Application Guide

Oligo 200 nt Purity and Integrity Kit

for the PA 800 Plus System

TPUB-CUST-16093-C

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Oligo 200 nt Purity and Integrity Kit

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 PA 800 Plus software and the Empower™ Chromatography Data System software,[1] and data analysis of data that is acquired with the PA 800 Plus 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: *Overview 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.

Workflow 1

The workflow has these steps:

1. Identify the number of samples to be analyzed and the number of replicates.
2. For PA 800 Plus software users:
    a. Download the required files. Refer to the section: Download the Required Files for the PA 800 Plus 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 and Start the Run.
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 PA 800 Plus software methods. Refer to the section: Import the Instrument Methods.
    c. Configure the Empower™ CDS software to use multiple plates. Refer to the section: Configure the Empower™ CDS Software to Use Multiple Plates.
    d. Create the sample set method and run the samples. Refer to the section: Create the Sample Set Method and Run the Samples.
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 PA 800 Plus System.
6. Start data acquisition:
    - PA 800 Plus software: Create the sequence and start the run. Refer to the section: Create the Sequence and Start the Run.
    - Empower™ CDS software: Start the sample set method from the Empower™ CDS software. Refer to the section: Create the Sample Set Method and Run the Samples.
7. After the run, stow the cartridge. Refer to the section: Stow the Cartridge After the Run.
8. Analyze the data:
    - PA 800 Plus software: When the sequence is complete, use the PA 800 Plus 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 2

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

Table 2-1 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 2-2 Other Supplies from SCIEX

Component	Quantity	Part Number
Capillary cartridge, blank	1	144738
Cartridge rebuild kit	1	144645
Filter, 254 nm, for use with UV detector	1	144438
nanoVials	100	5043467
Oligo 200 nt Ladder (10 nt to 200 nt)	1	5322663
PCR microvials (200 µL)	100	144709
Sample Loading Solution	6 mL	608082
Universal vial caps, blue	100	A62250
Universal vials	100	A62251

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
- Vortex mixer

Required Detector

A UV detector with a 254 nm filter is required.

Required Cartridge or Capillary

- Standard capillary cartridge assembly (PN 144738)
- Bare fused-silica capillary, 50 µm inner diameter (i.d.) × 67 cm (PN 338451)

Methods 3

The Oligo 200 nt Purity and Integrity kit requires a conditioning method, separation method, and shutdown method. To create the methods, refer to the sections: Initial Conditions, UV Detector Initial Conditions, and Time Programs.

Because this kit can be used in many different applications, use the methods that are given here as a place to start to create a method that is applicable for other relevant applications. For more application and workflow support, go to sciex.com/tech-notes or sciex.com/request-support.

Initial Conditions

Note: The values on the Initial Conditions tab are the same for all of the methods.

Figure 3-1 Initial Conditions Tab for the Oligo 200 nt Conditioning and Separation Method and Oligo 200 nt Shutdown Method 2 of 2

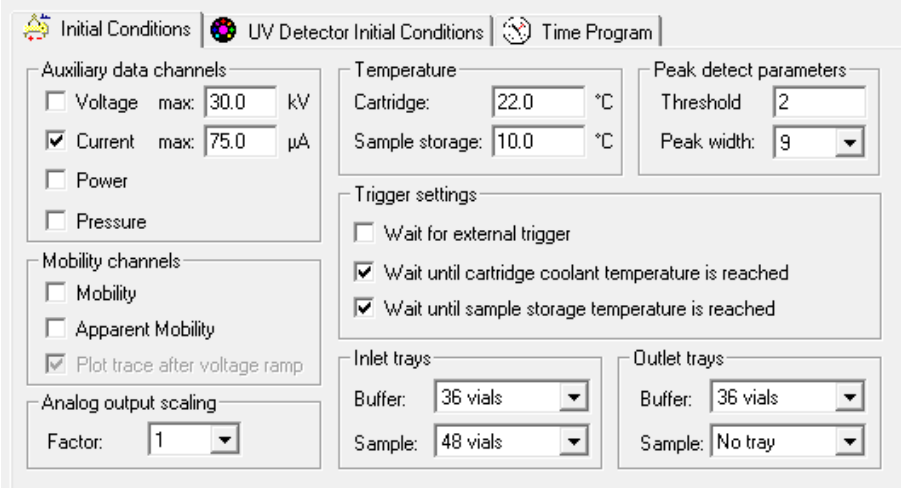
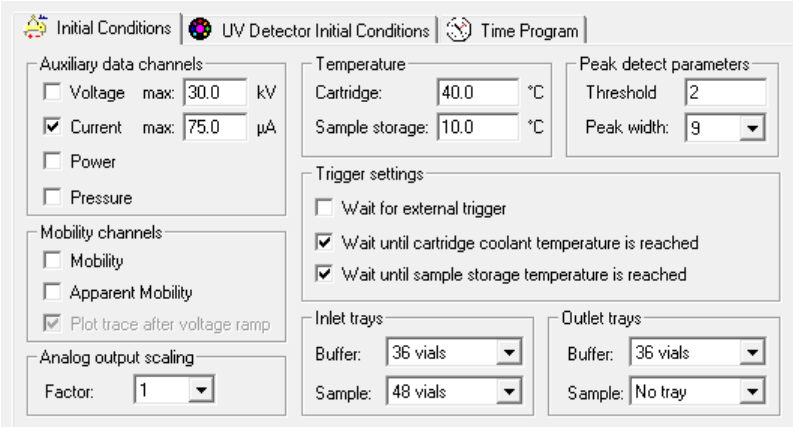


