

# RNA 9000 Purity & Integrity Kit

**For the BioPhase 8800 System**

Application Guide

---

This document is provided to customers who have purchased SCIEX equipment to use in the operation of such SCIEX equipment. This document is copyright protected and any reproduction of this document or any part of this document is strictly prohibited, except as SCIEX may authorize in writing.

Software that may be described in this document is furnished under a license agreement. It is against the law to copy, modify, or distribute the software on any medium, except as specifically allowed in the license agreement. Furthermore, the license agreement may prohibit the software from being disassembled, reverse engineered, or decompiled for any purpose. Warranties are as stated therein.

Portions of this document may make reference to other manufacturers and/or their products, which may contain parts whose names are registered as trademarks and/or function as trademarks of their respective owners. Any such use is intended only to designate such products as those manufacturers' products and does not imply any right and/or license to use or permit others to use such manufacturers' and/or their product names as trademarks.

SCIEX warranties are limited to those express warranties provided at the time of sale or license of its products and are the sole and exclusive representations, warranties, and obligations of SCIEX. SCIEX makes no other warranty of any kind whatsoever, expressed or implied, including without limitation, warranties of merchantability or fitness for a particular purpose, whether arising from a statute or otherwise in law or from a course of dealing or usage of trade, all of which are expressly disclaimed, and assumes no responsibility or contingent liability, including indirect or consequential damages, for any use by the purchaser or for any adverse circumstances arising therefrom.

(GEN-IDV-09-10816-E)

For Research Use Only. Not for use in Diagnostic Procedures.

Trademarks and/or registered trademarks mentioned herein, including associated logos, are the property of AB Sciex Pte. Ltd., or their respective owners, in the United States and/or certain other countries (see [sciex.com/trademarks](http://sciex.com/trademarks)).

AB Sciex™ is being used under license.

Made in USA with Foreign and Domestic Components.

© 2024 DH Tech. Dev. Pte. Ltd.

**EC Authorized Person** AB Sciex Netherlands B.V.  
1e Tochtweg 11,  
2913LN Nieuwerkerk aan den IJssel  
Netherlands



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

# Contents

---

<b>1 RNA 9000 Purity &amp; Integrity Kit</b> .....	<b>6</b>
Safety.....	6
Intended Use.....	6
Introduction.....	6
Workflow.....	7
<b>2 Required Equipment and Materials</b> .....	<b>9</b>
Storage Conditions.....	10
Customer-Supplied Equipment and Supplies.....	11
Required Detector.....	11
Required Cartridge.....	11
Condition the Capillaries.....	12
<b>3 Methods and Sequences</b> .....	<b>13</b>
Methods and Sequences for the BioPhase Software.....	13
Methods for the Waters Empower™ Software.....	13
Create the Sequence.....	14
<b>4 Prepare the Samples</b> .....	<b>17</b>
Best Practices for Working with RNA.....	17
Prepare the ssRNA Ladder.....	17
Prepare the RNA Sample.....	18
<b>5 Prepare the BioPhase 8800 System</b> .....	<b>20</b>
Load the Reagent Inlet and Outlet Plates.....	20
Load the Sample Inlet and Outlet Plates.....	22
Examine the Capillary Cartridge.....	24
Install the Capillary Cartridge.....	25
<b>6 Run the Samples</b> .....	<b>28</b>
Tips for Best Results.....	28
Use the Rinse Method.....	28
Guidance for Selection of the Injection Type.....	28
Start the Sequence from the Front Panel.....	31
Monitor the Run on the BioPhase 8800 Front Panel.....	33
Waste Disposal.....	41

## Contents

---

Stow the Cartridge After the Run .....	41
Stow the Cartridge for Less than 24 Hours .....	41
Stow the Cartridge for More than 24 Hours .....	41
Prepare the Cartridge After Storage .....	42
<b>7 Analyze the Data .....</b>	<b>43</b>
Use an Analysis Parameters File to Analyze the Data .....	43
Examine the Results .....	46
Review the Results on the Overlay Tab .....	47
Guidance for Acceptance Criteria .....	50
<b>8 Run the Samples with the Waters Empower™ Software .....</b>	<b>51</b>
Import the BioPhase Software Methods to Create Instrument Methods .....	51
Create the Sample Set Method .....	54
Start the Sample Set Method .....	57
Monitor the Run in the Waters Empower™ Software .....	63
<b>9 Troubleshooting .....</b>	<b>66</b>
Remove Blockage from a Capillary .....	78
Options for a Blocked or Damaged Capillary .....	78
<b>A Hazardous Substance Information .....</b>	<b>79</b>
<b>B Download Required Files .....</b>	<b>80</b>
Download and Configure the Required Files (BioPhase Software) .....	80
Download and Configure the Required Files (Waters Empower™ Software) .....	81
<b>C Reagents, Plate Layouts, and Methods .....</b>	<b>82</b>
Reagent Set .....	82
Plate Layouts .....	83
Methods .....	85
Conditioning Method .....	85
Separation Method with Electrokinetic Injection .....	86
Separation Method with Pressure Injection .....	88
Shutdown Method .....	89
Capillary Rinse Method .....	90
<b>Contact Us .....</b>	<b>92</b>
Customer Training .....	92
Online Learning Center .....	92
Purchase Supplies and Reagents .....	92
SCIEX Support .....	92

Cybersecurity .....	92
Documentation .....	92

# RNA 9000 Purity & Integrity Kit

# 1

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

This document gives instructions for sample preparation with the RNA 9000 Purity & Integrity kit. It also gives instructions for data acquisition with the BioPhase software and the Waters Empower™ software with the BioPhase 8800 driver for Empower™. This document also gives instructions for data analysis of data acquired with BioPhase software. The analysis of data acquired with the Waters Empower™ software must be done with the Waters Empower™ software.

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

---

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

---

## Safety

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

## Intended Use

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

## Introduction

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

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

The RNA sample is separated by size in a bare-fused silica capillary containing a replaceable polymer gel that provides sieving selectivity while concurrently limiting counter electroosmotic

---

flow (EOF). The fluorescent labeling dye SYBR™ Green II RNA Gel Stain<sup>1</sup> 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.

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

## Workflow

The workflow has the following steps:

1. Identify the number of samples to be analyzed and the number of replicates.
2. For BioPhase software users:
  - a. Create or change 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 the BioPhase software methods. Refer to the section: [Import the BioPhase Software Methods to Create 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. Refer to the section: [Plate Layouts](#).
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. After the run, stow the cartridge. Refer to the section: [Stow the Cartridge After the Run](#)

---

<sup>1</sup> SYBR™ is a trademark of the Life Technologies Corporation. SYBR™ Green II RNA Gel Stain is not available for resale.

### 9. Analyze the data.

- BioPhase software: When the sequence is complete, use the BioPhase Analysis software to analyze the data. Refer to the section: [Analyze the Data](#).
- Waters Empower™ software: When the sample set method is complete, use the Waters Empower™ software to analyze the data. For data analysis instructions, refer to the Waters Empower™ software guides and help file.



# 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
<b>Nucleic Acid Extended Range Purity &amp; Integrity Kit (PN 5087900)</b>		N/A
Acid wash/regenerating solution (100 mL)	1	5312349
CE Grade water (140 mL)	2	C48034
LIF Performance Test Mix (20 mL)	1	726022
Nucleic Acid Extended Range Gel (140 mL)	2	N/A
SYBR™ Green II RNA Gel Stain <sup>2</sup> (500×) (0.11 mL)	7	N/A
<b>ssRNA Ladder (0.05-9 kb) (PN 5088699)</b>		N/A
ssRNA Ladder (0.05 kb to 9 kb) (70 µL) (shipped separately)	2	N/A

**Table 2-2 Other Supplies from SCIEX**

Component	Quantity	Part Number
Capillary cartridge coolant (450 mL)	1	359976
Filter, 520 nm emission filter	1	144940
Sample Loading Solution	6 mL	608082

<sup>2</sup> SYBR™ is a trademark of the Life Technologies Corporation. SYBR™ Green II RNA Gel Stain is not available for resale.

## Required Equipment and Materials

---

**Table 2-2 Other Supplies from SCIEX (continued)**

Component	Quantity	Part Number
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 Other Required Reagents or Supplies**

Description	Vendor	Part Number
Acrodisc 32 mm syringe filter with a 0.45 µm pore-size membrane	Pall	4654
Luer-Lok tip disposable syringe (10 mL)	BD	309604
(Optional) Nuclease-free water (10 × 2 mL)	Integrated DNA Technologies	11-04-02-01
(Optional) RNaseZap RNase Decontamination Solution (250 mL)	Thermo Fisher Scientific	AM9780
X-Pierce Film	USA Scientific	2997-0100

## Storage Conditions

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

---

<sup>3</sup> SYBR™ is a trademark of the Life Technologies Corporation. SYBR™ Green II RNA Gel Stain is not available for resale.

## Customer-Supplied Equipment and Supplies

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

---

**Note:** For reagent plate preparation a repeater pipette or equivalent is recommended.

---

- Table-top mini centrifuge
- Vortex mixer
- Water bath or heat block capable of 37 °C to 100 °C

## Required Detector

An LIF detector with a 488 nm excitation source and a 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 used, then sample carryover, nonspecific binding, and poor separation can occur.**

---

A BioPhase 8800 bare-fused silica capillary cartridge with 50 µm inner diameter (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. The solution might negatively ionize the capillary wall and result in electro-osmotic flow detrimental to the separation of nucleic acids. These interactions might cause poor separation.**

---

- Before a new cartridge is used, condition the capillaries with the Conditioning Method-RNA 9000 method.

---

## Methods and Sequences for 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 website. 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 methods:
  - Separation Method with Electrokinetic Injection - RNA 9000: Does the separation of the sample with an electrokinetic injection of the sample.
  - Separation Method - Pressure Injection - RNA 9000: Does the separation of the sample with an pressure 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.
- Sequence templates:
  - RNA 9000 Test Sequence - Electrokinetic Sample Injection: A sequence with the separation method that uses electrokinetic sample injection.
  - RNA 9000 Test Sequence - Pressure Sample Injection: A sequence with the separation method that uses pressure sample injection.

## Methods for the Waters Empower™ Software

To create the required instrument methods, import the BioPhase software methods.

---

**Note:** If the methods are not included with the software, then they are available for download from the SCIEX website. 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, then this procedure is not applicable. Refer to the section: [Create the Sample Set Method](#).

---

**Note:** This procedure is for users who know how to use the BioPhase software. For detailed instructions, refer to the document: *Software Help System*.

---

This procedure gives instructions to create a sequence with a template that is supplied with the BioPhase software.

Two templates are supplied: one that uses a separation method with an electrokinetic injection, and the other that uses a separation method with a pressure injection. For information about the injection types, refer to the section: [Guidance for Selection of the Injection Type](#). The templates are set up with the ssRNA Ladder in the first column, followed by 16 samples in the second and third columns, and use 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, include separation methods, and then end with a shutdown method. Usually, the shutdown method should be identified 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 a sequence:
  - 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. In the table, click the sequence template, and then click **Open**.
    - To inject the samples with an electrokinetic injection, click **RNA 9000 Test Sequence - Electrokinetic Sample Injection**.
    - To inject the samples with a pressure injection, click **RNA 9000 Test Sequence - Pressure Sample Injection**.

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, then do any of the following actions:
  - Add or remove samples.
  - Clear a method that is 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. Usually, the shutdown method should be identified 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	<input type="checkbox"/>
⊞	2	1	Separation Method - Electrokinetic Injection - RNA 9000	1	<input type="checkbox"/>
⊞	3	2	Separation Method - Electrokinetic Injection - RNA 9000	1	<input type="checkbox"/>
⊞	4	3	Separation Method - Electrokinetic Injection - RNA 9000	1	<input type="checkbox"/>
	5	0	Shutdown Method - RNA 9000	1	<input checked="" type="checkbox"/>

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

## Methods and Sequences

**Figure 3-2 Sequence Summary Table Expanded**

Sequence Summary

Run #	Column	Method Name	Rep. #	Error Recovery
1	0	Conditioning Method-RNA 9000	1	<input type="checkbox"/>
⊖ 2	1	Separation Method with Electrokinetic Injection - RNA 9000	1	<input type="checkbox"/>
	<b>Well</b>	<b>Sample Id</b>	<b>Run Type</b>	<b>Data File</b>
	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	<input type="checkbox"/>
⊕ 4	3	Separation Method with Electrokinetic Injection - RNA 9000	1	<input type="checkbox"/>
5	0	Shutdown Method - RNA 9000	1	<input checked="" type="checkbox"/>

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

---

**Note:** If errors occurred, then the **SAVE** button is not available. Correct 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. Close the Print Preview dialog.



---

## 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  $\mu$ L aliquots into nuclease-free PCR vials.
  - d. Reserve one aliquot, and then store the remaining aliquots at  $-35\text{ }^{\circ}\text{C}$  to  $-15\text{ }^{\circ}\text{C}$ .

## Prepare the Samples

---

- Use one of the 8  $\mu\text{L}$  aliquots of the ssRNA Ladder. If it is frozen, then thaw it on ice before use.
- 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 $\mu\text{L}$	17.5 $\mu\text{L}$
Sample Loading Solution	48 $\mu\text{L}$	420 $\mu\text{L}$

- Heat the sample at 70 °C for 5 minutes.
- After 5 minutes, immediately put the mixture in an ice water bath for a minimum of 2 minutes.

---

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

---

- Add 50  $\mu\text{L}$  to 200  $\mu\text{L}$  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 related column on the sample outlet plate has 2.0 mL of gel buffer.
- Put the sample plate in the system.
- Make sure that the plate compartment temperature is set to 10 °C.

## Prepare the RNA Sample

- Thaw the RNA aliquot on ice.  
Keep the sample cool to help prevent the RNA from degrading.
- Prepare the RNA sample in Sample Loading Solution or nuclease-free water between 50 ng/mL to 50  $\mu\text{g/mL}$ .  
We recommend an RNA concentration between 1  $\mu\text{g/mL}$  and 5  $\mu\text{g/mL}$ .

---

**Note:** To use a higher sample concentration, decrease the **PMT Gain** in the Method Settings tab in the separation method.

---

- Heat the sample at 70 °C for 5 minutes.
- After 5 minutes, immediately put the mixture in an ice water bath for a minimum of 2 minutes.

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

---

5. Add between 50  $\mu$ L and 200  $\mu$ L to the sample plate well.
6. Make sure that, for any column on the sample plate with sample, the related column on the sample outlet plate has 2.0 mL of gel buffer.
7. Put the sample plate in the system.
8. 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.

