirty or plugged. 2. The capillary window or tip is broken. 3. There are dried reagents on the electrodes, insertion levers, capillary tips, 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 Electrodes, Insertion Levers, and 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 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 seconds to 20 seconds 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 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, insertion levers, capillary tips, 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 seconds to 20 seconds to remove air bubbles. 6. Clean the interface block daily or as needed. Refer to the section: Clean the Electrodes, Insertion Levers, and 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 tip 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. The tip 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 Methods. 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 tips under magnification. If a tip extends beyond the electrode, then cut the tip 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. • Prepare the sample at a lower ionic strength. 2. Prepare a new sample.
Slower migration time with or without concurrent low current	1. 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 newly prepared reagents, cover the vials with clean caps, and then replace the vials in the tray. Do not use vials or caps more than once.
Spikes in electropherogram	1. The gel buffer has air bubbles.	1. Sonicate the buffer from 10 seconds to 20 seconds to remove air bubbles. If air bubbles are still present, then prepare new gel buffer. Do not mix the buffer with a vortex mixer. Carefully invert the tube 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 in place 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 must be obeyed. For more information, refer to the respective 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.

Acid wash/regenerating solution

DANGER! Causes severe skin burns and eye damage.

Nucleic Acid Extended Range Gel

DANGER! May damage fertility or the unborn child.

SYBR™ Green II RNA Gel Stain[5]

WARNING! Combustible liquid.

Other Reagents

These components are not classified as hazardous:

- CE Grade water
- LIF Performance Test Mix

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

[5] 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

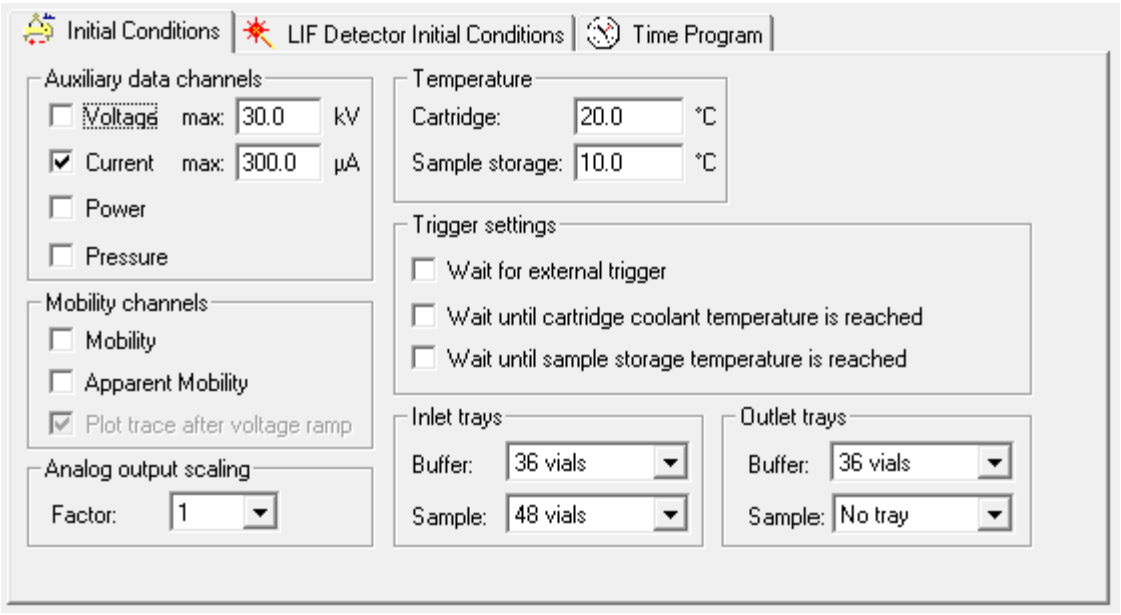


Figure B-2 LIF Detector Initial Conditions Tab

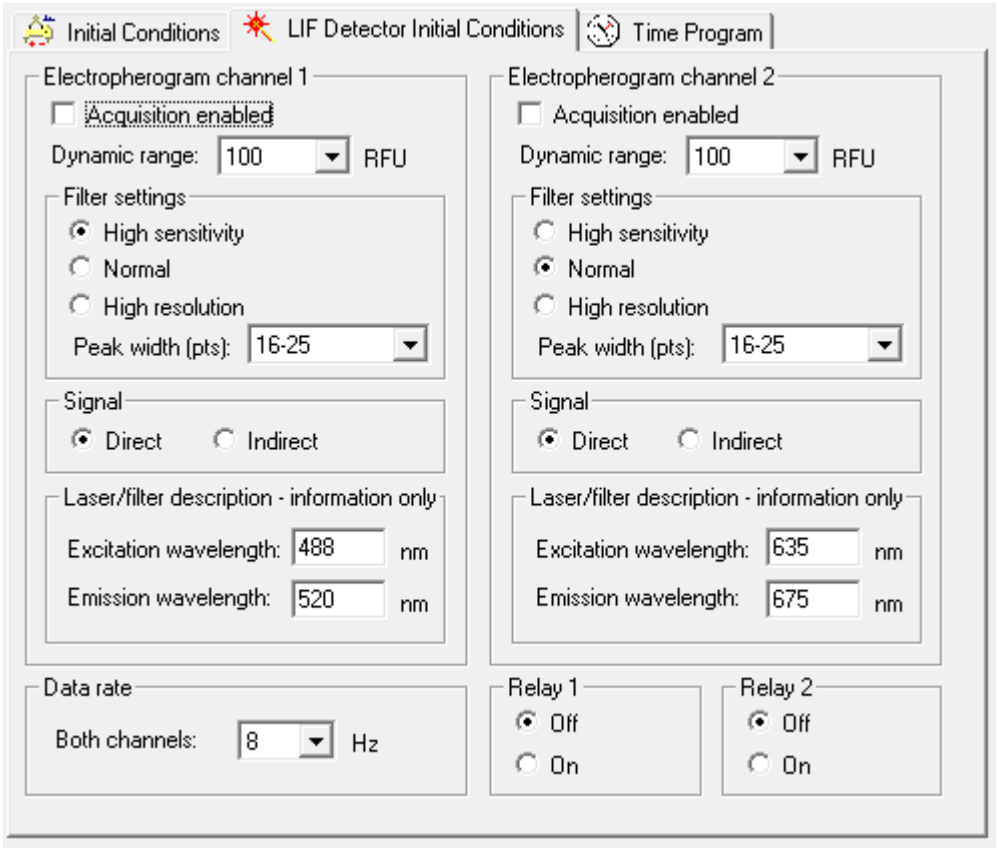


Figure B-3 Capillary Conditioning Method Time Program Tab

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 Methods

Two separation methods are available: one with an electrokinetic injection and one with a pressure injection. The parameters on the Initial Conditions and LIF Detector Initial Conditions tabs are the same for both methods. The parameters on the Time Program tab are different.

