

Application Guide

Oligo 200 nt Purity and Integrity Kit

for the BioPhase 8800 System

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Oligo 200 nt Purity and Integrity Kit 1

The Oligo 200 nt Purity and Integrity kit is a high-resolution analytical solution that is used to do the separation and sizing of single-stranded nucleic acids in the range from 10 bases to 200 bases. Single-base resolution has been done to a maximum of 141 bases. As a result, the kit is useful for capillary electrophoresis (CE) applications such as oligonucleotide purity assessment, fragment sizing, and quality control of synthetic DNA and RNA. A polymer-based sieving matrix and optimized capillary conditions let the kit supply sharp, reproducible peak profiles. The kit supplies the sensitivity and resolution that are required for the characterization of small nucleic acid fragments.

This document gives instructions for sample preparation with the Oligo 200 nt Purity and Integrity kit, data acquisition with the BioPhase software and the Empower™ Chromatography Data System software,¹ and analysis of data that is acquired with the BioPhase software. The analysis of data that is acquired with the Empower™ CDS software must be done with the Empower™ CDS software.

Use the information in this application guide as a place to start. If required, then 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

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

Intended Use

The Oligo 200 nt Purity and Integrity kit is for laboratory use only.

¹ Empower is a trademark of Waters Corporation used under license.

The workflow has these steps:

1. Identify the number of samples to be analyzed and the number of replicates.
2. For BioPhase software users:
 - a. Download the required files. Refer to the section: [Download the Required Files for the BioPhase Software](#).
 - b. Create or change the methods. Refer to the section: [Methods](#).
 - c. Create the sequence and the sample and reagent plate layouts. Refer to the section: [Create the Sequence](#).
3. For Empower™ CDS software users:
 - a. Download the required files. Refer to the section: [Download the Required Files for the Empower™ CDS Software](#).
 - b. Import the BioPhase software methods. Refer to the section: [Import BioPhase Software Methods to Create Instrument Methods](#).
 - c. Create the sample set methods and the sample and reagent plate layouts. Refer to the section: [Create the Sample Set Method](#).
4. Prepare the standards and samples. Refer to the section: [Prepare the Standards, Test Mix, and Samples](#).
5. Prepare the system. Refer to the section: [Prepare the BioPhase 8800 System](#). Use the sample and reagent plate layouts to prepare the plates. Refer to the section: [Plate Layouts](#).
6. Start data acquisition:
 - BioPhase software: Start the sequence from the Front Panel software. Refer to the section: [Run the Samples](#).
 - Empower™ CDS software: Start the sample set method from the Empower™ CDS software. Refer to the section: [Start the Sample Set Method](#).
7. After the run, stow the cartridge. Refer to the section: [Stow the Cartridge After the Run](#).
8. 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](#).
 - Empower™ CDS software: When the sample set method is complete, use the Empower™ CDS software to analyze the data. For analysis instructions, refer to the Empower™ CDS software guides and help file.

Required Equipment and Materials 3

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

Table 3-1 Oligo 200 nt Purity and Integrity Kit (PN 5321745)

Component	Quantity	Reorder Part Number
Acid Wash/Regenerating Solution (0.1 N HCl) (100 mL)	1	5312349
CE Grade Water (140 mL)	1	C48034
Oligo 200 nt Purity and Integrity Gel (200 mL)	1	5321746
Oligo 200 nt Purity and Integrity Sample Buffer (20×) (1,000 µL)	1	—
Oligo 200 nt Test Mix (50 nt to 141 nt) (40 µL)	1	5321748
Single-Stranded Oligo Standard (15 nt) (200 µL)	1	5321747

Table 3-2 Other Supplies from SCIEX

Component	Quantity	Part Number
Capillary cartridge coolant (450 mL)	1	359976
Filter, 254 nm, for use with UV detector	1	144438
nanoVials	100	5043467
Oligo 200 nt Ladder (10 nt to 200 nt)	1	5322663
Sample Loading Solution	6 mL	608082
BioPhase BFS capillary cartridge – 8 × 30 cm	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

Storage Conditions

- Upon receipt, keep the Single-Stranded Oligo Standard (15 nt) and Oligo 200 nt Test Mix (50 nt to 141 nt) at – 20 °C.
- Upon receipt, keep the Oligo 200 nt Purity and Integrity Gel at 2 °C to 8 °C.
- Keep the remaining contents of the kit at ambient temperature.

Customer-Supplied Equipment and Supplies

- Powder-free gloves, nitrile or chloroprene recommended
- Protective eyewear
- Laboratory coat
- Applicable centrifuge
- Microcentrifuge, or equivalent, and microcentrifuge tubes
- Pipettes and applicable tips

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

- Vortex mixer

Required Detector

A UV detector with a 254 nm filter is required.

Required Cartridge

CAUTION: Potential Incorrect Result. If a cartridge is used with the Oligo 200 nt Purity and Integrity kit, then do not use the same cartridge for another application. If the same cartridge is used with different buffers and sample types, then sample carryover, nonspecific binding, and poor separation can occur.

A BioPhase BFS capillary cartridge – 8 × 30 cm is required.

Refer to the table: [Table 3-2](#).

Methods and Sequences

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For Systems That Use the BioPhase Software

Note: If the validated methods and sequences are not included with the software, then download them. Refer to the section: [Download the Required Files for the BioPhase Software](#). The methods can also be created manually with the BioPhase software. Refer to the section: [Methods](#).

These methods and sequences are required:

- Oligo 200 nt Conditioning and Separation method: Rinses the capillaries with Acid Wash/Regenerating Solution (0.1 N HCl), rinses the capillaries with CE Grade Water, loads Oligo 200 nt Purity and Integrity Gel, and then separates oligonucleotides from 10 bases to 200 bases with single-base resolution to a maximum of 141 bases.
- Oligo 200 nt Shutdown Method 1 of 2 method: Rinses the capillaries with Acid Wash/Regenerating Solution (0.1 N HCl) at 40 °C to clean them.
- Oligo 200 nt Shutdown Method 2 of 2: Decreases the temperature to 22 °C and rinses the capillaries with CE Grade Water to remove the Acid Wash/Regenerating Solution (0.1 N HCl).
- Oligo 200 nt Separation sequence template: A template for the separation of small oligonucleotide samples.

For Systems That Use the Empower™ CDS Software

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

Note: If the methods are not included with the software, then download them. Refer to the section: [Download the Required Files for the Empower™ CDS Software](#). The methods can also be created manually with the Method Editors for BioPhase System software. Refer to the document: *Method Editors for BioPhase System Software Help*.

Create the Sequence

Note: If the Empower™ CDS 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 more information, refer to the document: *BioPhase Software Help*.

This procedure gives instructions to create a sequence with a template that is supplied with the BioPhase software. The template is set up for eight samples in a column and uses the validated methods that are supplied with the software.

Sequences can also be created without a template. Usually, a sequence starts with a conditioning method, includes separation methods, and is completed with a shutdown method. The shutdown method is usually identified as the error recovery method. The Oligo 200 nt Purity and Integrity kit uses Oligo 200 nt Shutdown Method 2 of 2 as the recovery method.

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

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 a sequence, and then select it:
 - a. (Optional) Type a **Start Date** and **End Date** for the search, or click the calendar icons and select the dates, and then click **Search**.
The available project folders are shown in the Folder Name pane.
 - b. Click the `Oligo 200 nt Project` folder.
The available sequences in the project are shown in the table to the right.
 - c. In the table, click **Oligo 200 nt Conditioning and Separation**, and then click **Open**.
This sequence template includes methods for UV detection and uses the traditional and lightning methods for one sample column each.
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 one of these 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.

Note: The recommended recovery method is Oligo 200 nt Shutdown Method 2 of 2.

For instructions, refer to the document: *BioPhase Software Help*.

6. If required, then edit the information in the Sequence Summary table.

Methods and Sequences

Note: The method names are different for each sequence.

Figure 4-1 Sequence Summary Table: Oligo 200 nt Test Sequence

Sequence Summary			Import	Export	
	Run #	Column	Method Name	Rep. #	Error Recovery
⊞ 1	1	1	Oligo 200 nt Conditioning and Separation Method	1	☐
⊞ 2	2	2	Oligo 200 nt Conditioning and Separation Method	1	☐
⊞ 3	3	3	Oligo 200 nt Conditioning and Separation Method	1	☐
⊞ 4	4	4	Oligo 200 nt Conditioning and Separation Method	1	☐
⊞ 5	5	5	Oligo 200 nt Conditioning and Separation Method	1	☐
⊞ 6	6	6	Oligo 200 nt Conditioning and Separation Method	1	☐
7	0	0	Oligo 200 nt Shutdown Method 1 of 2	1	☐
8	0	0	Oligo 200 nt Shutdown Method 2 of 2	1	☐

7. To see information about a run, click **+** in the row with the run.
8. If required, then change the information in the **Sample ID** and **Data File** columns.

Note: Do not use these characters in the sample ID name: < (less than), > (greater than), : (colon), " (double quote), / (forward slash), \ (backward slash), | (vertical bar or pipe), ? (question mark), or * (asterisk). If one of these characters is used in the sample ID name, then the data file will not be generated.

9. To see the sample plate and reagent plate layouts, open the Plates Layout tab.

Note: When samples in the sequence are added or removed, the reagent plate layout is updated automatically. Before the sequence is saved, make sure that the layout is correct. If required, then edit the reagent positions 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. To correct all of the errors in the Validation pane, click the side bar to expand it, and then click **SAVE**.

11. (Optional) To print the sample plate and reagent plate layouts, do this:
 - 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.

Import and Export Sequence Files

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

Note: To import or export sequence files, Microsoft Excel must be installed on the computer.

1. Select the sample wells to be analyzed.
2. Assign the method or methods to the sample wells.
3. To save the sequence template as a Microsoft Excel file, click **Export**.
4. Open the Excel file, and then update the **Sample ID** and **File Path** fields.
5. To open the Sequence Editor window, click **NEW**.
6. Click **Import**.
7. Select the file, and then click **Open**.
8. Select the sample wells in the **Method** column.
9. Click **OK**.
10. In the Sequence Summary tab, review the sequence.
11. Click **Save**.

Prepare the Standards, Test Mix, and Samples 5

The high-resolution separation of single-stranded oligonucleotides gives important purity and sizing information.

Before preparation of the standards, test mix, and samples starts, prepare a 20:1 solution of Oligo 200 nt Purity and Integrity Sample Buffer (20×) and CE Grade Water. In the procedures that follow, this solution is referred to as the *sample dilution buffer* (1×).

Prepare the Single-Stranded Oligo Standard (15 nt) Solution

1. Let the Single-Stranded Oligo Standard (15 nt) come to ambient temperature.
2. Add 1 µL of Single-Stranded Oligo Standard (15 nt) to 799 µL of sample dilution buffer (1×). The quantity of prepared solution is sufficient to use 50 µL in each sample plate well.
3. Use a vortex mixer to mix the solution.
4. Add 50 µL of the Single-Stranded Oligo Standard (15 nt) solution to the sample plate well.

Prepare the Oligo 200 nt Ladder (10 nt to 200 nt) Solution

The ladder standard that is selected for use in an experiment is related to the oligonucleotide samples to be analyzed and the goal of the analysis. If the Oligo 200 nt Ladder (10 nt to 200 nt) solution does not give satisfactory results, then a different lot of the standard might be required.

Note: The instructions that follow give quantities for one sample. For experiments with a different number of samples, change the quantities as applicable.

1. Let the Oligo 200 nt Ladder (10 nt to 200 nt) come to ambient temperature.
2. Add 40 µL of Oligo 200 nt Ladder (10 nt to 200 nt) to 760 µL of sample dilution buffer (1×).
3. Use a vortex mixer to mix the solution.
4. Add 50 µL of the Oligo 200 nt Ladder (10 nt to 200 nt) solution to the sample plate well.

Prepare the Oligo 200 nt Test Mix (50 nt to 141 nt) Solution

The test mix that is selected for use in an experiment is related to the oligonucleotide samples to be analyzed and the goal of the analysis. If the Oligo 200 nt Test Mix (50 nt to 141 nt) does not give satisfactory results, then a different lot of the test mix might be required.

Note: The instructions that follow give quantities for one sample. For experiments with a different number of samples, change the quantities as applicable.