---

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

---

## Load the Reagent Inlet and Outlet Plates

---

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

---

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

---

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

---

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

---

**CAUTION: Potential System Damage. Do not use a heated plate sealer to attach the film cover. If the heat causes damage to the surface of the plates, then issues with the pressure system might occur.**

---

**CAUTION: Potential Wrong Result. Before the plates are loaded in the system, spin them to remove air bubbles. Air bubbles might an unsatisfactory separation.**

---

**CAUTION: Potential System Damage. Before the plates are loaded in the system, remove the film cover. Do not load plates in the system before removing the film cover. If the film cover is not removed, then damage to the capillary tips might occur.**

---

1. Add the following reagents to a conical tube.

**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

---

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

Reagent	For 1 to 48 Samples	For 49 to 96 Samples
SYBR™ Green II RNA Gel Stain <sup>4</sup>	80 µL	80 µL

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

- Carefully invert the tube a minimum of 20 times.  
Make sure that air bubbles are not created when the tube is inverted.
- With a 0.45 µm Acrodisc syringe filter and a Luer-Lok syringe, filter the gel buffer.
- Use the reagent plate layout to add the following reagents to the reagent inlet and outlet plates. Refer to the figure: [Figure C-4](#).

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

Plate	Reagent
Inlet plate	800 µL per well
Outlet plate	<ul style="list-style-type: none"> <li>2.8 mL of reagent per well for separation or wait positions</li> <li>1.5 mL of CE Grade water per well for waste positions</li> </ul>

**Note:** For the outlet plate, make sure that the chamfered corner is on the top 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.

- Put a film cover on the plates.
- Put the plates in a swinging-bucket rotor, and then spin them for 4 minutes at 30 × g. Make sure that the buckets are balanced.
- Make sure that there are no air bubbles in the plates. To remove air bubbles, 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.
- On the front panel, touch **Eject Reagent**.

<sup>4</sup> SYBR™ is a trademark of the Life Technologies Corporation. SYBR™ Green II RNA Gel Stain is not available for resale.

## Prepare the BioPhase 8800 System

---

**Figure 5-1 Eject Reagent Button**



The plate compartment opens.

9. Remove the film cover from the plates.
10. If the plate compartment contains reagent plates, then remove them.
11. Align the notch in the reagent inlet plate with the tab, and then put the plate in the plate carrier.
12. 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.
13. Touch **Load Reagent**.

**Figure 5-2 Load Reagent Button**



The plate compartment closes.

## Load the Sample Inlet and Outlet Plates

---

**CAUTION: Potential System Damage. Do not use a heated plate sealer to attach the film cover. If the heat causes damage to the surface of the plates, then issues with the pressure system might occur.**

---

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

---

1. Use the sample plate layout to add the samples to the sample inlet plate. Refer to the figure: [Figure C-3](#).  
The recommended sample volume is 100  $\mu\text{L}$ . The minimum sample volume is 50  $\mu\text{L}$ . The maximum sample volume is 200  $\mu\text{L}$ .
2. To prevent damage to the capillaries, if there are columns where not every well has sample, then add between 100  $\mu\text{L}$  and 200  $\mu\text{L}$  of sample buffer to each empty well.  
In the sample plate layout, wells that should have sample buffer are shown in grey. If a column has no samples, then the wells can be left empty.

3. Use the sample plate layout to add the following reagents to the sample outlet plate. Refer to the figure: [Figure C-3](#).

The maximum volume is 2.0 mL.

**Note:** For the outlet plate, make sure that the chamfered corner is on the top 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	<ul style="list-style-type: none"> <li>• 2.0 mL of gel buffer per well</li> </ul>

4. Make sure that, for any column on the sample plate with sample, the related column on the outlet plate has 2.0 mL of separation gel.
5. Put a film cover on the plates.
6. Put the plates in a swinging-bucket rotor, and then spin them for 4 minutes at  $30 \times g$ . Make sure that the buckets are balanced.
7. Make sure that there are no air bubbles in the plates. To remove air bubbles, then spin the plates again at a higher relative centrifugal force (RCF).  
For the reagent plate, the maximum RCF is  $1,000 \times g$ . For the sample plate, the maximum RCF is  $375 \times g$ .
8. On the front panel, touch **Eject Sample**.

**Figure 5-3 Eject Sample Button**



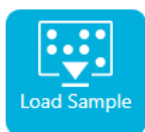
The plate compartment opens.

9. Remove the film cover from the plates.
10. If the plate compartment already contains sample plates, then remove the sample plates.
11. Align the notch in the sample plate with the tab, and then put the plate in the plate carrier.
12. Make sure that the chamfered corner of the sample outlet plate is in the top left, and then put the plate in the back of the plate carrier.
13. Touch **Load Sample**.

## Prepare the BioPhase 8800 System

---

Figure 5-4 Load Sample Button



The plate compartment closes.

## Examine the Capillary Cartridge



**WARNING! Puncture Hazard.** Touch the cartridge carefully. The capillary tips are extremely sharp.

---

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

---

**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 and make a residue on the capillary window that causes interference with the detector.

---

1. Examine the electrodes, capillary tips, cartridge seals, and cartridge body before use.
2. If gel or liquid has collected on the outside of the cartridge, then clean the cartridge with a moist 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 clean 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 have collected, then use short bursts of electronics-grade compressed air to remove them. Do not use water or other liquids to clean the capillary window.
5. Dampen a laboratory wipe or cotton swab with ethanol or isopropanol, and then clean the surface of the ID chip. Let the ID chip air-dry before the cartridge is installed.



## Install the Capillary Cartridge



**WARNING! Puncture Hazard.** Touch the cartridge carefully. The capillary tips are extremely sharp.

---



**WARNING! Pinching Hazard.** When the front panel is opened, do not 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 the cartridge is installed. Otherwise, damage to the cartridge might occur.

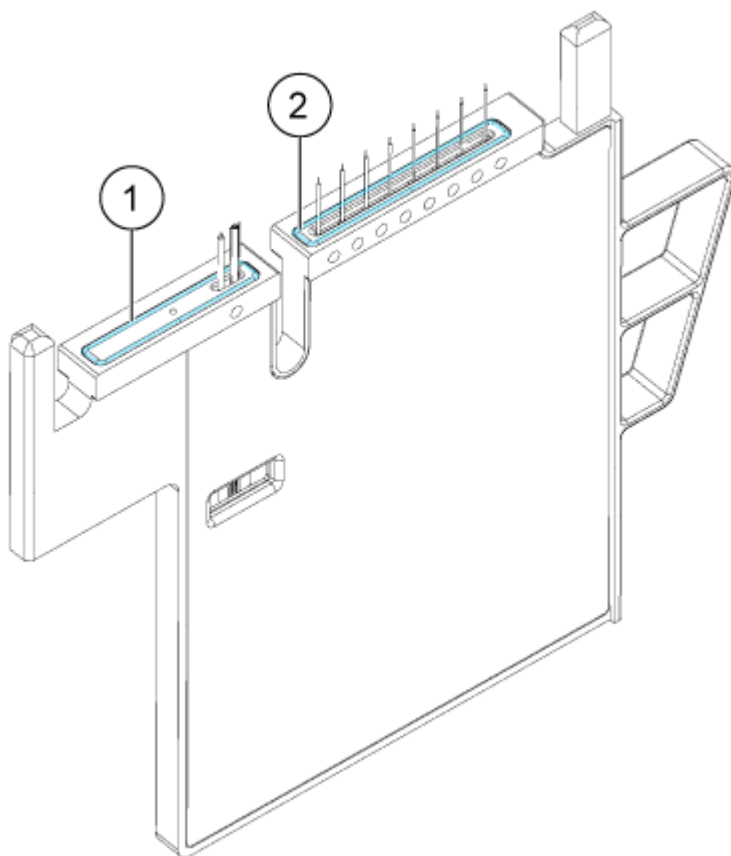
---

1. If the cartridge was kept in the refrigerator, then let the cartridge equilibrate to ambient temperature for approximately 30 minutes to prevent condensation in the system.
2. Remove the cartridge from the wetting tray.
3. To prevent arcing, 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 laboratory wipe to very carefully dry the area where the capillaries and electrodes come out of the cartridge. Do not touch or move the seals.

## Prepare the BioPhase 8800 System

---

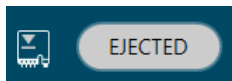
**Figure 5-5 Bottom of the Cartridge**



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**



The system moves the reagent plate until the capillaries are in position over column 1, and then lifts the plate until the capillary tips are immersed in CE Grade water.

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

## Tips for Best Results

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

If a specific characteristic is to be maximized, other temperatures can be used. Refer to the table: [Table 6-1](#).

**Table 6-1 Guidance for Setting the Capillary Temperature**

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

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

## Use the Rinse Method

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

To save time after the capillary has been conditioned, in the sequence, use the `Capillary Rinse - RNA 9000` method as an alternative to the `Conditioning Method-RNA 9000` method. The rinse method is approximately 30 minutes shorter than the conditioning method.

## Guidance for Selection of the Injection Type

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

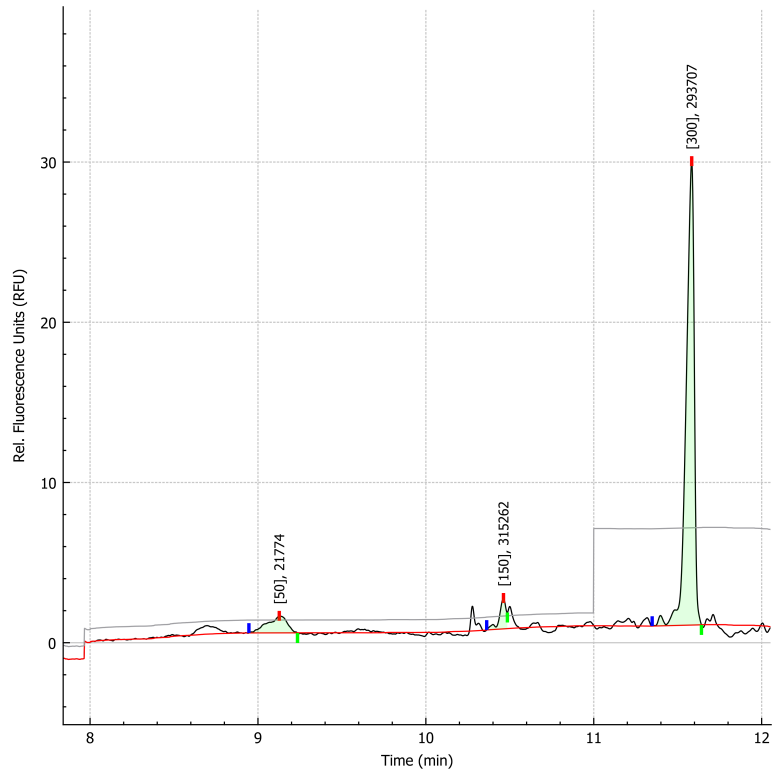
- Electrokinetic injection (EKI) uses an electric field to introduce the negatively charged RNA into the capillary.
  - Advantages: Assay sensitivity throughout the full separation range can be increased with a larger injection volume or more concentrated samples.
  - Disadvantages: Samples with higher ionic strength will have a lower signal intensity as a result of injection competition between nucleic acids and anions in solution. ssRNA ladder and sample peaks shorter than 300 bases might be more difficult to interpret and integrate as a result of decreased peak selectivity during electrokinetic sample introduction.
- Hydrodynamic (pressure) injection uses pressure-over-liquid to introduce the sample to the capillary.
  - Advantages: Peak height is more consistent because the ionic strength in the sample does not change from injection to injection. Peaks below 300 bases keep peak selectivity which simplifies integration and analysis.
  - Disadvantages: Assay sensitivity is decreased compared to electrokinetic injection.

The following figures show electropherograms of the separation of the ssRNA Ladder with the different sample injection methods. (Only the peaks from 50 bases to 300 bases are shown.) With electrokinetic injection the peak labeled 50 is much broader and thus has lower theoretical plates as compared to the same peak from a pressure injection. Refer to the figures: [Figure 6-1](#) and [Figure 6-2](#).

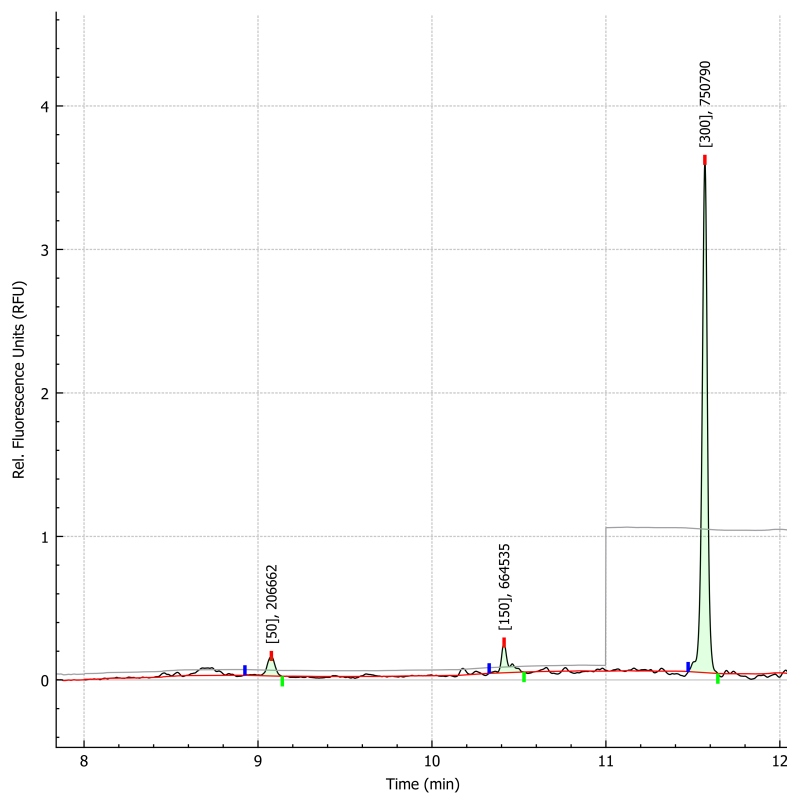
## Run the Samples

---

**Figure 6-1 ssRNA Ladder Sample with Electrokinetic Injection, Annotated with Length and Theoretical Plates**



**Figure 6-2 ssRNA Ladder Sample with Pressure Injection, Annotated with Length and Theoretical Plates**

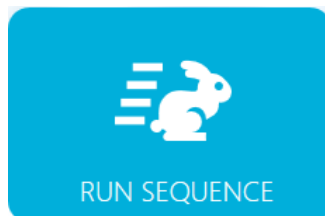


## Start the Sequence from the Front Panel

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 6-3 RUN SEQUENCE Button**



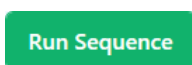
3. In the Projects pane, touch **RNA 9000**.
4. In the Available Sequences pane, touch the applicable sequence in the list.