Figure B-4 Initial Conditions Tab

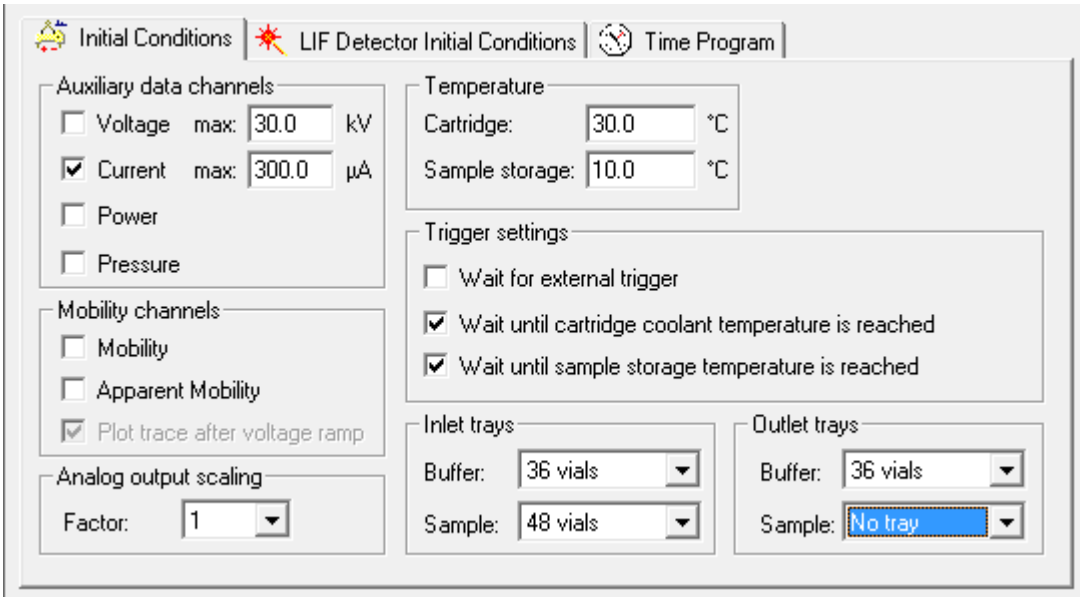


Figure B-5 LIF Detector Initial Conditions Tab

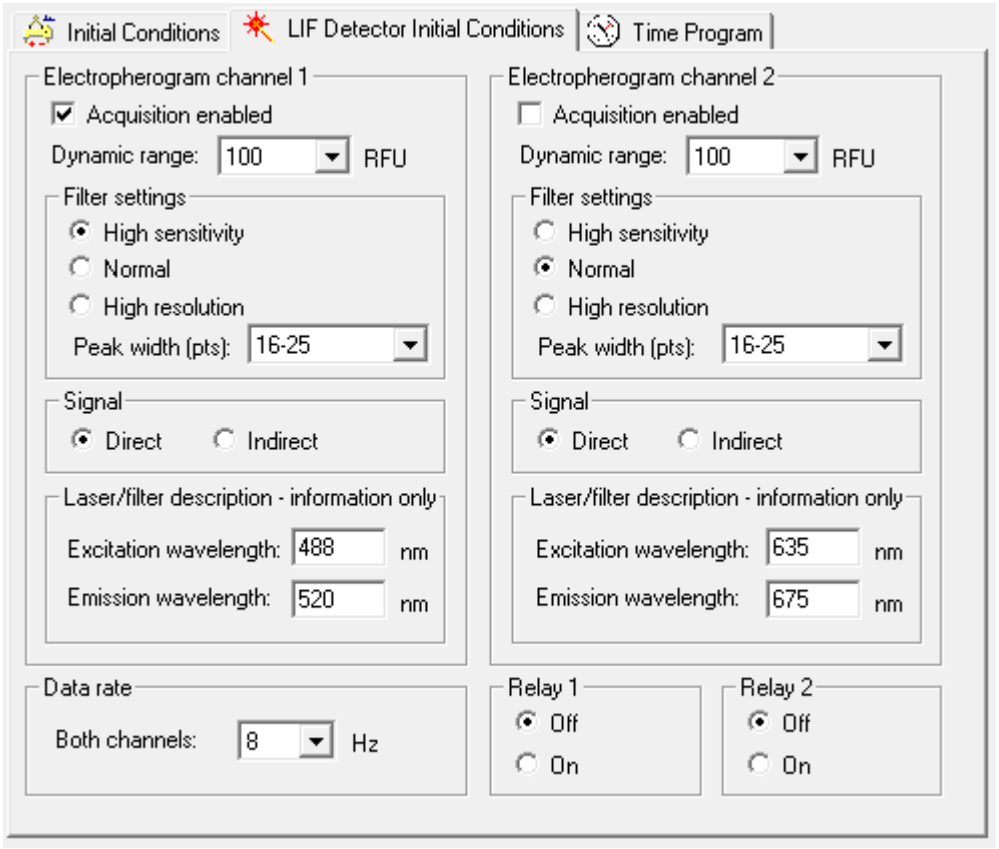


Figure B-6 Time Program Tab for Electrokinetic Injection

	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								

Figure B-7 Time Program Tab for Pressure Injection

Initial Conditions | LIF Detector Initial Conditions | Time Program

	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 - Pressure	0.5 psi	5.0 sec	BI:A1	BO:B1	No override, forward, In / Out vial inc 8	water plug
7		Inject - Pressure	1.0 psi	5.0 sec	SI:A1	BO:C1	Override, forward	Sample Pressure Injection
8		Wait		0.00 min	BI:D1	BO:D1	In / Out vial inc 8	water dip
9	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
10	8.00	Autozero						
11	22.00	End						
12								