1. Let the Oligo 200 nt Test Mix (50 nt to 141 nt) come to ambient temperature.
2. Add 1 μL of Oligo 200 nt Test Mix (50 nt to 141 nt) to 799 μL of sample dilution buffer (1 $\times$). The quantity of prepared solution is sufficient to use 50 μL in each sample plate well.
3. Use a vortex mixer to mix the solution.
4. Add 50 μL of the Oligo 200 nt Test Mix (50 nt to 141 nt) solution to the sample plate well.

Prepare the Sample

Note: The instructions that follow give quantities for one sample. For experiments with a different number of samples, change the quantities as applicable.

1. Add CE Grade Water or sample dilution buffer (1 $\times$) to the sample to get an oligonucleotide concentration between 5 $\text{pg}/\mu\text{L}$ and 500 $\text{pg}/\mu\text{L}$.

Note: For the best results, the recommended concentration is 100 pg/mL .

2. Add 50 μL of the sample to the sample plate well.
3. (Optional) For quantitation, do this:
 - a. Add 2.5 μL of Single-Stranded Oligo Standard (15 nt) to 22.5 μL of CE Grade Water.
 - b. Add 3 μL of the Single-Stranded Oligo Standard (15 nt) solution to the 50 μL of sample in the sample plate well.

Prepare the BioPhase 8800 System 6

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 the light source become warm.

Load the Reagent 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.

CAUTION: Potential Incorrect Result. Before the plates are loaded in the system, put them in a centrifuge to remove air bubbles. Air bubbles might cause an unsatisfactory separation.

CAUTION: Potential System Damage. Before the plates are loaded in the system, remove the film cover. If the film cover is not removed, then damage to the capillary tips 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 reagent plate layout to add the reagents in the table that follows to the reagent inlet and outlet plates. Refer to the figure: [Figure A-4](#).

Table 6-1 Reagents for the Reagent Inlet and Outlet Plates

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

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.

2. Put a film cover on the plates.

3. Put the inlet plates in a centrifuge at $30 \times g$ for 4 minutes.
4. Make sure that there are no air bubbles in the plates. To remove air bubbles, put the reagent inlet plates in the centrifuge again at a higher relative centrifugal force (RCF).

Note: Do not put the reagent outlet plate in a centrifuge. To remove large bubbles, tap the plate lightly on the work surface.

The maximum RCF for reagent inlet plates is $1,000 \times g$.

5. On the touchscreen, touch **Direct Control**, and then touch **Eject Reagent**.
The plate compartment opens.
6. Remove the film cover from the plates.
7. If the plate compartment contains reagent plates, then remove them.
8. Align the notch in the reagent inlet plate with the tab, and then put the plate in the plate carrier.
9. 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.
10. Touch **Load Reagent**.
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 A-3](#).
The recommended sample volume is 50 μL . The minimum sample volume is 50 μL . The maximum sample volume is 200 μL .
2. To prevent damage to the capillaries, for columns that do not have sample in every well, add 50 μL to 200 μL of CE Grade Water to each empty well.
If a column has no samples, then all of the wells can stay empty.
3. Use the sample plate layout to add the reagents to the sample outlet plate. For a column on the sample plate that has sample, make sure that the related column on the outlet plate has 2.0 mL of Oligo 200 nt Purity and Integrity Gel. Refer to the figure: [Figure A-3](#).

Prepare the BioPhase 8800 System

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.

4. If required, then do this to remove air bubbles:
 - a. Put a film cover on the plates.
 - b. Put the inlet plates in a centrifuge at $30 \times g$ for 4 minutes.
 - c. Make sure that there are no air bubbles in the plates. To remove air bubbles, put the plates in the centrifuge again at a higher relative centrifugal force (RCF).
The maximum RCF for sample plates is $375 \times g$.
 - d. On the touchscreen, touch **Direct Control**, and then touch **Eject Sample**.
The plate compartment opens.
 - e. Remove the film cover from the plates.
5. If the plate compartment already contains sample plates, then remove the sample plates.
6. Align the notch in the sample plate with the tab, and then put the plate in the plate carrier.
7. 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.
8. Touch **Load Sample**.
The plate compartment closes.

Examine the Capillary Cartridge



WARNING! Puncture Hazard. Touch the cartridge carefully. The capillary tips are very sharp. Personal injury might occur.

CAUTION: Potential System Damage. Do not let the gel buffer or other reagents dry into crystals on the electrodes, capillary tips, cartridge seals, or cartridge body. Electrolyte salt crystals or precipitate can cause blocked capillaries, incorrect pressure seals, errors when samples are injected, arcing, or electrical current leaks.

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 use a lint-free wipe that is dampened with CE Grade Water to clean the cartridge. Use a dry lint-free wipe to dry the cartridge fully.
-

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 external surface of the capillary inlets.
 - b. Use a lint-free 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 lint-free 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.
6. (Optional) If a cartridge that is loaded in the system is not identified, then dampen a lint-free 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 very sharp. Personal injury might occur.



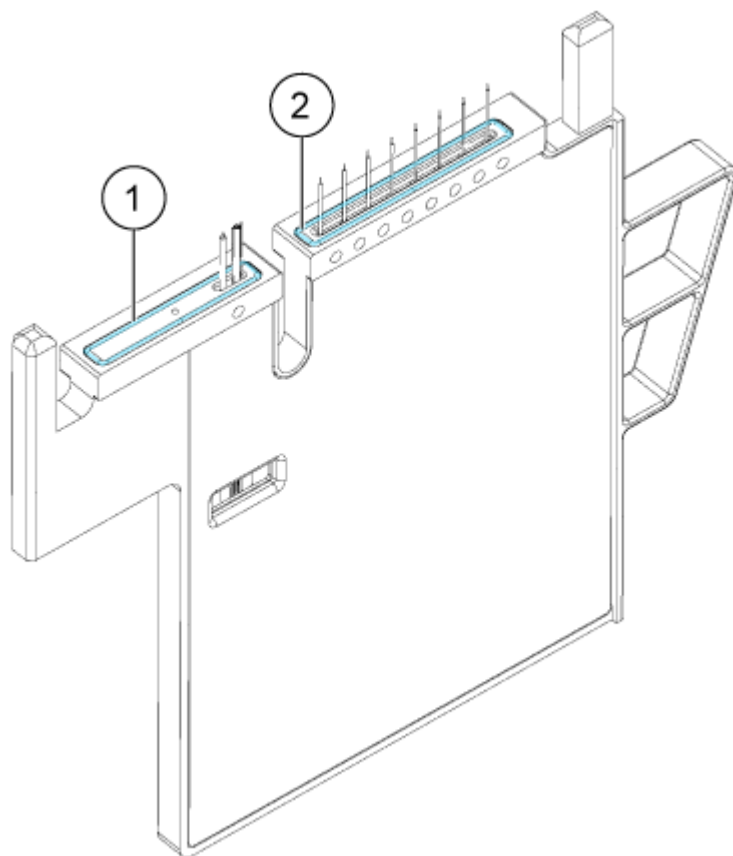
WARNING! Pinching Hazard. When the front panel is opened, do not put fingers to the left of the front panel. Personal injury might occur.

CAUTION: Potential System Damage. Make sure that the reagent plates are installed in the system before the cartridge is installed. If the cartridge is installed first, then 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 lint-free wipe to dry the cartridge body.
4. Turn the bottom of the cartridge up.
5. Use a lint-free wipe to very carefully dry the area where the capillaries and electrodes come out of the cartridge. Do not touch or move the seals. Make sure that the seals are in the correct position.

Prepare the BioPhase 8800 System

Figure 6-1 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.
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 fully in CE Grade Water, which is used as the Capillary Protect reagent.
9. On the touchscreen, go to the Home page, and then look at the icon for the coolant level in the status panel. If required, then add coolant.
Refer to the document: *Operator Guide*.

Download the Required Files

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To download, extract, and copy the required files to the applicable location, do the procedure for the software that is in use:

- BioPhase software: Refer to the section: [Download the Required Files for the BioPhase Software](#).
- Empower™ CDS software: Refer to the section: [Download the Required Files for the Empower™ CDS Software](#).

Download the Required Files for the BioPhase Software

1. Go to sciex.com/software-downloads.
2. In the More software downloads section, click **BioPhase Resources**.
3. Click the latest BioPhase `Project` file.
4. In File Explorer, right-click the latest BioPhase file, and then click **Extract All**.
5. Browse to the location to save the extracted files, click **Select Folder**, and then click **Extract**.
The extracted files are copied to the selected file path.
6. Move the extracted files to the correct location:

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

- New installations: Configure access to the `Project` folder.
- Updates to installed software: Copy the files to the `projects\oligo-200` folder.

Download the Required Files for the Empower™ CDS Software

For instructions to restore project or method files, refer to the document: *BioPhase 8800 Driver for Empower™ Software Release Notes*.

1. Go to sciex.com/software-downloads.

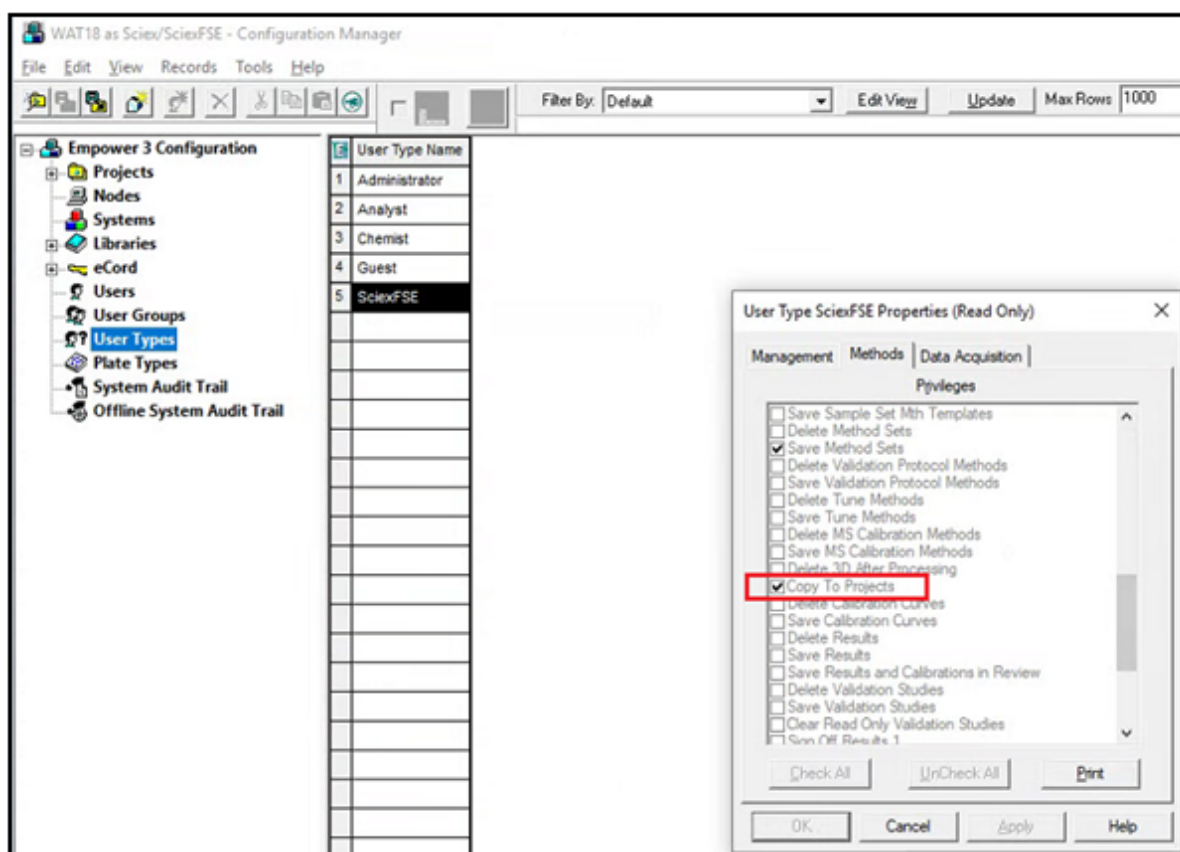
Download the Required Files

2. In the More software downloads section, click **BioPhase Driver Resources**.
3. Click **BioPhase Empower Method Files**.
4. In File Explorer, right-click the `BioPhase-Empower-Method-Files.zip` file, and then click **Extract All**.
5. 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.

