SCIEX
The Power of Precision

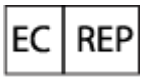
RNA 9000 Purity & Integrity Kit

For the BioPhase 8800 System

Application Guide

RUO-IDV-05-13438-C

April 2023



Leica Microsystems CMS GmbH
Ernst-Leitz-Strasse 17-37
35578 Wetzlar
Germany

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

Contents

1 RNA 9000 Purity & Integrity Kit 5
Safety 5
Intended Use 5
Introduction 5
Workflow 6

2 Required Equipment and Materials 7
Storage Conditions 8
Customer-Supplied Equipment and Supplies 9
Required Detector 9
Required Cartridge 9
Condition the Capillaries 9

3 Methods and Sequences 11
Create the Sequence 11

4 Prepare the Samples 15
Best Practices for Working with RNA 15
Prepare the ssRNA Ladder 15
Prepare the RNA Sample 16

5 Prepare the BioPhase 8800 System 18
Load the Reagent Inlet and Outlet Plates 18
Load the Sample Inlet and Outlet Plates 20
Examine the Capillary Cartridge 22
Install the Cartridge 23

6 Run the Samples 26
Tips for Best Results 26
Use the Rinse Method 26
Start the Sequence from the Front Panel 26
Monitor the Run on the BioPhase 8800 Front Panel 28
Waste Disposal 36
Store the Cartridge After the Run 36
Store the Cartridge for Less than 24 Hours 36
Store the Cartridge for More than 24 Hours 36

Prepare the Cartridge After Storage 36

7 Analyze the Data 37
Analyze the Data with an Analysis Parameters File 37
Examine the Results 40
Review the Results on the Overlay Tab 40
Guidance for Developing Acceptance Criteria 44

8 Run the Samples with the Waters Empower™ Software 45
Import the BioPhase Software Methods to Create the Instrument Methods 45
Create the Sample Set Method 49
Start the Sample Set Method 52
Monitor the Run in the Waters Empower™ Software 58

9 Troubleshooting 61
Remove Blockage from a Capillary 73
Options for a Blocked or Damaged Capillary 73
Condition the Capillaries 73

A Hazardous Substance Information 75

B Download Required Files 76
Download and Configure the Required Files (BioPhase Software) 76
Download and Configure the Required Files (Waters Empower™ Software) 77

C Reagents, Plate Layouts, and Methods 78
Reagent Set 78
Plate Layouts 79
Methods 81
Conditioning Method 81
Separation Method with Electrokinetic Injection 82
Shutdown Method 84
Capillary Rinse Method 85

Contact Us 86
Customer Training 86
Online Learning Center 86
Purchase Supplies and Reagents 86
SCIEX Support 86
CyberSecurity 86
Documentation 86

RNA 9000 Purity & Integrity Kit 1

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

This document provides instructions for sample preparation with the RNA 9000 Purity & Integrity Kit. It also provides instructions for data acquisition with the BioPhase software and the Waters Empower™ software with the BioPhase 8800 driver for Empower™. This document also provides instructions for analysis of data acquired with BioPhase software. Analysis of data acquired with the Waters Empower™ software must be done using the Waters Empower™ 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: *Operator 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

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

The workflow consists of the following steps:

1. Determine the number of samples to be analyzed and the number of replicates.
2. For BioPhase software users:
    a. Create or modify the methods. Refer to the section: Methods.
    b. Create the sequence and the sample and reagent plate layouts. Refer to the section: Create the Sequence.
3. For Waters Empower™ software users:
    a. Import BioPhase software methods. Refer to the section: Import the BioPhase Software Methods to Create the Instrument Methods.
    b. Create the sample set methods and the sample and reagent plate layouts. Refer to the section: Create the Sample Set Method.
4. Prepare the samples. Refer to the section: Prepare the Samples.
5. Use the sample and reagent plate layouts to prepare the plates.
6. Put the plates in the BioPhase 8800 system. Refer to the sections: Load the Sample Inlet and Outlet Plates and Load the Reagent Inlet and Outlet Plates.
7. Start data acquisition.
    - (BioPhase software) Start the sequence from the front panel. Refer to the section: Run the Samples.
    - (Waters Empower™ software) Start the sample set method from the Waters Empower™ software with the BioPhase 8800 driver for Empower™. Refer to the section: Start the Sample Set Method.
8. Analyze the data.
    - (BioPhase software) When the sequence is complete, analyze the data with the BioPhase Analysis software. Refer to the section: Analyze the Data.
    - (Waters Empower™ software) When the sample set method is complete, analyze the data with the Waters Empower™ software. For data analysis instructions, refer to the Waters Empower™ software guides and help file.

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

Required Equipment and Materials 2

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 2-1 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 Mixture (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 2-2 Additional Supplies from SCIEX

Component	Quantity	Part Number
(Optional) Capillary Performance Run Buffer A	1	338426
Capillary cartridge coolant (450 mL)	1	359976
Filter, 520 nm emission filter	1	144940

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

Table 2-2 Additional Supplies from SCIEX (continued)

Component	Quantity	Part Number
Sample Loading Solution	6 mL	608082
BioPhase 8800 bare fused-silica capillary cartridge (50 µm i.d. × 30 cm capillaries)	1	5080121
BioPhase 8800 outlet plates	20	5080315
BioPhase 8800 reagent plates	20	5080314
BioPhase 8800 sample plates	20	5080313
BioPhase 8800 Starter Plate Pack (4 sample plates, 4 reagent plates, 8 outlet plates)	1	5080311

Table 2-3 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
X-Pierce Film	USA Scientific	2997-0100

Storage Conditions

- Upon receipt, store the following at 2 °C to 8 °C:
  - Nucleic Acid Extended Range Gel
  - LIF Performance Test Mixture
- 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.

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

- 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)
- Table-top mini centrifuge
- Microcentrifuge, or equivalent, and nuclease-free microcentrifuge tubes
- Vortex mixer
- Pipettes and appropriate nuclease-free tips

  For reagent plate preparation a repeater pipette or equivalent is recommended.
- Water bath or heat block capable of 37 °C to 100 °C temperature
- Analytical balance
- Ice

Required Detector

An LIF detector with the 488 nm excitation source and the 520 nm emission filter is required.

Required Cartridge

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

A BioPhase 8800 BFS capillary cartridge with 50 µm i.d. × 30 cm capillaries is required.

Refer to the table: Table 2-2.

Condition the Capillaries

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

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

Methods and Sequences 3

For Systems That Use the BioPhase Software

Note: If the validated methods and sequences are not included with the software, then they are available for download from the SCIEX web site. Refer to the section: Download and Configure the Required Files (BioPhase Software). The methods can also be created manually with the BioPhase software. Refer to the section: Methods.

The following methods and sequence are required.

- Conditioning Method-RNA 9000: Conditions the capillaries before first use.
- Separation Method - Electrokinetic Injection - RNA 9000: Does the separation of the sample with an electrokinetic injection of the sample.
- Shutdown Method - RNA 9000: Cleans the capillaries at the end of a sequence, rinses the capillaries for storage, and then turns off the lamp.
- Capillary Rinse - RNA 9000: Rinses the capillary between sequences, if required.
- RNA 9000 Test Sequence - Electrokinetic Sample Injection: A sequence with the separation method that uses electrokinetic sample injection.

For Systems That Use the Waters Empower™ Software

Create the required instrument methods by importing the BioPhase software methods.

Note: If the methods are not included with the software, then they are available for download from the SCIEX web site. Refer to the section: Download and Configure the Required Files (Waters Empower™ Software). The methods can also be created manually with the Method Editors for BioPhase System software. Refer to the section: Methods.

Create the Sequence

Note: If the Waters Empower™ software will be used to acquire the data, this procedure does not apply. Refer to the section: Create the Sample Set Method.

Note: This procedure assumes familiarity with the BioPhase software. For detailed instructions, refer to the document: *Software Help System*.

This procedure gives instructions for creating a sequence with a template supplied with the BioPhase software. The template is set up with the ssRNA Ladder in the first column, followed

by 16 samples in the second and third columns, and uses the validated methods supplied with the software.

Sequences can also be created without a template. In most cases, a sequence should start with a conditioning method, followed by separation methods, and then ending with a shutdown method. The shutdown method should be assigned as the error recovery method. Refer to the document: *Software Help System*.

Note: If the sequence includes replicate samples, then make that sure the replicates are in the same row of the sample plate to decrease any capillary-to-capillary variation.

1. On the Home page of the BioPhase software, click **Sequence Editor**.
2. Click **Open Sequence**.
   The Open a Sequence dialog opens.
3. Search for, and then select **RNA 9000 Test Sequence - Electrokinetic Sample Injection**.
   a. (Optional) Type a **Start Date** and **End Date** for the search or click the calendar icons, select the dates, and then click **Search**.
      The available project folders are shown in the Folder Name pane.
   b. Click the **RNA 9000** project folder.
      The available sequences in the project are shown in the table to the right.
   c. Click **RNA 9000 Test Sequence - Electrokinetic Sample Injection** in the table, and then click **Open**.
      The Open a Sequence dialog closes, and then the Sequence Summary tab opens.
4. Above the Sequence Summary pane, click **Edit**.
   The Sample Plate Setup tab opens.
5. If required, do any of the following:
   - Add or remove samples.
   - Clear a method assigned to a sample well.
   - Assign a different method to a sample well.
   - In the Sequence Summary table, assign the recovery method to a method in the sequence. Typically, the shutdown method should be assigned as the recovery method.

   For detailed instructions, refer to the document: *Software Help System*.
6. If required, edit the information in the Sequence Summary table.

Figure 3-1 Sequence Summary Table: RNA 9000 Test Sequence - Electrokinetic Sample Injection

Sequence Summary

	Run #	Column	Method Name	Rep. #	Error Recovery
	1	0	Conditioning Method - RNA 9000	1	☐
⊞	2	1	Separation Method - Electrokinetic Injection - RNA 9000	1	☐
⊞	3	2	Separation Method - Electrokinetic Injection - RNA 9000	1	☐
⊞	4	3	Separation Method - Electrokinetic Injection - RNA 9000	1	☐
	5	0	Shutdown Method - RNA 9000	1	☑

7. To view details of a run, click the **+** in the row with the run.

Figure 3-2 Sequence Summary Table Expanded

Sequence Summary

	Run #	Column	Method Name	Rep. #	Error Recovery
	1	0	Conditioning Method-RNA 9000	1	☐
⊟	2	1	Separation Method with Electrokinetic Injection - RNA 9000	1	☐

Well	Sample Id	Run Type	Data File
A01	ssRNA_<WP>	Unknown	<Prj>\<SN>\<DT>\<Cap>_<SID>
B01	ssRNA_<WP>	Unknown	<Prj>\<SN>\<DT>\<Cap>_<SID>
C01	ssRNA_<WP>	Unknown	<Prj>\<SN>\<DT>\<Cap>_<SID>
D01	ssRNA_<WP>	Unknown	<Prj>\<SN>\<DT>\<Cap>_<SID>
E01	ssRNA_<WP>	Unknown	<Prj>\<SN>\<DT>\<Cap>_<SID>
F01	ssRNA_<WP>	Unknown	<Prj>\<SN>\<DT>\<Cap>_<SID>
G01	ssRNA_<WP>	Unknown	<Prj>\<SN>\<DT>\<Cap>_<SID>
H01	ssRNA_<WP>	Unknown	<Prj>\<SN>\<DT>\<Cap>_<SID>

⊞	3	2	Separation Method with Electrokinetic Injection - RNA 9000	1	☐
⊞	4	3	Separation Method with Electrokinetic Injection - RNA 9000	1	☐
	5	0	Shutdown Method - RNA 9000	1	☑

8. If required, change the information in the **Sample Id** and **Data File** columns.
9. To view the sample plate and reagent plate layouts, open the Plates Layout tab. If required, change the reagent locations in the Reagent Plate.
10. To save the sequence, click **SAVE**, and then add the required information.

Note: The **SAVE** button is not available if there are errors. Resolve all of the errors in the Validation pane, and then click **SAVE**.

11. (Optional) To print the sample plate and reagent plate layouts, do the following:
    a. Click **PRINT**.
       The Print Preview dialog opens.
    b. Click **Plate Layout Report**, and then click **Print**.
    c. Select the printing options, and then click **OK**.
       The report is printed.
    d. Click the close box, the × in the top right corner.
       The Print Preview dialog closes.

Prepare the Samples 4

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. Add the reagents in the following table to dilute the ssRNA Ladder.

Table 4-1 ssRNA Ladder Dilution

Reagent	For 1 Sample	For Up to 8 Samples
ssRNA Ladder	2 µL	17.5 µL
Sample Loading Solution	48 µL	420 µL

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. Using a pipette, add 50 µL to 200 µL of the cooled diluted ssRNA Ladder to the sample plate well.

Note: In the sequence templates supplied with the BioPhase software, the ssRNA Ladder must be present in all eight wells of column 1.

Make sure that, for any column on the sample plate with sample, the corresponding column on the sample outlet plate has 2.0 mL of gel buffer.

7. Put the sample plate in the system. Make sure that the plate compartment temperature is set to 10 °C.

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, decrease the **PMT Gain** in the Method Settings 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. With a pipette, add 50 µL to 200 µL to the sample plate well.

   Make sure that, for any column on the sample plate with sample, the corresponding column on the sample outlet plate has 2.0 mL of gel buffer.

6. Put the sample plate in the system. Make sure that the plate compartment temperature is set to 10 °C.

Prepare the BioPhase 8800 System 5

Use the procedures in this section to prepare the BioPhase 8800 system to acquire data.

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

Tip! To save time, turn on the light source 30 minutes before starting the run so it can warm up.

Load the Reagent Inlet and Outlet Plates

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, leading to lower intensity peaks.

   Table 5-1 Gel Buffer (Nucleic Acid Extended Range Gel with SYBR™ Green II RNA Gel Stain)

Reagent	For 1 to 48 Samples	For 49 to 96 Samples
Nucleic Acid Extended Range Gel	40 mL	60 mL
SYBR™ Green II RNA Gel Stain	80 µL	120 µL

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

   Tip! Wrap the vial containing 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. Add the reagents to the reagent inlet and outlet plates according to the reagent plate layout. Refer to the figure: Figure C-4.

   Use the volumes in the following table.

   Note: For the outlet plate, make sure that the chamfered corner is on the upper right, and then fill only the wells on the left side of the plate. The wells on the right side are for overflow and must be empty.

   Table 5-2 Reagents for the Reagent Inlet and Outlet Plates

Plate	Reagent
Inlet plate	800 µL per well
Outlet plate	• 2.8 mL per well of reagent for separation or wait actions • 1.5 mL per well of CE Grade Water for waste positions

4. Put a film cover on the plates.

   CAUTION: Potential System Damage. Do not use a heated plate sealer to apply the seal. The heat might damage the surface of the plates, which might cause issues with the pressure system.

   Note: Only X-Pierce film from USA Scientific is validated. If a different film is used, then test it before use.

5. Put the plates in a swinging-bucket rotor, and then spin them for 4 min at 30 *g*. Make sure that the buckets are balanced.

   CAUTION: Potential Wrong Result. Do not load the plates in the system without spinning them to remove air bubbles. The presence of air bubbles might cause the separation to fail.

6. Make sure that there are no air bubbles present in the plates. If air bubbles are present, then spin the plates again at a higher relative centrifugal force (RCF).

   For the reagent plate, the maximum RCF is 1,000 *g*. For the sample plate, the maximum RCF is 375 *g*.

7. On the front panel, touch **Eject Reagent**.

Figure 5-1 Eject Reagent Button

The plate compartment opens.

8. Remove the film cover from the plates.

CAUTION: Potential System Damage. Do not load plates in the system before removing the film cover. The presence of the film cover during a run might damage the capillary tips.

9. If the plate compartment already contains reagent plates, then remove the reagent plates.
10. Align the notch in the reagent inlet plate with the tab, and then put the plate in the plate carrier.
11. Make sure that the chamfered corner of the reagent outlet plate is in the top left, and then put the plate in the back of the plate carrier.
12. Touch **Load Reagent**.

Figure 5-2 Load Reagent Button

The plate compartment closes.

Load the Sample Inlet and Outlet Plates

1. Add the samples to the sample inlet plate according to the sample plate layout. Refer to the figure: Figure C-3.

   The recommended sample volume is 100 µL.

   The minimum sample volume is 50 µL. The maximum sample volume is 200 µL.
2. To prevent damage to the capillary, if there are columns where not every well has sample, then add between 100 µL and 200 µL of sample buffer to each empty well.

   If a column has no samples, then the wells can be left empty.
3. Add the reagents to the sample outlet plate according to the sample plate layout. Refer to the figure: Figure C-3.

   The maximum volume is 2.0 mL.

Use the volume in the following table.

Make sure that, for any column on the sample plate with sample, the corresponding column on the outlet plate has 2.0 mL of separation gel.

Note: For the outlet plate, make sure that the chamfered corner is on the upper right, and then fill only the wells on the left side of the plate. The wells on the right side are for overflow and must be empty.

Table 5-3 Reagents for the Sample Outlet Plate

Plate	Reagent
Outlet plate	• 2.0 mL of gel buffer per well

4. Put a film cover on the plates.

CAUTION: Potential System Damage. Do not use a heated plate sealer to apply the seal. The heat might damage the surface of the plates, which might cause issues with the pressure system.