## Run the Samples

---

- For an electrokinetic injection, click **RNA 9000 Test Sequence - Electrokinetic Sample Injection**.
  - For a pressure injection, click **RNA 9000 Test Sequence - Pressure Sample Injection**.
5. (Optional) To see 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 6-4 Run Sequence Button**



If the sequence contains a method that is incompatible with the system configuration, then **Run Sequence** is not available.

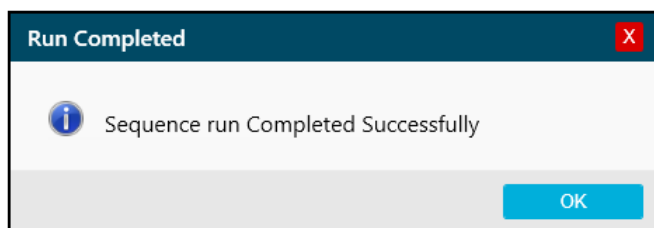
The data files are saved at the location specified in the sequence. If this location has a data file with the same name, then, to prevent the older data file from being overwritten, a date stamp is automatically added to the file name.

If an error occurs during a run and an error recovery method is included 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 6-5 Run Completed Dialog**



7. To close the Run Completed dialog, touch **OK**.
8. If required, stow the cartridge. Refer to the section: [Stow the Cartridge After the Run](#).



---

## Monitor the Run on the BioPhase 8800 Front Panel

---

**CAUTION: Potential System Damage.** If the run is stopped, then sample or reagent might be lost and damage to the cartridge might occur.

---

**CAUTION: Potential System Damage.** If the run is stopped and will not be started again, then use the shutdown method to rinse the capillaries before the cartridge is stowed. If the capillaries are not rinsed, then electrolyte salt crystals or precipitate can collect and might cause blocked capillaries, incorrect pressure sealing, errors when the samples are injected, arcing, or current leakage.

---

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

---

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

---

**CAUTION: Potential Wrong Result.** If the samples have been inside of the system for more than 24 hours, sample degradation might have occurred. Discard the samples before the run is started again.

---


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](#).

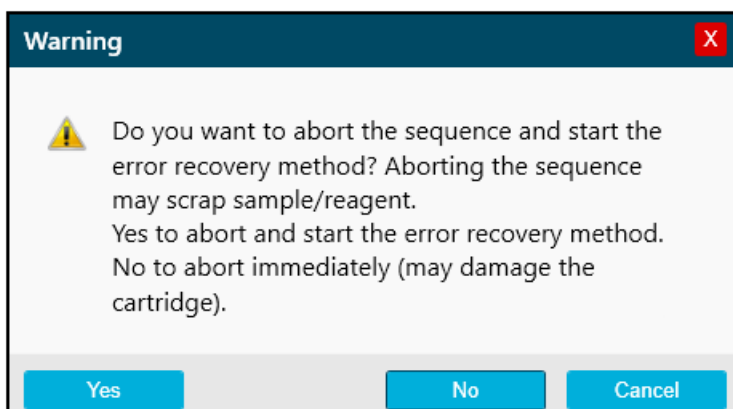
---

**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.

---

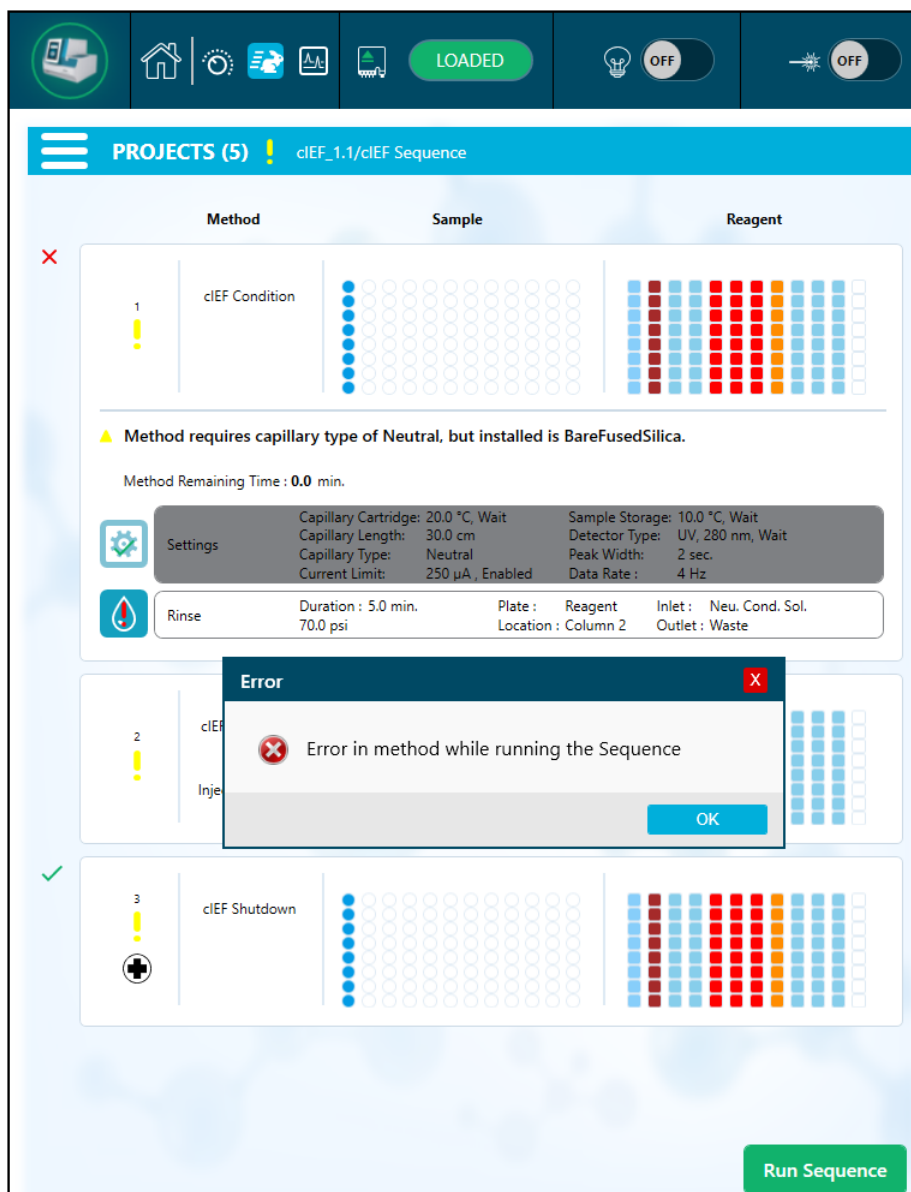
1. Monitor the traces for the detector and the current to make sure that the sequence is in progress.
2. To stop the run, touch .


**Figure 6-6 Warning Dialog**




3. Touch one of the following options:
  - **Yes:** To start the error recovery method, if one is assigned.
  - **No:** If an error recovery method is not assigned.
  - **Cancel:** To continue the run.
4. If an error occurs, then touch **OK** in the error dialog that opens.

Figure 6-7 Run Sequence Error Dialog



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

5. Review the error:
  - a. On the **Events** tab of the front panel log, touch .
  - b. Touch **Initialize System** to initialize the system and change the system status to idle.

## Run the Samples

---

**Figure 6-8 Sequence Error Events Log**

Events		System
2058	4/8/2022 5:40:24 PM	Unable to complete error recovery method, moving trays to Home positions.
2057	4/8/2022 5:38:49 PM	Sequence run is cancelled, error recovery method initiated.

INI Initialize System

6. If required, then touch **Pause Run**.

Figure 6-9 Run Sequence in Progress

Method Remaining Time : 1.1 minutes

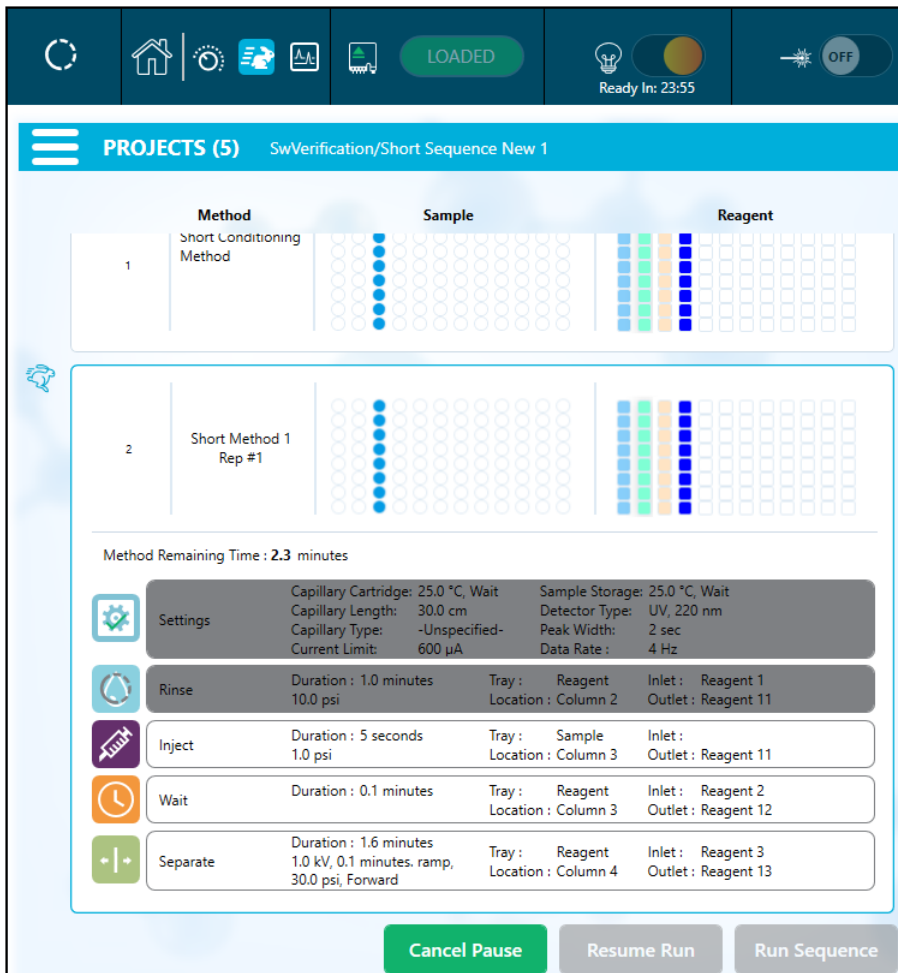
Settings	Capillary Cartridge: 20.0 °C, Wait	Capillary Length: 30.0 cm	Capillary Type: -Unspecified-	Current Limit: 600 µA	Sample Storage: 18.0 °C, Wait	Detector Type: UV, 220 nm, Wait	Peak Width: 2 sec	Data Rate: 4 Hz
Rinse	Duration : 1.0 minutes	0.1 psi	Tray : Reagent	Location : Column 2	Inlet : Water	Outlet : Water		
Inject	Duration : 5 seconds	0.5 psi	Tray : Sample	Location : Column 3	Inlet : Catholyte	Outlet : Catholyte		
Separate	Duration : 1.0 minutes	1.0 kV, 0.1 minutes. ramp	Tray : Reagent	Location : Column 3	Inlet : Chemical Mobilizer	Outlet : Water		

Pause Run Run Sequence

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

## Run the Samples

Figure 6-10 Run Sequence Paused



PROJECTS (5) SwVerification/Short Sequence New 1

Method	Sample	Reagent
1 Short Conditioning Method		
2 Short Method 1 Rep #1		

Method Remaining Time : 2.3 minutes

Settings	Capillary Cartridge: 25.0 °C, Wait	Sample Storage: 25.0 °C, Wait
	Capillary Length: 30.0 cm	Detector Type: UV, 220 nm
	Capillary Type: -Unspecified-	Peak Width: 2 sec
	Current Limit: 600 µA	Data Rate : 4 Hz

Rinse	Duration : 1.0 minutes	Tray : Reagent	Inlet : Reagent 1
	10.0 psi	Location : Column 2	Outlet : Reagent 11

Inject	Duration : 5 seconds	Tray : Sample	Inlet :
	1.0 psi	Location : Column 3	Outlet : Reagent 11

Wait	Duration : 0.1 minutes	Tray : Reagent	Inlet : Reagent 2
		Location : Column 3	Outlet : Reagent 12

Separate	Duration : 1.6 minutes	Tray : Reagent	Inlet : Reagent 3
	1.0 kV, 0.1 minutes. ramp, 30.0 psi, Forward	Location : Column 4	Outlet : Reagent 13

Buttons: Cancel Pause, Resume Run, Run Sequence

7. To see the data during acquisition, touch  in the ribbon.

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

Figure 6-11 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 see the electropherogram.
  - c. Use the hand icon to move the electropherogram.