Copy the Method Files to the Project Folder

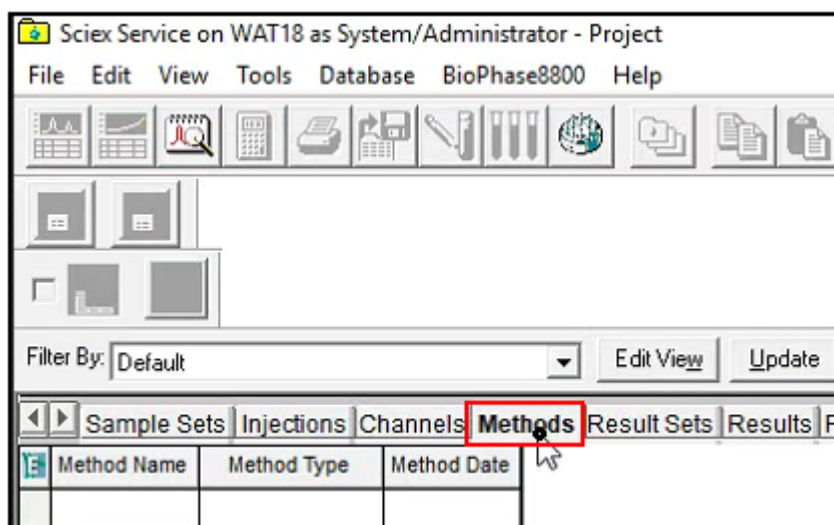
1. In the Configuration Manager, make sure that the **Copy To Projects** check box is selected.

Figure 7-1 Configuration Manager Window



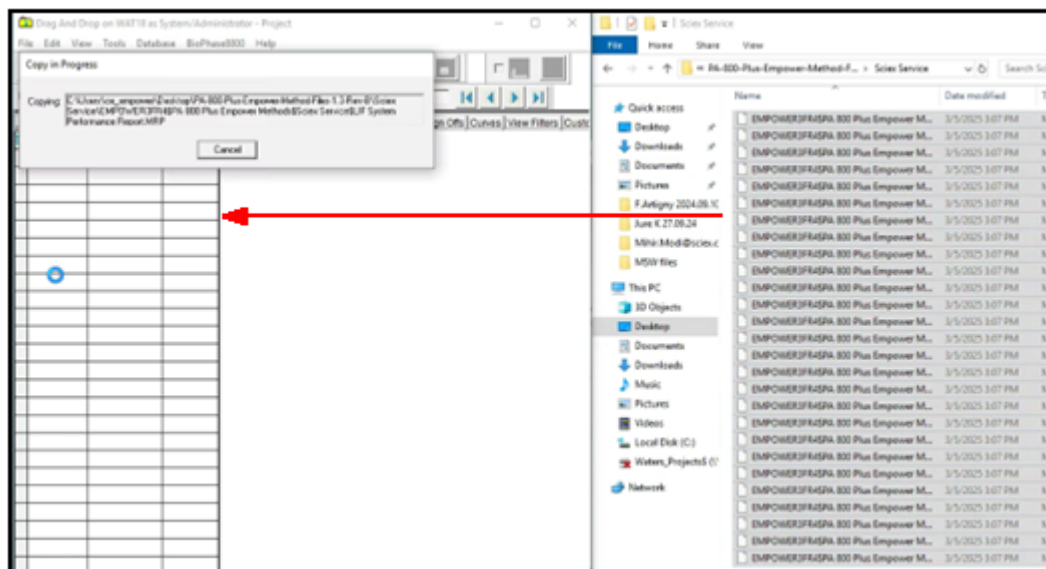
2. Browse to the new project, right-click, and then click **Open**.

Figure 7-2 Methods Tab



3. Open the Methods tab.
4. Open the Project and Method folders in different windows in a side-by-side layout.

Figure 7-3 Project and Method Folders Side by Side



5. Select all of the files in the Method folder, and then drag (copy) them to the Project folder.

Start the Sequence from the Front Panel Software

To use the Empower™ CDS software, refer to the section: [Start the Sample Set Method](#).

1. If required, then load the cartridge, reagent plates, and sample plates.
2. On the touchscreen, touch **RUN SEQUENCE**.
3. In the Projects pane, touch **Oligo 200 nt Project**.
4. In the Available Sequences pane, touch the applicable sequence in the list.
5. (Optional) To see information about the method, sample plates, or reagent plates, touch anywhere in the **Method** column.
To hide the information, touch the column or box again.

6. Touch **Run Sequence**.

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

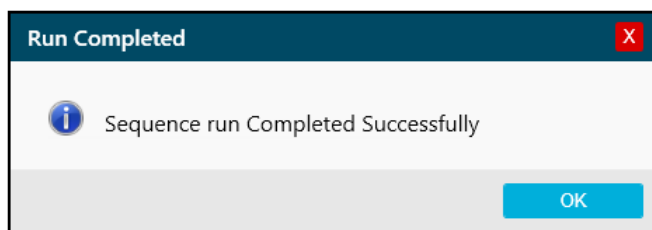
If the sequence contains a method with information that does not agree, then a yellow exclamation mark is shown. Refer to the figure: [Figure 8-3](#).

The data files are saved at the location that is identified 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 system starts the error recovery method.

During the run, actions are available. Refer to the section: [Monitor the Run in the Front Panel Software](#).

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

Figure 8-1 Run Completed Dialog

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

Monitor the Run in the Front Panel Software

CAUTION: Potential System Damage. Do not stop the run. 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 cause blocked capillaries, incorrect pressure seals, errors when the samples are injected, arcing, or electrical current leaks.

CAUTION: Potential System Damage. Before a run that has been paused or stopped is started again, make sure that the volume in the outlet plate has not increased. An increase in volume might cause reagents to overflow and subsequent damage to the system.

CAUTION: Potential Incorrect Result. Before a run that has been paused or stopped is started again, make sure that a sufficient volume of serviceable reagent is available to complete the run. If the reagent volume is not sufficient or the quality is unserviceable, then the results might not be correct.

CAUTION: Potential Incorrect Result. Before a run that has been paused or stopped is started again, remove and discard samples that have been in the system for more than 24 hours. Sample degradation might have occurred and the results might not be correct.

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

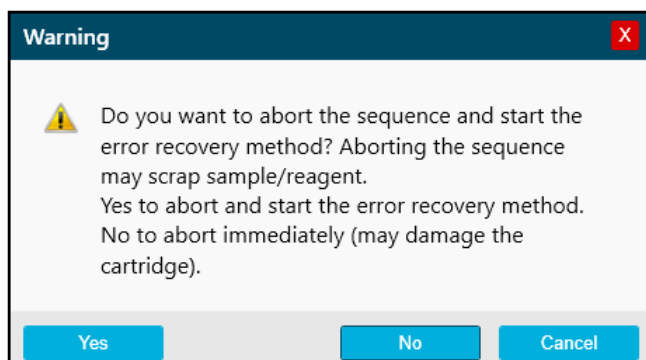
To use the Empower™ CDS software, refer to the section: [Monitor the Run in the Empower™ CDS Software](#).

Run the Samples

Note: The sequence shown in the figures that follow is for illustration only. The figures do not show a sequence for the Oligo 200 nt Purity and Integrity kit.

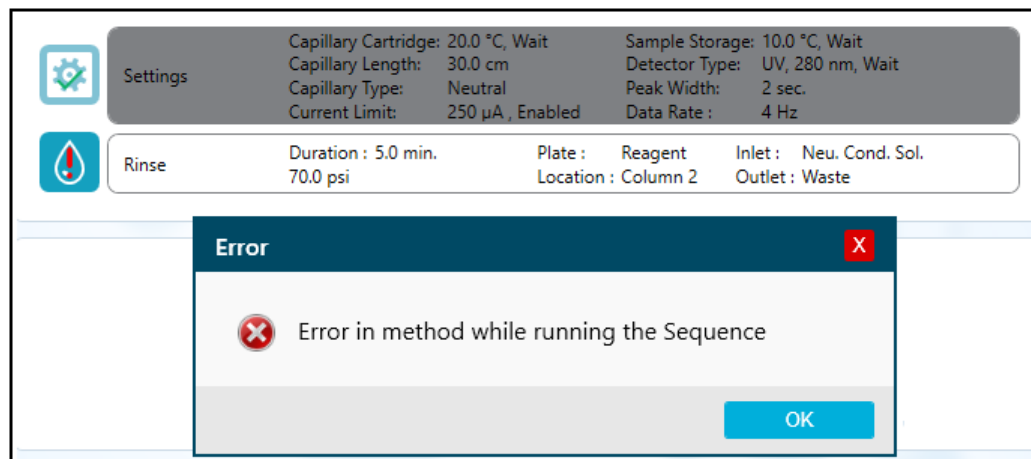
1. To make sure that the sequence is in progress, monitor the traces for the detector and current.
2. To stop the run, touch .

Figure 8-2 Warning Dialog



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

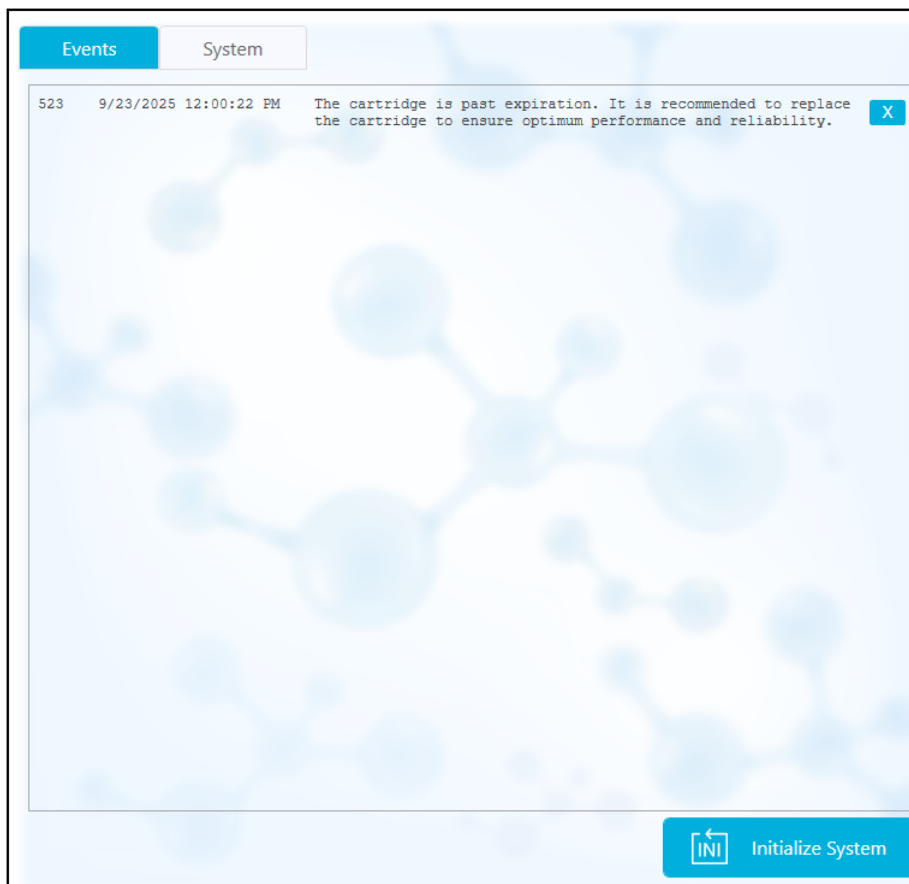
Figure 8-3 Run Sequence Error Dialog



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. Go to the Home page, and then touch **Log**.
6. Open the Events tab.