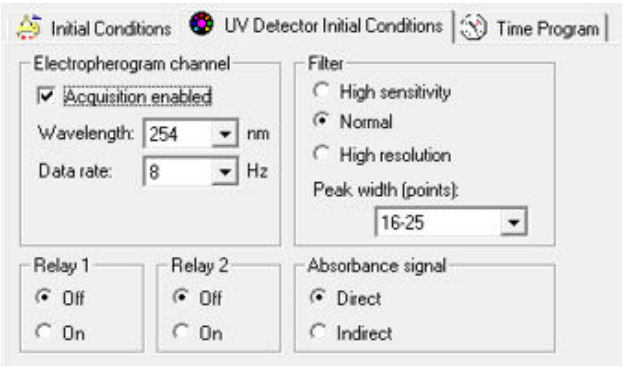
Figure 3-2 Initial Conditions Tab for the Oligo 200 nt Shutdown Method 1 of 2



UV Detector Initial Conditions

Note: The values on the UV Detector Initial Conditions tab are the same for all of the methods.

Figure 3-3 UV Detector Initial Conditions Tab



Time Programs

Figure 3-4 Time Program Tab for the Oligo 200 nt Conditioning and Separation Method

Initial Conditions | UV Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	70.0 psi	2.00 min	BI:E1	BO:E1	forward, In / Out vial inc 12	acidic rinse
2		Rinse - Pressure	70.0 psi	2.00 min	BI:F1	BO:F1	forward, In / Out vial inc 12	water rinse
3		Rinse - Pressure	50.0 psi	15.00 min	BI:B1	BO:B1	forward, In / Out vial inc 12	gel rinse
4		Wait		0.50 min	BI:A4	BO:A4	In / Out vial inc 12	water dip 1
5		Wait		0.50 min	BI:B4	BO:B4	In / Out vial inc 12	water dip 2
6		Wait		0.00 min	BI:C4	BO:C4	In / Out vial inc 12	water dip 3
7		Inject - Voltage	8.0 KV	4.0 sec	SI:A1	BO:C1	Override, reverse polarity	sample injection
8		Wait		0.00 min	BI:D1	BO:D1	In / Out vial inc 12	water dip 4
9	0.00	Separate - Voltage	8.3 KV	40.00 min	BI:C1	BO:C1	0.17 Min ramp, reverse polarity, In	separation
10	15.00	Autozero						
11	40.00	End						
12								

Figure 3-5 Time Program Tab for the Oligo 200 nt Shutdown Method 1 of 2

Initial Conditions | UV Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	70.0 psi	7.00 min	BI:E1	BO:E1	forward	acidic rinse
2		Rinse - Pressure	50.0 psi	20.00 min	BI:F4	BO:F1	forward	water rinse
3								

Figure 3-6 Time Program Tab for the Oligo 200 nt Shutdown Method 2 of 2

Initial Conditions | UV Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	50.0 psi	20.00 min	BI:F4	BO:F1	forward	water rinse
2		Lamp - Off						
3								

Prepare the Standards, Test Mix, and Samples 4

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 2 µL of Single-Stranded Oligo Standard (15 nt) to 798 µL of sample dilution buffer (1×).
3. Use a vortex mixer to mix the solution.
4. Add 100 µL of the Single-Stranded Oligo Standard (15 nt) solution to a PCR microvial.

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 10 µL of Oligo 200 nt Ladder (10 nt to 200 nt) to 90 µL of sample dilution buffer (1×).
3. Use a vortex mixer to mix the solution.
4. Add 100 µL of the Oligo 200 nt Ladder (10 nt to 200 nt) solution to a PCR microvial.