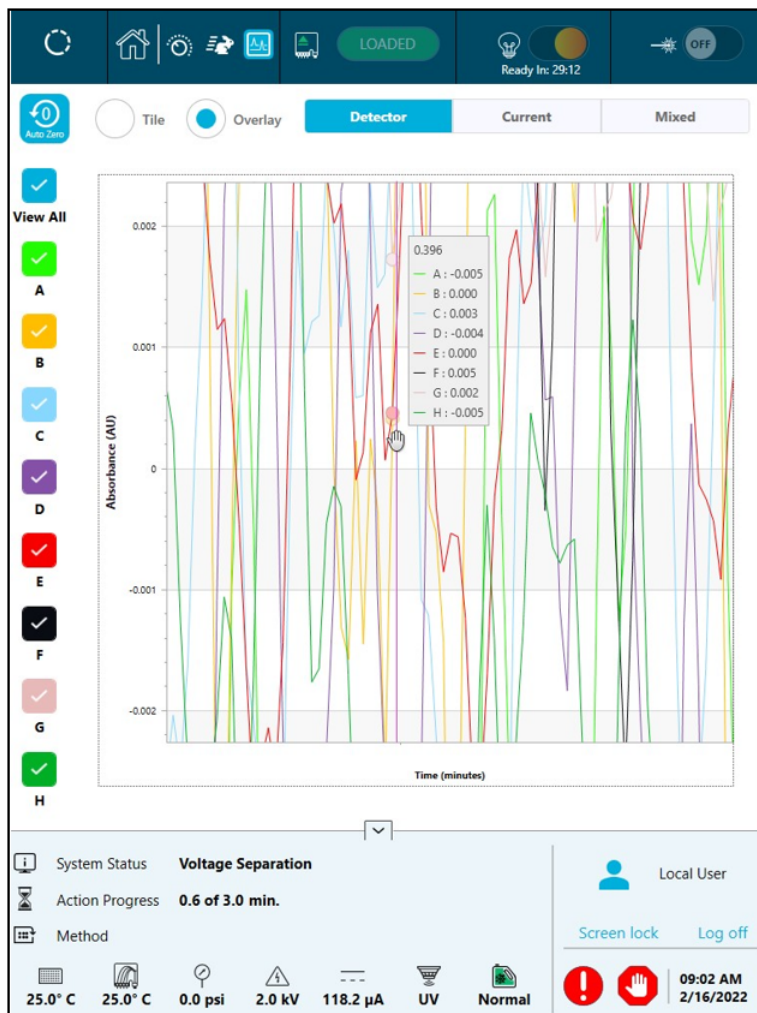
---

**Note:** The zoom feature can only be used with the Overlay view for **Detector** and **Current**.

---

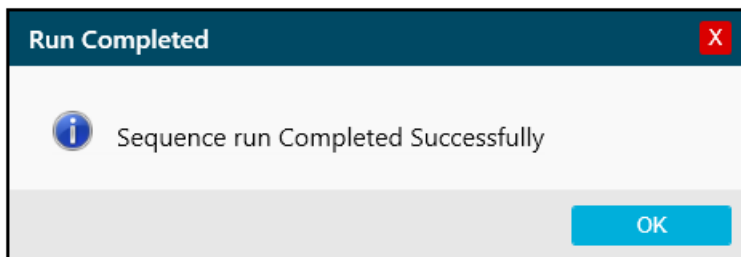
## Run the Samples

Figure 6-12 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 6-13 Run Completed Dialog





If the system has been idle for 24 hours, then the cooler for the sample storage compartment turns off.

## Waste Disposal



---

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

---

## Stow the Cartridge After the Run



---

**WARNING! Puncture Hazard. Touch the cartridge carefully. The capillary tips are extremely sharp.**

---

## Stow the Cartridge for Less than 24 Hours


1. If the sequence or sample set method does not include the shutdown method, then use the shutdown method to clean the capillaries.  
The shutdown method fills the capillaries with CE Grade water and decreases the cartridge temperature to 15 °C.
2. Keep the cartridge in the system for as long as 24 hours with the capillary tips immersed in CE Grade water.

---

**Note:** If the cartridge has not been used for 3 hours or longer, then run the conditioning method before a separation is done.

---

## Stow the Cartridge for More than 24 Hours

1. If the sequence or sample set method does not include the shutdown method, then use the shutdown method to clean the capillaries.  
The shutdown method fills the capillaries with CE Grade water and decreases the cartridge temperature to 15 °C.
2. On the ribbon on the BioPhase 8800 system front panel, touch  (**Loaded**), and then wait for approximately 1 minute to let the coolant go back to the coolant reservoir before the cartridge is removed.
3. Remove the cartridge from the system. Stow the cartridge upright in the cartridge box at 2 °C to 8 °C with the capillary tips immersed in CE Grade water.

---

**Note:** Replace the CE Grade water in the tray regularly to prevent microbial growth in the tray.

---

## Run the Samples

---

### Prepare the Cartridge After Storage

- If the cartridge has not been used for more than a day, then use the Capillary Rinse - RNA 9000 method to rinse the capillaries.

---

**Note:** To prevent arcing, and before the cartridge is installed in the system, carefully remove any water from around the electrodes and cartridge body.

---

---

## Use an Analysis Parameters File to Analyze the Data

---

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

---

An analysis parameters file contains all of the information required to integrate the data and then identify peaks.


---


**Note:** This procedure is for users who know how to use 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. On the **Project** toolbar, click , browse to the analysis parameters files, and then click **Open**.  
Two analysis parameters files are available. Click the applicable file:
  - **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 on 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 red to show 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 red and the threshold is a grey horizontal line. Any peaks identified in the analysis have a blue

## Analyze the Data

---



marker at the peak start, a red marker at the peak apex, and a green marker at the peak end.

Peaks in the graph have the following shading:

- Green: The peak is identified as a peak in the Marker Table on the Library tab.
- Blue: The peak is identified as 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 include 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 see 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 use the same zoom settings to all of the data files, click .


---


7. Make sure that the integration is satisfactory. If required, then adjust the integration parameters, and then analyze the data again.
8. Examine the electropherogram for the peaks in the Marker Table and Peak Table.
  - a. For each peak in the Marker Table and Peak Table, make sure that the correct peak has a label in the graph.
  - b. If required, adjust **MT** in the Marker Table and **MT** (or **Cal MT**) in the Peak Table.
  - c. If required, adjust **Tol** and **Crit**, and then click .
    - **Tol** is the tolerance used to find a match between a peak in the graph and a peak in the Marker Table or the Peak Table. To use the tolerance as a percentage, type % after the value.

- **Crit** is the peak characteristic used to find a match.
  - **Ctr**: The peak closest to the center of the range is used to find a match.
  - **Ht**: The tallest peak in the range is used to find a match.
  - **Area**: The largest peak in the range is used to find a match.
- 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) On 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) On the **File** toolbar, right-click , and then select **Print (all)**.

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

All changes to the results are saved to the data files. The analysis parameters are included.
- 12. On the **File** toolbar, right-click , and then select **Close (all)**.

All of the data files close.

## Examine the Results

Figure 7-1 ssRNA Ladder: Data Collected with Electrokinetic Injection

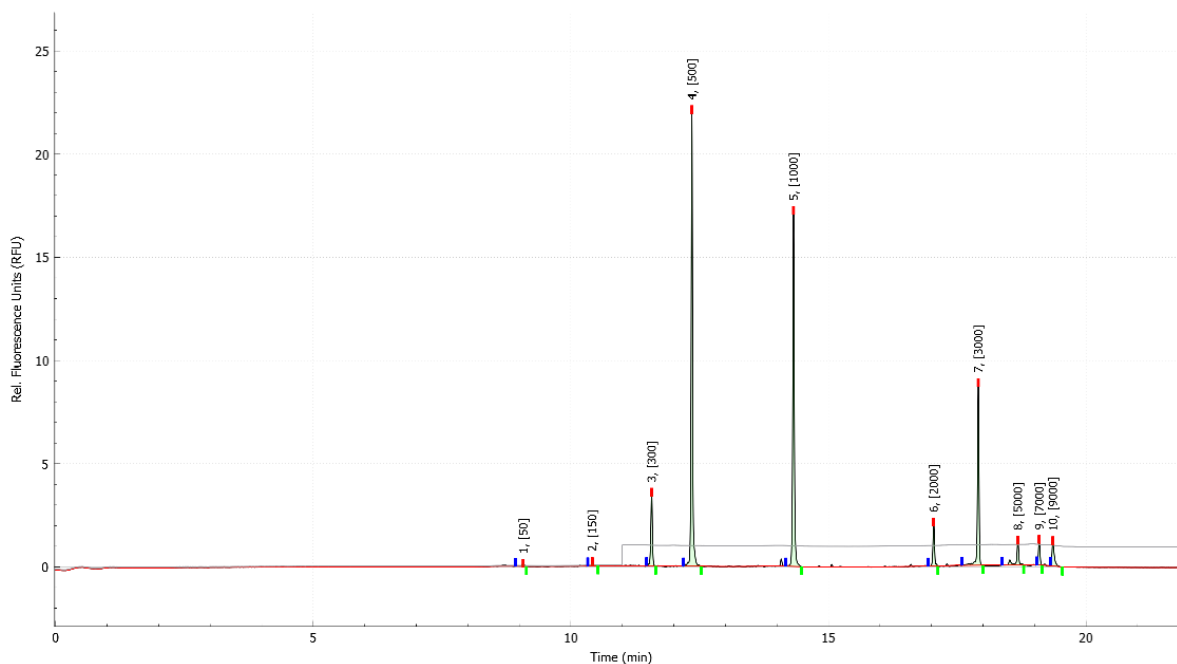
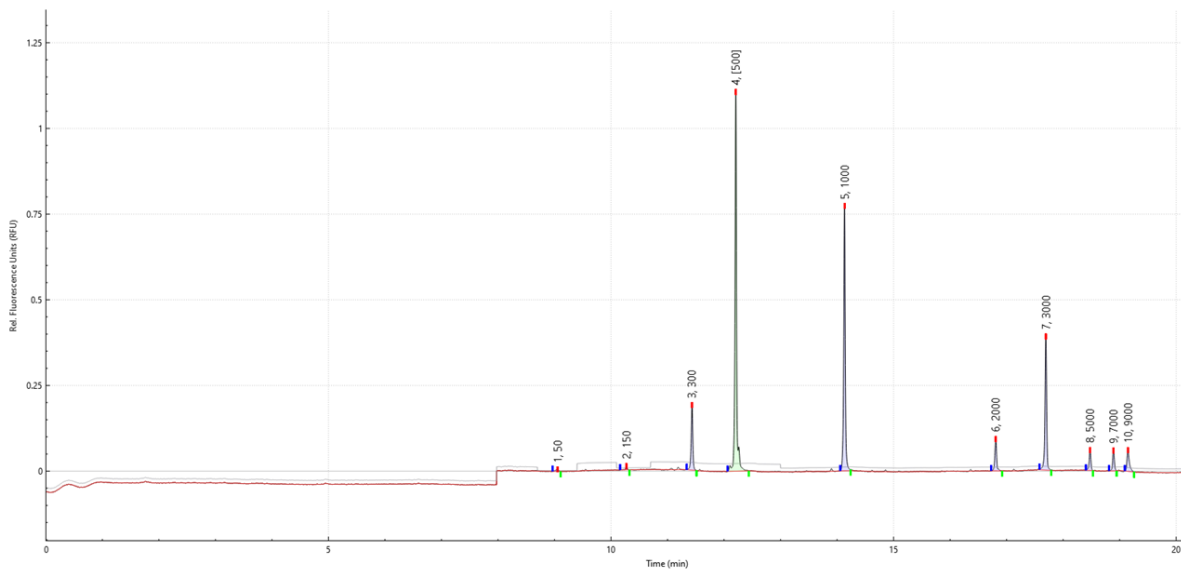


Figure 7-2 ssRNA Ladder: Data Collected with Pressure Injection



---


## Review the Results on the Overlay Tab

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

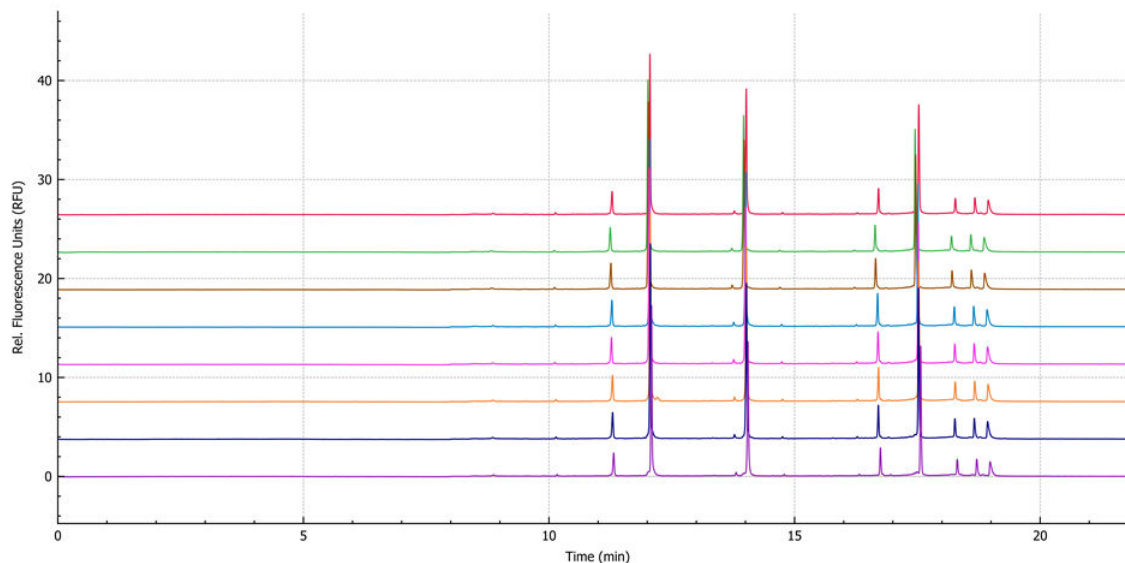
---

**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 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 7-3 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 for the file selected in the Files pane.

3. To change the order of the files in the overlay, in the Files pane, click the file to be moved, and then drag it up or down the list of files.