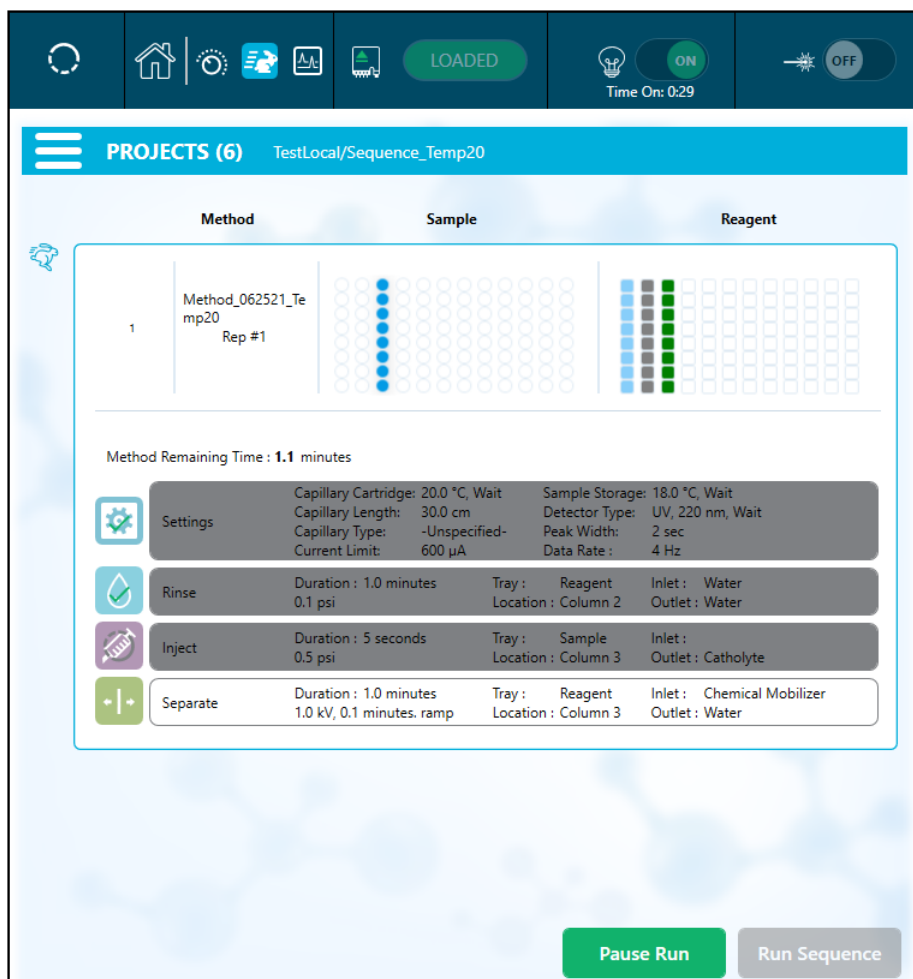
Figure 8-4 Events Log



7. To initialize the system and change the system status to `Idle`, touch **Initialize System**.
8. If required, then touch **Pause Run**.

Run the Samples

Figure 8-5 Run Sequence in Progress



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

Figure 8-6 Run Sequence Paused



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

Note: The data in the figure that follows is for illustration only.

Run the Samples

Figure 8-7 Capillary View



10. (Optional) To zoom in on the data, do this:
 - 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**.

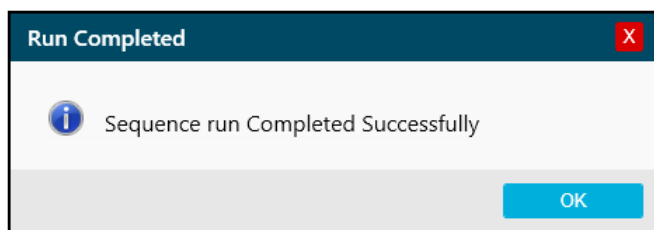
Figure 8-8 Zoom In or Out



11. When the run is completed, make sure that the message Sequence run Completed Successfully is shown, and then touch **OK**.

Run the Samples

Figure 8-9 Run Completed Dialog



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

Run Samples with the Empower™ CDS Software

9

This section gives instructions to use the Empower™ CDS software with the BioPhase 8800 system.

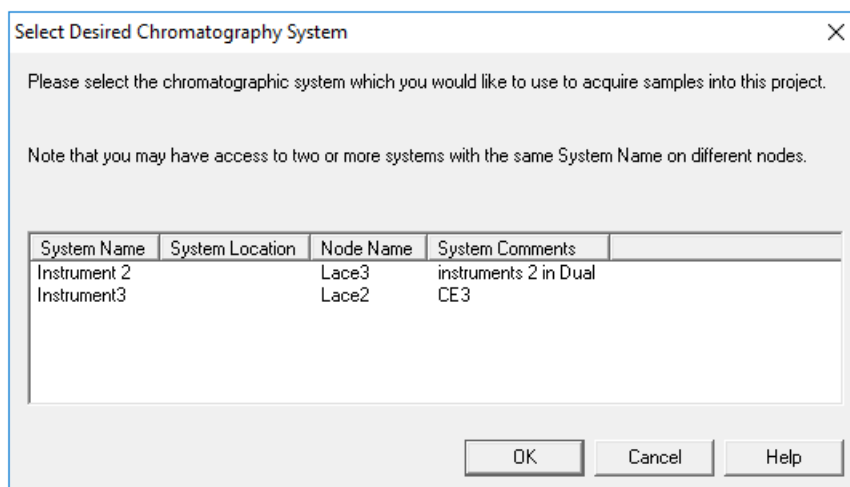
Import BioPhase Software Methods to Create Instrument Methods

Note: Methods created with the BioPhase software are supplied with the Empower™ CDS software. The methods can also be downloaded. Refer to the section: [Download the Required Files for the Empower™ CDS Software](#).

Instrument methods can be created in the Method Editors for BioPhase System software. Refer to the documents: *BioPhase 8800 System for Empower™ CDS Software Users Operator Guide* and *Method Editors for BioPhase System Software Help*.

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

Figure 9-1 Select Desired Chromatography System Dialog



2. Click the name of the system to be used, and then click **OK**.
The Instrument Method Editor opens.

Run Samples with the Empower™ CDS Software

- Click **Import**, and then browse to the separation 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 document: *Method Editors for BioPhase System Software Help*.

Figure 9-2 Instrument Method Editor: Method Settings

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

Figure 9-3 Instrument Method Editor: Method Program

Method Settings 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
1	Rinse	7.0 min	70.0		Water Rinse	Waste							
2	Rinse	7.0 min	70.0		Acid Wash	Waste							
3	Rinse	50.0 min	50.0		Gel	Waste							
4	Separate	5.0 min			Gel	Gel							
5	Wait	0.5 min			Water Dip 1	Water Dip 1							
6	Inject	5 sec				Gel	10	0	Reverse	0 actions		True	
7	Wait	0.5 min			Water Dip 2	Water Dip 2				0 actions			

Parameters - Rinse

Duration: 7.0 min

Pressure: 70.0 psi

Reagent Type:

Inlet: Water Outlet: Waste

Comments:

6. Save the instrument method:

- Click **File > Save with Method Set**.
The Save current Instrument Method dialog opens.
- 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 @ (at sign), _ (underscore), and % (percent). Some versions of the Empower™ CDS 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.

- (Optional) In the **Method Comments** field, type the information.
- If a prompt is shown, then, in the **Password** field, type the Empower™ CDS software password for the current user, and then click **Save**.

The instrument method and method set are saved to the selected 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 shutdown instrument method and method set, do step 3 to step 7 again.

Create the Sample Set Method

Note: A sample set method requires method sets. Make sure that the required instrument methods are part of a method set.

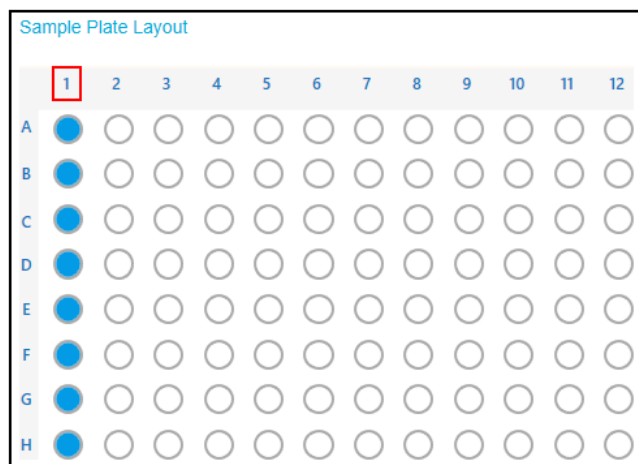
The instructions that follow create a sample set method for eight samples (the number of wells in one column in a sample plate).

1. In the Empower™ CDS 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 the applicable separation method set.
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 9-4 Sample Plate Layout Pane



5. In the Sample Set Summary table, add the required sample information. In row 2 to row 9, do these steps:
 - a. In the **Sample Name** cell, type a name.
 - b. Click the **Method Set Name** cell, and then select the applicable separation method set.

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

6. In the last row, click the **Method Set Name** cell, and then select the applicable shutdown method set.

Figure 9-5 Sample Set Summary Table

Sample Set Summary

Column	# of Injs	Plate/Well	Sample Name	Method Set Name	Run Time (Minutes)	Run Number
1	1	1:A,1	Washington	Oligo 200 nt_ Cond and Sep	60.0	
1	1	1:B,1	Hoover	Oligo 200 nt_ Cond and Sep	60.0	
1	1	1:C,1	Polk	Oligo 200 nt_ Cond and Sep	60.0	
1	1	1:D,1	Coolidge	Oligo 200 nt_ Cond and Sep	60.0	
1	1	1:E,1	Jackson	Oligo 200 nt_ Cond and Sep	60.0	
1	1	1:F,1	Eisenhower	Oligo 200 nt_ Cond and Sep	60.0	
1	1	1:G,1	Kennedy	Oligo 200 nt_ Cond and Sep	60.0	
1	1	1:H,1	Truman	Oligo 200 nt_ Cond and Sep	60.0	
				Oligo 200 nt Shutdown 1 of 2	27.0	

7. If the Validation pane is shown as a blue panel on the right, then click it to see the errors. To find an error, click it, and then make the required change.

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

8. 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 @ (at sign), _ (underscore), and % (percent). Some versions of the Empower™ CDS 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**, and then click **OK**.

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

9. To see, save, or print the plate layouts, do this:

- a. Open the Plate Layouts tab.

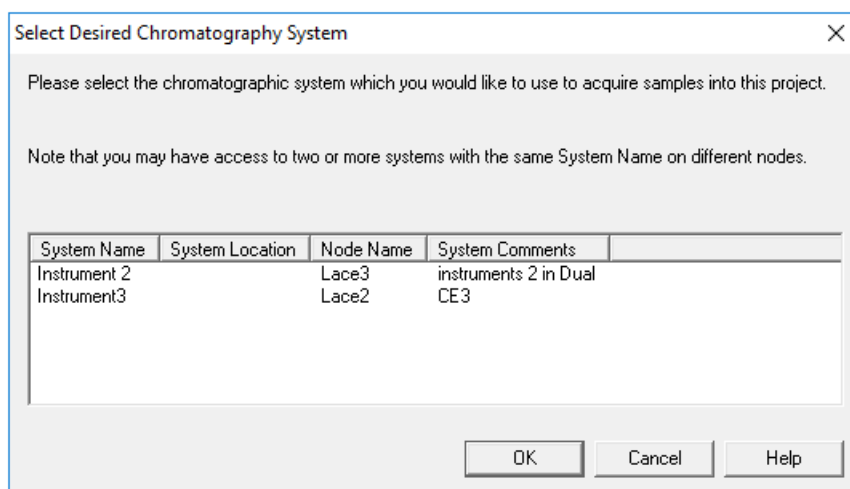
Run Samples with the Empower™ CDS Software

- b. (Optional) Click **PRINT**.
The Print Preview dialog opens.
 - c. If required, then click the applicable button to print or save the plate layouts.
 - d. Close the Print Preview dialog.
10. Close the Method Editors for BioPhase System software.
The Run Samples window opens.