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 2 µL of Oligo 200 nt Test Mix (50 nt to 141 nt) to 798 µL of sample dilution buffer (1×).
3. Use a vortex mixer to mix the solution.
4. Add 100 µL of the Oligo 200 nt Test Mix (50 nt to 141 nt) solution to a PCR microvial.

Prepare the Sample

1. Dilute the oligonucleotide sample with CE Grade Water, Sample Loading Solution, or sample dilution buffer (1×) to make a concentration between 5 pg/µL to 500 pg/µL.
2. (Optional) For samples that require heat denaturation, do this:
   a. Use heat to increase the temperature of the sample to 70 °C for 5 minutes.
   b. Immediately put the sample on ice for a minimum of 2 minutes.
3. Add 100 µL of the sample to a microvial.
   If the available sample volume is low, then add 5 µL to 10 µL of the sample to a nanoVial.

Prepare the PA 800 Plus System 5

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

Install the UV Detector

1. Turn off the PA 800 Plus system.
2. Install the UV detector. Refer to the document: *Maintenance Guide*.
3. Turn on the system.
4. Let the lamp become warm for a minimum of 30 minutes.

Clean the Electrodes, Insertion Levers, and Interface Block

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

Clean the electrodes, opening levers, and interface block every week or when chemistries are changed. For detailed instructions, refer to the section: "Clean the Electrodes, Insertion Levers, and Interface Block" in the document: *Maintenance Guide*.

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

Install the Capillary

CAUTION: Potential System Damage. Do not let the capillary become dehydrated. The coating inside the capillary starts to dehydrate 5 minutes to 10 minutes after the tip of the capillary is cut.

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

1. Install the capillary in a capillary cartridge. Refer to the section: "Rebuild a Capillary Cartridge" in the document: *Maintenance Guide*.

The recommended capillary length is 20 cm to the window and 30 cm total length. Use an aperture that is 100 µm × 200 µm.

2. To keep damage to the capillary coating to a minimum, do this:
   a. Fill two universal vials with 1.5 mL of CE Grade Water, and then attach blue caps.
   b. Cut the end-cap from the inlet side of the capillary.
   c. Install the capillary in the cartridge.
   d. Cut the end-cap from the outlet side of the capillary.
   e. Complete the cartridge assembly.
   f. Cut the capillary tips to the recommended length.
   g. Put the capillary tips in vials that are filled with CE Grade Water.

Install the Cartridge

1. Remove the cartridge from the box.
2. Install the cartridge in the PA 800 Plus system. For instructions, refer to the document: *Maintenance Guide*.

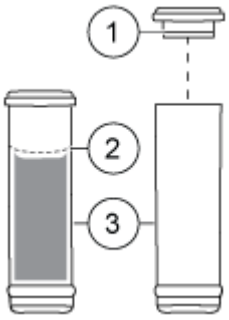
Load the Buffer Trays

CAUTION: Potential System Damage. Do not fill the vials with more than 1.8 mL of liquid or let more than 1.8 mL of liquid collect in the waste vials. If a vial is filled with more than 1.8 mL of liquid, then damage to the pressure system might occur.

Note: For this application, all of the vials and caps are made to be used for a maximum of five runs. Because the vials and caps might be contaminated with dried gel and other chemicals, do not use them again.

1. Fill a vial for each sample to be run, and then attach caps to the vials.

Figure 5-1 Universal Vial and Cap

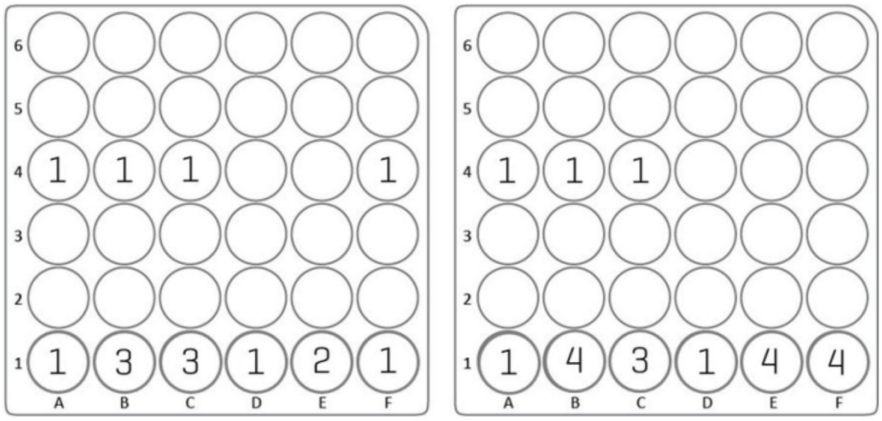


Item	Description
1	Universal vial cap
2	Maximum fill line
3	Universal vial

2. Put the vials in the buffer trays as shown in the figure that follows.

Note: Each row is sufficient for five runs.