---

**Note:** The order of the colors assigned to the files does not change after a file is moved.

---

4. To adjust the distance between the traces, move the slider on the right side of the graph up or down.

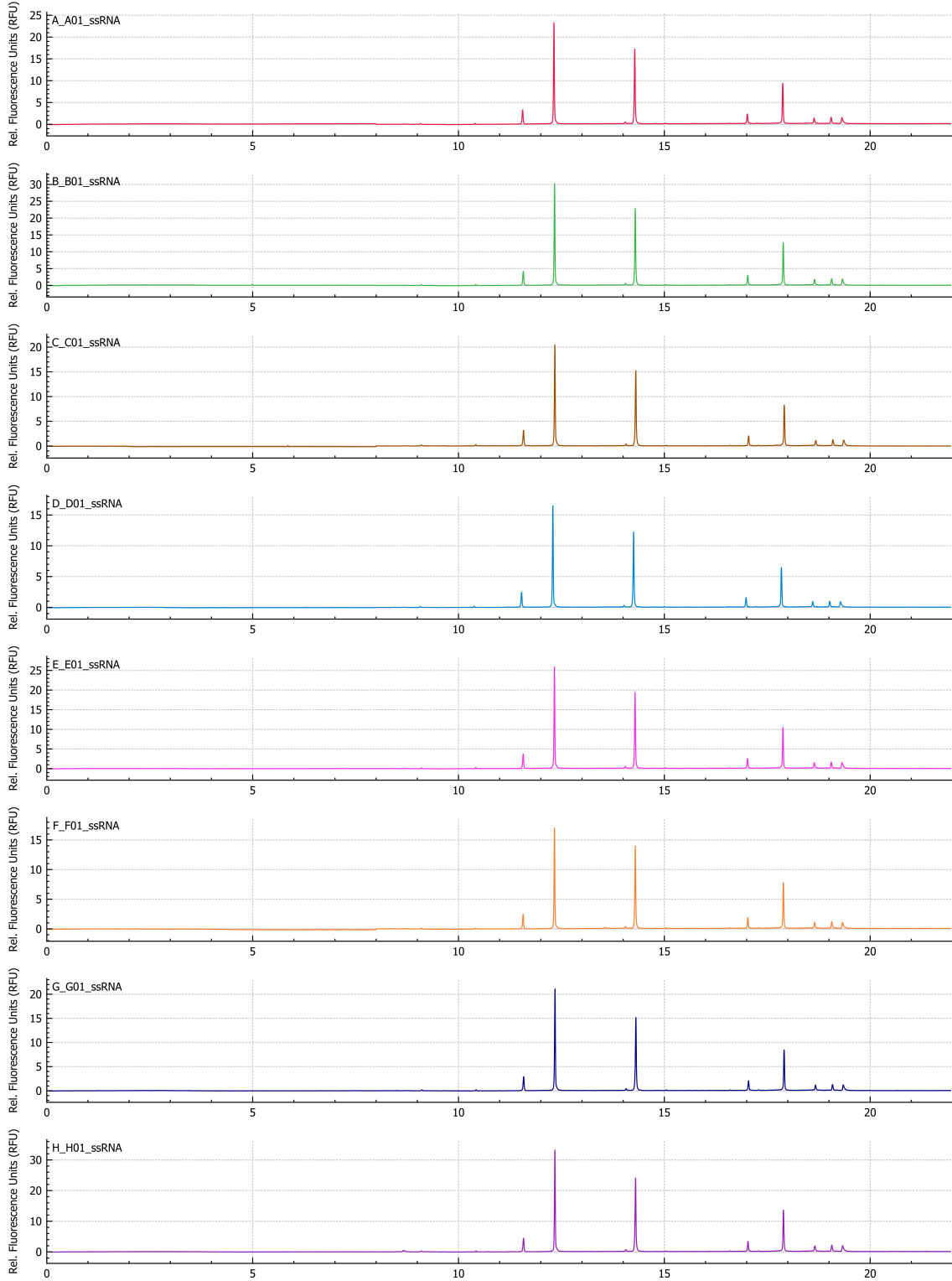
---

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

---

## Analyze the Data

Figure 7-4 Tiled Results





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

**Figure 7-5 Results Table**

ssRNA_A01	Reference - All	Name	Cal MT	Height	Corr. Area	Corr. Area%	Rel. Area	Width 50%	N	Asym.	Res.	μ	S/N P-P	S/N
ssRNA_A01	[1000]	2437	831.02	15.1995	0.60	29.98	0.00	0.0232	2093...	0.93	52.5635	4.6804	311.39	
ssRNA_B01	[1000]	14.2688	830.50	13.7542	0.54	29.70	0.00	0.0230	2134...	0.93	52.9354	4.6722	276.78	1315.42
ssRNA_C01	[1000]	14.2604	829.84	11.6767	0.45	30.29	0.00	0.0229	2143...	0.91	53.0907	4.6749	273.31	1296.15
ssRNA_D01	[1000]	14.2750	829.08	13.4779	0.53	30.22	0.00	0.0228	2163...	0.92	53.1329	4.6702	322.88	1481.81
ssRNA_E01	[1000]	14.2667	831.82	13.0368	0.50	30.75	0.00	0.0227	2189...	0.96	53.3796	4.6729	281.51	1300.50
ssRNA_F01	[1000]	14.3208	830.54	12.9790	0.50	29.83	0.00	0.0226	2222...	0.93	53.7531	4.6552	294.43	1331.49
ssRNA_G01	[1000]	14.2937	830.53	13.0456	0.50	31.13	0.00	0.0229	2162...	0.97	53.1809	4.6640	296.68	1350.21
ssRNA_H01	[1000]	14.2958	831.34	11.4817	0.45	30.64	0.00	0.0228	2178...	0.93	53.3576	4.6634	282.05	1307.22
Mean		14.2781	830.58	13.0814	0.51	30.32	0.00	0.0229	2161...	0.94	53.1742	4.6692	292.38	1355.85
SD		0.0242	0.85	1.1737	0.05	0.49	0.00	0.0002	38696.9	0.02	0.3484	0.0079	17.51	74.47
RSD		0.17	0.10	8.97	9.42	1.62	nan	0.76	1.79	1.98	0.66	0.17	5.99	5.49
Min-Max		14.2437-14.3208	829.08-831.82	11.4817-15.1995	0.45-0.60	29.70-31.13	0.00-0.00	0.0226-0.0232	2093...	0.91-0.97	52.5635-5...	4...	273.31-322.88	1296.15-1481.81
Min-Max		14.2437-14.3208	829.08-831.82	11.4817-15.1995	0.45-0.60	29.70-31.13	0.00-0.00	0.0226-0.0232	2093...	0.91-0.97	52.5635-5...	4...	273.31-322.88	1296.15-1481.81

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

- a. To select the type of analysis, click the list on the right side of the Results Table header.

These options are available:

- **Reference - All:** In the Results Table, show statistics for every peak in the reference file that is 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 in all of the other data files.
- **Named Peaks:** In the Results Table, show statistics for all of the named peaks in 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:** System suitability testing is different than the other reports. For more information, refer to the section "System Suitability Testing" in the documents: *Operator Guide* or *Software Help System*.

If the migration times of the peak apexes in the data file are within 5% of the peak apexes in the reference file, then a match is made.

- b. To select the reference file, click the list on the left.

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

## Analyze the Data

---

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

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



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

6. (Optional) To use a different reference file or see a different type of analysis, do step 5 again.
7. (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.

---

8. (Optional) Click **File > Print**.  
The contents of the Overlay tab are printed in the current report template.
9. (Optional) On 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.
10. On the **File** toolbar, right-click , and then select **Close (all)**.  
All of the data files close.

## Guidance for Acceptance Criteria

To create acceptance criteria for use with this kit for SOPs or other purposes, use parameters that are inherent to the quality of the separation and attributes that show critical sample qualities. Differences between gel and capillary lots and different systems might cause variations in absolute migration times.

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

# 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 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 website. Refer to the section: [Download and Configure the Required Files \(Waters Empower™ Software\)](#).

---

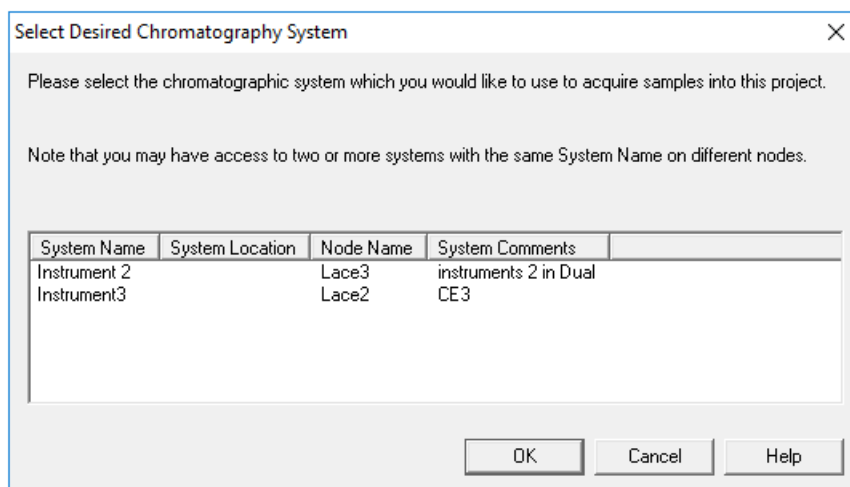
Instrument methods can also be created in the Method Editors for BioPhase System software. Refer to the documents: *Operator Guide* and *Software Help System*.

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

The following methods are available.

- Conditioning Method-RNA 9000: Conditions the capillaries before first use.
  - Separation Method with Electrokinetic Injection - RNA 9000: Does the separation of the sample with an electrokinetic injection of the sample.
  - Separation Method - Pressure Injection - RNA 9000: Does the separation of the sample with a pressure 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.
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 name of 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 with the Method Settings tab in front.

---

**Note:** The Instrument Method Editor 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 Instrument Method Editor: Method Settings

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

Figure 8-3 Instrument Method Editor: Method Program

#	Action	Duration	Pressure (psi)	Pressure Direction	Inlet	Outlet	Voltage (kV)	Ramp Time (min)	Voltage Polarity	Advance After	Auto Zero (min)	Data Collection	Mode	Cor
1	Rinse	5.0 min	50.0		Water	Waste								
2	Rinse	5.0 min	20.0		Acidic Condit...	Waste								
3	Rinse	2.0 min	20.0		Water	Waste								
4	Rinse	10.0 min	50.0		Nucleic Acid ...	Waste								
5	Wait	0.0 min			Water Dip 1	Water Dip 1				0 actions				
6	Wait	0.0 min			Water Dip 2	Water Dip 2				0 actions				
7	Separate	20.0 min	0.0	None	Nucleic Acid ...	Nucleic Acid ...	6.0	2.0	Reverse	0 actions	8.0	False		

## Run the Samples with the Waters Empower™ Software

---

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 %. 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 a prompt is shown, then 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 is not available unless the window is closed and then opened again.

---

8. To create the other instrument methods and method sets, repeat steps 3 through 7.

## Create the Sample Set Method

---

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

---

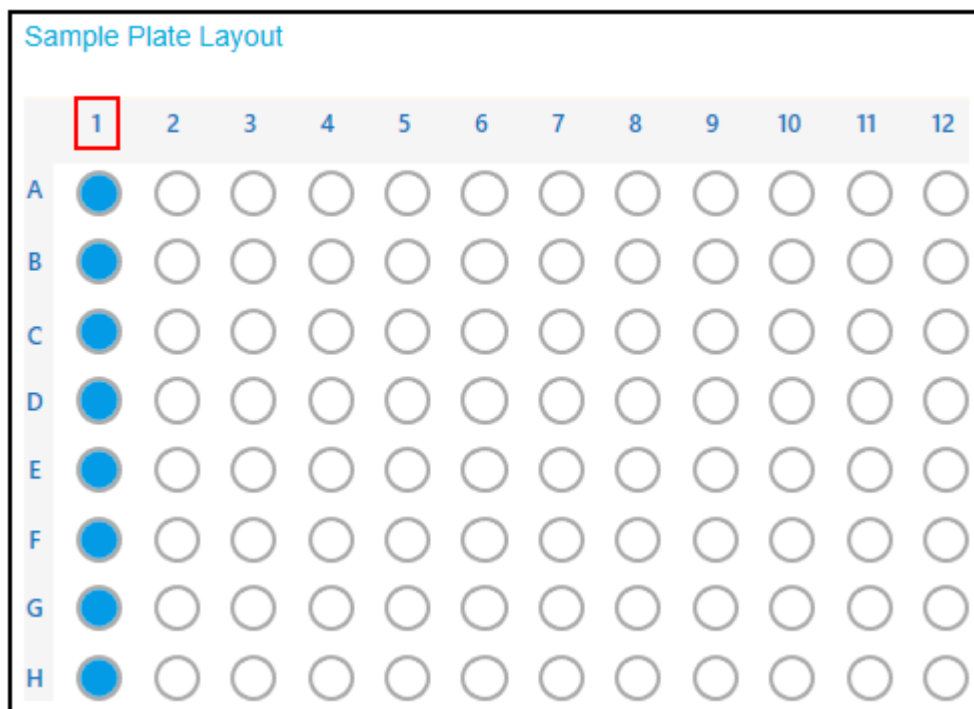
This procedure gives instructions for creating a sample set method with the ssRNA Ladder in the first column, followed by 16 samples in the second and third columns.

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

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

2. Click **New Sample Set Method**.  
The Sample Set Method Editor opens to the Sample Plate Setup tab.
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 changes to show the selected wells.

Figure 8-4 Sample Plate Layout Pane



5. In the Sample Set Summary table add the required sample information. In row 2 through row 9, do the following:
  - a. In the **Sample Name** cell, type a name.
  - b. Click the **Method Set Name** cell, and then select **Separation Method with Electrokinetic Injection - RNA 9000** or another separation method.

---

**Tip!** After the method set is selected 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 identify where it occurs, and then make the required change. If there are no errors, then the Validation pane is not shown.
9. Save the sample set method:
  - a. Click **SAVE AS**.

---

**Note:** If errors occurred, then the **SAVE AS** button is not available. Correct all of the errors in the Validation pane, and then click **SAVE AS**.

---

The Save Sample Set dialog opens.

- b. In the **Sample Set 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 %. 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 **Description** field, type information.
  - d. Click **Save**.
  - e. To acknowledge the saved method, click **OK**.
- 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. If required, click the applicable button to print or save the plate layouts.  
Refer to the section: "Print Preview Dialog" in the document: *Software Help System*.
    - d. Close the Print Preview dialog.
  11. Close the Method Editors for BioPhase System software.  
The Run Samples window is shown.


## Start the Sample Set Method

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



**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 changes 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. To set the order of access for the wells during the run, click .
  - e. To save the changes, click **OK**.
  - f. Close the dialog.
- 

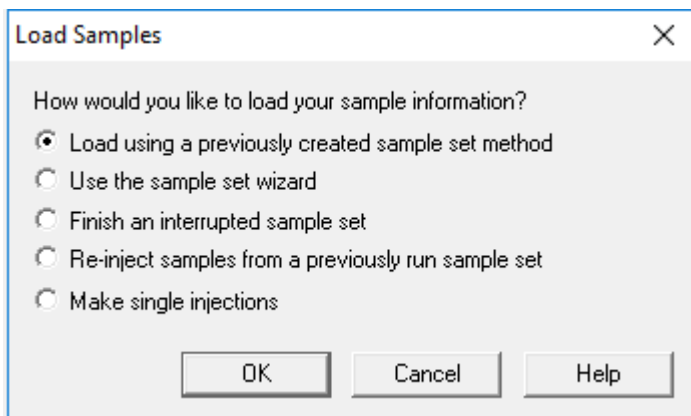
**Tip!** To permanently configure the plate type, click **Customize > Defaults**, click **Plates**. In the Define Plates For Sample Set Method dialog, do steps 4.b through 4.e, and then, in the Run Samples Defaults dialog, click **OK**.