Start the Sample Set Method

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

Figure 9-6 Select Desired Chromatography System Dialog



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

Figure 9-7 Define Plates for Sample Set Method Dialog

	Plate Type Name	Plate Layout Position

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

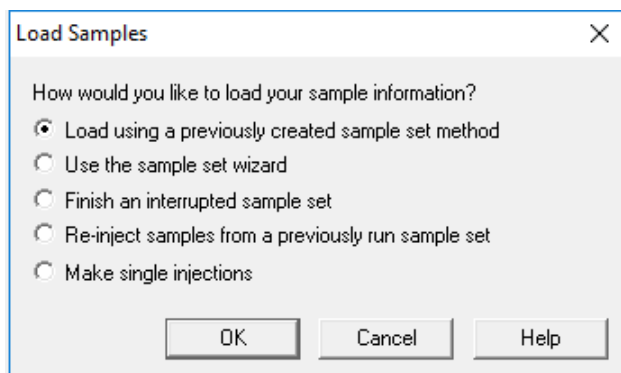
- b. Click the **Plate Type Name** cell, and then select **ANSI-96well2mL**.
The dialog changes to an image of the plate and buttons for the plate sequencing mode.
- c. Click the **Plate Layout Position** cell, and then type 1.
- d. To set the order of access for the wells during the run, click .
- e. To save the changes, click **OK**.
- f. Close the dialog.

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

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

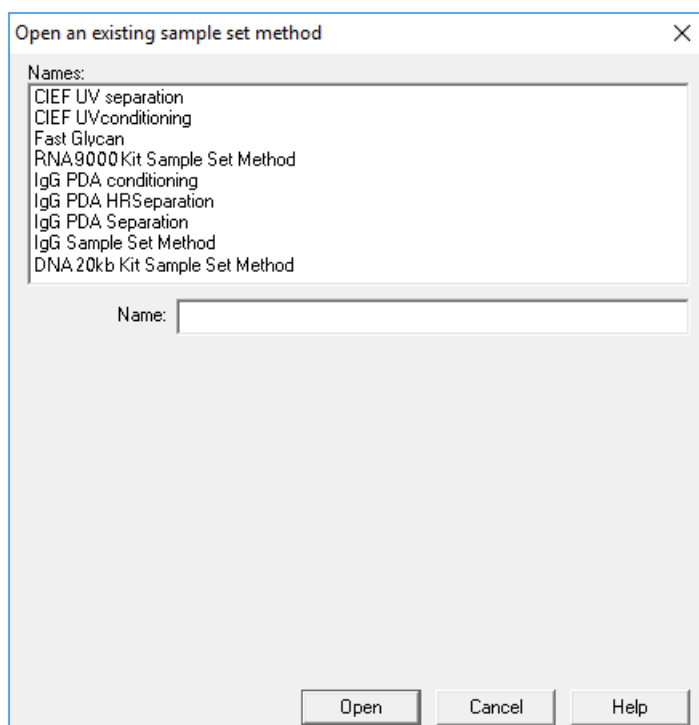
5. Click  (**Load Sample Set**).

Figure 9-8 Load Samples Dialog



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

Figure 9-9 Open an existing sample set method Dialog



7. Click **Oligo 200 nt 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 applicable to the BioPhase 8800 system:
 - a. Right-click, and then click **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**.

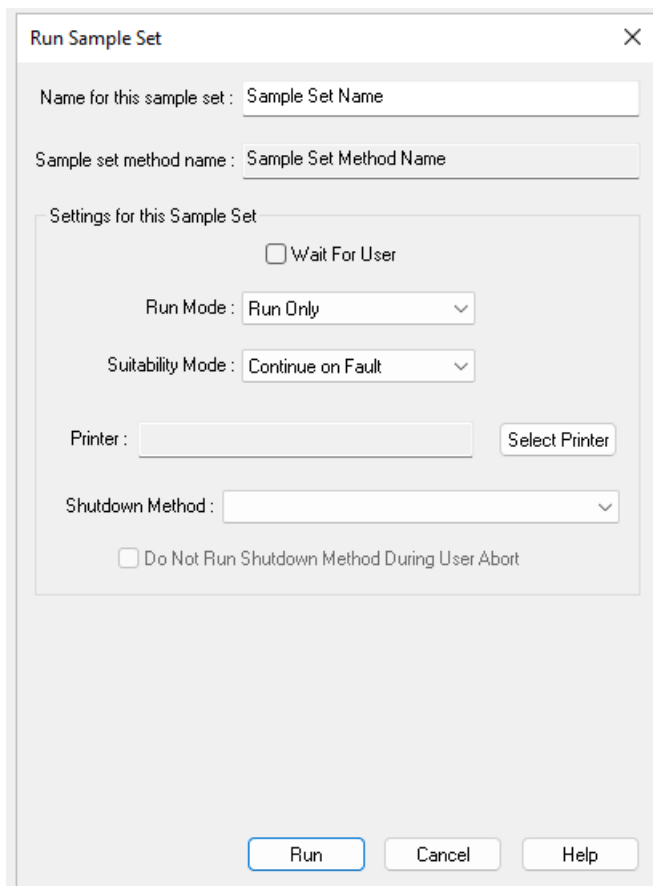
The table changes to show the selected columns.

Figure 9-10 Samples Tab

	Plate/Well	Inj Vol (uL)	# of Injs	SampleName	Function	Method Set / Report or Export Method	Processing	Run Time (Minutes)
1	1:A,1	10.0	1	Washington	Inject Samples	Oligo 200 nt_ Cond and Sep	Normal	60.00
2	1:B,1	10.0	1	Hoover	Inject Samples	Oligo 200 nt_ Cond and Sep	Normal	60.00
3	1:C,1	10.0	1	Polk	Inject Samples	Oligo 200 nt_ Cond and Sep	Normal	60.00
4	1:D,1	10.0	1	Coolidge	Inject Samples	Oligo 200 nt_ Cond and Sep	Normal	60.00
5	1:E,1	10.0	1	Jackson	Inject Samples	Oligo 200 nt_ Cond and Sep	Normal	60.00
6	1:F,1	10.0	1	Eisenhower	Inject Samples	Oligo 200 nt_ Cond and Sep	Normal	60.00
7	1:G,1	10.0	1	Kennedy	Inject Samples	Oligo 200 nt_ Cond and Sep	Normal	60.00
8	1:H,1	10.0	1	Truman	Inject Samples	Oligo 200 nt_ Cond and Sep	Normal	60.00
9					Condition Column	Oligo 200 nt Shutdown 1 of 2		27.00

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 Empower™ CDS software Project window, click  (**Start**).

Figure 9-11 Run Sample Set Dialog



The dialog box is titled "Run Sample Set" and contains the following fields and controls:

- Name for this sample set :** A text field containing "Sample Set Name".
- Sample set method name :** A text field containing "Sample Set Method Name".
- Settings for this Sample Set** (grouped in a box):
 - ☐ Wait For User
 - Run Mode :** A dropdown menu showing "Run Only".
 - Suitability Mode :** A dropdown menu showing "Continue on Fault".
 - Printer :** A text field with a "Select Printer" button next to it.
 - Shutdown Method :** A dropdown menu.
 - ☐ Do Not Run Shutdown Method During User Abort
- Buttons:** "Run", "Cancel", and "Help" at the bottom.

11. If required, then edit the information:

- a. If required, then edit the value in the **Name for this sample set** field.
- b. (Optional) In the **Shutdown Method** list, select an instrument method that rinses the capillaries.

If a SCIEX-supplied rinse method is available, then use it. If a rinse method is not available, then create one. Use the same reagent set as the sample set method and these parameters:

- Pressure: 50 psi
- Duration: 2 minutes
- Inlet: Water
- Outlet: Waste

If an error occurs during a run, then this instrument method is done, and then the run stops.

- c. If required, then select the **Do Not Run Shutdown Method During User Abort** check box.
- d. Click **Run**.

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

CAUTION: Potential Data Loss. During a run, although the system status might be **Idle**, do not start an operation from the Direct Control pane in the Empower™ CDS software. Operations that are started might cause interference with data acquisition.

Monitor the Run in the Empower™ CDS 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 cause blocked capillaries, incorrect pressure seals, errors when the samples are injected, arcing, or electrical current leaks.

CAUTION: Potential System Damage. Before a run that has been paused or stopped is started again, make sure that the volume in the outlet plate has not increased. An increase in volume might cause reagents to overflow and subsequent damage to the system.

CAUTION: Potential Incorrect Result. Before a run that has been paused or stopped is started again, make sure that a sufficient volume of serviceable reagent is available to complete the run. If the reagent volume is not sufficient or the quality is unserviceable, then the results might not be correct.

CAUTION: Potential Incorrect Result. Before a run that has been paused or stopped is started again, remove and discard samples that have been in the system for more than 24 hours. Sample degradation might have occurred and the results might not be correct.

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

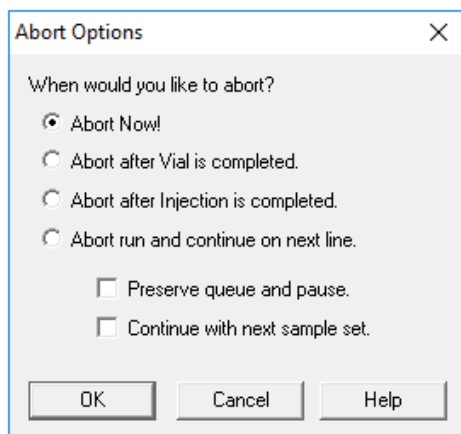
Note: Most of the panes in the Empower™ CDS software are made for chromatography. Use the steps that follow to monitor the progress of the capillary electrophoresis separation and ignore the information in the Time Remaining and Solvent Required panes.

Run Samples with the Empower™ CDS Software

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.

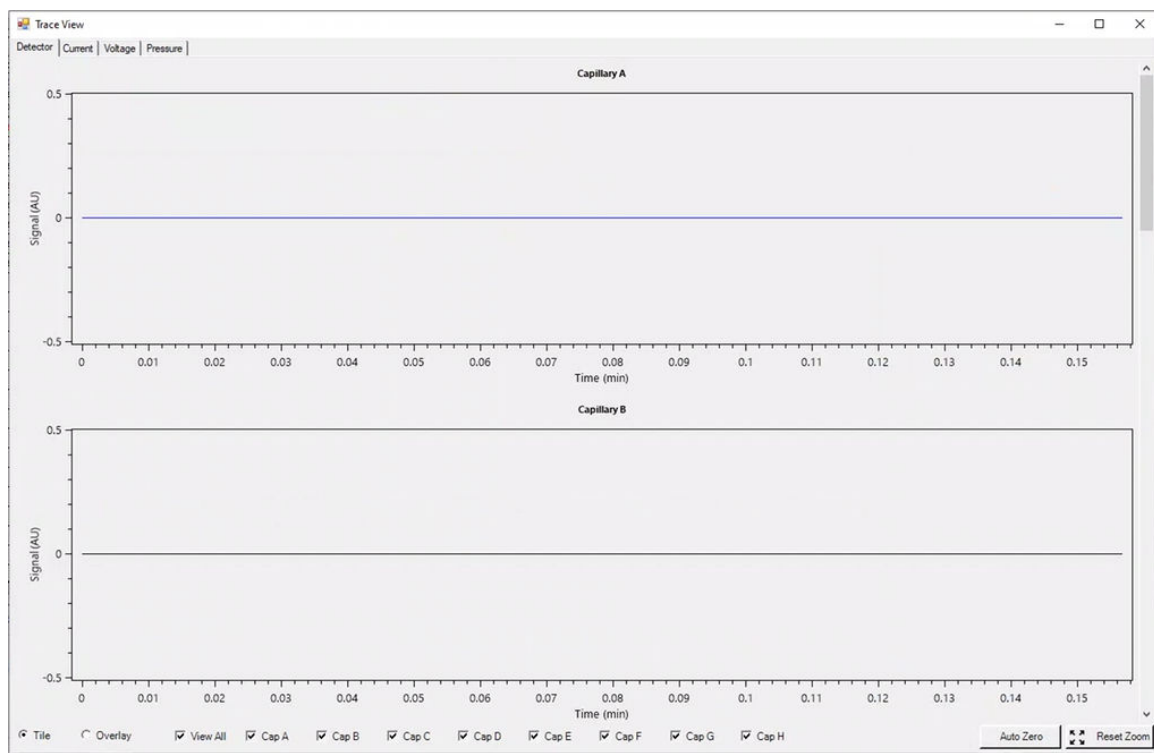
Figure 9-12 Abort Options Dialog



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 9-13 Trace View Window



3. If required, then do the applicable actions that follow:
 - 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 cursor 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-axis or Y-axis, and then drag the cursor.
4. If required, then right-click **Auto Zero** at the bottom right.
The detector signal is set to zero.
5. Wait until  (**Abort**) changes to  (**Start**).