Figure 5-2 Buffer Tray Inlet (BI), Left and Buffer Tray Outlet (BO), Right



Item	Description
1	1.5 mL CE Grade Water
2	1.5 mL Acid Wash/Regenerating Solution (0.1 N HCl)
3	1.5 mL Oligo 200 nt Purity and Integrity Gel
4	1.0 mL CE Grade Water

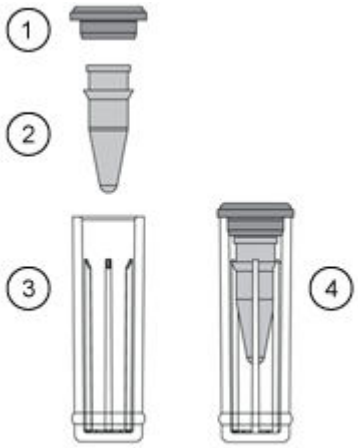
Note: The Oligo 200 nt Purity and Integrity Gel is stable for 24 hours at ambient temperature. If a sequence is longer than 24 hours, then replace the Oligo 200 nt Purity and Integrity Gel.

Load the Sample Tray

Note: Because the vials and caps might be contaminated with dried gel and other chemicals, do not use them again.

1. Prepare the samples:
   - For samples that are in PCR microvials, put the PCR microvial in a universal vial, and then attach a cap.
   - For samples that are in nanoVials, attach a cap.

Figure 5-3 Microvial in a Universal Vial

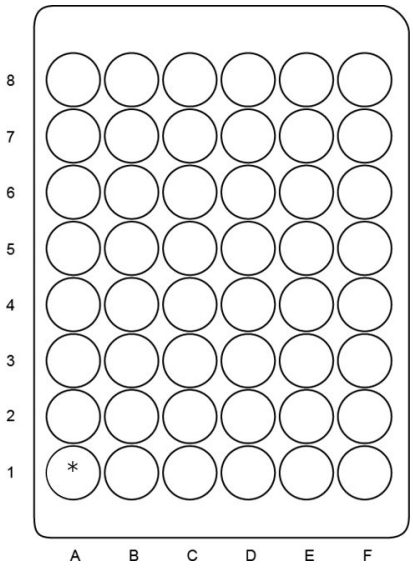


Item	Description
1	Universal vial cap
2	PCR microvial
3	Universal vial
4	Microvial inside a universal vial

2. Put the sample vials in the sample inlet tray:
   a. Fill the well at position A1.
   b. Fill the remaining wells in column A.

c. Fill the other wells.

Figure 5-4 Sample Tray Inlet (SI)



Download the Required Files 6

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

- PA 800 Plus software: Refer to the section: Download the Required Files for the PA 800 Plus Software.
- Empower™ CDS software: Refer to the section: Download the Required Files for the Empower™ CDS Software.

Download the Required Files for the PA 800 Plus Software

1. Go to sciex.com/software-downloads.
2. In the More software downloads section, click **PA 800 Plus Resources**.
3. Click the latest PA 800 Plus `Project` file.
4. In File Explorer, right-click the latest PA 800 Plus 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 `oligo-200` folder to the `C:\32Karat\projects` folder.