Note: Only X-Pierce film from USA Scientific is validated. If a different film is used, then test it before use.

5. Put the plates in a swinging-bucket rotor, and then spin them for 4 min at 30 *g*. Make sure that the buckets are balanced.

CAUTION: Potential Wrong Result. Do not load the plates in the system without spinning them to remove air bubbles. The presence of air bubbles might cause the separation to fail.

6. Make sure that there are no air bubbles present in the plates. If air bubbles are present, then spin the plates again at a higher relative centrifugal force (RCF).

   For the reagent plate, the maximum RCF is 1,000 *g*. For the sample plate, the maximum RCF is 375 *g*.

7. On the front panel, touch **Eject Sample**.

Figure 5-3 Eject Sample Button

The plate compartment opens.

8. Remove the film cover from the plates.

CAUTION: Potential System Damage. Do not load plates in the system before removing the film cover. The presence of the film cover during a run might damage the capillary tips.

9. If the plate compartment already contains sample plates, then remove the sample plates.
10. Orient the sample plate so that the alignment notch in the plate aligns with the tab, and then put the plate in the plate carrier.
11. Orient the sample outlet plate so that the chamfered corner is in the upper left, and then put the plate in the back of the plate carrier.
12. Touch **Load Sample**.

Figure 5-4 Load Sample Button

The plate compartment closes.

Examine the Capillary Cartridge

WARNING! Puncture Hazard. Be careful when handling the cartridge. The tips of the capillaries are extremely sharp.

CAUTION: Potential System Damage. Do not let the gel buffer or other reagents crystallize on the electrodes, capillary ends, cartridge seals, or cartridge body. Electrolyte salt crystals or precipitate can cause plugged capillaries, improper pressure sealing, errors when injecting samples, arcing, or current leakage.

1. Examine the electrodes, capillary tips, cartridge seals, and cartridge body interface before use.
2. If there is gel or liquid on the outside of the cartridge, then clean the cartridge with a damp lint-free laboratory wipe. After cleaning, make sure to dry the cartridge.

Note: Do not use soap or detergent to clean the cartridge.

3. If the capillary tips are blocked, then do this:
   a. Use CE Grade Water to clean the capillary inlets.
   b. Use a lint-free laboratory wipe to wipe the capillary inlets carefully in an outward direction.

4. Use a magnifying glass to examine both sides of the capillary window. If lint or other particles are present, then use short bursts of electronics-grade compressed air to remove them. Do not use water or other liquids to clean the capillary window.

   CAUTION: Potential System Damage. Do not use organic solvents, such as methanol or acetone, to clean the capillary window. Organic solvents can dissolve the adhesives, leaving residue on the capillary window that might interfere with the detector.

5. Moisten a lint-free laboratory wipe or cotton swab with ethanol or isopropyl alcohol, and then wipe the surface of the chip. Let the chip air dry before installing the cartridge.

Install the Cartridge

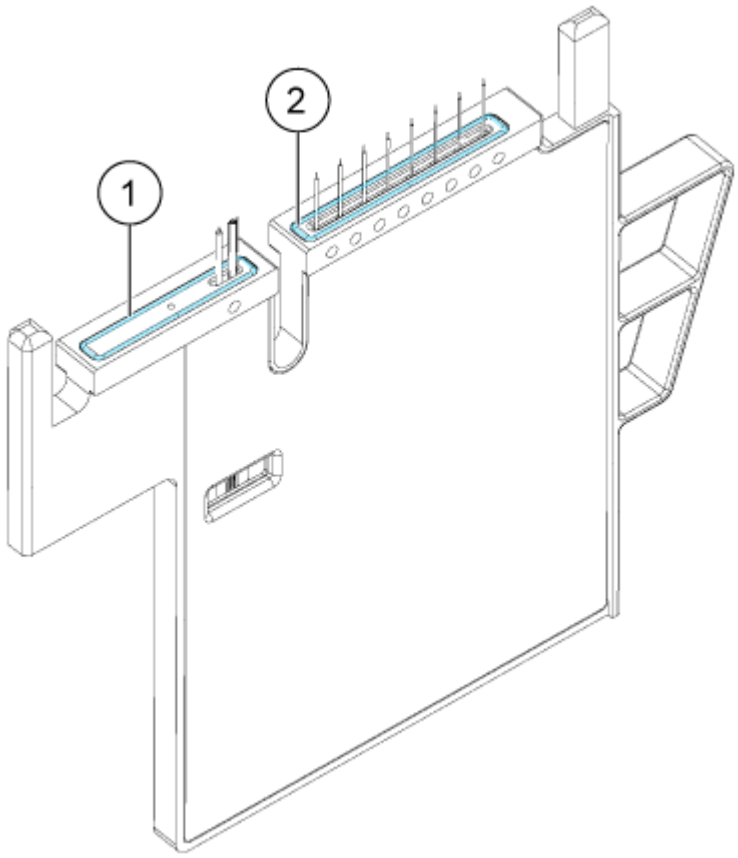
WARNING! Puncture Hazard. Be careful when handling the cartridge. The tips of the capillaries are extremely sharp.

WARNING! Pinching Hazard. When opening the front panel, be careful not to put fingers to the left of the front panel.

CAUTION: Potential System Damage. Make sure that the reagent plates are installed in the system before installing the cartridge. Failure to do so might damage the cartridge.

1. If the cartridge was stored in the refrigerator, then let the cartridge equilibrate to room temperature for approximately 30 min to prevent condensation in the system.
2. Remove the cartridge from the wetting tray.
3. Use a disposable laboratory wipe to dry the cartridge body to prevent arcing.
4. Turn the bottom of the cartridge up.
5. Use a disposable lint-free laboratory wipe to very gently dry the area where the capillaries and electrodes emerge from the cartridge. Do not disturb the seals.

Figure 5-5 Bottom of the Cartridge



Item	Description
1	Outlet plate seal
2	Inlet plate seal

6. If the reagent plates are not installed in the system, then install them. Refer to the section: Load the Reagent Inlet and Outlet Plates.
7. Open the front panel, and then put the cartridge in the system.
8. Close the front panel, and then touch **EJECTED** to lock the cartridge.

Figure 5-6 EJECTED Button

If the cartridge run life has been exceeded, then a warning message is added to the front panel log. To view the warning message, touch  on the front panel status area. The cartridge can still be used or a new one can be installed.

The system moves the reagent plate so that the capillaries are in position over column 1, and then raises the plate so that the capillary ends are immersed in CE Grade Water.

9. Examine the coolant level on the front panel. If required, add coolant into the fill port on the system.
   Refer to the section: "Add Capillary Cartridge Coolant" in the document: *Operator Guide*.

Run the Samples 6

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 6-1.

Table 6-1 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.

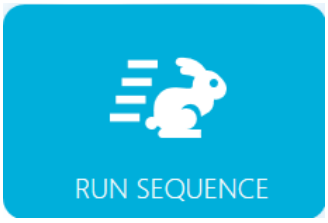
Start the Sequence from the Front Panel

To use the Waters Empower™ software, refer to the section: Start the Sample Set Method.

1. If required, load the cartridge, reagent plates, and sample plates.

2. On the front panel, touch **RUN SEQUENCE**.

Figure 6-1 RUN SEQUENCE Button



3. In the Projects pane, touch **RNA 9000**.
4. In the Available Sequences pane, touch **RNA 9000 Test Sequence - Electrokinetic Sample Injection** in the list.
5. (Optional) To view the details of the method, sample plates, or reagent plates, touch anywhere in the **Method** column.
   To hide the details, touch the column or box again.
6. Touch **Run Sequence**.

Figure 6-2 Run Sequence Button

Run Sequence is not enabled if the sequence contains a method that is incompatible with the system configuration.

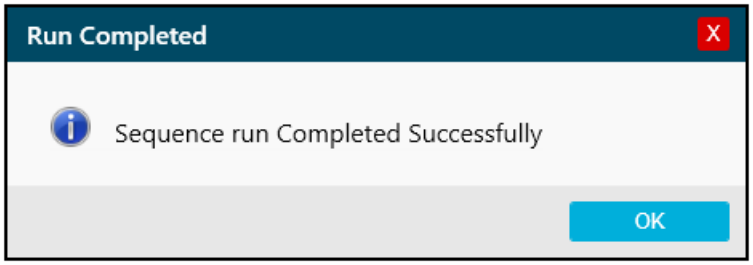
The data files are saved at the location specified in the sequence.

If an error occurs during a run and an error recovery method is present in the sequence, then the BioPhase 8800 system starts the error recovery method.

During the run, various actions are available. Refer to the section: Monitor the Run on the BioPhase 8800 Front Panel.

When the run is complete, the Run Completed dialog opens.

Figure 6-3 Run Completed Dialog



7. Touch **OK** to close the Run Completed dialog.
8. As required, store the cartridge. Refer to the section: Store the Cartridge After the Run.

Monitor the Run on the BioPhase 8800 Front Panel

Use this procedure to monitor the sequence progress, and then, if required, pause or stop the sequence.

To use the Waters Empower™ software, refer to the section: Monitor the Run in the Waters Empower™ Software.

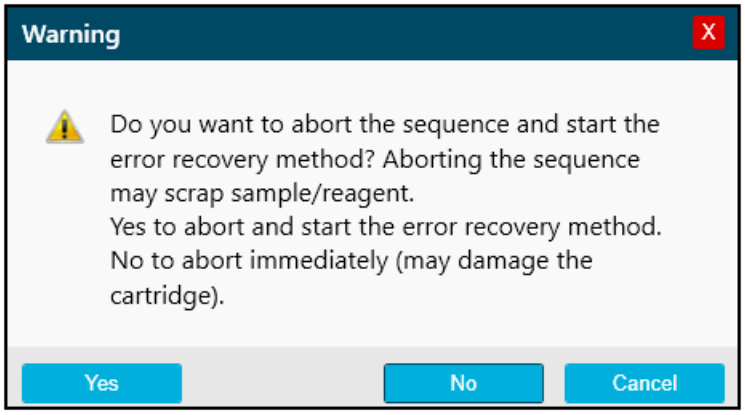
Note: The sequence shown in the following figures is for the purpose of illustration. It does not show a sequence for the RNA 9000 Purity & Integrity Kit.

1. Monitor the traces for the detector and the current to make sure that the sequence is running.
2. If a problem is detected, then touch  to stop the run, and then, in the Warning dialog, touch one of the following:
   - **Yes**: Touch to start the error recovery method, if one is assigned.
   - **No**: Touch if an error recovery method is not assigned.

     Note: Stopping the run might result in loss of sample or reagent and damage to the cartridge.

   - To continue the run, touch **Cancel**.

Figure 6-4 Warning Dialog



CAUTION: Potential System Damage. If the run is stopped and will not be resumed, then use the shutdown method to rinse the capillaries before storing the cartridge. If the capillaries are not rinsed, electrolyte salt crystals or precipitate can accumulate and might cause plugged capillaries, improper pressure sealing, errors when injecting samples, arcing, or current leakage.

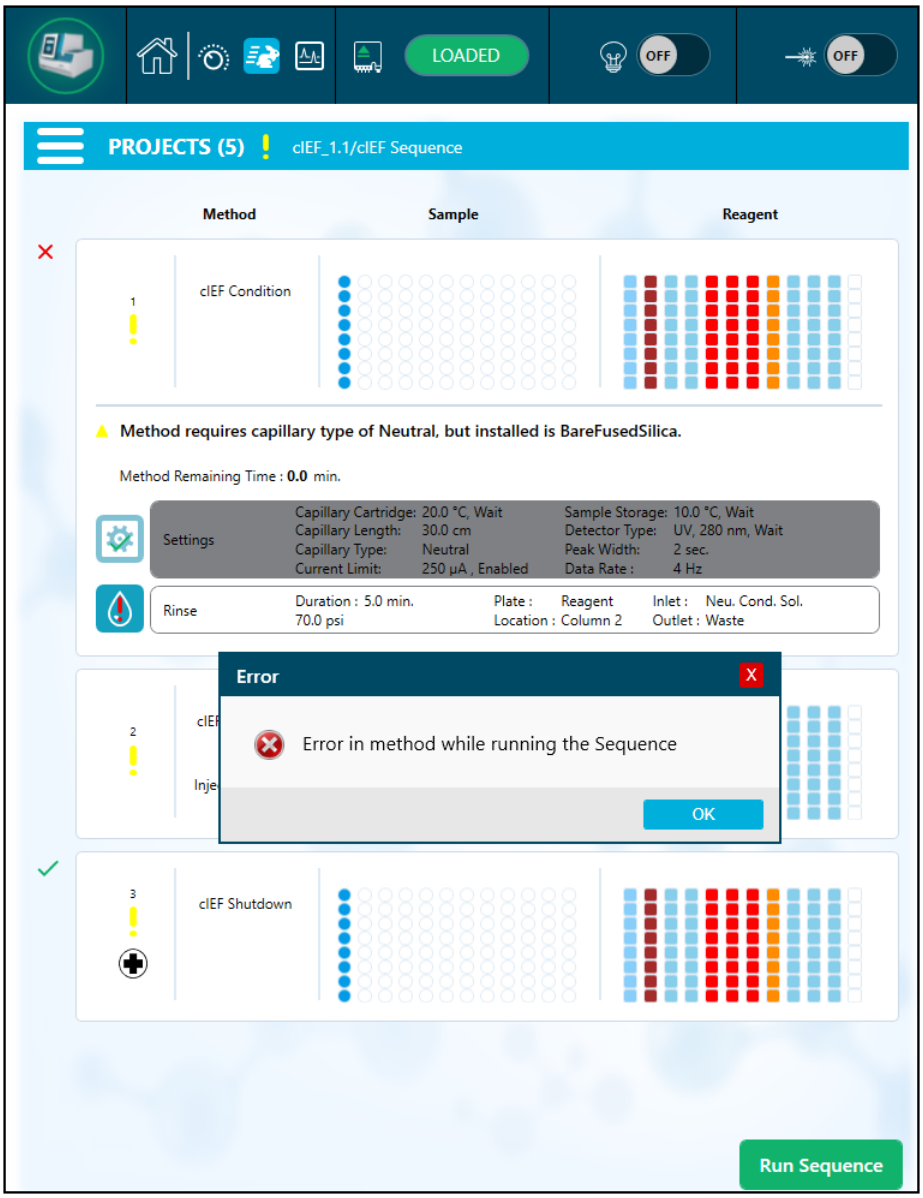
CAUTION: Potential System Damage. Before starting the run again, make sure to empty or replace the outlet plate to prevent overflow of reagent and possible damage to the instrument.

CAUTION: Potential Wrong Result. Before starting the run again, prepare new reagent plates. If the run has been stopped, then there might not be enough reagents available to complete the run.

CAUTION: Potential Wrong Result. If the samples have been inside of the system for more than 24 hours, then before starting the run again, discard them. The samples might have degraded.

3. If an error occurs, then touch **OK** in the error dialog that is shown.

Figure 6-5 Run Sequence Error



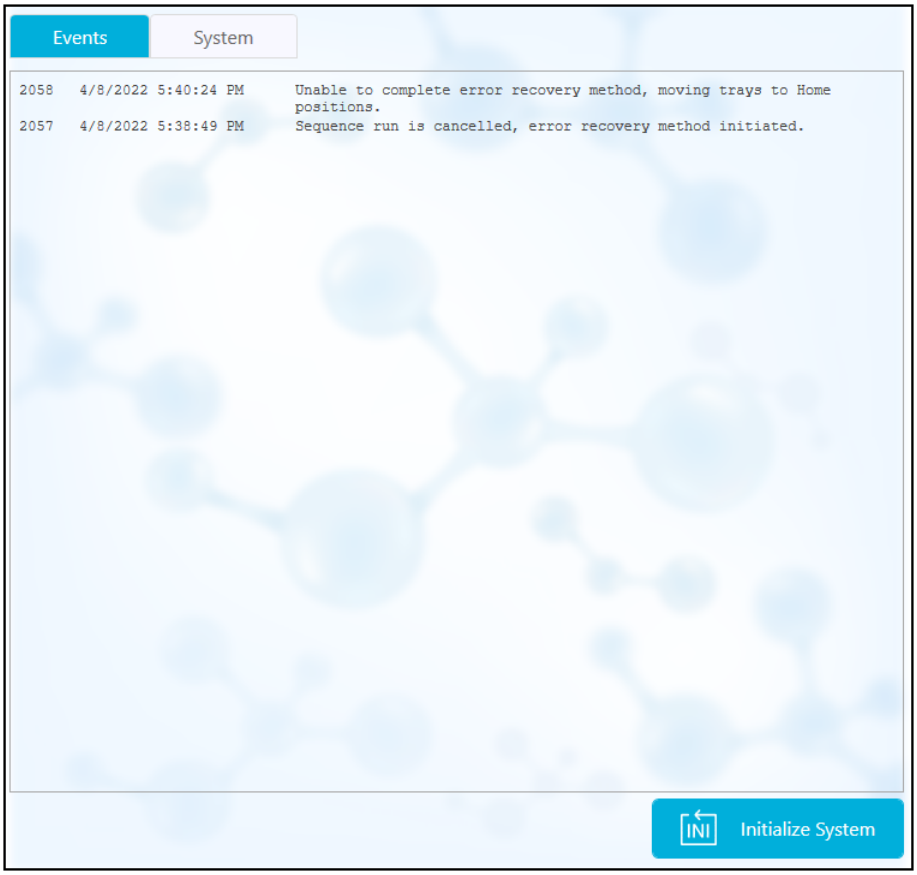
Note: The  shows an error at the **Rinse** action. The grey shading in the row above the **Rinse** action indicates that the action is in progress or completed.