Shutdown Method

Figure B-8 Initial Conditions Tab

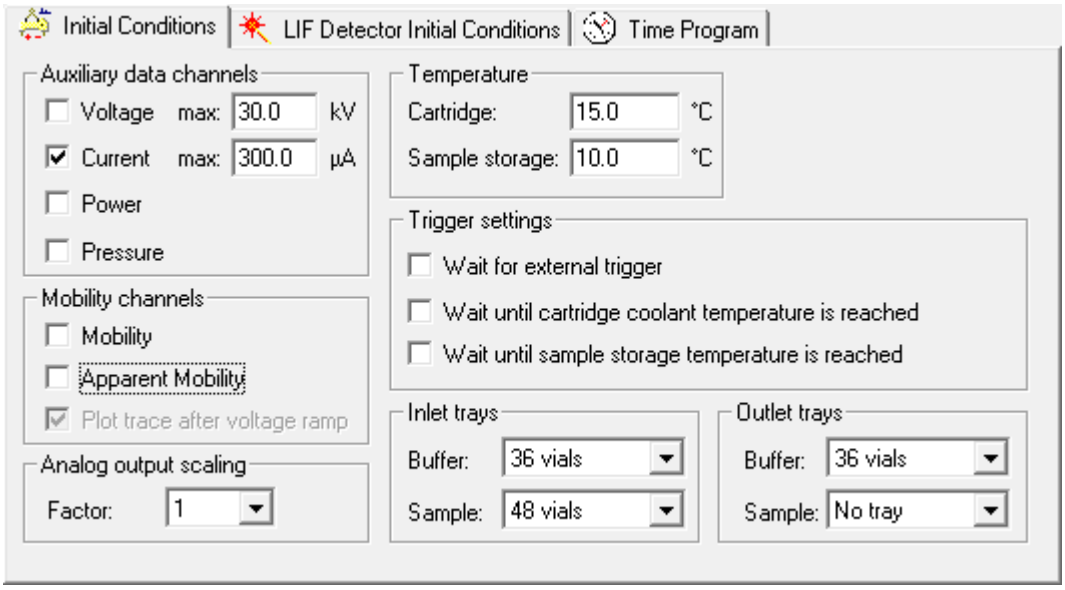


Figure B-9 LIF Detector Initial Conditions Tab

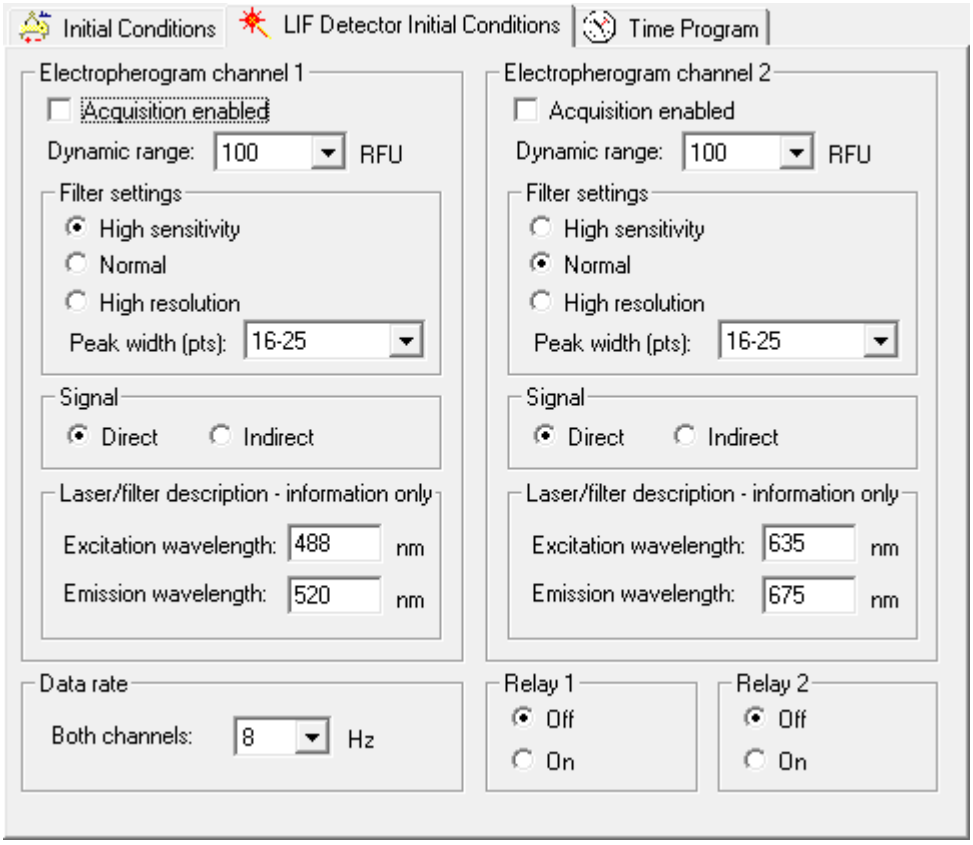
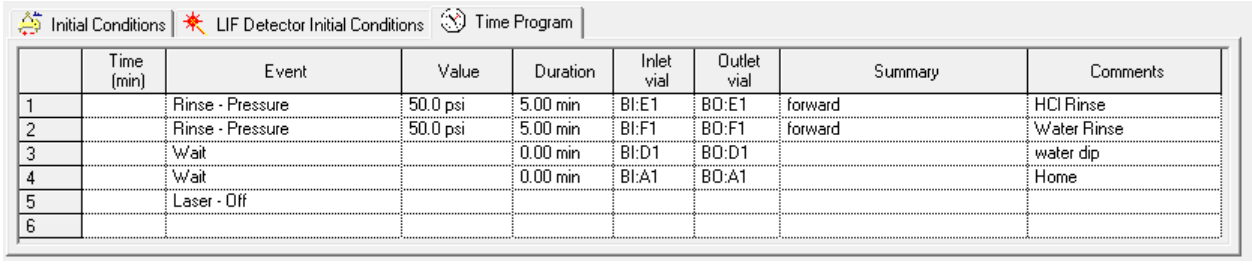