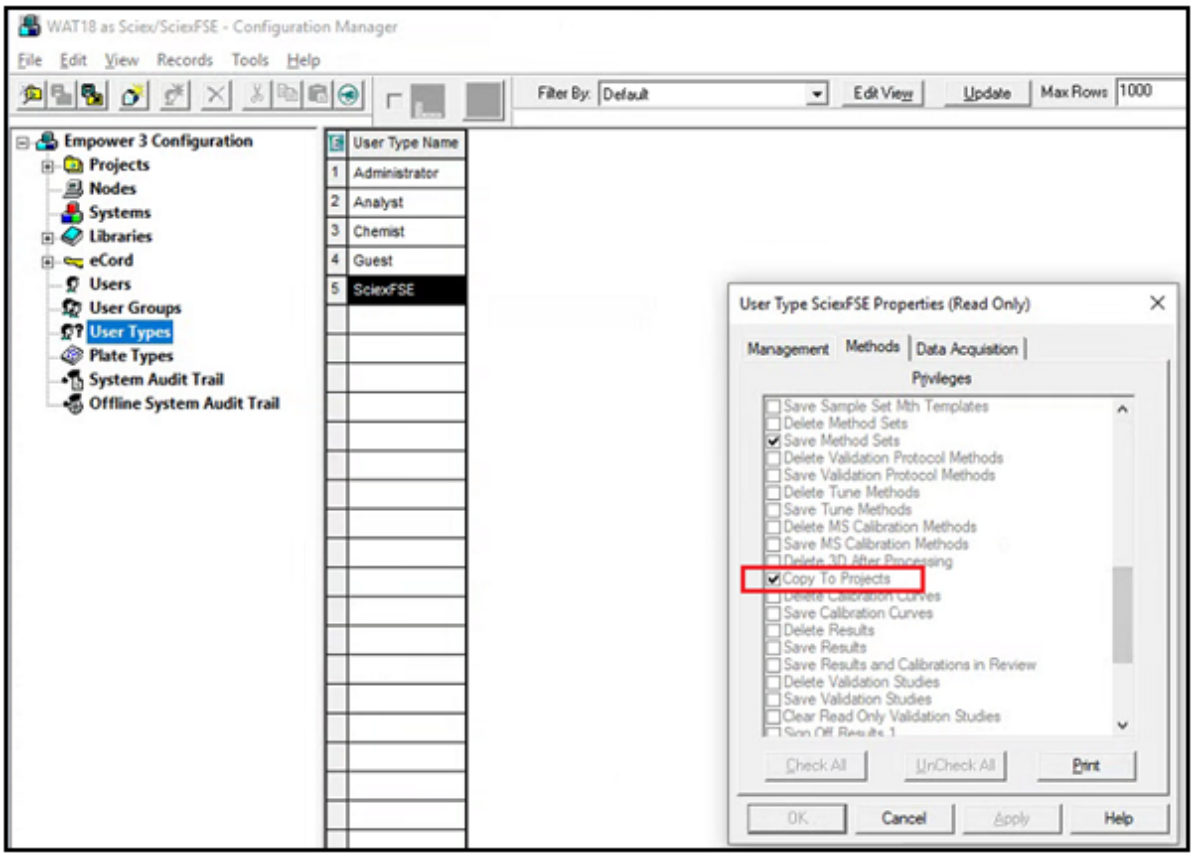
Download the Required Files for the Empower™ CDS Software

1. Go to sciex.com/software-downloads.
2. In the More software downloads section, click **PA 800 Plus Driver Resources**.
3. Click **PA 800 Plus Empower Method Files**.
4. In File Explorer, right-click the `PA-800-Plus-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

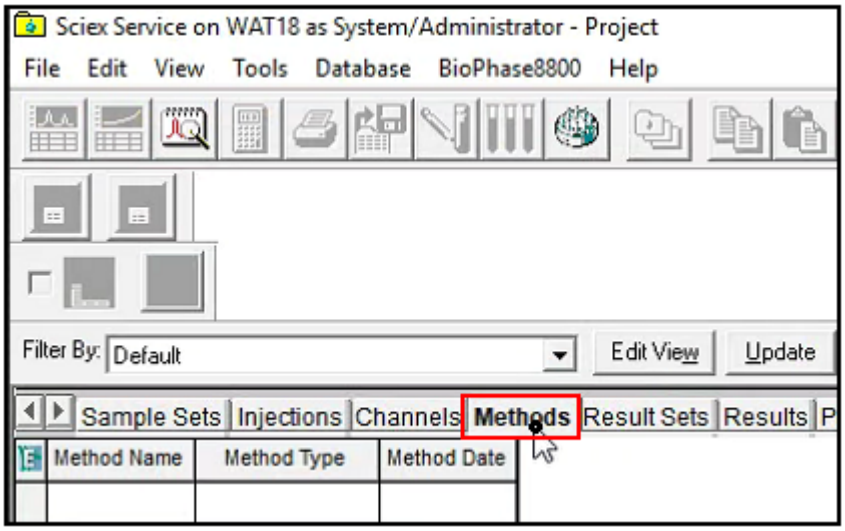
1. In the Configuration Manager, make sure that the **Copy To Projects** check box is selected.