---

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

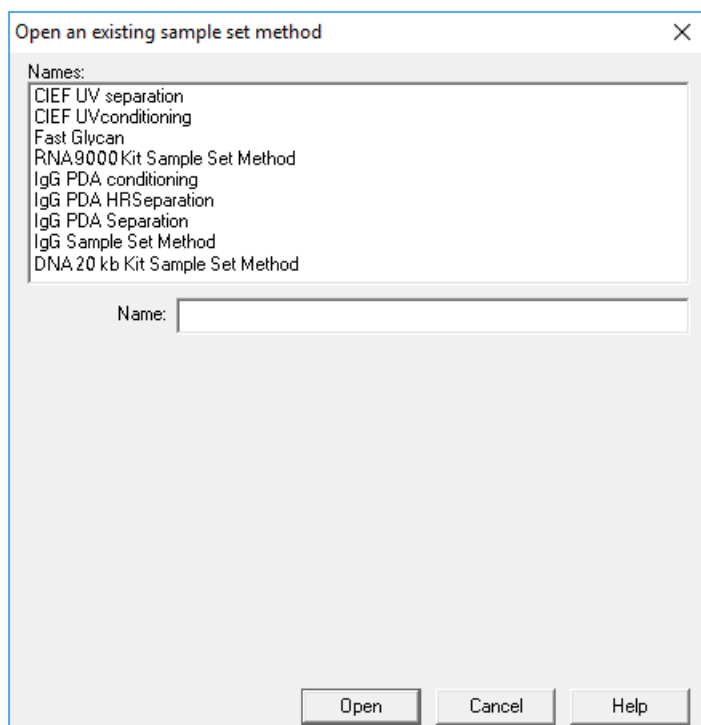
5. Click  (**Load Sample Set**).

**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**, 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 **Plate/Well**, **# of Injs**, **SampleName**, **Function**, and **Method Set / Report or Export Method** check boxes.
  - c. Click **OK**.  
The table changes 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 changes are required, then edit the method in the Method Editors for BioPhase System software. Changes to the instrument methods or method sets are automatically transmitted to the sample set method.
10. In the Waters Empower™ Software Project window, click  (Start).

**Figure 8-11 Run Sample Set Dialog**

Run Sample Set

Name for this sample set : One column cIEF

Sample set method name : One column cIEF

Settings for this Sample Set

Wait For User

Run Mode : Run Only

Suitability Mode : Continue on Fault

Printer :

Shutdown Method : Capillary Rinse

Do Not Run Shutdown Method During User Abort

11. If required, edit the information:

- 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, in the Sample Set Method window, the text in the row for the sample being acquired is red.

---

**CAUTION: Potential Data Loss. During a run, do not start any actions from the BioPhase 8800 driver for Empower™ Direct Control pane, even if the system status is idle. Any actions that are started might cause interference with data acquisition.**

---

## Monitor the Run in the Waters Empower™ Software

---

**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.

---

**CAUTION: Potential System Damage.** If the run is stopped and will not be started again, then use the shutdown method to rinse the capillaries before the cartridge is stowed. If the capillaries are not rinsed, then electrolyte salt crystals or precipitate can collect and might cause blocked capillaries, incorrect pressure sealing, errors when the samples are injected, arcing, or current leakage.

---

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

---

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

---

**CAUTION: Potential Wrong Result.** If the samples have been inside of the system for more than 24 hours, sample degradation might have occurred. Discard the samples before the run is started again.


---

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

---

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

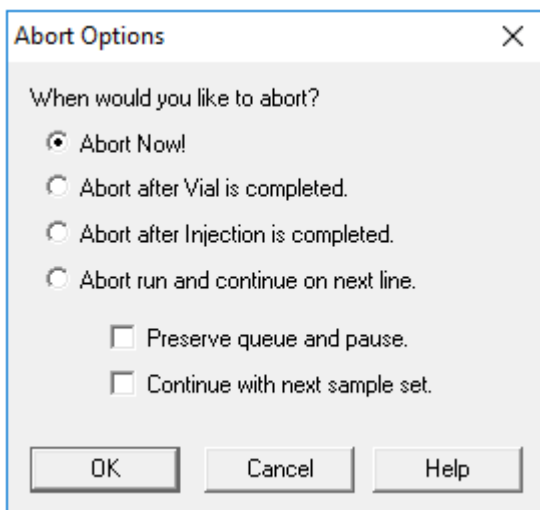
---

1. To stop the run, click  (**Abort**).
- 


**Note:** Do not use the **Stop** button in the Direct Control pane. That button is only applicable to functions that are started from the Direct Control pane.

---

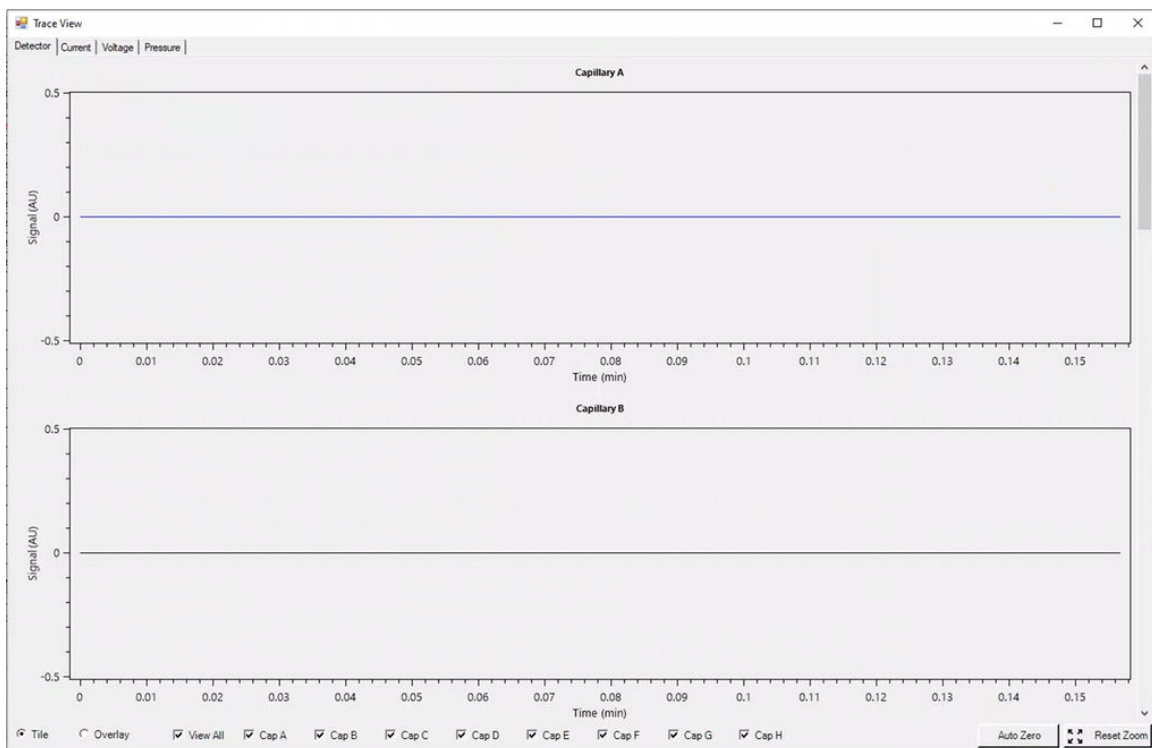
**Figure 8-12 Abort Options Dialog**





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

2. To see the data during acquisition, in the Direct Control pane, click  (**Monitor**).

**Figure 8-13 Trace View Window**





3. If required, do any of the following:
  - To see current, voltage, or pressure, open the applicable tab in the top left.
  - To see one graph with the data for all of the capillaries, in the bottom left, click **Overlay**.
  - To see data for some of the capillaries, select or clear the check boxes at the bottom of the window for the capillaries of interest.
  - To see 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 go back to the original data dimensions, in the bottom right, click **Reset Zoom**.
  - To see a different area of a zoomed plot, right-click the X- or Y-axis, and then drag.
4. If required, at the bottom right, right-click **Auto Zero**.  
The detector signal is set to zero.
5. Wait until  (**Abort**) changes to  (**Start**).  
A delay between data acquisition and when all of the data is saved might occur. The green button identifies that all of the data is saved.
6. If required, discard the samples and reagents. Refer to the section: [Waste Disposal](#).
7. If required, stow the cartridge. Refer to the section: [Stow the Cartridge After the Run](#).

# Troubleshooting

# 9

After each corrective action is complete, we recommend that the analysis be done 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	<ol style="list-style-type: none"><li>1. The ID chip on the cartridge is not clean.</li><li>2. The contact pins on the system are not clean.</li><li>3. The BioPhase 8800 system firmware is not up to date.</li></ol>	<ol style="list-style-type: none"><li>1. Moisten a lint-free laboratory wipe or cotton swab with ethanol or isopropanol, and then clean the surface of the ID chip. Let the ID chip air-dry before the cartridge is installed.</li><li>2. Moisten a lint-free laboratory wipe or cotton swab with ethanol or isopropanol, and then clean the contact pins. Let the pins air-dry before the cartridge is installed.</li><li>3. Do the following:<ol style="list-style-type: none"><li>a. On the BioPhase 8800 system front panel, touch the icon in the top left corner.</li><li>b. Record the firmware version.</li><li>c. Contact <a href="http://sciex.com/request-support">sciex.com/request-support</a>.</li></ol></li></ol>

Symptom	Possible Cause	Corrective Action
Error found at start of run	<ol style="list-style-type: none"> <li>1. The optical scan at the beginning of the run failed because of condensation on the cartridge window.</li> <li>2. When the optics door was opened and closed, a sensor error occurred.</li> </ol>	<ol style="list-style-type: none"> <li>1. In the shutdown method, increase the <b>Sample Storage</b> temperature to 20 °C to prevent condensation.</li> <li>2. Turn off, and then turn on the BioPhase 8800 system.  To prevent this from occurring again, always use the applicable procedure to change the filters. Refer to the sections: "Install a UV Filter" and "Install the Filters for the LIF Detector" in the document: <i>Operator Guide</i>.</li> </ol>

## Troubleshooting

Symptom	Possible Cause	Corrective Action
Broad peaks, poor resolution	<ol style="list-style-type: none"> <li>1. The capillary tip is damaged.</li> <li>2. The sample concentration is too high.</li> <li>3. The capillary is blocked.</li> <li>4. The internal surface of the capillary is contaminated.</li> </ol>	<ol style="list-style-type: none"> <li>1. Do the following actions: <ul style="list-style-type: none"> <li>• Use a magnifying lens to examine the capillary tip.</li> <li>• Use a lint-free laboratory wipe to clean the capillary inlets carefully in an outward direction.</li> <li>• Make sure that the capillary tip extends approximately 2 mm from the gold cannula electrode.</li> <li>• Make sure that the capillary tip has a straight cut and is not broken. If the capillary cannot be used, then refer to the section: <a href="#">Options for a Blocked or Damaged Capillary</a>.</li> </ul> </li> <li>2. Do one or all of the following: <ul style="list-style-type: none"> <li>• Decrease the <b>Duration</b> in the <b>Inject</b> action in the separation method to inject less sample. If the results are not satisfactory, then decrease the <b>Pressure</b> or <b>Voltage</b>.</li> <li>• Dilute the sample again with the sample diluent.</li> </ul> </li> <li>3. Refer to the section: <a href="#">Options for a Blocked or Damaged Capillary</a>.</li> <li>4. Edit the sequence to omit the contaminated capillary or replace the cartridge.</li> </ol>

Symptom	Possible Cause	Corrective Action
Broad peaks, poor resolution (continued)	<ol style="list-style-type: none"> <li>1. The cartridge is no longer serviceable.</li> <li>2. The SYBR™ Green II RNA Gel Stain concentration in the gel buffer is too high.</li> <li>3. The Nucleic Acid Extended Range Gel was kept at ambient temperature too long.</li> <li>4. The capillary cartridge was kept at ambient temperature for more than a week.</li> </ol>	<ol style="list-style-type: none"> <li>1. Do a test separation of the ssRNA Ladder. If the peak widths are consistently wider than previous runs, then replace the cartridge.</li> <li>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: <a href="#">Load the Reagent Inlet and Outlet Plates</a></li> <li>3. Prepare new Nucleic Acid Extended Range Gel. Make sure to keep it between 2 °C and 8 °C. Minimize the time that the gel is at ambient temperature.</li> <li>4. Replace the cartridge.</li> </ol>

## Troubleshooting

Symptom	Possible Cause	Corrective Action
Carryover	<ol style="list-style-type: none"> <li>1. The sample concentration is too high.</li> <li>2. The reagent plate is contaminated with sample.</li> </ol>	<ol style="list-style-type: none"> <li>1. Do one or all of the following: <ul style="list-style-type: none"> <li>• Decrease the <b>Duration</b> in the <b>Inject</b> action in the separation method to inject less sample. If the results are not satisfactory, then decrease the <b>Pressure</b> or <b>Voltage</b>.</li> <li>• Dilute the sample again with the sample diluent.</li> </ul> </li> <li>2. In the separation method, add one or more water dip steps after sample injection. With the edited method, do the following steps: <ol style="list-style-type: none"> <li>a. Create a new sequence that uses this separation method.</li> <li>b. Prepare new reagent plates for the new sequence. Refer to the section: <a href="#">Load the Reagent Inlet and Outlet Plates</a>.</li> </ol> </li> </ol>
Extra peaks	<ol style="list-style-type: none"> <li>1. Non-nucleic acid components of the sample interacted with the dye.</li> <li>2. The plasticware used during sample preparation or the sample plate is contaminated with materials that interact with the dye.</li> <li>3. Light scattering caused by particulates larger than 1 µm in the gel buffer occurred.</li> </ol>	<ol style="list-style-type: none"> <li>1. Prepare the sample again. Make sure that it is pure.</li> <li>2. Use clean plasticware and a clean sample plate to prepare the sample again.</li> <li>3. Before the gel buffer is added to the reagent plates, put the sample through a syringe filter with a 0.45 µm pore-size membrane.</li> </ol>

Symptom	Possible Cause	Corrective Action
High current	<ol style="list-style-type: none"> <li>1. The gel buffer is contaminated.</li> <li>2. The positions of the reagents in the reagent plate do not agree with the plate layouts in the sequence.</li> <li>3. The Nucleic Acid Extended Range Gel was kept at ambient temperature too long.</li> </ol>	<ol style="list-style-type: none"> <li>1. Prepare the inlet and outlet reagent plates again to replace the gel buffer.</li> <li>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 use the plate layout to prepare the plates again. Refer to the section: <a href="#">Plate Layouts</a>.</li> <li>3. Prepare new Nucleic Acid Extended Range Gel. Make sure to keep it between 2 °C and 8 °C. Minimize the time that the gel is at ambient temperature.</li> </ol>