Run Samples with the Empower™ CDS Software

A delay between data acquisition and when all of the data is saved might occur. The green button identifies when all of the data is saved.

6. If required, then discard the samples and reagents. Refer to the section: [Waste Disposal](#).
7. If required, then stow the cartridge. Refer to the section: [Stow the Cartridge After the Run](#).

Stow the Cartridge After the Run

10

Stow the Cartridge for Less than 3 Days



WARNING! Puncture Hazard. Touch the cartridge carefully. The capillary tips are very sharp. Personal injury might occur.

- 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.

Stow the Cartridge for More than 3 Days



WARNING! Puncture Hazard. Touch the cartridge carefully. The capillary tips are very sharp. Personal injury might occur.

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.
2. Rinse the capillaries with CE Grade Water for 5 minutes at 70 psi to clean them.
3. Before the cartridge is removed, in the Front Panel software, touch  (**Loaded**) on the ribbon, and then wait approximately 1 minute for the coolant to go back to the reservoir.
4. Remove the cartridge from the system.
5. Stow the cartridge upright in the cartridge box at ambient temperature.
6. Put the capillary tips fully in vials of CE Grade Water.

Note: To prevent microbial growth, replace the CE Grade Water regularly.

Prepare the Cartridge After Storage

- The Oligo 200 nt Conditioning and Separation method includes a conditioning method. The capillaries are conditioned automatically before each sample run in a sequence.

We recommend the use of Direct Control to rinse the capillaries for 30 minutes at 50 psi with CE Grade Water. Refer to the document: *Operator Guide*.

Stow the Cartridge After the Run

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

Use an Analysis Parameters File to Analyze the Data

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

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

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

Note: The analysis parameters file in this procedure is an example. 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 opens to the main window.
2. Click **File > Open**, select the data files to analyze, and then click **Open**.
3. On the **Project** toolbar, click , browse to the Oligo 200 nt Analysis file, and then click **Open**.
4. Right-click , and then click **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 was 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. Peaks that are identified in the analysis have a blue marker at the peak start, a red marker at the peak apex, and a green marker at the peak end.

Integrated Peaks in the graph have this shading:

- Green: Identified as a peak in the Marker Table on the Library tab.

Analyze the Data

- Blue: Identified as a peak in the Peak Table on the Library tab.
- Red: 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 other applicable 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: *BioPhase Software Help*.

6. In the Files pane, click a file, and then push the **Up** and **Down** arrow keys to see the data for each file in the Data pane.
7. If required, then drag the cursor over a region on the graph to zoom in to see information about the identified peaks in that region.

Tip! To use the same zoom settings for all of the data files, click .

8. Make sure that the integration is satisfactory. If required, then adjust the integration parameters, and then analyze the data again.
9. 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, then adjust **MT** in the Marker Table and **MT** (or **Cal MT**) in the Peak Table.
 - c. If required, then 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 that is nearest 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 click **Apply & Analyze (all)**.

The software makes the changes in all of the data files.

10. (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.

11. (Optional) On the **File** toolbar, right-click , and then click **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: *BioPhase 8800 System Operator Guide*.

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

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

Examine the Results

Figure 11-1 Oligo 200 nt Test Mix (50 nt to 141 nt)

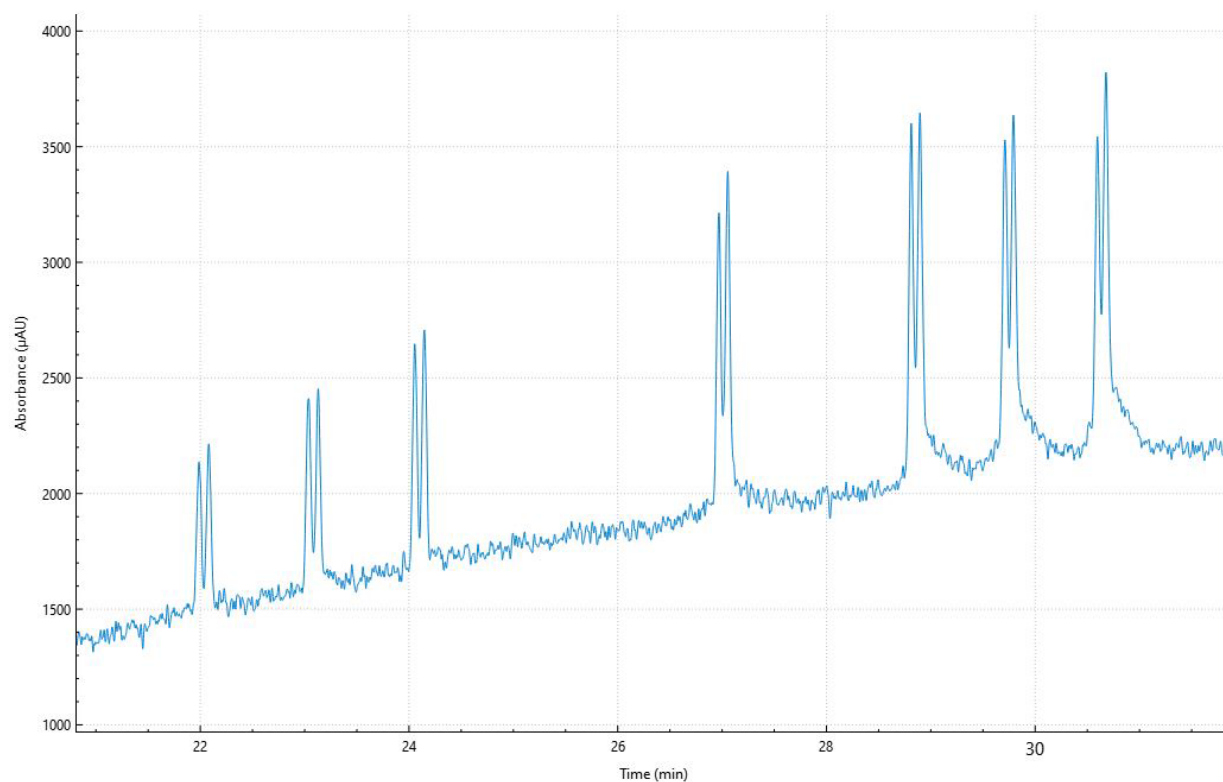
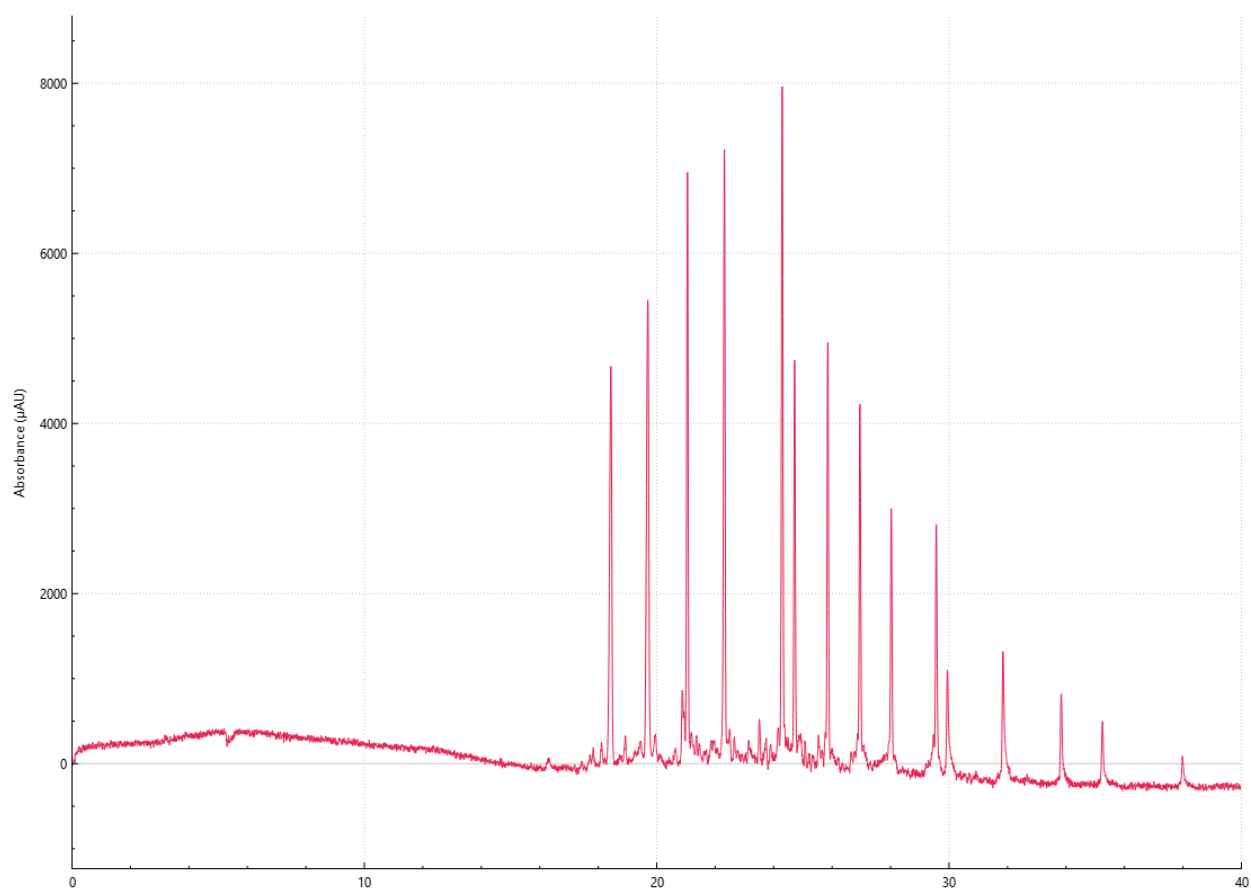
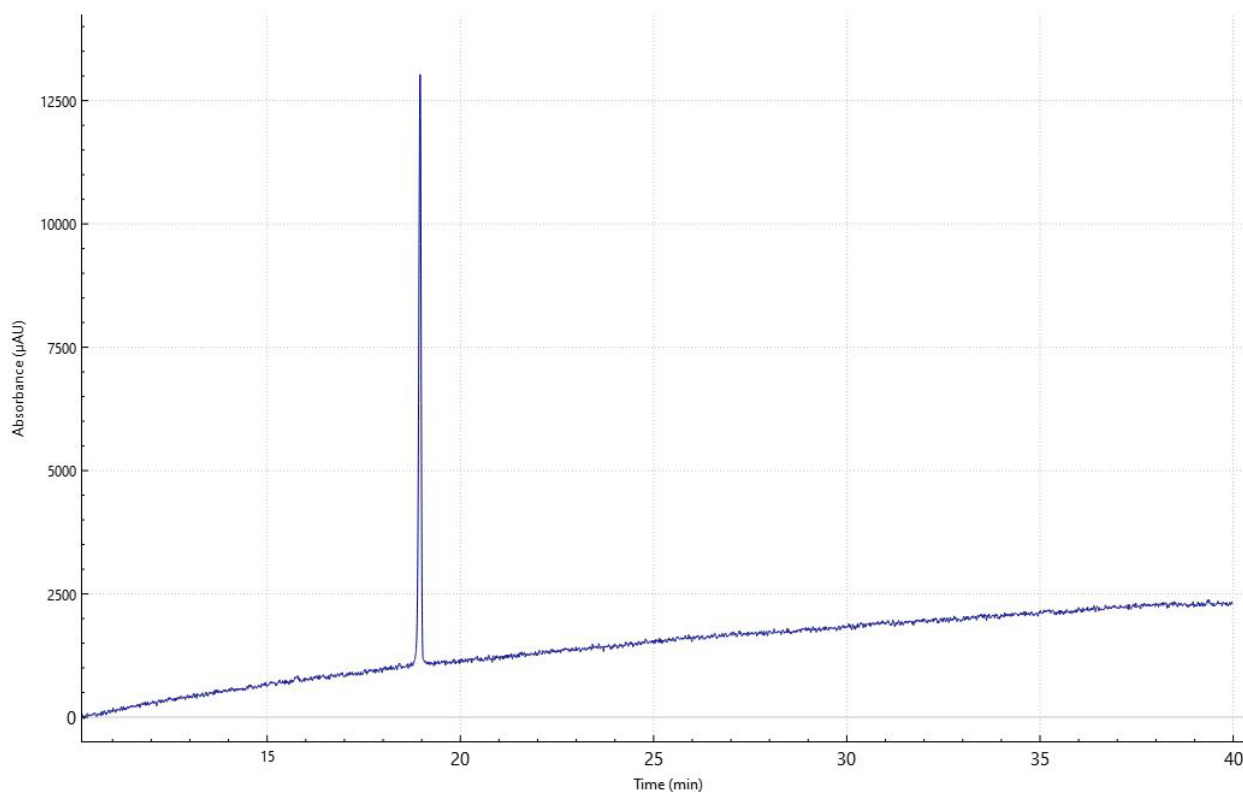