Figure 6-1 Configuration Manager Window



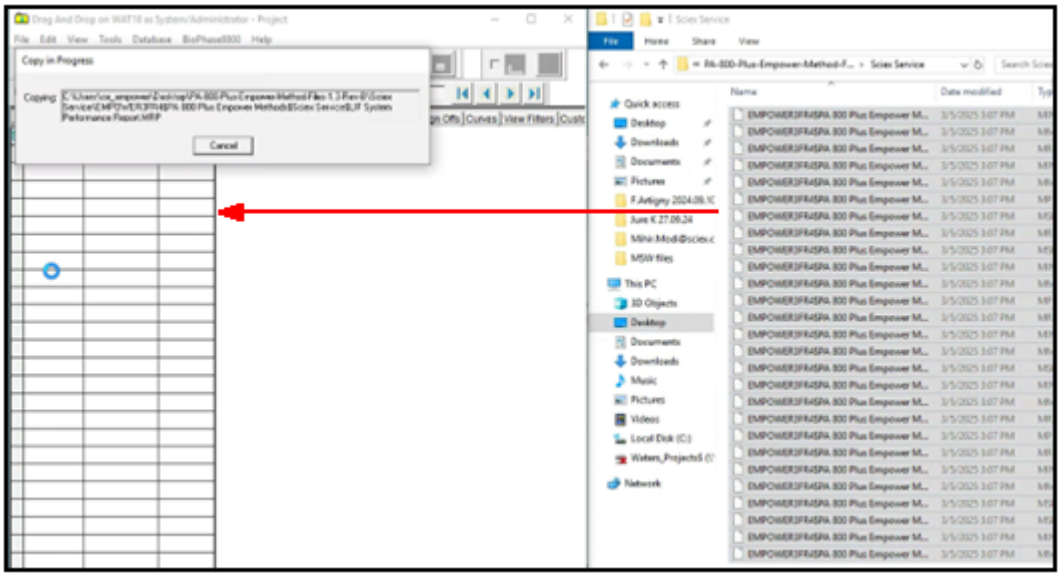
2. Browse to the new project, right-click, and then click **Open**.

Figure 6-2 Methods Tab



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

Figure 6-3 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.

Run the Samples 7

Create the Sequence and Start the Run

To do this procedure, the user must know how to use the 32 Karat software to create and run a sequence. For instructions, refer to the document: *Methods Development Guide*.

1. Open the 32 Karat software.
2. Select an instrument with a UV detector or create a new instrument, and then open the instrument.
3. To run only the test mix, create a sequence with three rows:
   - Row 1: Separation method
   - Row 2: Shutdown method 1
   - Row 3: Shutdown method 2
4. To run more samples, add another row after the conditioning method for each sample.
5. Fill the vials in each of the buffer trays.

   Note: Each buffer vial row is sufficient for five samples.
6. Make sure that the UV lamp is turned on.
7. Make sure that the sample and buffer trays are loaded.
8. Click .
   The Run Sequence dialog opens.
9. Make the required changes, and then click **Start**.

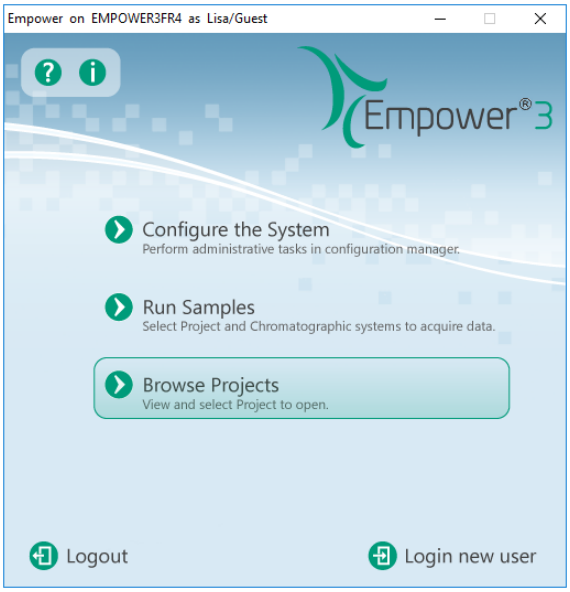
Run the Samples with the Empower™ CDS Software 8

This section gives instructions to acquire data with the Empower™ CDS software.

Import the Instrument Methods

1. Browse to the location where the downloaded method files were saved.
2. In the Empower™ CDS Software Pro Interface window, click **Browse Projects**, click the project of interest, and then click **OK**.

Figure 8-1 Empower™ CDS Software Pro Interface Window



The Project window opens.