## Troubleshooting

Symptom	Possible Cause	Corrective Action
Low signal	<ol style="list-style-type: none"> <li>1. The capillary tip is dirty or plugged.</li> <li>2. The sample volume is too low.</li> <li>3. The salt concentration in the sample is too high.</li> <li>4. The initial nucleic acid concentration is too low.</li> <li>5. Degradation of the nucleic acids in the sample occurred because of exposure to RNase or other nucleases.</li> </ol>	<ol style="list-style-type: none"> <li>1. Rinse the capillary. Refer to the section: <a href="#">Remove Blockage from a Capillary</a>. Do a blank separation run to equilibrate the capillary surface.</li> <li>2. Make sure that there is a minimum of 50 µL of sample in the sample well.</li> <li>3. Do one or all of the following: <ul style="list-style-type: none"> <li>• If the separation method uses an electrokinetic injection, then use a pressure injection.</li> <li>• Prepare the sample at a lower ionic strength.</li> </ul> </li> <li>4. Do one or all of the following: <ul style="list-style-type: none"> <li>• Increase the <b>Duration</b> up to 15 seconds in the <b>Inject</b> action in the separation method to inject more sample. If the results are not satisfactory, then increase the <b>Pressure</b> or <b>Voltage</b>.</li> <li>• Prepare the sample again, with the recommended concentration. The recommended concentration is 50 ng/mL to 50 µg/mL.</li> </ul> </li> <li>5. Prepare the sample again. Make sure that the sample is not exposed to RNase. Refer to the section: <a href="#">Best Practices for Working with RNA</a>.</li> </ol>



Symptom	Possible Cause	Corrective Action
Low current	<ol style="list-style-type: none"> <li>1. The capillary is blocked.</li> <li>2. The position of the gel buffer in the reagent plate does not agree with the sequence.</li> </ol>	<ol style="list-style-type: none"> <li>1. Refer to the section: <a href="#">Remove Blockage from a Capillary</a>.</li> <li>2. Make sure that the position of the gel buffer during the voltage separation step is correct. If the positions are not correct, then use the plate layout to prepare the plates again. Refer to the section: <a href="#">Load the Reagent Inlet and Outlet Plates</a>.</li> </ol>
Low or unsteady current	<ol style="list-style-type: none"> <li>1. The capillary tip is blocked or contaminated.</li> <li>2. The gel buffer has air bubbles.</li> </ol>	<ol style="list-style-type: none"> <li>1. Refer to the section: <a href="#">Remove Blockage from a Capillary</a>. If the current is low or unsteady, then replace the cartridge.</li> <li>2. Do one or all of the following: <ul style="list-style-type: none"> <li>• Use a centrifuge to spin the plate at <math>30 \times g</math> for 5 minutes to remove air bubbles.</li> <li>• De-gas the gel buffer with 5 inches Hg to 15 inches Hg vacuum for 5 minutes.</li> </ul> </li> </ol>

## Troubleshooting

Symptom	Possible Cause	Corrective Action
No electrical current during separation	<ol style="list-style-type: none"> <li>1. The capillary is damaged.</li> <li>2. The electrode is broken or bent.</li> <li>3. The capillary tip is blocked or contaminated.</li> <li>4. The positions of the reagents in the reagent plate do not agree with the plate layouts in the sequence.</li> <li>5. A capillary is filled with air bubbles.</li> </ol>	<ol style="list-style-type: none"> <li>1. Refer to the section: <a href="#">Options for a Blocked or Damaged Capillary</a>.</li> <li>2. Replace the cartridge.</li> <li>3. Refer to the section: <a href="#">Remove Blockage from a Capillary</a>.</li> <li>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 use the plate layout to prepare the plates again. Refer to the section: <a href="#">Plate Layouts</a>.</li> <li>5. Do one or all of the following: <ul style="list-style-type: none"> <li>• Make sure that the wells of the sample and reagent plates contain sufficient solution.</li> <li>• Make sure that the positions of the reagents in the reagent plates agree with the plate layouts. If the positions are not correct, then use the plate layout to prepare the plates again. Refer to the section: <a href="#">Plate Layouts</a>.</li> <li>• Use a centrifuge to spin the plate at <math>30 \times g</math> for 5 minutes to remove air bubbles.</li> </ul> </li> </ol>

Symptom	Possible Cause	Corrective Action
No peaks	<ol style="list-style-type: none"> <li>1. The lifetime of the LIF detector laser has been exceeded.</li> <li>2. The wavelength of the emission filter is not correct.</li> <li>3. The method parameters are incorrect.</li> <li>4. Air bubbles in a well of the sample plate prevent sample injection.</li> <li>5. The capillary window is blocked.</li> <li>6. A capillary is blocked and has a low current.</li> <li>7. The sample volume is too low.</li> <li>8. The capillary was cleaned with a basic solution such as 0.1 N NaOH.</li> </ol>	<ol style="list-style-type: none"> <li>1. Contact SCIEX Technical Support at <a href="http://sciex.com/request-support">sciex.com/request-support</a>.</li> <li>2. Make sure that the emission filter wavelength is 520 nm.</li> <li>3. Do the following: <ul style="list-style-type: none"> <li>• Open the separation method in the software, and then make sure that the method is correct. Refer to the section: <a href="#">Separation Method with Pressure Injection</a> or <a href="#">Separation Method with Electrokinetic Injection</a>.</li> <li>• 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 use the plate layout to prepare the plates again. Refer to the section: <a href="#">Plate Layouts</a>.</li> </ul> </li> <li>4. Use a centrifuge to spin the plate at <math>30 \times g</math> for 5 minutes to remove air bubbles.</li> <li>5. Examine the capillary window. Make sure that the window is clean and the path is clear. Refer to the section: <a href="#">Examine the Capillary Cartridge</a>.</li> <li>6. Refer to the section: <a href="#">Options for a Blocked or Damaged Capillary</a>.</li> <li>7. Make sure that there is 100 <math>\mu\text{L}</math> of sample in the sample well.</li> <li>8. Replace the cartridge.</li> </ol>

## Troubleshooting

Symptom	Possible Cause	Corrective Action
No peaks (continued)	<ol style="list-style-type: none"> <li>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.</li> <li>2. A pipetting error occurred during preparation of the sample.</li> <li>3. The salt concentration in the sample is too high.</li> </ol>	<ol style="list-style-type: none"> <li>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 use the plate layout to prepare the plates again. Refer to the section: <a href="#">Plate Layouts</a>.</li> <li>2. Prepare a new sample.</li> <li>3. Do one or all of the following: <ul style="list-style-type: none"> <li>• If the separation method uses an electrokinetic injection, then use a pressure injection.</li> <li>• Prepare the sample again at a lower ionic strength.</li> </ul> </li> </ol>
Slower migration time, with or without concurrent low current	<ol style="list-style-type: none"> <li>1. The capillary tip is blocked or contaminated.</li> </ol>	<ol style="list-style-type: none"> <li>1. Refer to the section: <a href="#">Remove Blockage from a Capillary</a>.</li> </ol>
Dramatic shift in migration time between runs on the same day	<ol style="list-style-type: none"> <li>1. The capillary has not been conditioned sufficiently.</li> <li>2. The gel buffer has evaporated.</li> </ol>	<ol style="list-style-type: none"> <li>1. Condition the capillary. Refer to the section: <a href="#">Condition the Capillaries</a>. Do a blank separation run to equilibrate the capillary surface.</li> <li>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.</li> </ol>

Symptom	Possible Cause	Corrective Action
Spikes in electropherogram	1. The gel buffer has air bubbles.	<p>1. Do one or all of the following:</p> <ul style="list-style-type: none"> <li>• Use a centrifuge to spin the plate at <math>30 \times g</math> for 5 minutes to remove air bubbles.</li> <li>• De-gas the gel buffer with 5 inches Hg to 15 inches Hg vacuum for 5 minutes.</li> </ul> <p>If air bubbles are still present, then prepare new gel buffer. Do not mix the buffer with a vortex mixer. Carefully invert the tube a minimum of 20 times.</p>
Broad or split peaks	<p>1. After the denaturation step, there is still secondary structure in the nucleic acid.</p> <p>2. The cooling step after denaturation was too slow.</p> <p>3. The SYBR™ Green II RNA Gel Stain concentration in the gel buffer is too high.</p> <p>4. The cartridge is no longer serviceable.</p>	<p>1. Dilute the samples with Sample Loading Solution in place of nuclease-free water.</p> <p>2. Cool the samples immediately to prevent the formation of secondary structure.</p> <p>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: <a href="#">Load the Reagent Inlet and Outlet Plates</a>.</p> <p>4. Do a test separation of the ssRNA Ladder. If the peak widths are consistently wider than previous runs, then replace the cartridge.</p>

## Troubleshooting

---

Symptom	Possible Cause	Corrective Action
Saturated peaks	<ol style="list-style-type: none"><li>1. In the <b>Method Settings</b> for the separation method, the <b>PMT Gain</b> is too high.</li><li>2. The sample concentration is too high.</li></ol>	<ol style="list-style-type: none"><li>1. Decrease the value for the <b>PMT Gain</b>.</li><li>2. Do one or all of the following:<ul style="list-style-type: none"><li>• Decrease the <b>Duration</b> in the <b>Inject</b> action in the separation method to inject less sample. If the results are not satisfactory, then decrease the <b>Pressure</b> or <b>Voltage</b>.</li><li>• Dilute the sample again with the sample diluent.</li></ul></li></ol>
Unstable baseline	<ol style="list-style-type: none"><li>1. The concentration of dye in the inlet reagent plate is not the same as the concentration in the outlet reagent plate.</li></ol>	<ol style="list-style-type: none"><li>1. Prepare sufficient gel buffer for both the inlet and outlet reagent plates.</li></ol>

## Remove Blockage from a Capillary

1. Rinse the capillary with CE Grade water at 75 psi for 10 minutes.
2. Use CE Grade water to clean the capillary inlets.
3. Use a lint-free laboratory wipe to clean the capillary inlets carefully in an outward direction.
4. If the blockage cannot be removed, then edit the sequence to ignore 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](#).

# Hazardous Substance Information

---

# A

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

Hazard classification according to HCS 2012.

## Acid wash/regenerating solution



**DANGER! Causes severe skin burns and eye damage.**

## Nucleic Acid Extended Range Gel



**DANGER! May damage fertility or the unborn child.**

## SYBR™ Green II RNA Gel Stain<sup>5</sup>

**WARNING! Combustible liquid.**

## Other Reagents

These components are not classified as hazardous:

- CE Grade water
- LIF Performance Test Mix

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

---

<sup>5</sup> SYBR™ is a trademark of the Life Technologies Corporation. SYBR™ Green II RNA Gel Stain is not available for resale.

# Download Required Files

---

# B

Do the procedure for the software in use:

- BioPhase software: Refer to the section: [Download and Configure the Required Files \(BioPhase Software\)](#).
- Waters Empower™ software: 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 at [sciex.com](http://sciex.com). Use the following instructions to download, extract, and then copy the files to the applicable location.

---

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

---

1. Go to [sciex.com/software-downloads](http://sciex.com/software-downloads). In the More software downloads section, click **BioPhase Resources**.
2. Click **BioPhase Project Files 1.4**.
3. In File Explorer, right-click the `BioPhase 1.4.zip` file, and then click **Extract All**.
4. Browse to a location, click **Select Folder**, and then click **Extract**. The extracted files are copied to the selected file path.
5. Move the extracted files to the correct locations:

---

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

---

- a. Drag the `BioPhase 1.4\Projects\RNA 9000` folder to the `C:\BioPhase\Projects` folder.
- b. Drag the `BioPhase 1.4\Reagents\RNA 9000` folder to the `C:\BioPhase\Reagents` folder.
- c. Drag the `BioPhase 1.4\Data Analysis\RNA 9000` folder to the `C:\BioPhase\Data Analysis` folder.



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

Files for the RNA 9000 Purity & Integrity kit are available on [sciex.com](https://sciex.com). Use the following instructions to download, extract, and then copy the files to the applicable location.

1. Go to [sciex.com/software-downloads](https://sciex.com/software-downloads). In the More software downloads section, click **BioPhase Driver Resources**.
2. Click **BioPhase Method Files 1.4**.
3. In File Explorer, right-click the `BioPhase-Empower-Method-Files-1.4.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 files are extracted and then 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.