4. Review the error:

   a. Touch  in the **Events** tab of the front panel log.

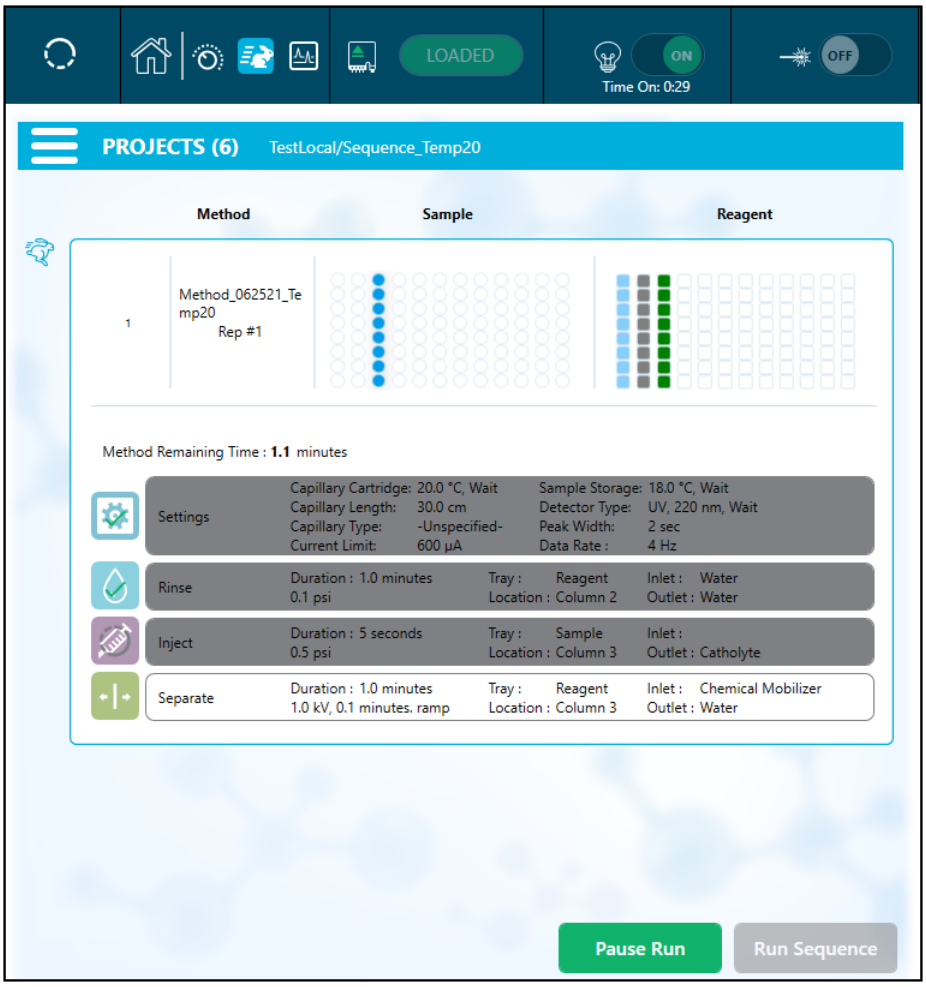
b. Touch **Initialize System** to reinitialize the system, and then change the system status to idle.

Figure 6-6 Sequence Error Events Log



5. If required, pause the run by touching **Pause Run**.

Figure 6-7 Run Sequence in Progress



6. To continue the run, touch **Cancel Pause**.

Figure 6-8 Restart the Run Sequence

7. To view the data while it is being acquired, touch  in the ribbon.

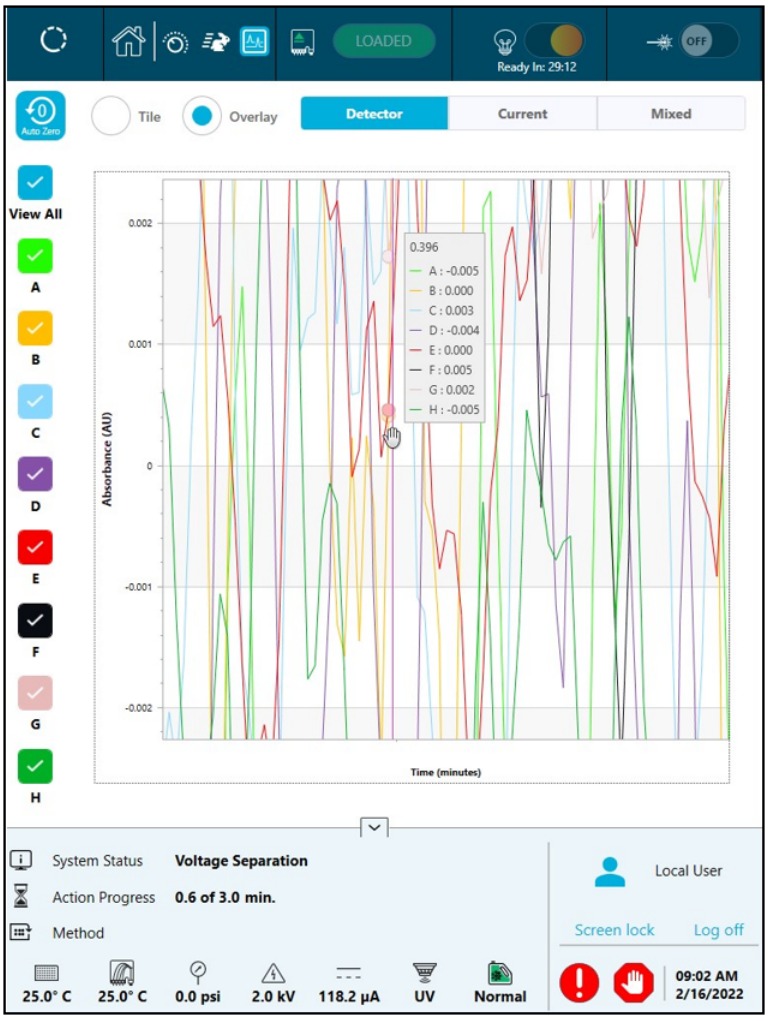
Note: The data in the following figure is for the purposes of illustration. It does not show results for samples prepared with the RNA 9000 Purity & Integrity Kit.

Figure 6-9 Capillary View

8. (Optional) To zoom in on the data, do the following:
   a. Touch **Overlay**.
   b. Use two fingers to zoom in or out to view the electropherogram.
   c. Use the hand icon to move the electropherogram.

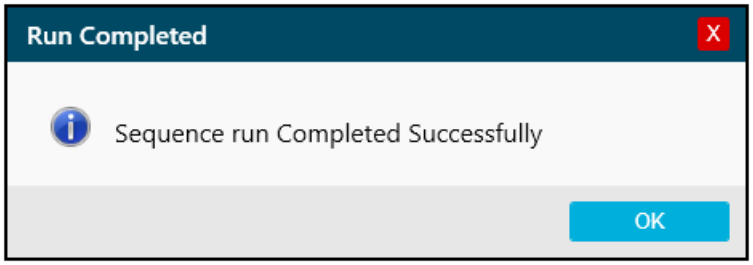
Note: The zoom feature only works with the overlay view for detector and current.

Figure 6-10 Zoom In or Out



9. Make sure that the message, `Sequence run Completed Successfully`, is shown when the run completes. In the dialog, touch **OK**.

Figure 6-11 Run Completed



Waste Disposal

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

Store the Cartridge After the Run

WARNING! Puncture Hazard. Be careful when handling the cartridge. The tips of the capillaries are extremely sharp.

Store the Cartridge for Less than 24 Hours

1. If the sequence or sample set method does not include a shutdown method, then use the shutdown method to clean the capillary.
2. Store the cartridge for up to three days in the system with the capillary ends immersed in CE Grade Water.

Note: If the cartridge has not been used for three hours or longer, then run the conditioning method before doing a separation.

Store the Cartridge for More than 24 Hours

1. If the sequence or sample set method does not include a shutdown method, then use the shutdown method to clean the capillary.
2. On the ribbon on the BioPhase 8800 system front panel, touch 

(**Loaded**) and then wait for about one minute.

   Waiting lets the coolant return to the coolant reservoir before the cartridge is removed.
3. Remove the cartridge from the system, and then store it upright in the cartridge box at 2 °C to 8 °C with the capillary ends immersed in CE Grade Water.

Note: Replace the CE Grade Water in the tray regularly to avoid microbial growth in the tray.

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 use the Conditioning Method-RNA 9000 method to condition the capillary.

Note: To prevent arcing, and before installing the cartridge in the system, carefully wipe off any water from around the electrodes and cartridge body.

Analyze the Data 7

Analyze the Data with an Analysis Parameters File

Note: If the Waters Empower™ software will be used to acquire the data, then this procedure does not apply.

The following instructions explain how to analyze data with the BioPhase Analysis software with an analysis parameters file. An analysis parameters file contains all of the information required to integrate the peaks and identify peaks in the data.

Note: This procedure assumes familiarity with the BioPhase software. For detailed instructions, refer to the document: *Software Help System*.

Note: The analysis parameters files in this procedure are examples. The parameters might not be optimal for all data files.

1. On the Home page of the BioPhase software, click **Data Analysis**.
   The BioPhase Analysis software main window opens.
2. Click **File** > **Open**, select the data files to analyze, and then click **Open**.
3. In the **Project** toolbar, click  , navigate to the analysis parameters files, and then click **Open**.
   Two analysis parameters files are available. Click the file that is appropriate:
   - **data analysis with 50b as IST**: Analyze the RNA ladder sample.
   - **data analysis with 50b point to point**: Estimate the size of the unknown RNA sample.
4. Right-click  , and then select **Apply & Analyze (all)**.

   The software applies all of the parameters in the Integration, Library, and Post Analysis tabs to all of the data files, and then shows the results.

   In the Files pane, the file name is shown in red to indicate that the data has been analyzed. The number of peaks identified is shown in the **Peaks** column.

   In the Data pane, the analysis results are shown in the table below the graph. At the top of the table, **RMS Noise**, **P-P Noise**, and **Drift** are shown. In the graph, the baseline is shown in red and the threshold is shown as a grey horizontal line. Any peaks identified in

the analysis have a blue marker at the peak start, a red marker at the peal apex, and a green marker at the peak end.

Peaks in the graph are shaded as follows:

- Green: The peak corresponds to a peak in the Marker Table on the Library tab.
- Blue: The peak corresponds to a peak in the Peak Table on the Library tab.
- Red: The peak is not a named peak.

Refer to the section: Examine the Results.

5. Show the peak names on the graph.

   a. Right-click  .
   The Information Setup dialog opens.

   b. Select **Name** and any other information to view on the graph, such as **MT**, and then click **OK**.

   c. Click  .

   The peak names are shown on the graph. Refer to the section: Examine the Results.

   The names are part of the analysis parameters file. To use different names, refer to the section "Identify the Peaks" in the document: *Software Help System*.

6. Click in the Files pane below the list of files, and then press the **Up** and **Down** arrow keys to view the data for each file in the Data pane.
   If required, drag over a region on the graph to zoom in to see the details of the identified peaks in that region.

Tip! To prevent having to zoom in on each data file, click  to apply the same zoom settings to all of the data files.

---

7. Make sure that the integration is satisfactory. Adjust the integration parameters, and then analyze the data again as required.
8. Examine the electropherogram for the peaks in the Marker Table and the Peak Table.

   a. For each peak in the Marker Table and the Peak Table, make sure that the correct peak is labeled in the graph.

   b. As required, adjust **MT** in the Marker Table and **MT** (or **Cal MT**) in the Peak Table.

   c. As required, adjust **Tol** and **Crit**, and then click  .

      - **Tol** is the tolerance for matching a peak in the graph to a peak in the Marker Table or the Peak Table. Type % to use the tolerance as a percentage.

- **Crit** is the peak characteristic to be matched.
  - **Ctr**: The peak closest to the center of the range is matched.
  - **Ht**: The tallest peak in the range is matched.
  - **Area**: The largest peak in the range is matched.

d. When the peak assignments are satisfactory, right-click  , and then select **Apply & Analyze (all)**.

The software applies the changes to all of the data files.

9. (Optional) In the **Project** toolbar, click  , type a name, select a location, and then click **OK**.
   The analysis parameters are saved to a file for later use.

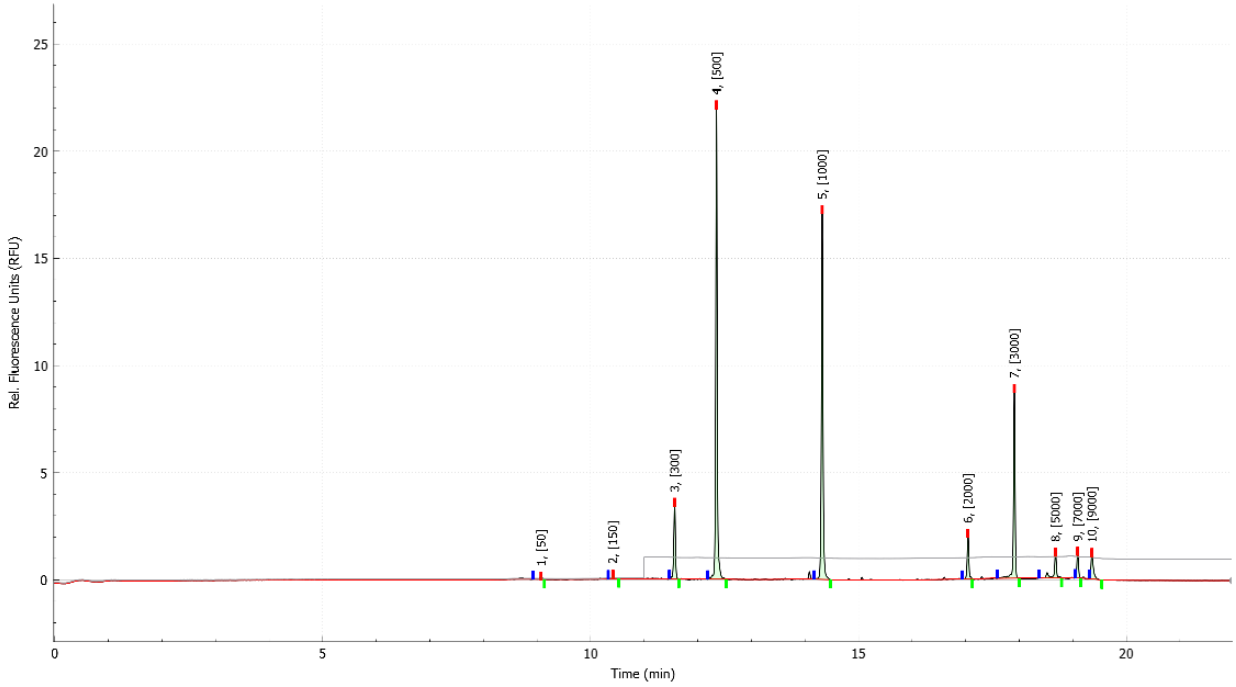
10. (Optional) In the **File** toolbar, right-click  , and then select **Print (all)**.
    The contents of the Data pane are printed in the current report template. For instructions to create a report template, refer to the section "Configure a Report" in the document: *Operator Guide*.

11. In the **File** toolbar, right-click  , and then select **Save (all)**.
    All changes to the results, including the analysis parameters, are saved to the data files.

12. In the **File** toolbar, right-click  , and then select **Close (all)**.
    All of the data files close.

Examine the Results

Figure 7-1 ssRNA Ladder



Review the Results on the Overlay Tab

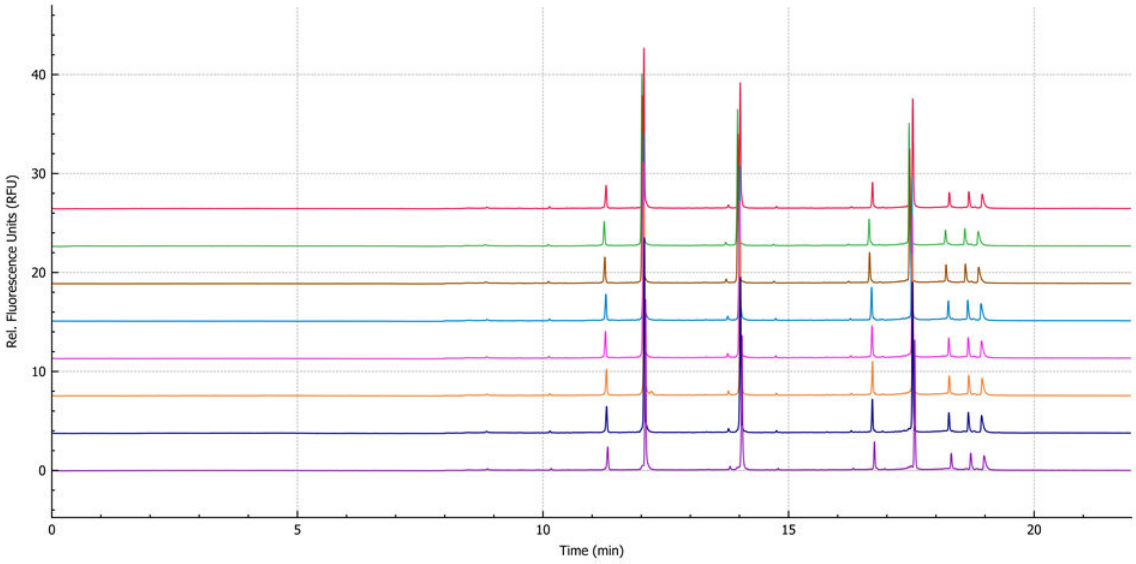
The Overlay tab shows the graphs for the selected data files. This tab contains the statistics for the selected data files, as well as the system suitability report.