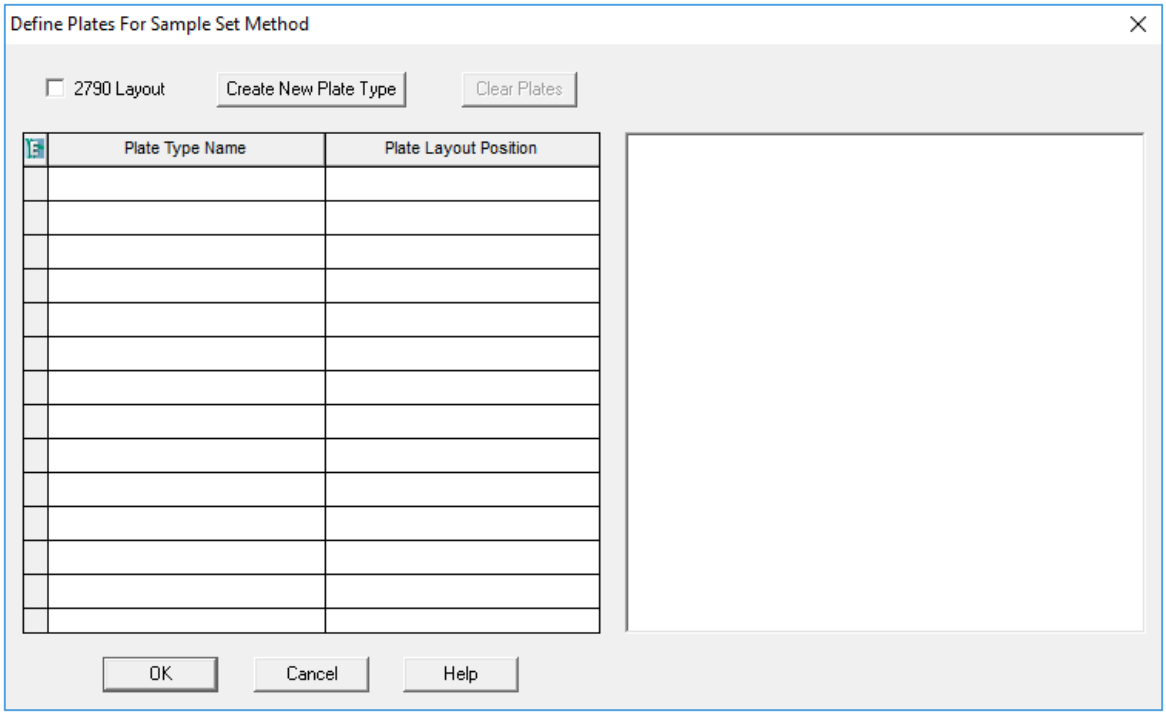
3. Open the Methods tab.
4. On the Windows desktop, click each `min` file in the `Methods` folder, and then drag it to the Project window.
   The instrument method is added to the project. It can be edited and added to a method set.

Configure the Empower™ CDS Software to Use Multiple Plates

The Empower™ CDS software is made for chromatography systems that do not have buffer trays. To change the configuration to use the buffer trays, do this procedure.

1. In the Empower™ CDS software Run Samples window, click **Edit** > **Plates**.

Figure 8-2 Define Plates for Sample Set Method Dialog



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

2. In the first row, configure the buffer inlet tray:
   a. Click the **Plate Type Name** cell, and then select **PA 800 Plus Buffer Tray**.

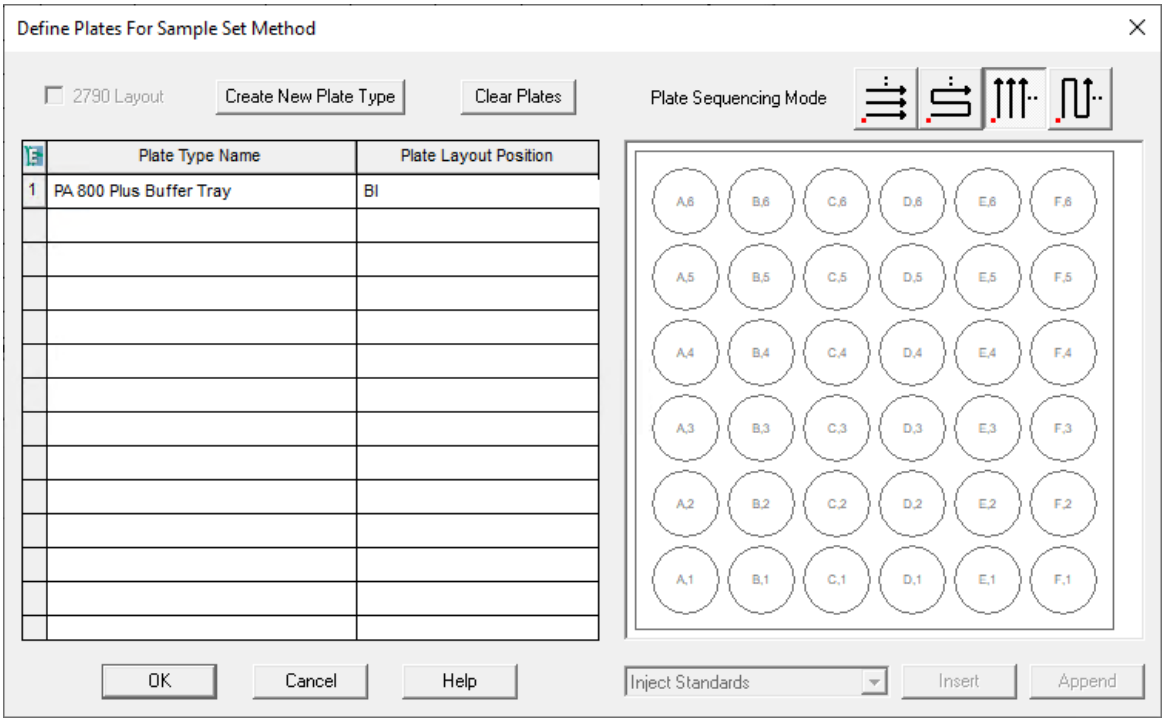
      Note: If **PA 800 Plus Buffer Tray** is missing, then the definition for the buffer and sample trays might not have been set. Refer to the document: *PA 800 Plus Empower™ Driver Software User Guide*.