Figure B-10 Shutdown Method Time Program Tab



	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-11 Initial Conditions Tab

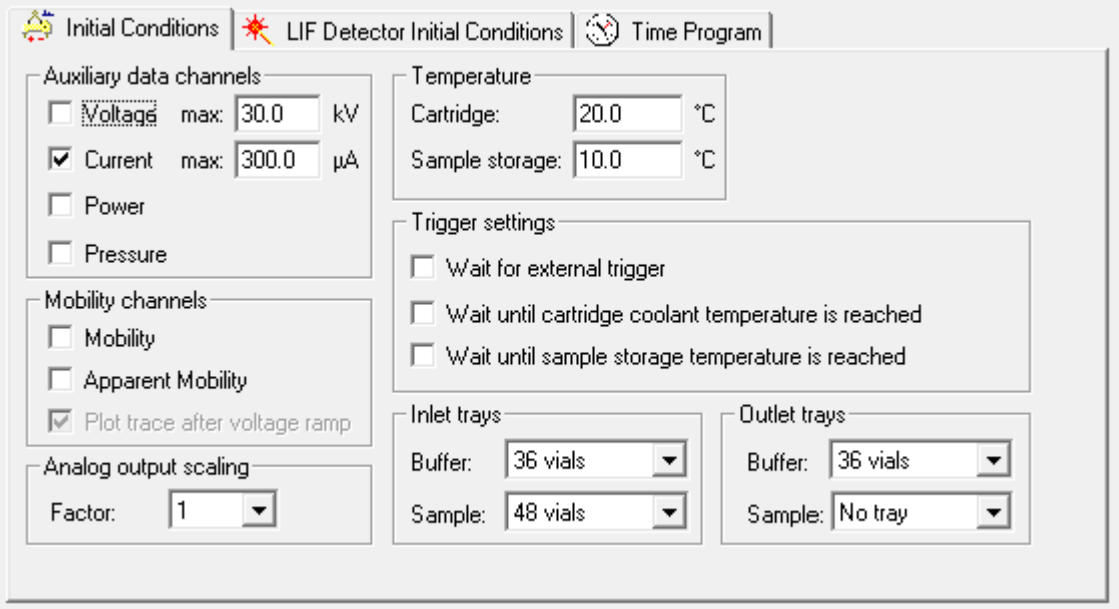


Figure B-12 LIF Detector Initial Conditions Tab

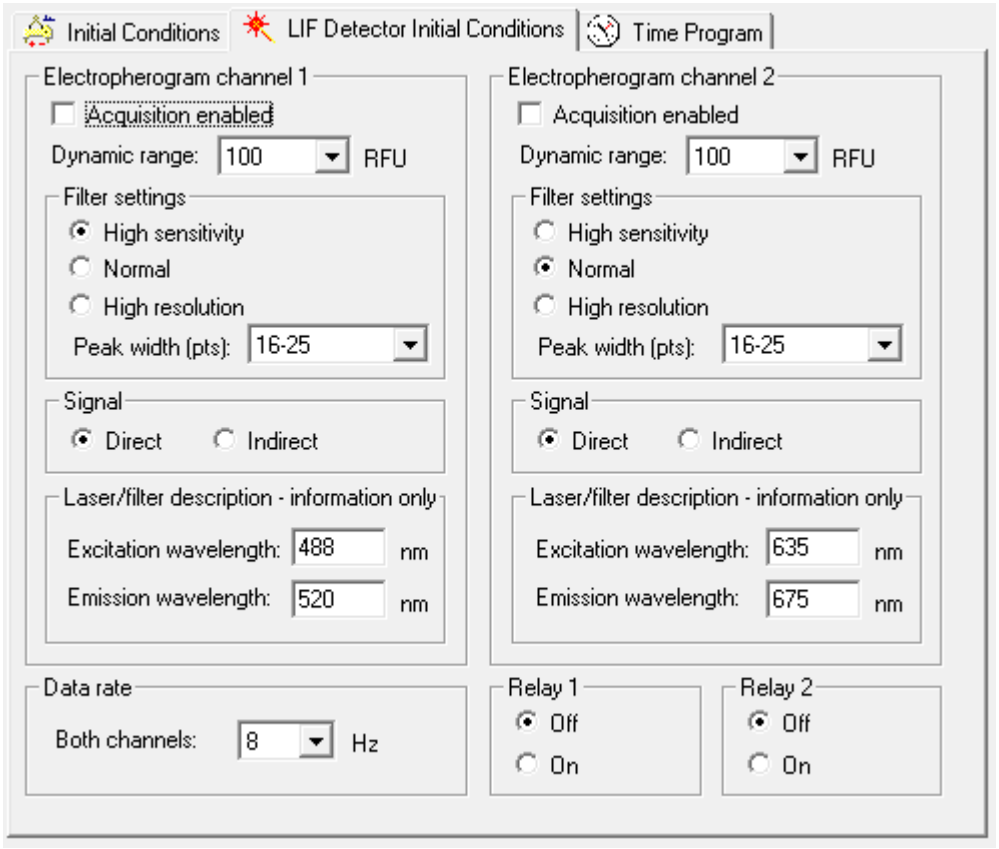
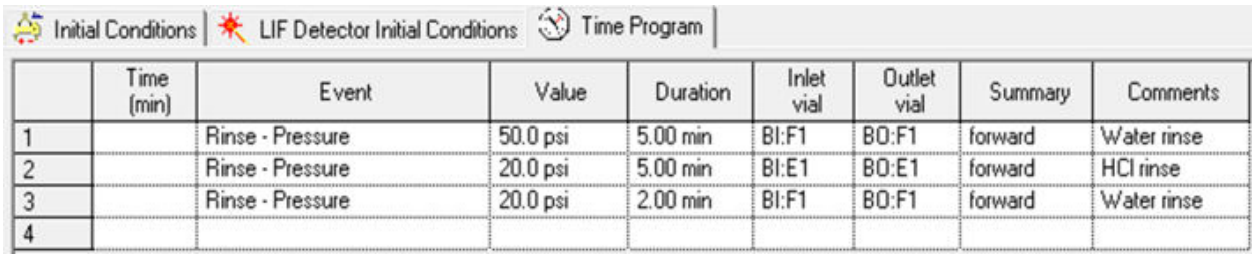


Figure B-13 Capilllary Rinse Method Time Program Tab



	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 (Optional) C

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*.