Note: This section does not describe the system suitability function. For information about system suitability, refer to the document: *Operator Guide*.

1. Open a set of data files and the appropriate analysis parameters file, and then analyze the data. If required, adjust the analysis parameters until the results are satisfactory.

2. In the Files pane, click  , and then open the Overlay tab.

Figure 7-2 Overlay Tab



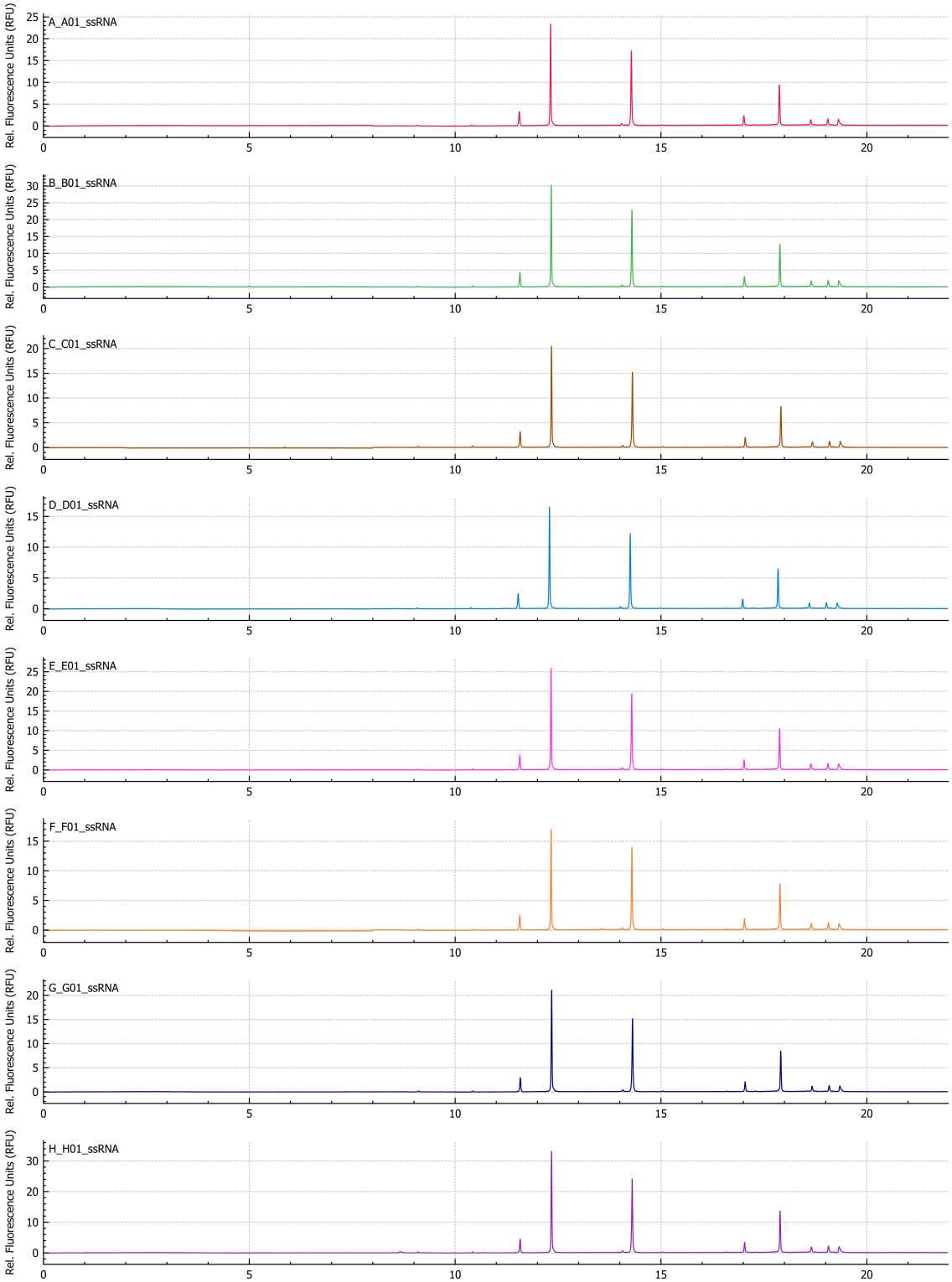
The color of the trace in the graph corresponds to the color in the circle next to the file name in the Files pane.

The thicker line is the trace that corresponds to the file selected in the Files pane.

3. Move the slider on the right side of the graph up or down to adjust the traces.

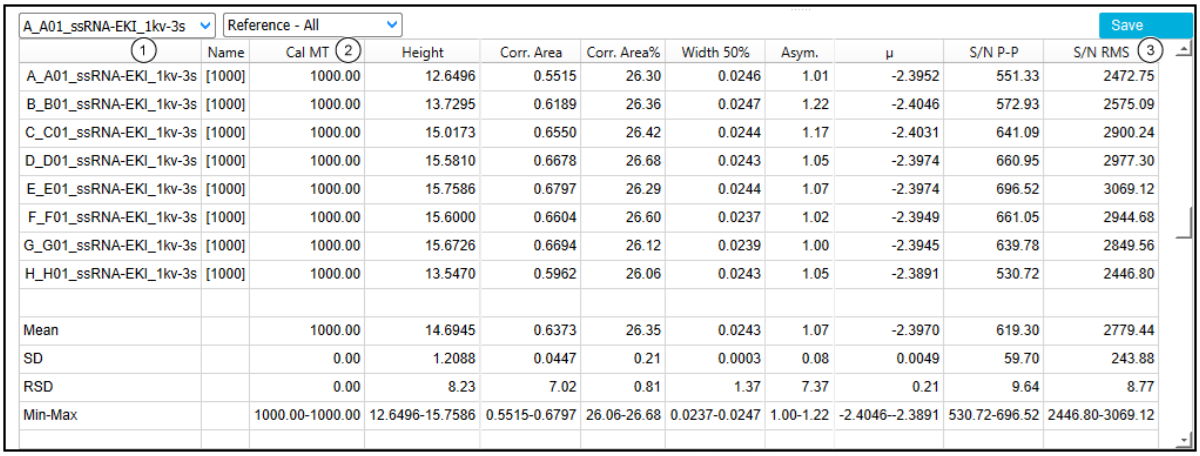
Note: To view the traces as a series of tiled graphs, move the slider all of the way to the top.

Figure 7-3 Tiled Results



4. Calculate the results for all of the files on the Overlay tab.

Figure 7-4 Results Table



Item	Description
1	Reference file
2	Type of analysis
3	Save the results to a comma-separated file

a. Click the list on the right side of the Results Table header to select the type of analysis.

These options are available:

- **Reference - All**: In the Results Table, show statistics for every peak in the reference file that is present in all of the other data files.
- **Reference - Peak Table**: In the Results Table, show statistics for every named peak in the reference file that is present in all of the other data files.
- **Named Peaks**: In the Results Table, show statistics for all of the named peaks in any of the data files.
- **All Data (not displayed)**: Calculate but do not show statistics for all of the peaks in all of the data files.
- **System Suitability**: If system suitability was enabled when the data was analyzed, then show the system suitability report.

A peak in a data file is considered to be matched to a peak in the reference file if the migration times of the peak apexes match within 5%.

b. Click the list on the left, and then select the reference file.

The reference file is the file against which all of the other files are compared.

Only the **Reference - All** and **Reference - Peak Table** analyses use a reference file.

The Results Table updates to show the selected analysis or the system suitability report.

If **All Data (not displayed)** is selected, then the Results Table is empty. To view the results, click **Save** to save the results to a comma-separated file, and then open the file in another program.

5. (Optional) Repeat step 4 to use a different reference file or view a different type of analysis.
6. (Optional) Click **Save**.
   The Results Table is saved to a comma-separated text file. Only the columns that are shown in the table are saved.

   Note: To save the system suitability results, click **File** > **Save Report**. The results are saved as a PDF.
7. (Optional) Click **File** > **Print**.
   The contents of the Overlay tab are printed in the current report template.
8. (Optional) In the **File** toolbar, right-click  , and then select **Save (all)**.
   All changes to the results and the analysis parameters are saved to the data files.
9. In the **File** toolbar, right-click  , and then select **Close (all)**.
   All of the data files close.

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.

Run the Samples with the Waters Empower™ Software 8

This section gives instructions for the use of the Waters Empower™ software and the BioPhase 8800 driver for Empower™ with the BioPhase 8800 system.

Import the BioPhase Software Methods to Create the Instrument Methods

Note: Methods created with the BioPhase software are supplied with the BioPhase 8800 driver for Empower™. The methods are also available for download from the SCIEX web site. Refer to the section: Download and Configure the Required Files (Waters Empower™ Software).

Instrument methods can also be created in the Method Editors for BioPhase System software. Refer to the documents: *Operator Guide* and *Software Help System*.

Typically, three types of methods are required: a conditioning method, a separation method, and a shutdown method. For some workflows, there are additional methods.

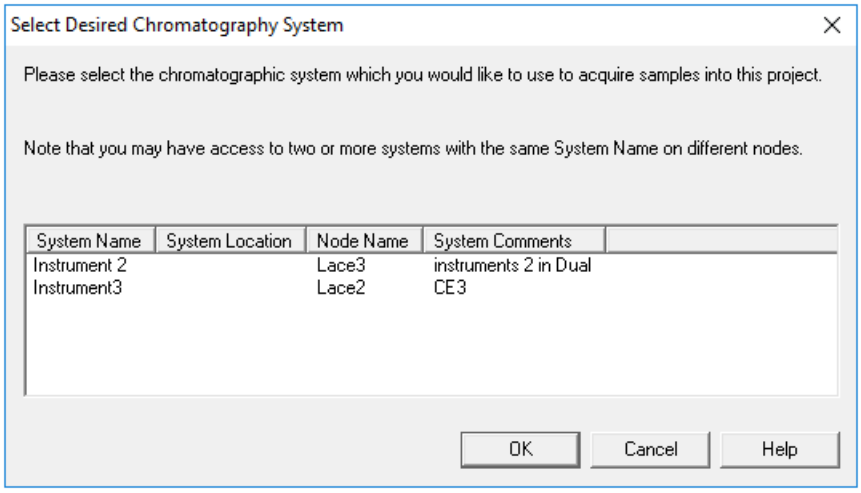
The following methods are available.

- Conditioning Method-RNA 9000: Conditions the capillaries before first use.
- Separation Method - Electrokinetic Injection - RNA 9000: Does the separation of the sample with an electrokinetic injection of the sample.
- Shutdown Method - RNA 9000: Cleans the capillaries at the end of a sequence, rinses the capillaries for storage, and then turns off the lamp.
- Capillary Rinse - RNA 9000: Rinses the capillary between sequences, if required.

Use the following steps to import the BioPhase software methods and create instrument methods and method sets to be used with the Waters Empower™ software.

1. In the Waters Empower™ Software Project window, click **File** > **New Method** > **Instrument Method**.

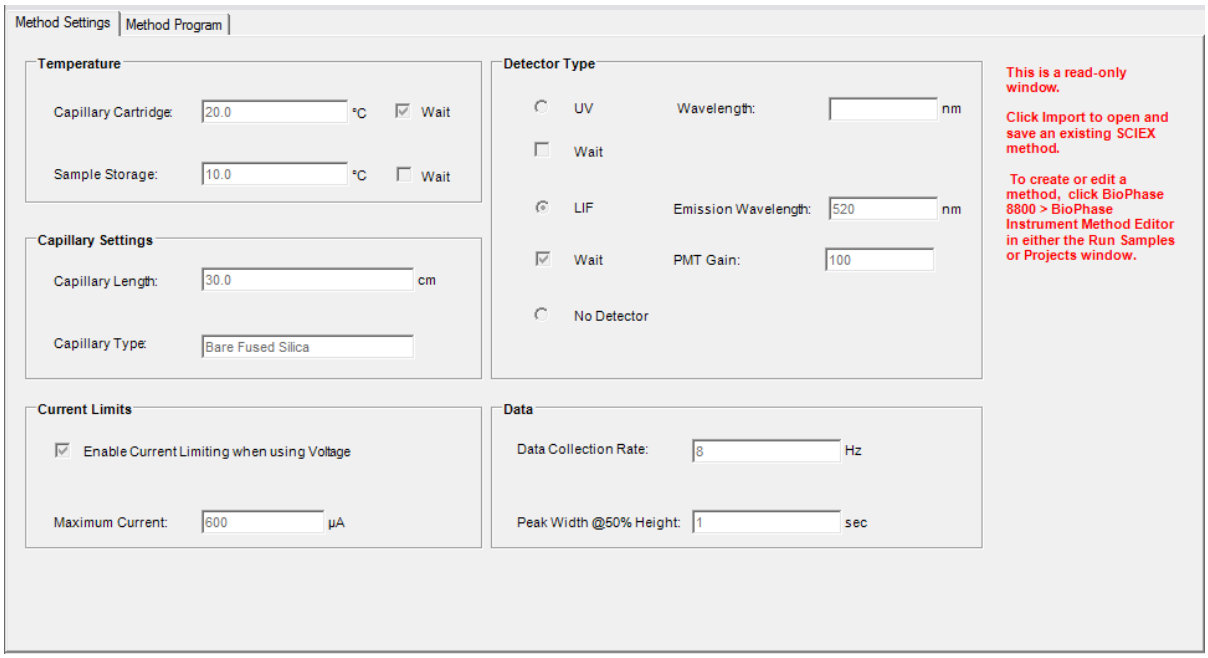
Figure 8-1 Select Desired Chromatography System Dialog



2. Click the system to be used, and then click **OK**.
   Make sure that the instrument is configured with an LIF detector.
   The Instrument Method Editor opens.
3. Click **Import**, and then browse to the conditioning method.
   The method opens in the Instrument Method Editor window with the Method Settings tab in front.

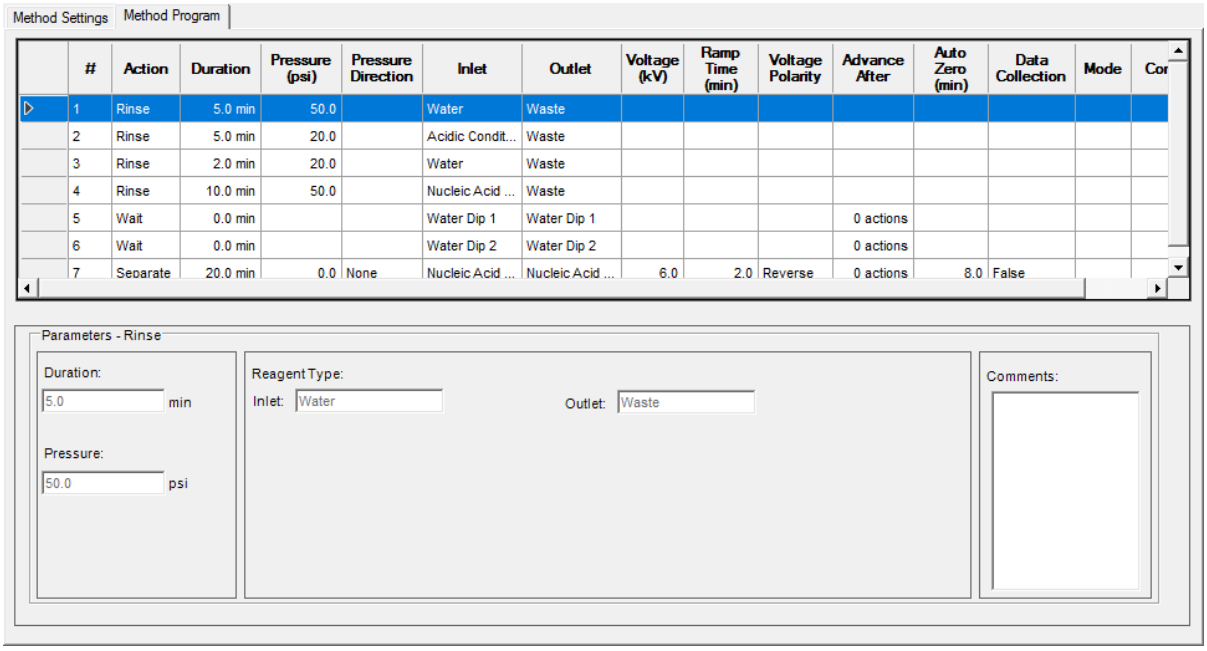
Note: This window is read-only. If changes to the method are required, then save the instrument method, and then edit the method in the Method Editors for BioPhase System software. Refer to the section: "Edit an Existing Instrument Method" in the document: *Software Help*.

Figure 8-2 Method Settings Tab in the Instrument Method Editor



4. (Optional) Open the Method Program tab to see the actions.
5. To see the parameters for an action, click the row in the table.
   The Parameters pane updates to show the parameters.