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


Inlet Reagents from Reagent Set:








Name	Viscosity	Color
Water	0.89	 SkyBlue
Acidic Conditioning Solution (0.1M HCl)	0.89	 Red
Water Dip 1	0.89	 SkyBlue
Water Dip 2	0.89	 SkyBlue
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**

Outlet Reagents from Reagent Set:



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

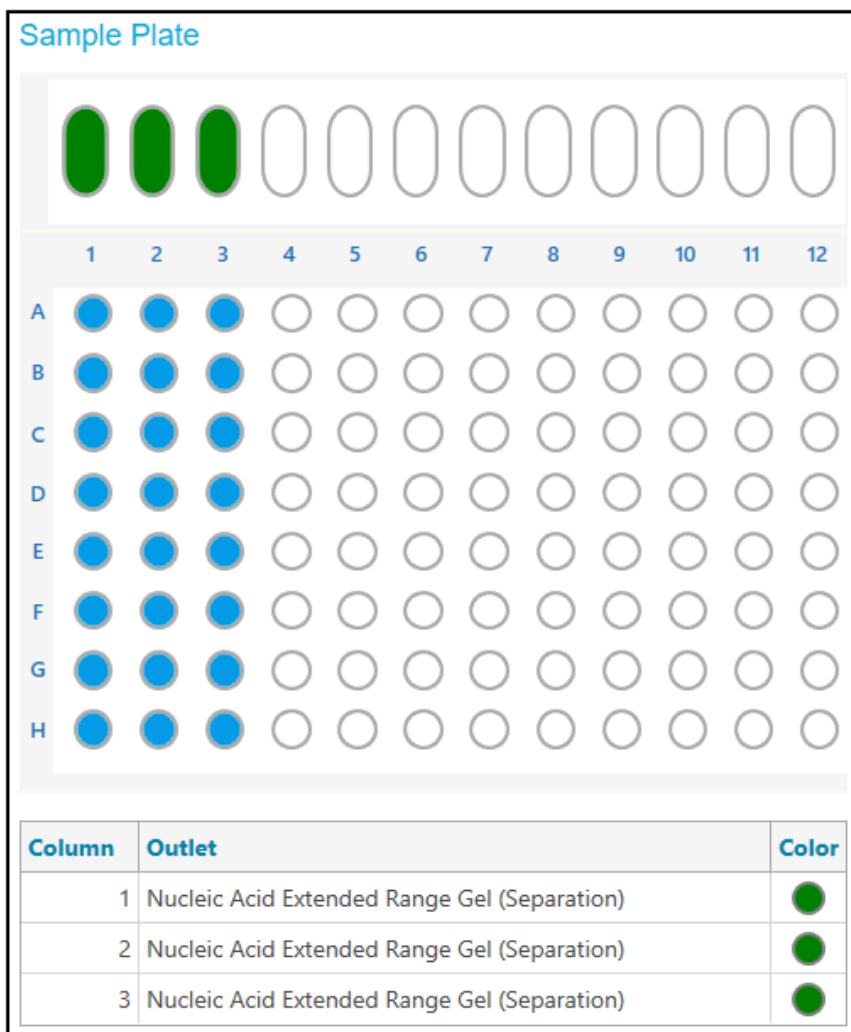
## Plate Layouts

**Note:** The following figures show the plate layouts for the sequence supplied with the software. If more samples have been added or the reagent positions have been changed, 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



## Reagents, Plate Layouts, and Methods

### 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



# Methods

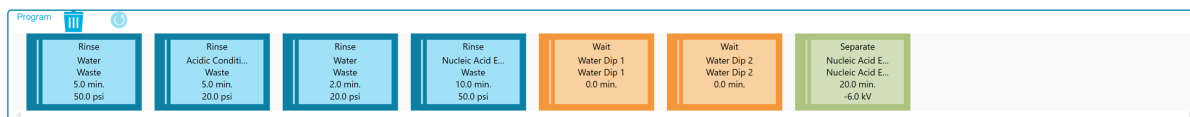
For instructions to create a method, refer to the document: *Software Help System*.

## Conditioning Method

Figure C-5 Method Settings for the Conditioning Method

<b>Temperature</b>		<b>Detector Type</b>	
Capillary Cartridge	<input type="text" value="20.0"/> °C <input checked="" type="checkbox"/> Wait	<input type="radio"/> UV	Wavelength <input type="text" value="220"/> nm
Sample Storage	<input type="text" value="10.0"/> °C <input type="checkbox"/> Wait	<input type="checkbox"/> Wait	
<b>Cartridge Settings</b>		<input checked="" type="radio"/> LIF	Emission Wavelength <input type="text" value="520"/> nm
Capillary Length	<input type="text" value="30.0"/> cm	<input checked="" type="checkbox"/> Wait	PMT Gain <input type="text" value="100"/>
Capillary Type	<input type="text" value="Bare Fused Silica"/>	<input type="radio"/> No Detector	
<b>Current Limits</b>		<b>Data</b>	
<input checked="" type="checkbox"/> Enable current limiting when using voltage		Data Collection Rate	<input type="text" value="8"/> Hz
Maximum Current	<input type="text" value="600"/> μA	Peak Width @ 50% Height	<input type="text" value="1"/> sec









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



## Reagents, Plate Layouts, and Methods

**Figure C-7 Summary of Actions in the Conditioning Method**

Method Duration: 42.0 min.    Number of Actions: 7

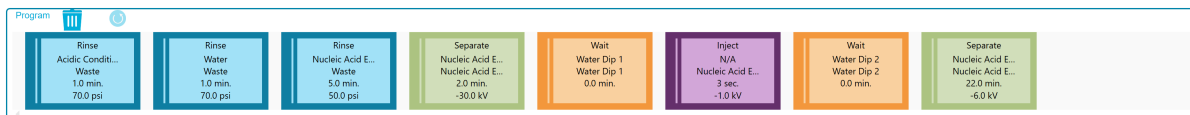
	Settings	Capillary Cartridge: 20.0 °C, Wait Capillary Length: 30.0 cm Capillary Type: Bare Fused Silica Current Limit: 600 µA, Enabled	Sample Storage: 10.0 °C Detector Type: LIF, 520 nm, Wait, PMT Gain: 100 Peak Width: 1 sec. Data Rate: 8 Hz
	Rinse	Duration: 5.0 min. 50.0 psi	Inlet: Water Outlet: Waste
	Rinse	Duration: 5.0 min. 20.0 psi	Inlet: Acidic Conditioning Solution (0.1M HCl) Outlet: Waste
	Rinse	Duration: 2.0 min. 20.0 psi	Inlet: Water Outlet: Waste
	Rinse	Duration: 10.0 min. 50.0 psi	Inlet: Nucleic Acid Extended Range Gel (Rinse) Outlet: Waste
	Wait	Duration: 0.0 min.	Inlet: Water Dip 1 Outlet: Water Dip 1
	Wait	Duration: 0.0 min.	Inlet: Water Dip 2 Outlet: Water Dip 2
	Separate	Duration: 20.0 min. -6.0 kV Ramp Time: 2.0 min. Autozero: 8.0 min. Disable Data Collection	Inlet: Nucleic Acid Extended Range Gel (Separation) Outlet: Nucleic Acid Extended Range Gel (Separation)

## Separation Method with Electrokinetic Injection

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

<b>Temperature</b>		<b>Detector Type</b>	
Capillary Cartridge	<input type="text" value="30.0"/> °C <input checked="" type="checkbox"/> Wait	<input type="radio"/> UV	Wavelength <input type="text" value="220"/> nm
Sample Storage	<input type="text" value="10.0"/> °C <input checked="" type="checkbox"/> Wait	<input type="checkbox"/> Wait	
<b>Cartridge Settings</b>		<input checked="" type="radio"/> LIF	Emission Wavelength <input type="text" value="520"/> nm
Capillary Length	<input type="text" value="30.0"/> cm	<input checked="" type="checkbox"/> Wait	PMT Gain <input type="text" value="100"/>
Capillary Type	<input type="text" value="Bare Fused Silica"/>	<input type="radio"/> No Detector	
<b>Current Limits</b>		<b>Data</b>	
<input checked="" type="checkbox"/> Enable current limiting when using voltage		Data Collection Rate	<input type="text" value="8"/> Hz
Maximum Current	<input type="text" value="600"/> µA	Peak Width @ 50% Height	<input type="text" value="1"/> sec

**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)

## Separation Method with Pressure Injection

Figure C-11 Method Settings for the Separation Method with Pressure Injection

<b>Temperature</b>		<b>Detector Type</b>	
Capillary Cartridge	<input type="text" value="30.0"/> °C <input checked="" type="checkbox"/> Wait	<input type="radio"/> UV	Wavelength <input type="text" value="220"/> nm
Sample Storage	<input type="text" value="10.0"/> °C <input checked="" type="checkbox"/> Wait	<input type="checkbox"/> Wait	
<b>Cartridge Settings</b>		<input checked="" type="radio"/> LIF	Emission Wavelength <input type="text" value="520"/> nm
Capillary Length	<input type="text" value="30.0"/> cm	<input checked="" type="checkbox"/> Wait	PMT Gain <input type="text" value="100"/>
Capillary Type	<input type="text" value="Bare Fused Silica"/>	<input type="radio"/> No Detector	
<b>Current Limits</b>		<b>Data</b>	
<input checked="" type="checkbox"/> Enable current limiting when using voltage		Data Collection Rate	<input type="text" value="8"/> Hz
Maximum Current	<input type="text" value="100"/> μA	Peak Width @ 50% Height	<input type="text" value="1"/> sec

Figure C-12 Actions in Program Pane for the Separation Method with Pressure Injection

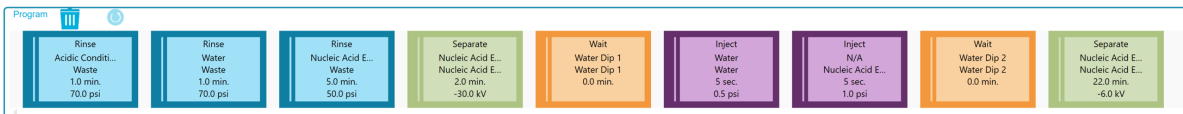














Figure C-13 Summary of Actions in the Separation Method with Pressure Injection

Method Duration: 31.0 min. Number of Actions: 9

	Settings	Capillary Cartridge: 30.0 °C, Wait Capillary Length: 30.0 cm Capillary Type: Bare Fused Silica Current Limit: 100 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: 5 sec. 0.5 psi	Plate: Reagent Inlet: Water Outlet: Water
	Inject	Duration: 5 sec. 1.0 psi	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)

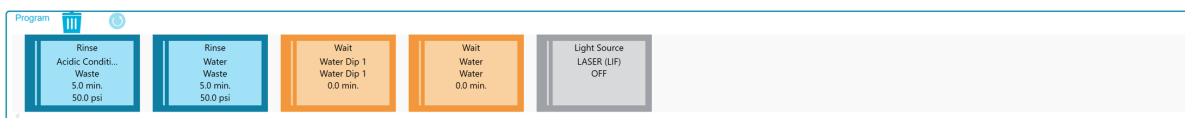
## Shutdown Method

Figure C-14 Method Settings for the Shutdown Method

<b>Temperature</b>		<b>Detector Type</b>	
Capillary Cartridge	<input type="text" value="15.0"/> °C <input checked="" type="checkbox"/> Wait	<input type="radio"/> UV	Wavelength <input type="text" value="220"/> nm
Sample Storage	<input type="text" value="10.0"/> °C <input checked="" type="checkbox"/> Wait	<input type="checkbox"/> Wait	
<b>Cartridge Settings</b>		<input checked="" type="radio"/> LIF	Emission Wavelength <input type="text" value="520"/> nm
Capillary Length	<input type="text" value="30.0"/> cm	<input checked="" type="checkbox"/> Wait	PMT Gain <input type="text" value="100"/>
Capillary Type	<input type="text" value="Bare Fused Silica"/>	<input type="radio"/> No Detector	
<b>Current Limits</b>		<b>Data</b>	
<input checked="" type="checkbox"/> Enable current limiting when using voltage		Data Collection Rate	<input type="text" value="8"/> Hz
Maximum Current	<input type="text" value="600"/> µA	Peak Width @ 50% Height	<input type="text" value="1"/> sec







## Reagents, Plate Layouts, and Methods

**Figure C-15 Actions in the Program Pane for the Shutdown Method**



**Figure C-16 Summary of Actions in the Shutdown Method**

Method Duration: 20.0 min. Number of Actions: 5

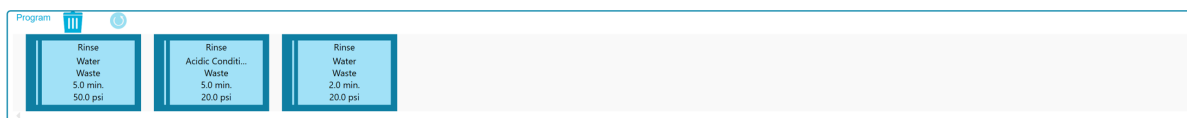
	Settings	Capillary Cartridge: 15.0 °C, Wait Capillary Length: 30.0 cm Capillary Type: Bare Fused Silica Current Limit: 600 µA, Enabled	Sample Storage: 10.0 °C, Wait Detector Type: LIF, 520 nm, Wait, PMT Gain: 100 Peak Width: 1 sec. Data Rate: 8 Hz
	Rinse	Duration: 5.0 min. 50.0 psi	Inlet: Acidic Conditioning 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-17 Method Settings for the Capillary Rinse Method**

<b>Temperature</b>		<b>Detector Type</b>	
Capillary Cartridge	<input type="text" value="20.0"/> °C <input type="checkbox"/> Wait	<input type="radio"/> UV	Wavelength <input type="text" value="220"/> nm
Sample Storage	<input type="text" value="10.0"/> °C <input type="checkbox"/> Wait	<input type="checkbox"/> Wait	
<b>Cartridge Settings</b>		<input checked="" type="radio"/> LIF	Emission Wavelength <input type="text" value="520"/> nm
Capillary Length	<input type="text" value="50.0"/> cm	<input checked="" type="checkbox"/> Wait	PMT Gain <input type="text" value="100"/>
Capillary Type	<input type="text" value="Bare Fused Silica"/>	<input type="radio"/> No Detector	
<b>Current Limits</b>		<b>Data</b>	
<input type="checkbox"/> Enable current limiting when using voltage		Data Collection Rate	<input type="text" value="8"/> Hz
Maximum Current	<input type="text" value="600"/> µA	Peak Width @ 50% Height	<input type="text" value="1"/> sec

**Figure C-18 Actions in the Program Pane for the Capillary Rinse Method**



**Figure C-19 Summary of Actions in the Capillary Rinse Method**

	Settings	Capillary Cartridge: 20.0 °C, Wait Capillary Length: 50.0 cm Capillary Type: Bare Fused Silica Current Limit: 600 µA	Sample Storage: 10.0 °C, Wait Detector Type: LIF, 520 nm, Wait, PMT Gain: 10 Peak Width: 1 sec. Data Rate: 8 Hz
	Rinse	Duration: 5.0 min. 80.0 psi	Inlet: Acid Wash/Regenerating Solution (0.1N HCl) Outlet: Waste
	Rinse	Duration: 5.0 min. 80.0 psi	Inlet: Water - Rinse Outlet: Waste
	Wait	Duration: 0.0 min.	Inlet: Water Dip 1 Outlet: Water Dip 1

# Contact Us

---

## Customer Training

- In North America: [NA.CustomerTraining@sciex.com](mailto:NA.CustomerTraining@sciex.com)
- In Europe: [Europe.CustomerTraining@sciex.com](mailto:Europe.CustomerTraining@sciex.com)
- Outside the EU and North America, visit [sciex.com/education](http://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](http://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, Canada, United Kingdom, Belgium, Netherlands, France, Germany, and Switzerland 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](http://sciex.com) or contact us in one of the following ways:

- [sciex.com/contact-us](http://sciex.com/contact-us)
- [sciex.com/request-support](http://sciex.com/request-support)

## Cybersecurity

For the latest guidance on cybersecurity for SCIEX products, visit [sciex.com/productsecurity](http://sciex.com/productsecurity).

## Documentation

This version of the document supersedes 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](https://sciex.com/customer-documents).

---

**Note:** To request a free, printed version of this document, contact [sciex.com/contact-us](https://sciex.com/contact-us).

---