Figure 11-2 Oligo 200 nt Ladder (10 nt to 200 nt)

Analyze the Data

Figure 11-3 Single-Stranded Oligo Standard (15 nt)



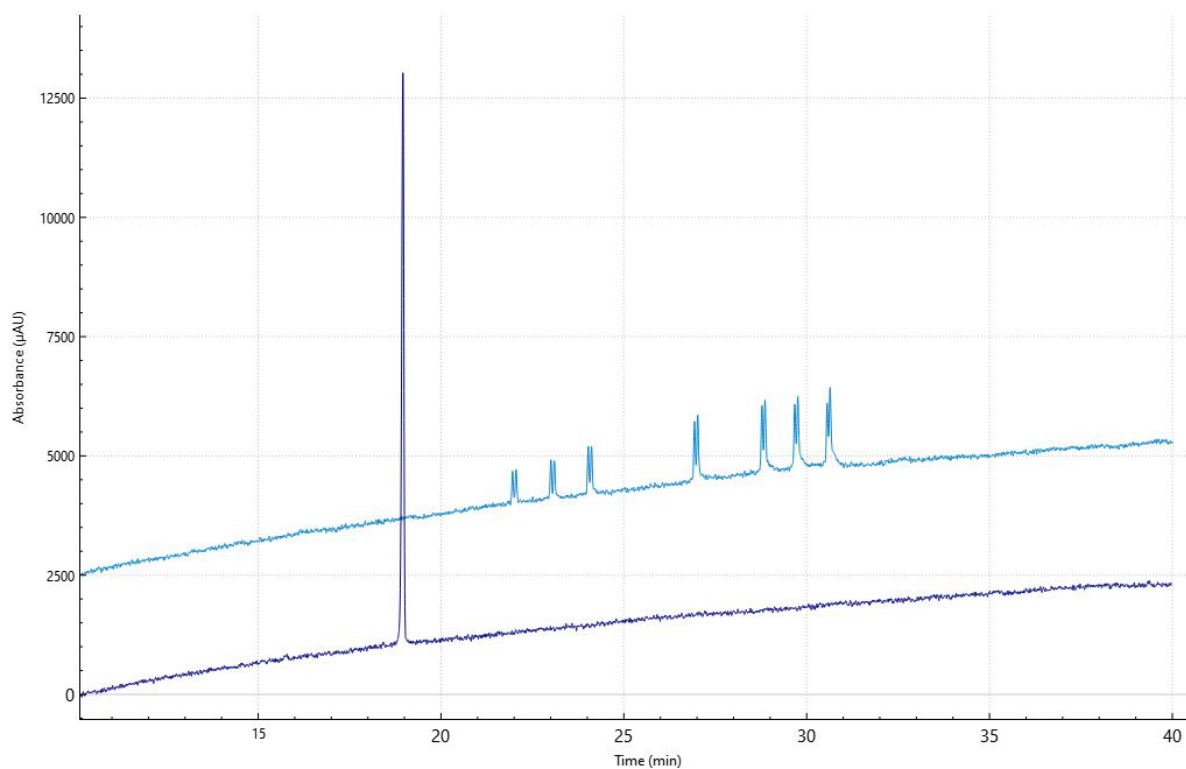
Review the Results on the Overlay Tab

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

Note: This section does not describe the system suitability function. For information about system suitability, refer to the document: *BioPhase 8800 System Operator Guide*.

1. Open a set of data files and the analysis parameters file, and then analyze the data. If required, then adjust the analysis parameters until the results are satisfactory.
2. In the Files pane, click , and then open the Overlay tab.

Figure 11-4 Overlay Tab



The color of the trace in the graph is related to the color in the circle adjacent 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.

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

Analyze the Data

Figure 11-5 Results Table

No.	Name	MT	MT*	Cal MT	Height	Area	Area%	Width 50%
93		24.2875	24.2875	24.28	920.8604	4258.8130	0.73	0.0511
94		24.8500	24.8500	24.85	7687.7227	18858.3320	3.22	0.0371
95		25.0282	25.0282	25.03	6854.6323	20271.1289	3.46	0.0430
96		25.1250	25.1250	25.13	1225.5273	9504.5029	1.11	0.0627
97		25.7782	25.7782	25.78	3225.0591	8366.8952	1.43	0.0492
98		25.8500	25.8500	25.85	4374.9521	13058.3682	2.23	0.0445
99		25.9500	25.9500	25.95	772.8219	4230.8364	0.72	0.0557
100		26.5833	26.5833	26.58	2776.5762	7533.0815	1.29	0.0439
101		26.6583	26.6583	26.66	4558.4105	14472.8085	2.47	0.0462
102		26.7458	26.7458	26.75	897.4018	4881.8320	0.83	0.0555
103		27.3782	27.3782	27.38	3083.2100	8853.4717	1.51	0.0473
104		27.4500	27.4500	27.45	4910.4814	17105.8242	2.82	0.0512
105		27.5282	27.5282	27.53	1276.3547	7658.6953	1.31	0.0587

- 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: *BioPhase 8800 System Operator Guide* or *BioPhase Software Help*.

- 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.

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 to 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 report template.
9. (Optional) On the **File** toolbar, right-click , and then click **Save (all)**.
All of the changes to the results and the analysis parameters are saved to the data files.
10. On the **File** toolbar, right-click , and then click **Close (all)**.

For more reporting, saving, and printing options, refer to the document: *BioPhase 8800 System Operator Guide*.

Guidance for Acceptance Criteria

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

For single-stranded oligonucleotide analysis, the ratio of the migration time between the Single-Stranded Oligo Standard (15 nt) and the analytical peak can be used as a system suitability criterion. If Oligo 200 nt Test Mix (50 nt to 141 nt) is used as a system suitability sample, then the resolution between peaks pairs in the test mix can be used. Different lots of the Oligo 200 nt Test Mix (50 nt to 141 nt) might give different results. The resolution between resolved peaks can also be used. We strongly recommend that peak height, peak area, and absolute migration time not be used as acceptance criteria.



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

After each corrective action is completed, we recommend that the analysis be done again to make sure that the symptom was corrected.

Symptom	Possible Cause	Corrective Action
Cartridge not detected error	1. The ID chip on the cartridge is not clean. 2. The contact pins on the system are not clean. 3. The BioPhase 8800 system firmware is not up to date.	1. Moisten a lint-free 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. 2. Moisten a lint-free wipe or cotton swab with ethanol or isopropanol, and then clean the contact pins. Let the pins air-dry before the cartridge is installed. 3. Do the actions that follow: a. On the touchscreen, touch the icon in the top left corner. b. Record the firmware version. c. Contact sciex.com/request-support.
Capillary not found at start of separation	The optical scan at the beginning of the run failed because of condensation on the cartridge window.	In the shutdown method, increase the Sample Storage temperature to 20 °C to prevent condensation.
Error message: Access door is opened	When the optics door was opened and closed, a sensor error occurred.	Turn off, and then turn on the BioPhase 8800 system. To prevent another occurrence of this issue, always use the applicable procedure to change the filters. Refer to the document: <i>Operator Guide</i>.

Troubleshooting

Symptom	Possible Cause	Corrective Action
Broad peaks, poor resolution	1. The capillary tip is damaged. 2. The sample concentration is too high. 3. The capillary is blocked. 4. The internal surface of the capillary is contaminated. 5. The capillary coating is damaged.	1. Do the actions that follow: a. Use a magnifying lens to examine the capillary tip. b. Use a lint-free wipe to clean the capillary inlets carefully in an outward direction. c. Make sure that the capillary tip extends approximately 2 mm from the cannula electrode. d. 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: Options for a Blocked or Damaged Capillary. 2. Do one or all of the actions that follow: • Decrease the Duration in the Inject action in the separation method to inject less sample. If the results are not satisfactory, then decrease the Pressure or Voltage. • Dilute the sample again with the sample diluent. 3. Refer to the section: Options for a Blocked or Damaged Capillary. 4. Edit the sequence to ignore the contaminated capillary or replace the cartridge.
Carryover	1. The sample concentration is too high.	1. Do one or all of the actions that follow:

Symptom	Possible Cause	Corrective Action
	The reagent plate is contaminated with sample.	- Decrease the Duration in the Inject action in the separation method to inject less sample. If the results are not satisfactory, then decrease the Pressure or Voltage. - Dilute the sample again with the sample diluent. - In the separation method, add one or more water dip steps after sample injection. With the edited method, do this: - Create a new sequence that uses this separation method. - Prepare new reagent plates for the new sequence. Refer to the section: Load the Reagent Inlet and Outlet Plates.
High current	- The gel buffer is contaminated. - The positions of the reagents in the reagent plate do not agree with the plate layouts in the sequence.	- Prepare the inlet and outlet reagent plates again to replace the gel buffer. - 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: Plate Layouts.
Low or unsteady current	- The capillary tip is blocked or contaminated. - The gel buffer has air bubbles.	Refer to the section: Remove Blockage from a Capillary. If the current is low or unsteady, then replace the cartridge.

Troubleshooting

Symptom	Possible Cause	Corrective Action
		- Do one or all of the actions that follow: - Use a centrifuge to spin the plate at $30 \times g$ for 5 minutes. - De-gas the gel buffer with 5 inches Hg to 15 inches Hg vacuum for 5 minutes.
Missing peaks in electropherogram sample	- A pipetting error occurred during preparation of the sample. - The method parameters are incorrect. - The positions of the reagents in the reagent plate do not agree with the plate layouts in the sequence.	- Prepare a new sample. - Do the actions that follow: - In Method Settings, make sure that the value for Detector Type is UV or NFD. - In the separation method, make sure that the values for Type of Injection and Duration are correct. - Make sure that the positions of the samples in the sample plate 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: Reagents, Plate Layouts, and Methods.
No electrical current during separation	- The capillary is damaged. - The electrode is broken or bent. - The capillary tip is blocked or contaminated. - The positions of the reagents in the reagent plate do not agree with the plate layouts in the sequence.	- Refer to the section: Options for a Blocked or Damaged Capillary. - Replace the cartridge. - Refer to the section: Remove Blockage from a Capillary. - Make sure that the positions of the samples and reagents in the plates agree with the plate layouts. If the positions are

Symptom	Possible Cause	Corrective Action
	5. A capillary is filled with air bubbles.	not correct, then use the plate layout to prepare the plates again. Refer to the section: Plate Layouts. 5. Do one or all of the actions that follow: • Make sure that the wells of the sample and reagent plates contain sufficient solution. • Make sure that the positions of the reagents in the reagent plates agree with the plate layouts. If the positions are not correct, then use the plate layout to prepare the plates again. Refer to the section: Plate Layouts. • Use a centrifuge to spin the plate at $30 \times g$ for 5 minutes.
No peaks	1. UV detectors: The life of the UV lamp is more than the maximum. 2. The method parameters are incorrect. 3. Air bubbles in a sample well prevent sample injection. 4. The capillary window is blocked. 5. The capillary tip is blocked or contaminated. 6. The sample volume is too low. 7. There is no sample in a sample well, or the position of the samples in the sample	1. Replace the UV lamp. Replace the UV lamp. Refer to document: <i>Operator Guide</i>. 2. Do the actions that follow: - In Method Settings, make sure that the value for Detector Type is UV or NFD. - In the Inject and Separate actions, make sure that the value for Polarity is Reverse. - In the UV separation method, make sure that the value for Wavelength is

Troubleshooting

Symptom	Possible Cause	Corrective Action
	plate do not agree with the plate layouts in the sequence.	220 nm. If an NF detector is used, then make sure the 350 nm filter is installed. d. Make sure that pressure is applied at the inlet and the outlet during the separation. 3. Use a centrifuge to spin the plate at $30 \times g$ for 5 minutes. 4. Examine the capillary window. Make sure that the window is clean and the path is clear. Refer to the section: Examine the Capillary Cartridge. 5. Refer to the section: Options for a Blocked or Damaged Capillary. 6. Make sure that there is 100 μL of sample in the sample well. 7. Make sure that the position of the samples in the sample plate agrees with the plate layout. Refer to the section: Plate Layouts.
Spikes in electropherogram	1. The gel buffer has air bubbles.	1. Do one or all of the actions that follow: • Use a centrifuge to spin the plate at $30 \times g$ for 5 minutes. • De-gas the gel buffer with 5 inches Hg to 15 inches Hg vacuum for 5 minutes.