Figure 8-3 Method Program Tab in the Instrument Method Editor



6. Save the conditioning instrument method.

   a. Click **File** > **Save with Method Set**.
   The Save current Instrument Method dialog opens.

   b. In the **Name** field, type a name.

   Note: The name must be less than 30 characters and can contain alphanumeric characters, spaces, and the special characters @, _, and %. Although some versions of the Waters Empower™ software accept more than 30 characters and other special characters, if the method is edited in the Method Editors for BioPhase System software, then those characters might cause issues.

   c. (Optional) In the **Method Comments** field, type the information.

   d. If prompted, in the **Password** field, type the Waters Empower™ software password for the current user, and then click **Save**.

   The instrument method and method set are saved to the current project.

7. Click **File** > **Exit**.

   Note: After a method has been imported, the **Import** button in the Instrument Method Editor window is not available unless the window is closed and then opened.

8. Repeat steps 3 through 7 to create the other instrument methods and method sets.

Create the Sample Set Method

The following instructions create a sample set method for eight samples, the number of wells in one column in a sample plate.

Note: A sample set method requires method sets. Make sure that any required instrument method is part of a method set.

This procedure gives instructions for creating a sample set method with the ssRNA Ladder in the first column, followed by 16 samples in the second and third columns.

1. In the Waters Empower™ Software Run Samples window, click **BioPhase 8800** > **BioPhase Sample Set Editor**.

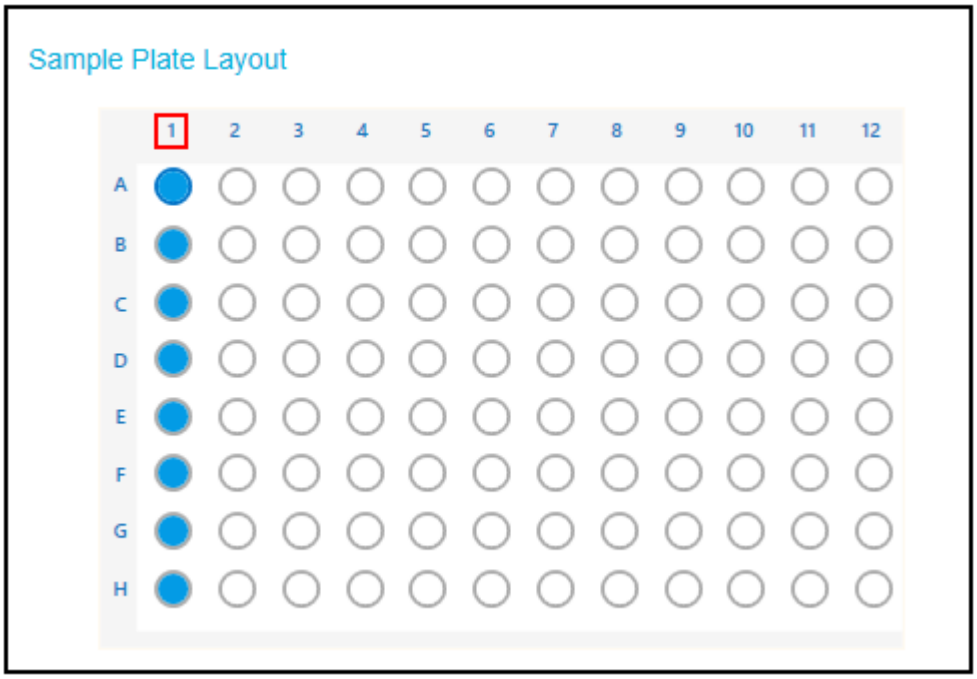
   The Method Editors for BioPhase System software opens, with the Sample Set Method Editor workspace shown.

2. Click **New Sample Set Method**.
   The Sample Set Method Editor opens with the Sample Plate Setup tab shown.

3. In first row of the Sample Set Summary table, click the **Method Set Name** cell, and then select **Conditioning Method-RNA 9000**.

4. In the Sample Plate Layout pane, click **1**.
   The first column in the sample plate is selected and the Sample Set Summary table updates to show the selected wells.

Figure 8-4 Sample Plate Layout Pane



5. Add the required sample information to the Sample Set Summary table. In rows 2 through 9, do the following:
   a. In the **Sample Name** cell, type a name for the sample.
   b. Click the **Method Set Name** cell, and then select **Separation Method - Electrokinetic Injection - RNA 9000** from the list.

   Tip! After selecting the method set for row 2, right-click and select **Apply method to all samples in column** to assign the method to all of the samples.

6. Repeat, steps 4 and 5 twice, for column **2** and then for column **3**.
7. In the last row, click the **Method Set Name** cell, and then select **Shutdown Method - RNA 9000**.

Note: The following figure does not show the entire sample set method.

Figure 8-5 Sample Set Summary Table

Sample Set Summary

Column	# of Injs	Plate/Well	Sample Na...	Method Set Name	Run Time (Minutes)
				Conditioning Method RNA 9000	42.0
1	1	1:A,1	Smith	Separation Method RNA 9000	31.0
1	1	1:B,1	Jones	Separation Method RNA 9000	31.0
1	1	1:C,1	Wang	Separation Method RNA 9000	31.0
1	1	1:D,1	Lee	Separation Method RNA 9000	31.0
1	1	1:E,1	Chavez	Separation Method RNA 9000	31.0
1	1	1:F,1	Robles	Separation Method RNA 9000	31.0
1	1	1:G,1	Jensen	Separation Method RNA 9000	31.0
1	1	1:H,1	Andersen	Separation Method RNA 9000	31.0
2	1	1:A,2	Smith	Separation Method RNA 9000	31.0
2	1	1:B,2	Jones	Separation Method RNA 9000	31.0
2	1	1:C,2	Wang	Separation Method RNA 9000	31.0
2	1	1:D,2	Lee	Separation Method RNA 9000	31.0
2	1	1:E,2	Chavez	Separation Method RNA 9000	31.0
2	1	1:F,2	Robles	Separation Method RNA 9000	31.0
2	1	1:G,2	Jensen	Separation Method RNA 9000	31.0
2	1	1:H,2	Andersen	Separation Method RNA 9000	31.0
3	1	1:A,3	Smith	Separation Method RNA 9000	31.0
3	1	1:B,3	Jones	Separation Method RNA 9000	31.0

8. If the Validation pane is shown, then click the pane to see the errors. Click an error to highlight the location where it occurs, and then make the required change.

   If no errors are present, then the Validation pane is not shown.

9. Save the sample set method.

   a. Click **SAVE AS**.

   Note: The **SAVE AS** button is not available if there are errors. Resolve all of the errors in the Validation pane, and then click **SAVE AS**.

   The Save Sample Set dialog opens.

   b. Type a name in the **Sample Set Name** field.

Note: The name must be less than 30 characters and can contain alphanumeric characters, spaces, and the special characters @, _, and %. Although some versions of the Waters Empower™ software accept more than 30 characters and other special characters, if the method is edited in the Method Editors for BioPhase System software, then those characters might cause issues.

c. (Optional) Type information in the **Description** field.

d. Click **Save**, and then click **OK** to acknowledge the saved method.

The sample set method is saved to the Waters Empower™ software database.

10. To see, save, or print the plate layouts:

    a. Open the Plate Layouts tab.

    b. (Optional) Click **PRINT**.
    The Print Preview window opens.

    c. As required, click the buttons to print or save the plate layouts.

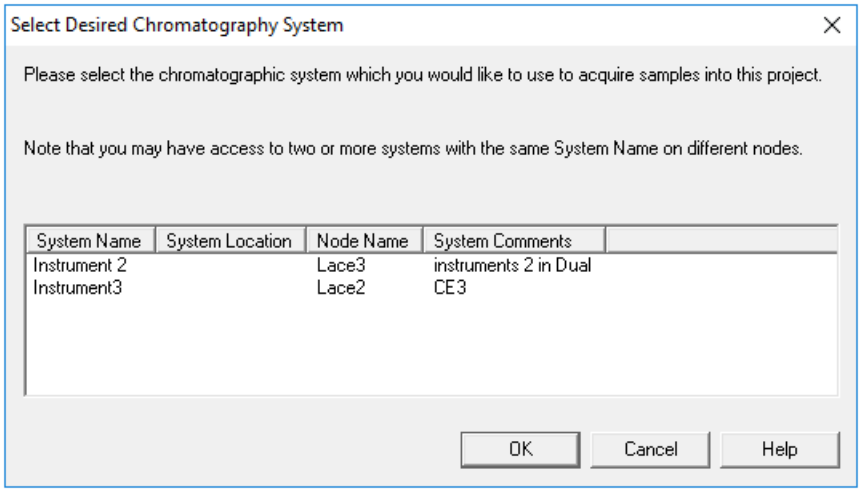
    d. Click the close box, the × in the top right corner.
    The Print Preview dialog closes.

11. In the Method Editors for BioPhase System window, click the close box, the × in the top right corner.
    The Method Editors for BioPhase System software closes and the Run Samples window is shown.

Start the Sample Set Method

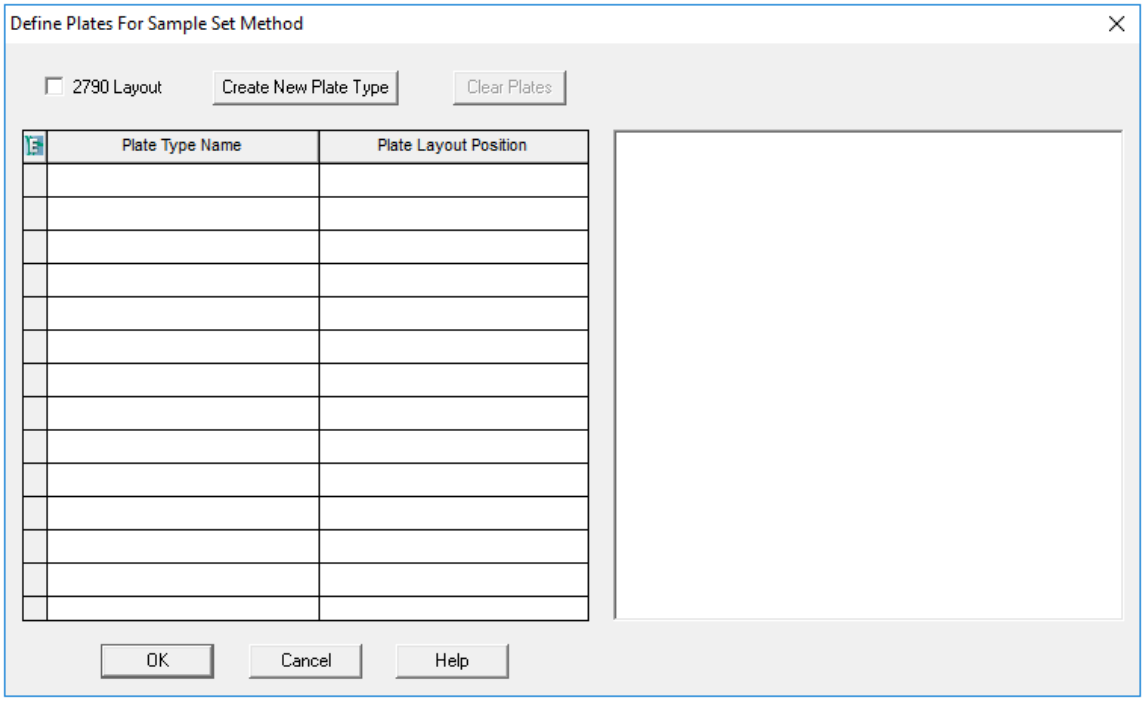
1. Load the cartridge and the plates. Refer to the section: Prepare the BioPhase 8800 System.
2. In the Waters Empower™ software Project window, click **Tools** > **Run Samples**.

Figure 8-6 Select Desired Chromatography System Dialog



3. Click the system to be used, and then click **OK**.
   The Run Samples window opens.
4. Configure the plate type.
   a. Click **Edit** > **Plates**.

Figure 8-7 Define Plates for Sample Set Method Dialog



Note: If the dialog does not look like the preceding figure, then clear the **2790 Layout** check box.

b. Click the **Plate Type Name** cell, and then select **ANSI-96well2mL**.
The dialog updates with an image of the plate and buttons for the plate sequencing mode.

c. Click the **Plate Layout Position** cell, and then type `1`.

d. Click  to indicate the order that the wells are accessed during the run.

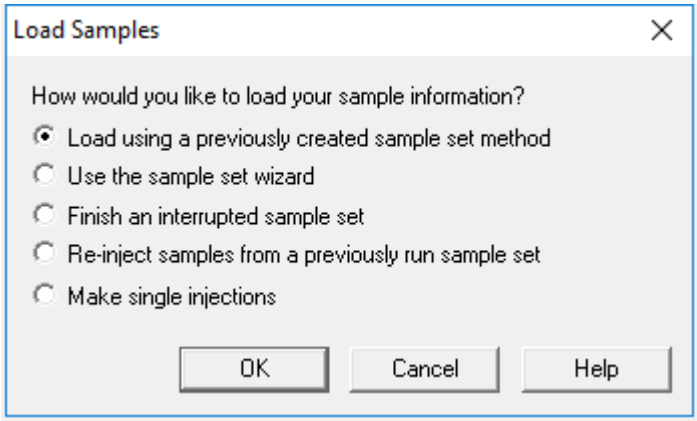
e. Click **OK** to save the changes, and then close the dialog.

Tip! To permanently configure the plate type, click **Customize** > **Defaults**, click **Plates**, do steps 4.b through 4.e, and then click **OK**.

In the Sample Set Method table, the heading for the **Vials** column changes to **Plate/Well**.

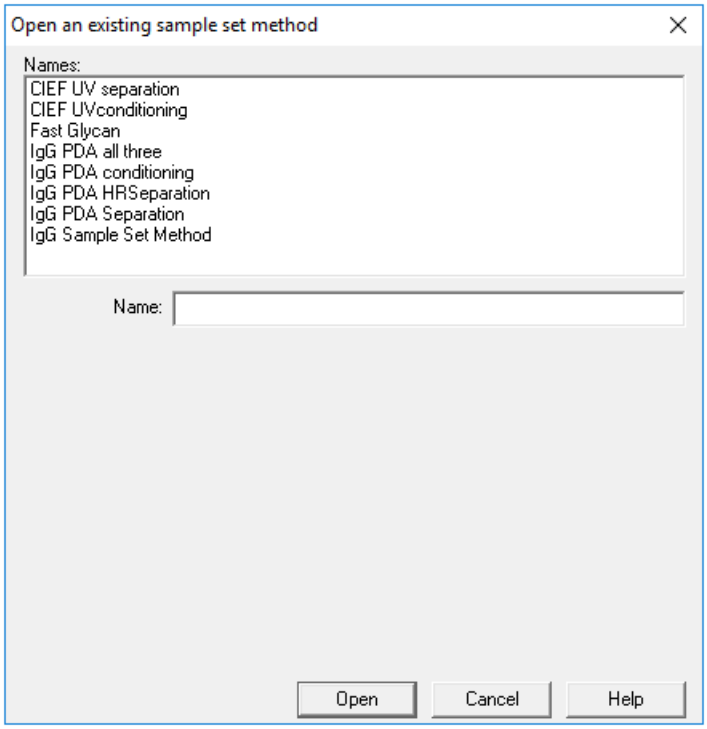
5. Click  (**Load Sample Set**).

Figure 8-8 Load Samples Dialog



6. Click **Load using a previously created sample set method**, and then click **OK**.

Figure 8-9 Open an existing sample set method Dialog



7. Click **RNA 9000 Kit Sample Set Method** in the list, and then click **Open**.
   The sample set method opens in the Samples tab.
8. (Optional) Configure the table to show only the columns that are relevant for the BioPhase 8800 system.
   a. Right-click, and then select **Table Properties**.
      The Table Properties dialog opens.
   b. Click **Hide All**, and then clear the check boxes for **Plate/Well**, **# of Injs**, **SampleName**, **Function**, and **Method Set / Report or Export Method**.
   c. Click **OK**.

   The table updates to show the selected columns.