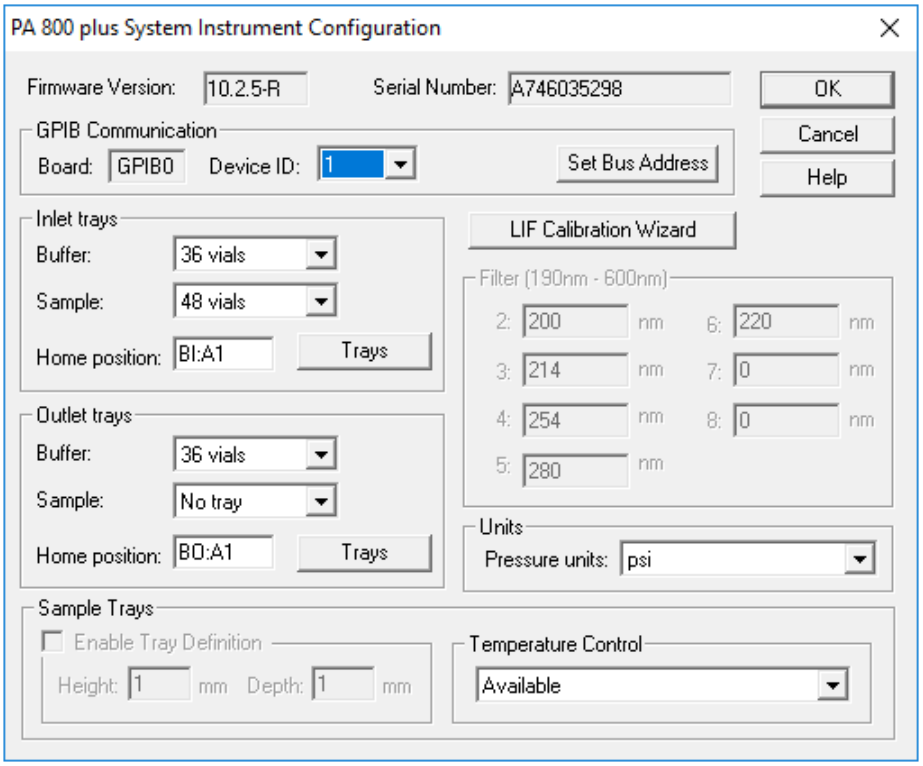
Calibrate the LIF detector after the LIF detector is installed, after a different cartridge is installed, or after a new capillary is installed in the cartridge.

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

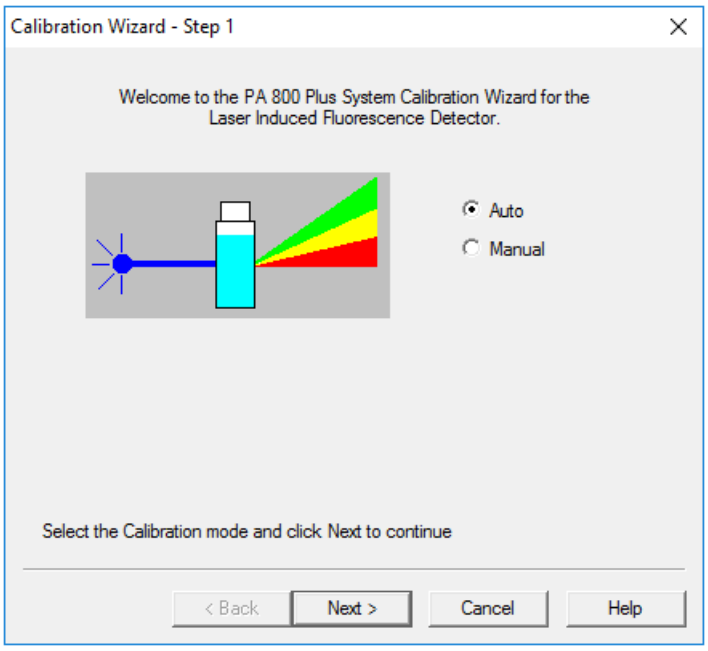
1. Turn on the PA 800 Plus system.
2. Open the 32 Karat software.
   The 32 Karat Software Enterprise window opens.
3. Open the LIF instrument, open the Direct Control window, and then turn on the laser.
4. In the 32 Karat Software Enterprise window, click **Tools** > **Enterprise Login**, and then log on as a user with Administrative privileges.
5. Right-click the **RNA 9000** 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 C-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 C-2 Calibration Wizard - Step 1