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 external surface of the capillary inlets.

3. Use a lint-free 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 ignore the blocked or damaged capillary. Refer to the section: [Create the Sequence](#).

Reagents, Plate Layouts, and Methods

A

Reagent Set

If the reagents are not available, then use the figures that follow to create a new reagent set.

Figure A-1 Oligo 200 nt Purity and Integrity Kit Inlet Reagents

Inlet Reagents from Reagent Set:



Name	Viscosity	Color
Water	0.89	 SkyBlue
Acidic Conditioning Solution	0.89	 Red
Water Dip 1	0.89	 SkyBlue
Water Dip 2	0.89	 SkyBlue
Water Dip 3	0.89	 SkyBlue
Water Dip 4	0.89	 SkyBlue
Oligo 200 nt Purity and Integrity Gel	200.00	 Green

Figure A-2 Oligo 200 nt Purity and 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
Water Dip 3	0.89	 SkyBlue
Water Dip 4	0.89	 SkyBlue
Waste	0.89	 Black
Oligo 200 nt Purity and Integrity Gel	200.00	 Green

Plate Layouts

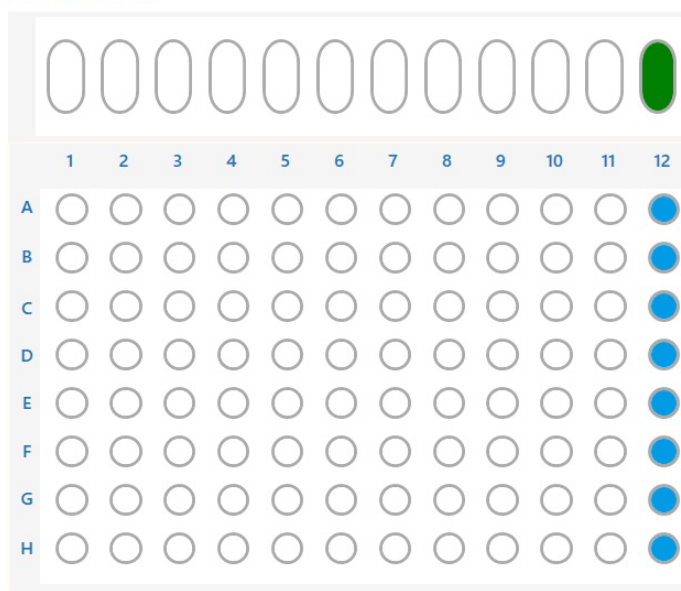
Note: The figures that follow show the plate layouts for the Oligo 200 nt Separation sequence that is supplied with the software. The plate layouts are automatically created in the software for each sequence.

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 A-3 Layouts for the Sample Inlet Plate and Sample Outlet Plate

Sample Plate



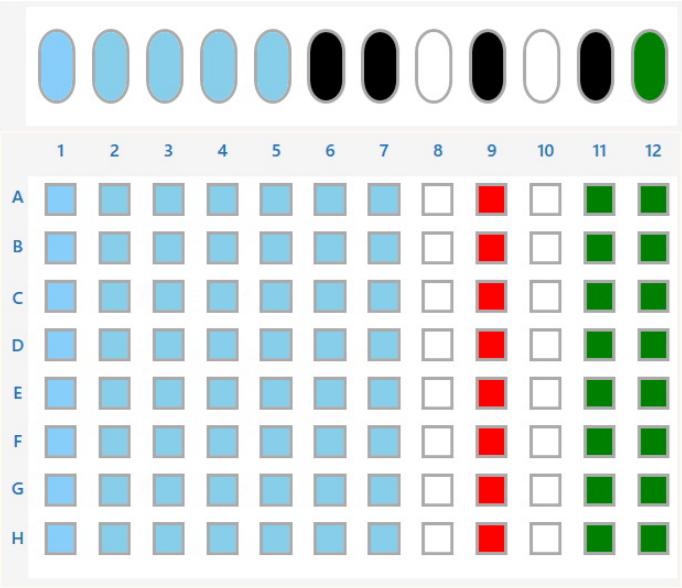
Column	Outlet	Col...
12	Oligo 200 nt Purity and Integrity Gel	

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 A-4 Layouts for the Reagent Inlet Plate and Reagent Outlet Plate

Reagent Plate



C..	Inlet	Color	Outlet	Color
2	Water Dip 1	Light Blue	Water Dip 1	Light Blue
3	Water Dip 2	Light Blue	Water Dip 2	Light Blue
4	Water Dip 3	Light Blue	Water Dip 3	Light Blue
5	Water Dip 4	Light Blue	Water Dip 4	Light Blue
6	Water	Light Blue	Waste	Black
7	Water	Light Blue	Waste	Black
9	Acidic Conditioning Solution	Red	Waste	Black
11	Oligo 200 nt Purity and Integri...	Green	Waste	Black
12	Oligo 200 nt Purity and Integri...	Green	Oligo 200 nt Purity and Integri...	Green

Methods

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

If a shorter run time and higher throughput are required, then refer to the other methods that are available for the Oligo 200 nt Purity and Integrity kit for the BioPhase 8800 system at sciex.com/tech-notes.

Method Settings

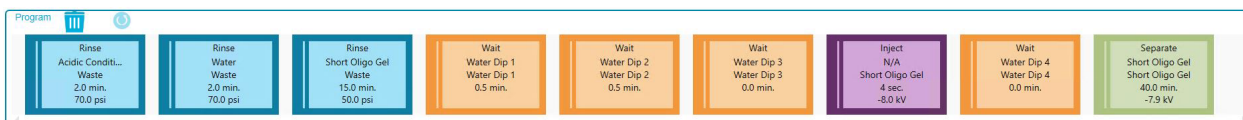
Note: Use the settings that follow for all of the methods with a **Detector Type** of **UV**.

Figure A-5 Settings for the Oligo 200 nt Purity and Integrity Method with UV Detection

Temperature Capillary Cartridge 22.0 °C ☒ Wait Sample Storage 10.0 °C ☒ Wait		Detector Type ☒ UV ☐ LIF ☐ No Detector		Wavelength 254 nm
Cartridge Settings Capillary Length 30.0 cm Capillary Type Bare Fused Silica		Emission Wavelength 520 nm PMT Gain 100		
Current Limits ☐ Enable current limiting when using voltage Maximum Current 600 µA		Data Data Collection Rate 8 Hz Peak Width @ 50% Height 2 sec		

Conditioning and Separation Method

Figure A-6 Actions in the Oligo 200 nt Conditioning and Separation Method



Reagents, Plate Layouts, and Methods

Figure A-7 Summary of Actions in the Oligo 200 nt Conditioning and Separation Method

Method Duration: 60.0 min. Number of Actions: 9			
	Settings	Capillary Cartridge: 22.0 °C, Wait Capillary Length: 30.0 cm Capillary Type: Bare Fused Silica Current Limit: 600 µA	Sample Storage: 10.0 °C, Wait Detector Type: UV, 254 nm, Wait Peak Width: 2 sec. Data Rate: 8 Hz
	Rinse	Duration: 2.0 min. 70.0 psi	Inlet: Acidic Conditioning Solution Outlet: Waste
	Rinse	Duration: 2.0 min. 70.0 psi	Inlet: Water Outlet: Waste
	Rinse	Duration: 15.0 min. 50.0 psi	Inlet: Short Oligo Gel Outlet: Waste
	Wait	Duration: 0.5 min.	Inlet: Water Dip 1 Outlet: Water Dip 1
	Wait	Duration: 0.5 min.	Inlet: Water Dip 2 Outlet: Water Dip 2
	Wait	Duration: 0.0 min.	Inlet: Water Dip 3 Outlet: Water Dip 3
	Inject	Duration: 4 sec. -8.0 kV	Plate: Sample Outlet: Short Oligo Gel
	Wait	Duration: 0.0 min.	Inlet: Water Dip 4 Outlet: Water Dip 4
	Separate	Duration: 40.0 min. -7.9 kV Ramp Time: 0.1 min. Autozero: 10.0 min.	Inlet: Short Oligo Gel Outlet: Short Oligo Gel

Shutdown Methods

Oligo 200 nt Shutdown Method 1 of 2

Figure A-8 Actions in the Oligo 200 nt Shutdown Method 1 of 2

Program	
	Rinse Acidic Condi... Waste 7.0 min. 70.0 psi
	Rinse Water Waste 20.0 min. 50.0 psi

Figure A-9 Summary of Actions in the Oligo 200 nt Shutdown Method 1 of 2

Method Duration: 27.0 min. Number of Actions: 2			
	Settings	Capillary Cartridge: 40.0 °C, Wait Capillary Length: 30.0 cm Capillary Type: Bare Fused Silica Current Limit: 600 µA	Sample Storage: 15.0 °C, Wait Detector Type: UV, 254 nm, Wait Peak Width: 2 sec. Data Rate: 8 Hz
	Rinse	Duration: 7.0 min. 70.0 psi	Inlet: Acidic Conditioning Solution Outlet: Waste
	Rinse	Duration: 20.0 min. 50.0 psi	Inlet: Water Outlet: Waste

Oligo 200 nt Shutdown Method 2 of 2

Figure A-10 Actions in the Oligo 200 nt Shutdown Method 2 of 2

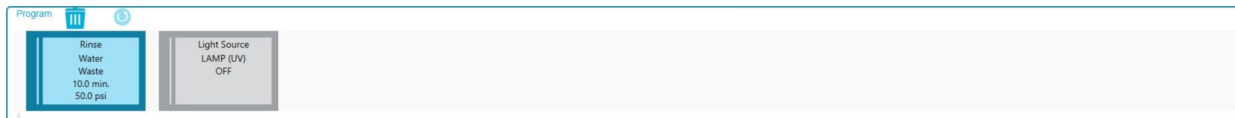


Figure A-11 Summary of Actions in the Oligo 200 nt Shutdown Method 2 of 2

Method Duration: 10.1 min. Number of Actions: 2

⚙️	Settings	Capillary Cartridge: 22.0 °C, Wait Capillary Length: 30.0 cm Capillary Type: Bare Fused Silica Current Limit: 600 µA	Sample Storage: 10.0 °C, Wait Detector Type: UV, 254 nm, Wait Peak Width: 2 sec. Data Rate: 8 Hz
💧	Rinse	Duration: 10.0 min. 50.0 psi	Inlet: Water Outlet: Waste
💡	UV Lamp	OFF	

Hazardous Substance Information

B

The information that follows must be noted and the related safety measures must be obeyed. For more information, refer to the related safety data sheets (SDS). The SDS are available on request or can be downloaded from our website, at sciex.com/tech-regulatory.

Hazard classification according to HCS 2012.

Acid Wash/Regenerating Solution (0.1 N HCl)



DANGER! Causes severe skin burns and eye damage.

Oligo 200 nt Purity and Integrity Gel



DANGER! Might damage fertility or the unborn child.

Other Reagents

- CE Grade Water
- Oligo 200 nt Purity and Integrity Sample Buffer (20×)
- Oligo 200 nt Test Mix (50 nt to 141 nt)
- Oligo 200 nt Ladder (10 nt to 200 nt)
- Single-Stranded Oligo Standard (15 nt)

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

Contact Us

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Customer Training

- Global: sciex.com/contact-us

Online Learning Center

- [SCIEX Now Learning Hub](#)

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- sciex.com/contact-us
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Documentation

This version of the document supersedes all of the 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.

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