Figure 8-10 Samples Tab

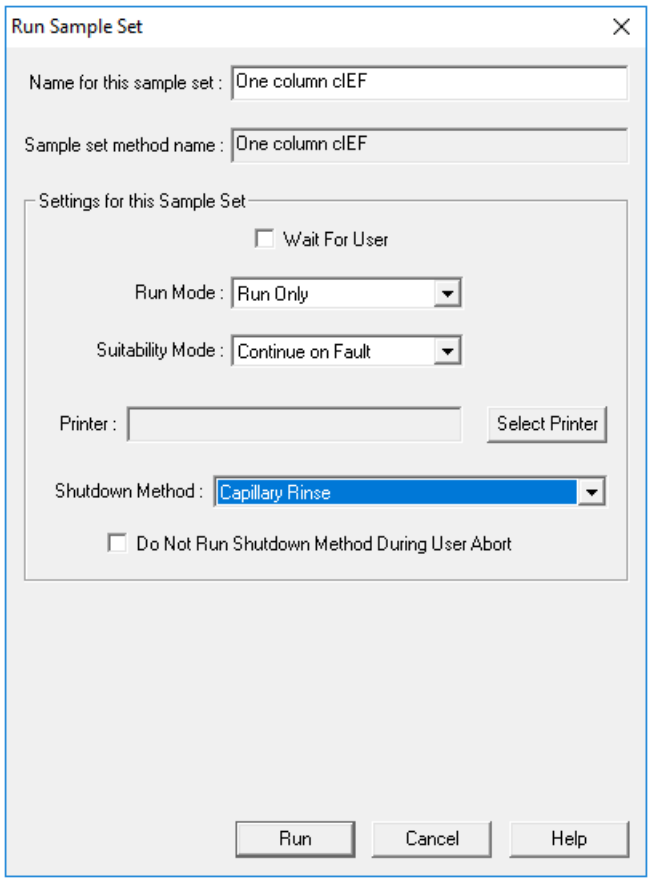
Sample Set Method: RNA 9000 Kit Sample Set Method

	Plate/Well	# of Injs	SampleName	Function	Method Set / Report or Export Method
1				Condition Column	Conditioning Method RNA 9000
2	1:A,1	1	Smith	Inject Samples	Separation Method RNA 9000
3	1:B,1	1	Jones	Inject Samples	Separation Method RNA 9000
4	1:C,1	1	Wang	Inject Samples	Separation Method RNA 9000
5	1:D,1	1	Lee	Inject Samples	Separation Method RNA 9000
6	1:E,1	1	Chavez	Inject Samples	Separation Method RNA 9000
7	1:F,1	1	Robles	Inject Samples	Separation Method RNA 9000
8	1:G,1	1	Jensen	Inject Samples	Separation Method RNA 9000
9	1:H,1	1	Andersen	Inject Samples	Separation Method RNA 9000
10	1:A,2	1	Smith	Inject Samples	Separation Method RNA 9000
11	1:B,2	1	Jones	Inject Samples	Separation Method RNA 9000
12	1:C,2	1	Wang	Inject Samples	Separation Method RNA 9000
13	1:D,2	1	Lee	Inject Samples	Separation Method RNA 9000
14	1:E,2	1	Chavez	Inject Samples	Separation Method RNA 9000
15	1:F,2	1	Robles	Inject Samples	Separation Method RNA 9000
16	1:G,2	1	Jensen	Inject Samples	Separation Method RNA 9000
17	1:H,2	1	Andersen	Inject Samples	Separation Method RNA 9000
18	1:A,3	1	Smith	Inject Samples	Separation Method RNA 9000

9. Review the sample set method. Make sure that the correct reagent plate layout was used. If any changes are required, then edit the method in the Method Editors for BioPhase System software. Any changes to the instrument methods or method sets automatically propagate to the sample set method.

10. In the Waters Empower™ Software Project window, click  (**Start**).

Figure 8-11 Run Sample Set Dialog



11. If required, edit the information in the Run Sample Set dialog.
    a. If required, edit the **Name for this sample set** field.
    b. (Optional) Click **Shutdown Method**, and then select **Capillary Rinse - RNA 9000**, an instrument method that rinses the capillaries.
       If the system encounters an error during a run, then it executes this instrument method, and then stops the run.
    c. If required, select **Do Not Run Shutdown Method During User Abort**.
    d. Click **Run**.

    The run starts. During the run, the text in the row in the Sample Set Method window for the sample being acquired is red.

CAUTION: Potential Data Loss. Do not initiate any actions from the BioPhase 8800 driver for Empower™ Direct Control pane during a run, even if the system status is idle. Any actions might interfere with data acquisition.

Monitor the Run in the Waters Empower™ Software

Use this procedure to monitor the progress of the sample set method, and then, if required, pause or stop it.

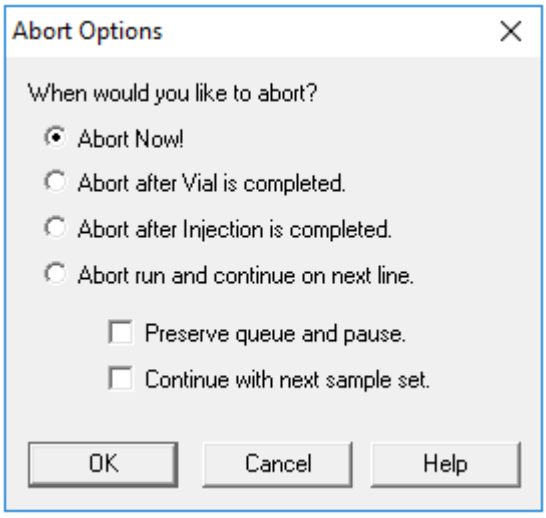
Note: Most of the panes in the Waters Empower™ software are designed for chromatography. Use the following steps to monitor the progress of the capillary electrophoresis separation and disregard information in the Time Remaining and Solvent Required panes.

1. If a problem is detected, to stop the run, click  (**Abort**).

   CAUTION: Potential Data Loss. Do not stop the run until all of the data is saved. The data is saved when the sample set method is on the next row.

   Note: Do not use the **Stop** button in the Direct Control pane. That button only operates on functions initiated from the Direct Control pane.

   Figure 8-12 Abort Options Dialog



   CAUTION: Potential System Damage. If the run is stopped and will not be resumed, then use the shutdown method to rinse the capillaries before storing the cartridge. If the capillaries are not rinsed, electrolyte salt crystals or precipitate can accumulate and might cause plugged capillaries, improper pressure sealing, errors when injecting samples, arcing, or current leakage.

CAUTION: Potential System Damage. Before starting the run again, make sure to empty or replace the outlet plate to prevent overflow of reagent and possible damage to the instrument.

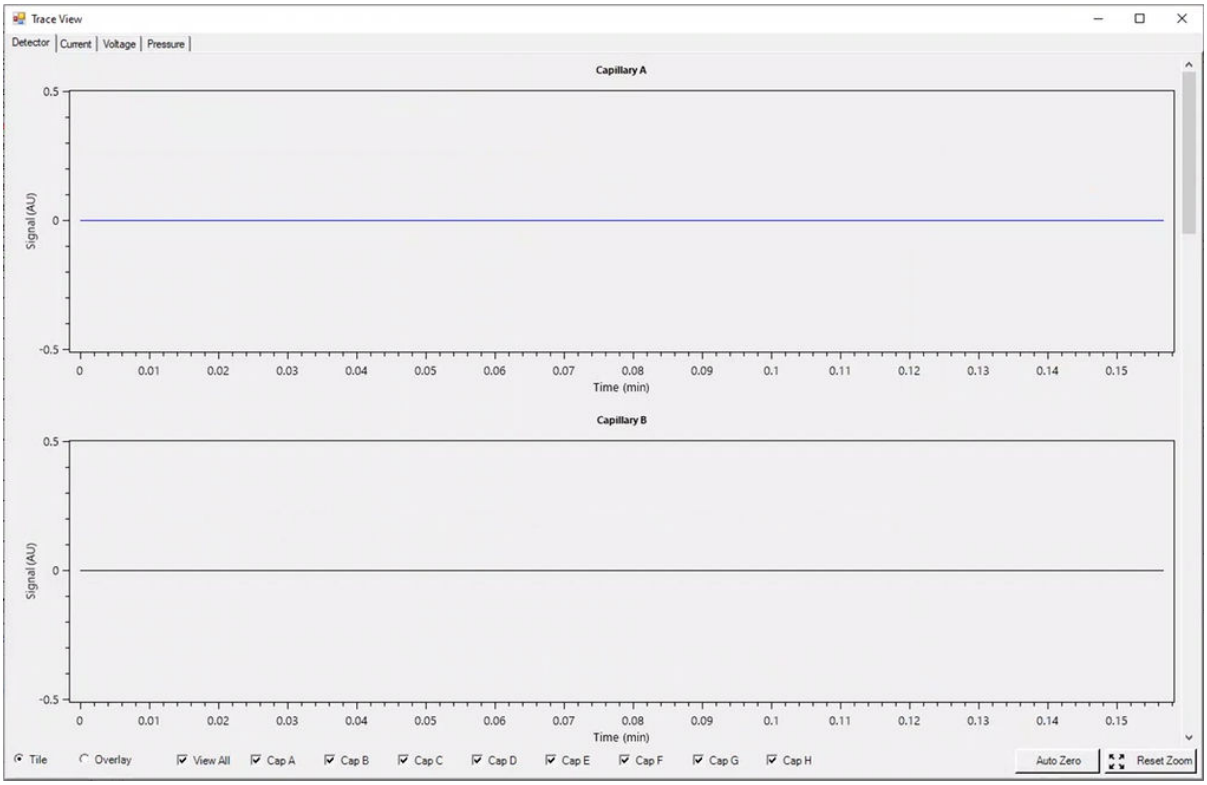
CAUTION: Potential Wrong Result. Before starting the run again, prepare new reagent plates. If the run has been stopped, then there might not be enough reagents available to complete the run.

CAUTION: Potential Wrong Result. If the samples have been inside of the system for more than 24 hours, then before starting the run again, discard them. The samples might have degraded.

When the run ends, the text in all of the rows in the Sample Set Method window is red.

2. To view the data while it is acquired, in the Direct Control pane, click  (**Monitor**). The Trace View window opens, and then the data is shown.

Figure 8-13 Trace View Window



3. If required, do any of the following:

- To view current, voltage, or pressure, open the applicable tab in the top left.
- To view one graph with the data for all of the capillaries, in the bottom left click **Overlay**.
- To view data for specific capillaries, select or clear the check boxes at the bottom of the window to select the capillaries of interest.
- To view the time and detector values for any point on a trace, click the trace at the position of interest.
- To zoom in on the data, make sure that **Overlay** is selected, and then drag to select the area to zoom. The mouse scroll wheel can also be used to zoom.
- To return the data to the original dimensions, in the bottom right click **Reset Zoom**.
- To view a different area of a zoomed plot, right-click the X- or Y-axis and then drag.

4. If required, at the bottom right click **Auto Zero**.
   The detector signal is set to zero.
5. Wait until the **Abort** button () changes from red to green ().
   There might be a delay between data acquisition and when all of the data is saved. The green button indicates when all of the data is saved.
6. As required, dispose of samples and reagents. Refer to the section: Waste Disposal.
7. As required, store the cartridge. Refer to the section: Store the Cartridge After the Run.

Troubleshooting 9

After each corrective action is complete, we recommend doing the analysis again to make sure that the symptom has been corrected.

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
Cartridge not detected error	1. The ID chip on the cartridge is not clean. 2. The contact pins on the system are not clean. 3. The BioPhase 8800 system firmware is not up to date.	1. Moisten a lint-free laboratory wipe or cotton swab with ethanol or isopropanol, and then wipe the surface of the ID chip. Let the ID chip air dry before installing the cartridge. 2. Moisten a lint-free laboratory wipe or cotton swab with ethanol or isopropanol, and then wipe the contact pins. Let the pins air dry before installing the cartridge. 3. Do the following: a. On the BioPhase 8800 system front panel, touch the icon in the upper left corner. b. Record the firmware version. c. Contact sciex.com/request-support.

Symptom	Possible Cause	Corrective Action
Error encountered at beginning of run	1. The optical scan at the beginning of the run failed because of condensation on the cartridge window. 2. Opening and closing the optics door caused a sensor error.	1. In the shutdown method, increase the **Sample Storage** temperature to 20 °C to prevent condensation. 2. Turn off, and then turn on the BioPhase 8800 system. Make sure to follow the procedure to change UV filters and do not open the optics door except as instructed. Refer to the section: "Install a UV Filter" in the document: *Operator Guide*.

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.	1. To assess the condition of the capillary end: • Use a magnifying lens to examine it. • Use a lint-free laboratory wipe to wipe the capillary inlets carefully in an outward direction. • Make sure that the capillary end extends approximately 2 mm from the gold cannula electrode. • Make sure that the capillary end has a straight cut. If the capillary cannot be used, then refer to the section: Options for a Blocked or Damaged Capillary. 2. Do one or all of the following: • Decrease the **Duration** in the **Inject** action 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. 3. Refer to the section: Options for a Blocked or Damaged Capillary. 4. Either edit the sequence to omit the contaminated capillary or replace the cartridge.

Symptom	Possible Cause	Corrective Action
Broad peaks, poor resolution (continued)	1. The lifetime of the cartridge has been exceeded. 2. The SYBR™ Green II RNA Gel Stain concentration in the gel buffer is too high. 3. The Nucleic Acid Extended Range Gel was left at room temperature too long. 4. The capillary cartridge was left at room temperature for more than a week.	1. Do a test separation of the ssRNA Ladder. If the peak widths are consistently wider than previous runs then replace the cartridge. 2. 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 Reagent Inlet and Outlet Plates 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. 4. Replace the cartridge.

Symptom	Possible Cause	Corrective Action
Carryover	1. The sample concentration is too high. 2. The reagent plate is contaminated with sample.	1. Do one or all of the following: • Decrease the **Duration** in the **Inject** action 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. In the separation method, add one or more water dip steps after sample injection. With the edited method: a. Create a new sequence that uses this separation method. b. Prepare new reagent plates for the new sequence. Refer to the section: Load the Reagent Inlet and Outlet Plates.
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 plate 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 with clean plasticware and a clean sample plate. 3. Filter the gel buffer with a syringe filter before adding it to the reagent plates.

Symptom	Possible Cause	Corrective Action
High current	1. The gel buffer is contaminated. 2. The positions of the reagents in the reagent plate do not agree with the plate layouts in the sequence. 3. The Nucleic Acid Extended Range Gel was left at room temperature too long.	1. Prepare the inlet and outlet reagent plates again to replace the gel buffer. 2. Make sure that the positions of the reagents in the reagent plates agree with the plate layouts. If the positions are not correct, then prepare the plates again according to the plate layout. Refer to the section: Plate Layouts. 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 volume is too low. 3. The salt concentration in the sample is too high. 4. The initial nucleic acid concentration is too low. 5. The nucleic acids in the sample have degraded due to the presence of RNAse or other nucleases.	1. Condition the capillary. Refer to the section: Condition the Capillaries. Do a blank separation run to equilibrate the capillary surface. 2. Make sure that there is a minimum of 50 µL of sample in the sample well. 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 again at a lower ionic strength. 4. Do one or all of the following: • Increase the **Duration** up to 15 s in the **Inject** action 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. 5. Prepare the sample again, making sure to limit exposure to RNase. Refer to the section: Best Practices for Working with RNA.

Symptom	Possible Cause	Corrective Action
Low current	1. The capillary is blocked. 2. The position of the gel buffer in the reagent plate does not agree with the sequence.	1. Refer to the section: Remove Blockage from a Capillary. 2. Make sure that the position of the gel buffer during the voltage separation step is correct. If the positions are not correct, then prepare the plates again according to the plate layout. Refer to the section: Load the Reagent Inlet and Outlet Plates.
Low or unsteady current	1. The capillary end is blocked or contaminated. 2. The gel buffer has air bubbles.	1. Refer to the section: Remove Blockage from a Capillary. If the current is low or unsteady, then replace the cartridge. 2. Do one or all of the following: • Use a centrifuge to spin the plate for 5 min at 30 *g* to remove air bubbles. • De-gas the gel buffer for 5 min with 5 inches Hg to 15 inches Hg vacuum.

Symptom	Possible Cause	Corrective Action
No electrical current during separation	1. The capillary is damaged. 2. The electrode is broken or bent. 3. The capillary end is blocked or contaminated. 4. The positions of the reagents in the reagent plate do not agree with the plate layouts in the sequence. 5. A capillary is filled with air bubbles.	1. Refer to the section: Options for a Blocked or Damaged Capillary. 2. Replace the cartridge. 3. Refer to the section: Remove Blockage from a Capillary. 4. Make sure that the positions of the samples and reagents in the plates agree with the plate layouts. If the positions are not correct, then prepare the plates again according to the plate layout. Refer to the section: Plate Layouts. 5. Do one or all of the following: • Make sure that the wells of the sample and reagent plates contain sufficient solution. • Make sure that the positions of the reagents in the reagent plates agree with the plate layouts. If the positions are not correct, then prepare the plates again according to the plate layout. Refer to the section: Plate Layouts. • Use a centrifuge to spin the plate for 5 min at 30 *g* to remove air bubbles.

Symptom	Possible Cause	Corrective Action
No peaks	1. The lifetime of the LIF detector laser has been exceeded. 2. The wavelength of the emission filter is not correct. 3. The method parameters are incorrect. 4. Air bubbles in a well of the sample plate prevent sample injection. 5. The capillary window is blocked. 6. A capillary is blocked and has a low current. 7. The sample volume is too low. 8. The capillary was cleaned with a basic solution such as 0.1 N NaOH.	1. Contact SCIEX Technical Support at sciex.com/request-support. 2. Make sure that the emission filter wavelength is 520 nm. 3. Do the following: • Open the separation method in the software and then make sure that the method is correct. Refer to the section: Separation Method with Electrokinetic Injection. • Make sure that the positions of the samples and reagents in the plates agree with the plate layouts. If the positions are not correct, then prepare the plates again according to the plate layout. Refer to the section: Plate Layouts. 4. Use a centrifuge to spin the plate for 5 min at 30 *g* to remove air bubbles. 5. Examine the capillary window. Make sure that the window is clean and the path is clear. Refer to the section: Examine the Capillary Cartridge. 6. Refer to the section: Options for a Blocked or Damaged Capillary. 7. Make sure that there is 100 µL of sample in the sample well. 8. Replace the cartridge.