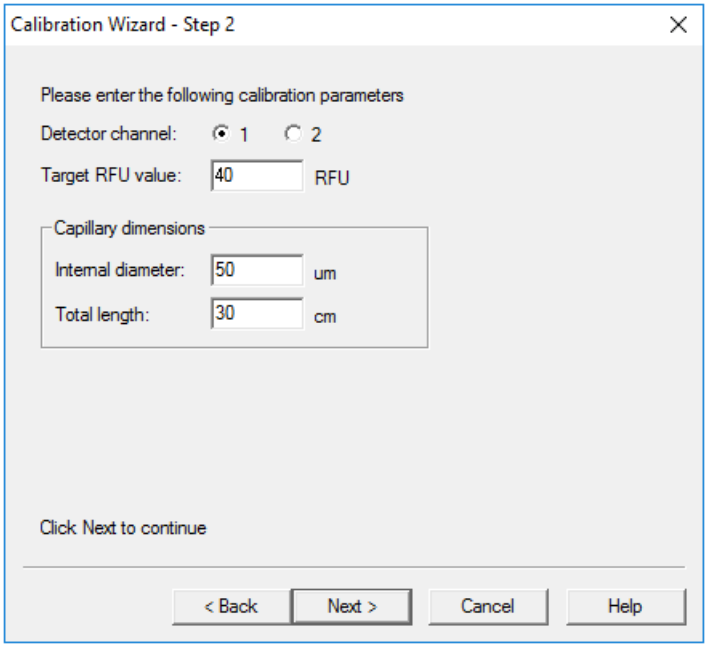


b. In the **Target RFU** field, type `40`.

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 is intended for transferability.

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

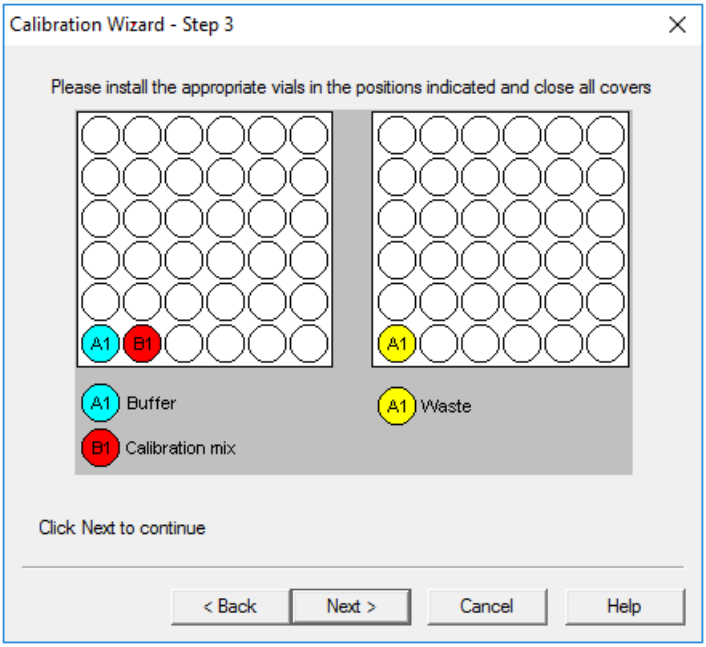
Figure C-3 Calibration Wizard - Step 2



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.

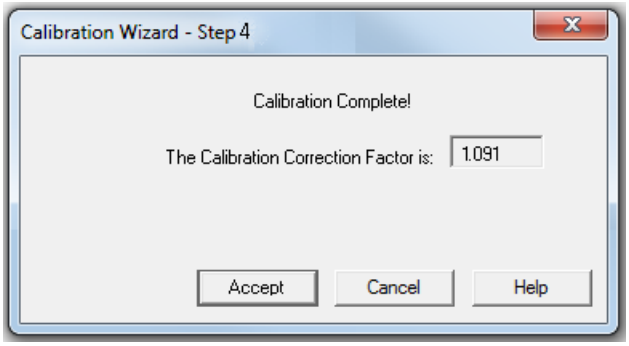
Figure C-4 Calibration Wizard - Step 3



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
    - 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 C-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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