Symptom	Possible Cause	Corrective Action
No peaks (continued)	1. There is no sample in a sample well, or the position of the samples in the sample plate do not agree with the plate layouts in the sequence. 2. A pipetting error occurred during preparation of the sample. 3. The salt concentration in the sample is too high.	1. Make sure that the position of the samples in the sample plate agrees with the plate layout. If the positions are not correct, then prepare the plates again according to the plate layout. Refer to the section: Plate Layouts. 2. Prepare a new sample. 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 again at a lower ionic strength.
Slower migration time, with or without concurrent low current	1. The capillary end is blocked or contaminated.	1. Refer to the section: Remove Blockage from a Capillary.
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 Capillaries. Do a blank separation run to equilibrate the capillary surface. 2. Prepare new reagent plates with fresh gel buffer. Decrease the interval between loading the new reagent plates and running a sample to minimize the on-instrument time for the reagent plates.

Symptom	Possible Cause	Corrective Action
Spikes in electropherogram	1. The gel buffer has air bubbles.	1. Do one or all of the following: • Use a centrifuge to spin the plate for 5 min at 30 *g* to remove air bubbles. • De-gas the gel buffer for 5 min with 5 inches Hg to 15 inches Hg vacuum. 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.
Broad or split peaks	1. After the denaturation step, there is still secondary structure in the nucleic acid. 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 cartridge 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 Reagent Inlet and Outlet Plates. 4. Do a test separation of the ssRNA Ladder. If the peak widths are consistently wider than previous runs then replace the cartridge.

Symptom	Possible Cause	Corrective Action
Saturated peaks	1. In the **Method Settings** for the separation method, the **PMT Gain** is too high. 2. The sample concentration is too high.	1. Decrease the value for the **PMT Gain**. 2. Do one or all of the following: • Decrease the **Duration** in the **Inject** action 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.
Unstable baseline	1. The concentration of dye in the inlet reagent plate is not the same as the concentration in the outlet reaget plate.	1. Make enough gel buffer for both the inlet and outlet reagent plates.

Remove Blockage from a Capillary

1. Rinse the capillary with CE Grade Water for 10 min at 75 psi.
2. Use CE Grade Water to clean the capillary inlets.
3. Use a lint-free laboratory wipe to wipe the capillary inlets carefully in an outward direction.
4. If the blockage cannot be removed, then either edit the sequence to omit the damaged capillary or replace the cartridge.

Options for a Blocked or Damaged Capillary

If blockage in a capillary cannot be removed or a capillary is damaged, then edit the sequence to omit the blocked or damaged capillary. Refer to the section: Create the Sequence.

Condition the Capillaries

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

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

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 Mixture
- 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.

Download Required Files B

Depending on the software in use, the steps are different.

- BioPhase software users, refer to the section: Download and Configure the Required Files (BioPhase Software).
- Waters Empower™ software users, refer to the section: Download and Configure the Required Files (Waters Empower™ Software).

Download and Configure the Required Files (BioPhase Software)

Files with methods, sequences, reagents, and analysis parameters for the RNA 9000 Purity & Integrity Kit are available on sciex.com. Use the following instructions to download the files, and then copy them to the appropriate location.

Note: The following procedure is only required when BioPhase software version 1.1 is being used. The required files for the RNA 9000 Purity & Integrity Kit are included as part of the BioPhase software version 1.2 or later.

1. Go to sciex.com/software-support/software-downloads, and then click **BioPhase Resources** in the More software downloads section.
2. Click `BioPhase Project Files 1.2`.
3. In File Explorer, right-click the `BioPhase 1.2.zip` file, and then click **Extract All** to extract the installation package.
4. Browse to a location, click **Select Folder**, and then click **Extract**.
   The extracted files are copied to the selected file path.
5. Put the extracted files in the correct locations. Do the following:

   Note: The following instructions assume that the BioPhase software project folders are located in the default location: `C:\Biophase`. If the project folders are in a different location, then put the extracted files in that location.

   a. Drag the `BioPhase 1.2\Projects\RNA 9000` folder to `C:\BioPhase\Projects`.
   b. Drag the `BioPhase 1.2\Reagents\RNA 9000` folder to `C:\BioPhase\Reagents`.
   c. Drag the `BioPhase 1.2\Data Analysis\RNA 9000` folder to `C:\BioPhase\Data Analysis`.

Download and Configure the Required Files (Waters Empower™ Software)

Required files for the RNA 9000 Purity & Integrity Kit are available on sciex.com. Use the following instructions to download the files, and then copy them to the appropriate location.

1. Go to sciex.com/software-support/software-downloads, and then, in the More software downloads section, click **BioPhase Driver Resources**.
2. Click **BioPhase Method Files 1.3**.
3. In File Explorer, right-click the `BioPhase-Empower-Method-Files-1.3.zip` file, and then click **Extract All**.
4. Browse to the location to save the method files, click **Select Folder**, and then click **Extract**. The extracted files are extracted and copied to the specified location.

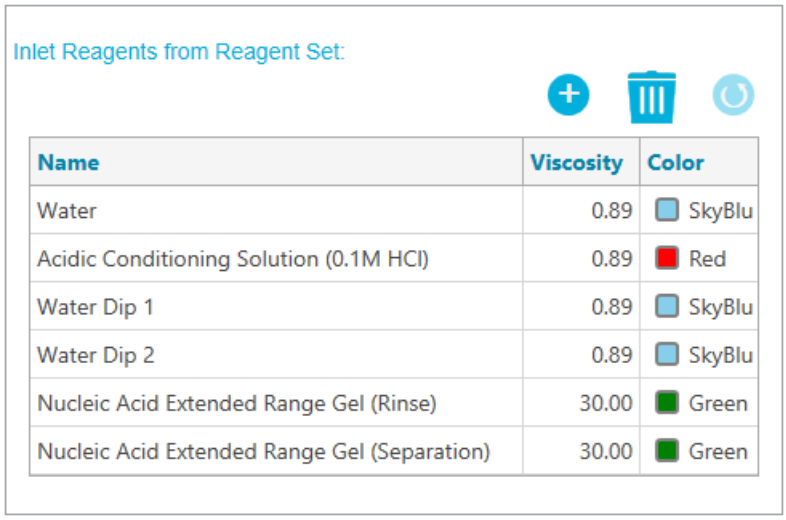
Reagents, Plate Layouts, and Methods

C

Reagent Set

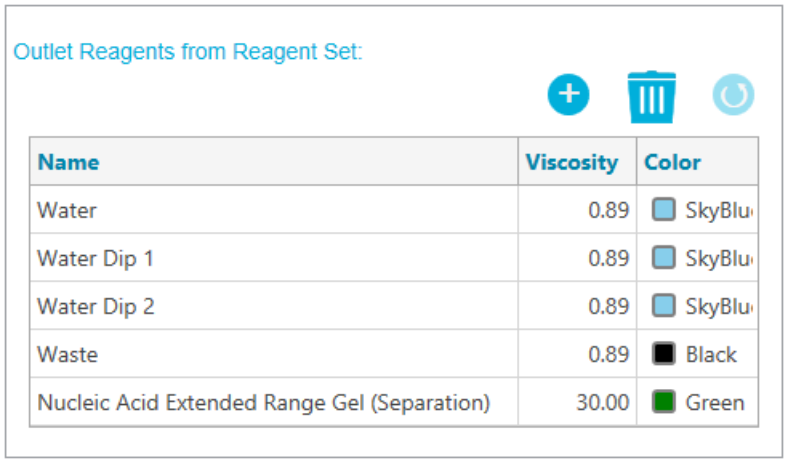
If the reagents are not available, then use the following figures to create a new reagent set. Refer to the figures: Figure C-1 and Figure C-2.

Figure C-1 RNA 9000 Purity & Integrity Kit Inlet Reagents



Name	Viscosity	Color
Water	0.89	SkyBlu
Acidic Conditioning Solution (0.1M HCl)	0.89	Red
Water Dip 1	0.89	SkyBlu
Water Dip 2	0.89	SkyBlu
Nucleic Acid Extended Range Gel (Rinse)	30.00	Green
Nucleic Acid Extended Range Gel (Separation)	30.00	Green

Figure C-2 RNA 9000 Purity & Integrity Kit Outlet Reagents



Name	Viscosity	Color
Water	0.89	SkyBlu
Water Dip 1	0.89	SkyBlu
Water Dip 2	0.89	SkyBlu
Waste	0.89	Black
Nucleic Acid Extended Range Gel (Separation)	30.00	Green

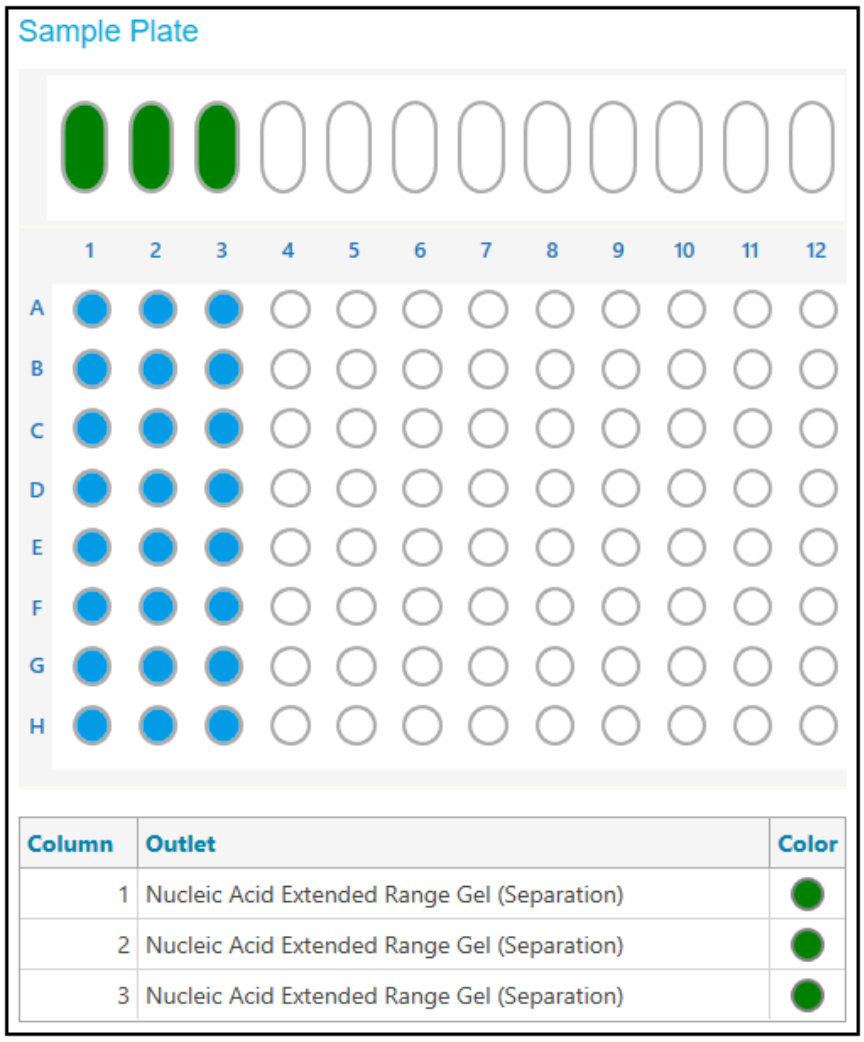
Plate Layouts

Note: The following figures show the plate layouts corresponding to the sequence supplied with the software. If additional samples have been added or the reagent positions have been edited, then the following layouts are not correct.

Sample Plates

Note: The top row shows the layout for the sample outlet plate. The bottom section shows the layout for the sample inlet plate.

Figure C-3 Layouts for the Sample Inlet Plate and Sample Outlet Plate

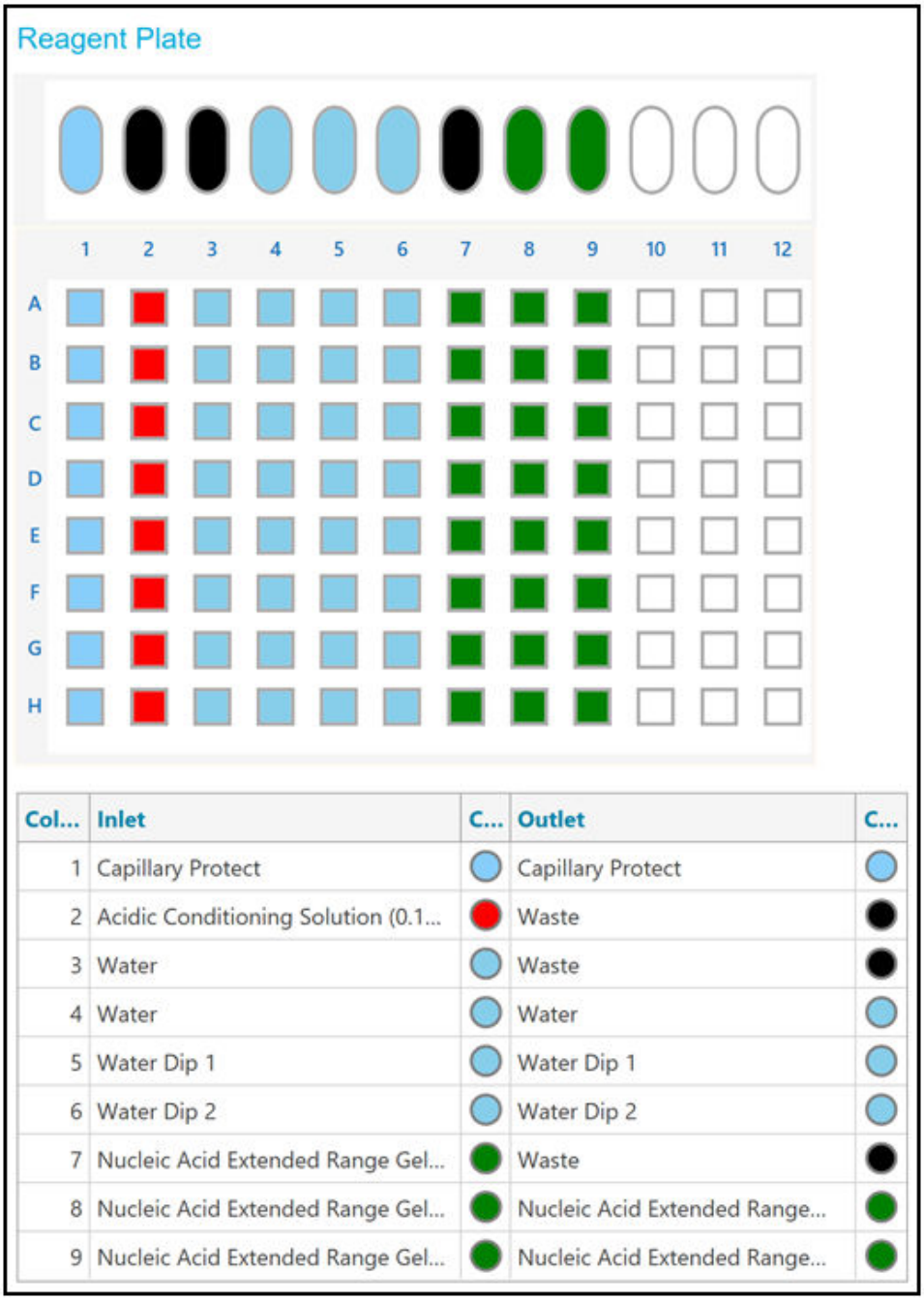


Column	Outlet	Color
1	Nucleic Acid Extended Range Gel (Separation)	
2	Nucleic Acid Extended Range Gel (Separation)	
3	Nucleic Acid Extended Range Gel (Separation)	

Reagent Plates

Note: The top row shows the layout for the reagent outlet plate. The bottom section shows the layout for the reagent inlet plate.

Figure C-4 Layouts for the Reagent Inlet Plate and Reagent Outlet Plate



Col...	Inlet	C...	Outlet	C...
1	Capillary Protect		Capillary Protect	
2	Acidic Conditioning Solution (0.1...		Waste	
3	Water		Waste	
4	Water		Water	
5	Water Dip 1		Water Dip 1	
6	Water Dip 2		Water Dip 2	
7	Nucleic Acid Extended Range Gel...		Waste	
8	Nucleic Acid Extended Range Gel...		Nucleic Acid Extended Range...	
9	Nucleic Acid Extended Range Gel...		Nucleic Acid Extended Range...	

Methods

For instructions for creating a method, refer to the document: *Software Help System*.

Conditioning Method

Figure C-5 Method Settings for the Conditioning Method

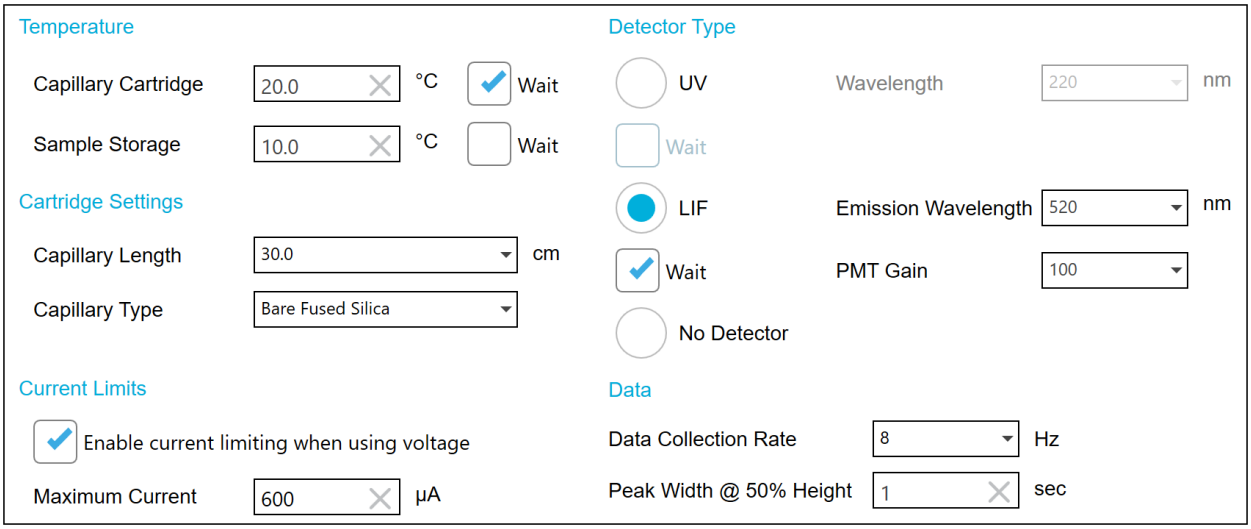


Figure C-6 Actions in the Program Pane for the Conditioning Method

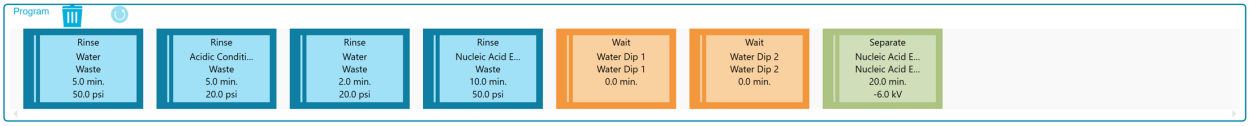
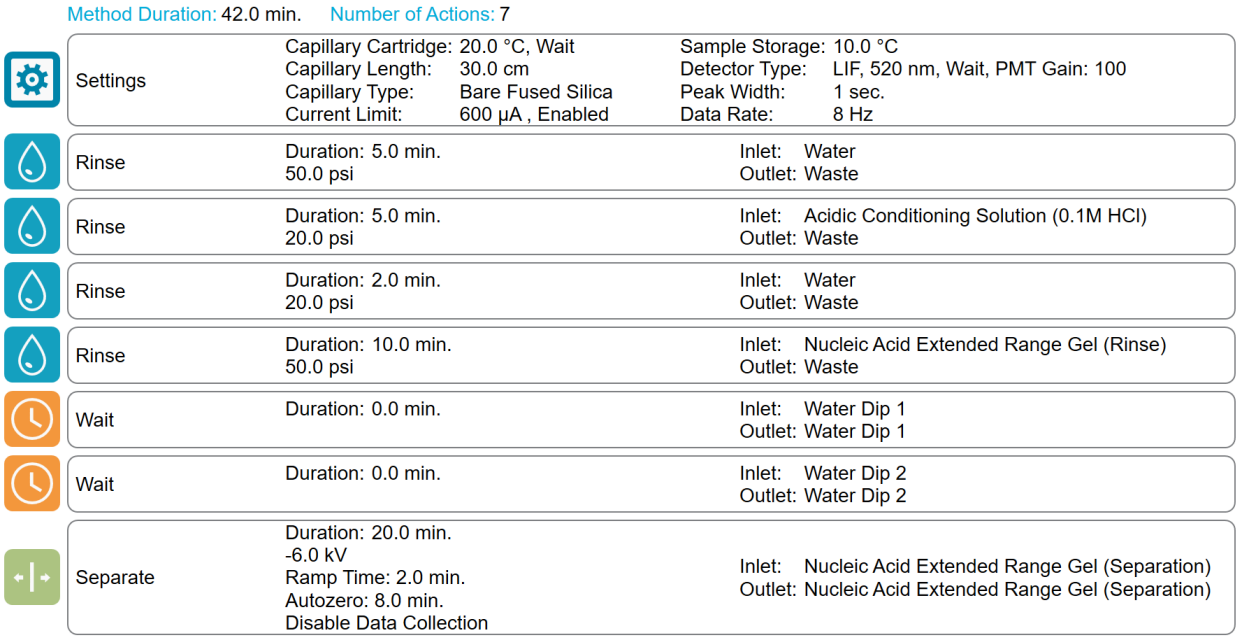


Figure C-7 Summary of Actions in the Conditioning Method



Separation Method with Electrokinetic Injection

Figure C-8 Method Settings for the Separation Method with Electrokinetic Injection

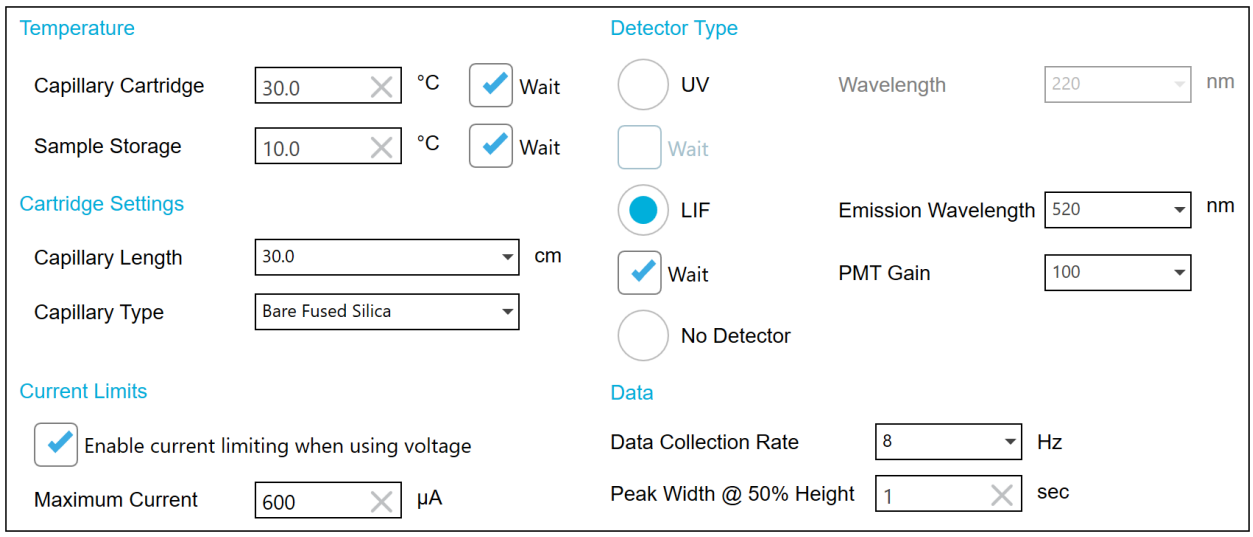


Figure C-9 Actions in Program Pane for the Separation Method with Electrokinetic Injection

Figure C-10 Summary of Actions in the Separation Method with Electrokinetic Injection

Method Duration: 31.0 min. Number of Actions: 8

Settings	Capillary Cartridge: 30.0 °C, Wait Capillary Length: 30.0 cm Capillary Type: Bare Fused Silica Current Limit: 600 µA , Enabled		Sample Storage: 10.0 °C, Wait Detector Type: LIF, 520 nm, Wait, PMT Gain: 100 Peak Width: 1 sec. Data Rate: 8 Hz
Rinse	Duration: 1.0 min. 70.0 psi		Inlet: Acidic Conditioning Solution (0.1M HCl) Outlet: Waste
Rinse	Duration: 1.0 min. 70.0 psi		Inlet: Water Outlet: Waste
Rinse	Duration: 5.0 min. 50.0 psi		Inlet: Nucleic Acid Extended Range Gel (Rinse) Outlet: Waste
Separate	Duration: 2.0 min. -30.0 kV Ramp Time: 0.2 min. Disable Data Collection		Inlet: Nucleic Acid Extended Range Gel (Separation) Outlet: Nucleic Acid Extended Range Gel (Separation)
Wait	Duration: 0.0 min.		Inlet: Water Dip 1 Outlet: Water Dip 1
Inject	Duration: 3 sec. -1.0 kV	Plate: Sample	Outlet: Nucleic Acid Extended Range Gel (Separation)
Wait	Duration: 0.0 min.		Inlet: Water Dip 2 Outlet: Water Dip 2
Separate	Duration: 22.0 min. -6.0 kV Ramp Time: 2.0 min. Autozero: 8.0 min., Advance after: 6 actions		Inlet: Nucleic Acid Extended Range Gel (Separation) Outlet: Nucleic Acid Extended Range Gel (Separation)

Shutdown Method

Figure C-11 Method Settings for the Shutdown Method

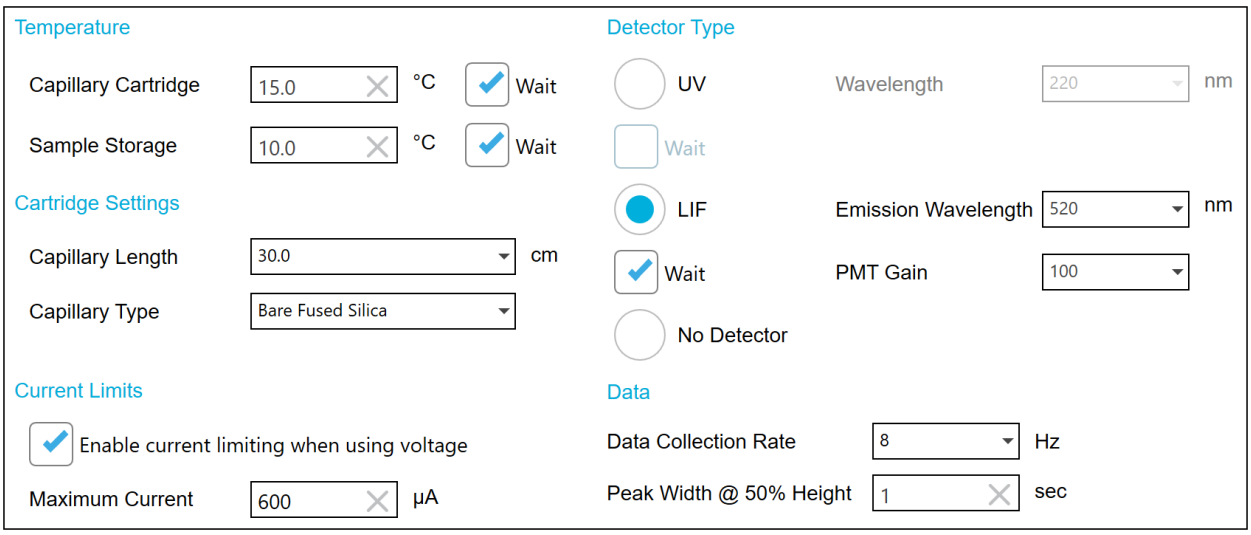


Figure C-12 Actions in the Program Pane for the Shutdown Method

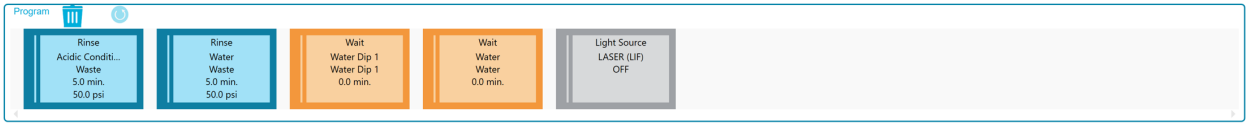


Figure C-13 Summary of Actions in the Shutdown Method

Method Duration: 20.0 min. Number of Actions: 5

Settings	Capillary Cartridge: 15.0 °C, Wait Capillary Length: 30.0 cm Capillary Type: Bare Fused Silica Current Limit: 600 µA , Enabled	Sample Storage: 10.0 °C, Wait Detector Type: LIF, 520 nm, Wait, PMT Gain: 100 Peak Width: 1 sec. Data Rate: 8 Hz
Rinse	Duration: 5.0 min. 50.0 psi	Inlet: Acidic Conditioning Solution (0.1M HCl) Outlet: Waste
Rinse	Duration: 5.0 min. 50.0 psi	Inlet: Water Outlet: Waste
Wait	Duration: 0.0 min.	Inlet: Water Dip 1 Outlet: Water Dip 1
Wait	Duration: 0.0 min.	Inlet: Water Outlet: Water
LIF Laser	OFF	

Capillary Rinse Method

Figure C-14 Method Settings for the Capillary Rinse Method

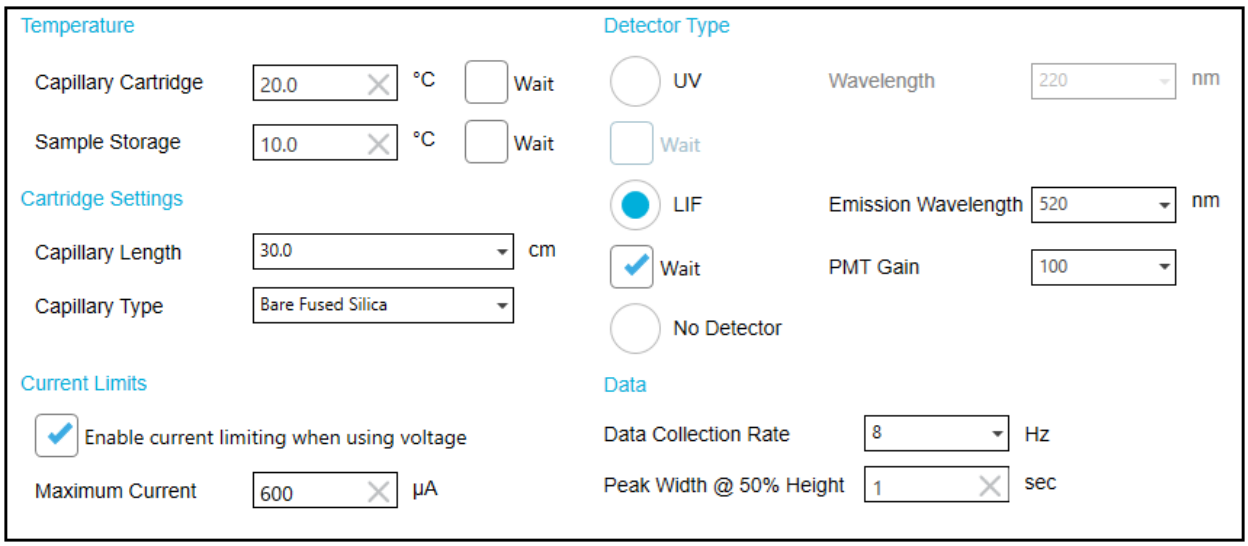
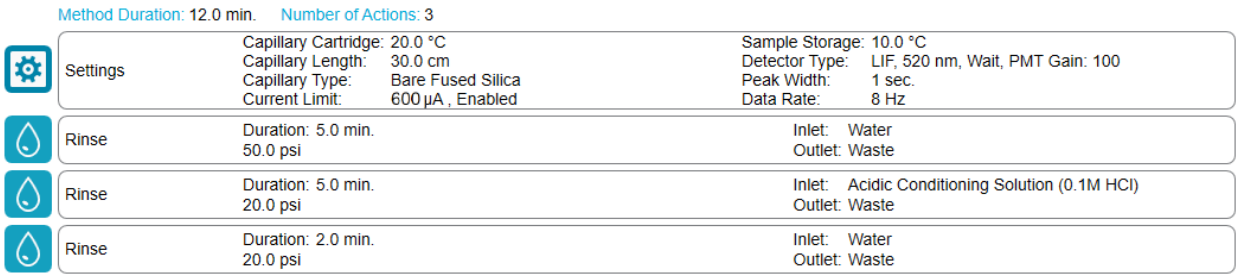


Figure C-15 Actions in the Program Pane for the Capillary Rinse Method

Figure C-16 Summary of Actions in the Capillary Rinse Method



Contact Us

Customer Training

- In North America: NA.CustomerTraining@sciex.com
- In Europe: Europe.CustomerTraining@sciex.com
- Outside the EU and North America, visit sciex.com/education for contact information.

Online Learning Center

- SCIEX Now Learning Hub

Purchase Supplies and Reagents

Reorder SCIEX supplies and reagents online at store.sciex.com. To set up an order, use the account number, found on the quote, order confirmation, or shipping documents. Currently, customers in the United States, United Kingdom, and Germany have access to the online store, but access will be extended to other countries in the future. For customers in other countries, contact a local SCIEX representative.

SCIEX Support

SCIEX and its representatives maintain a staff of fully-trained service and technical specialists located throughout the world. They can answer questions about the system or any technical issues that might arise. For more information, visit the SCIEX website at sciex.com or contact us in one of the following ways:

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

CyberSecurity

For the latest guidance on cybersecurity for SCIEX products, visit sciex.com/productsecurity.

Documentation

This version of the document supercedes all previous versions of this document.

To see this document electronically, Adobe Acrobat Reader is required. To download the latest version, go to https://get.adobe.com/reader.

To find software product documentation, refer to the release notes or software installation guide that comes with the software.

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

The latest versions of the documentation are available on the SCIEX website, at sciex.com/customer-documents.

Note: To request a free, printed version of this document, contact sciex.com/contact-us.