      The dialog updates with an image of the plate and buttons for the plate sequencing mode.

   b. Click the **Plate Layout Position** cell, and then type `BI`.

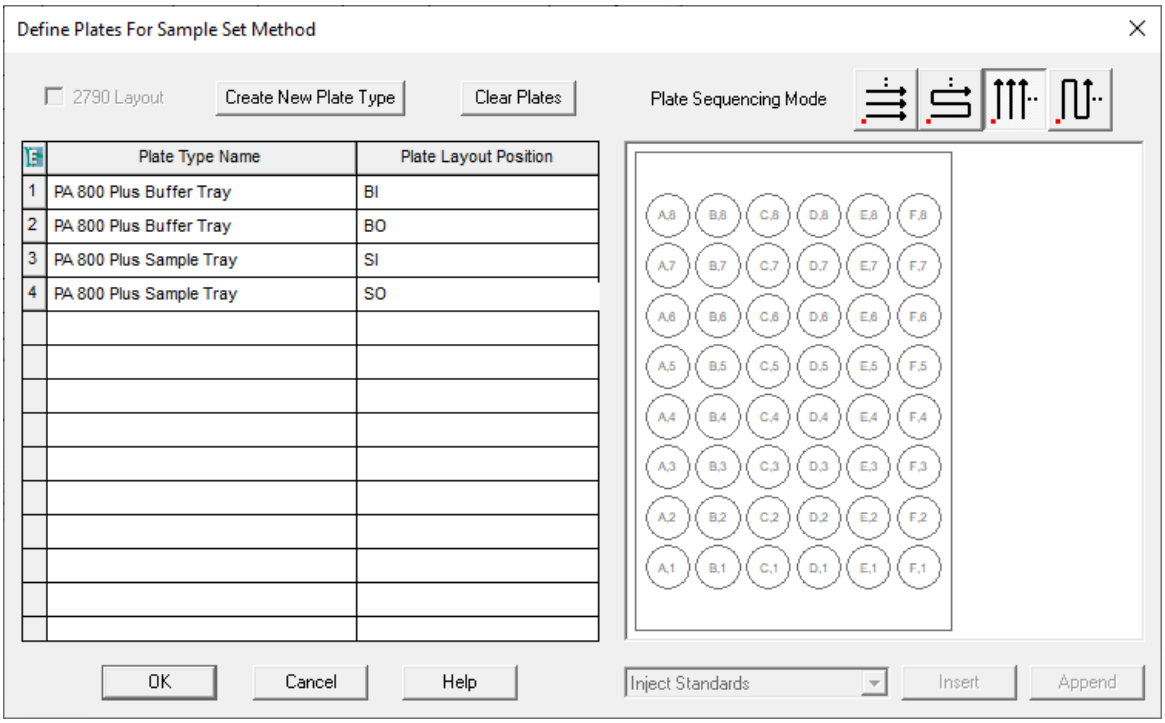
c. To identify the injection order for the vials during the run, click (**Vertical Discontinuous Plate Sequencing Mode**).

Figure 8-3 After the Buffer Inlet Plate Is Defined



3. To configure the buffer outlet tray in the second row, do step 2 again. Type `BO` for the **Plate Layout Position**.
4. In the third row, configure the sample inlet tray:
   a. Click the **Plate Type Name** cell, and then select the correct plate type:
      - **PA 800 Plus Sample Tray**
      - **PA 800 Plus 96 Well Sample Tray**
   b. Click the **Plate Layout Position** cell, and then type `SI`.
   c. To identify the injection order for the vials during the run, click (**Vertical Discontinuous Plate Sequencing Mode**).
5. To configure the sample outlet tray in the fourth row, do step 4 again. Type `SO` for the **Plate Layout Position**.

Figure 8-4 After Defining All Plate Types

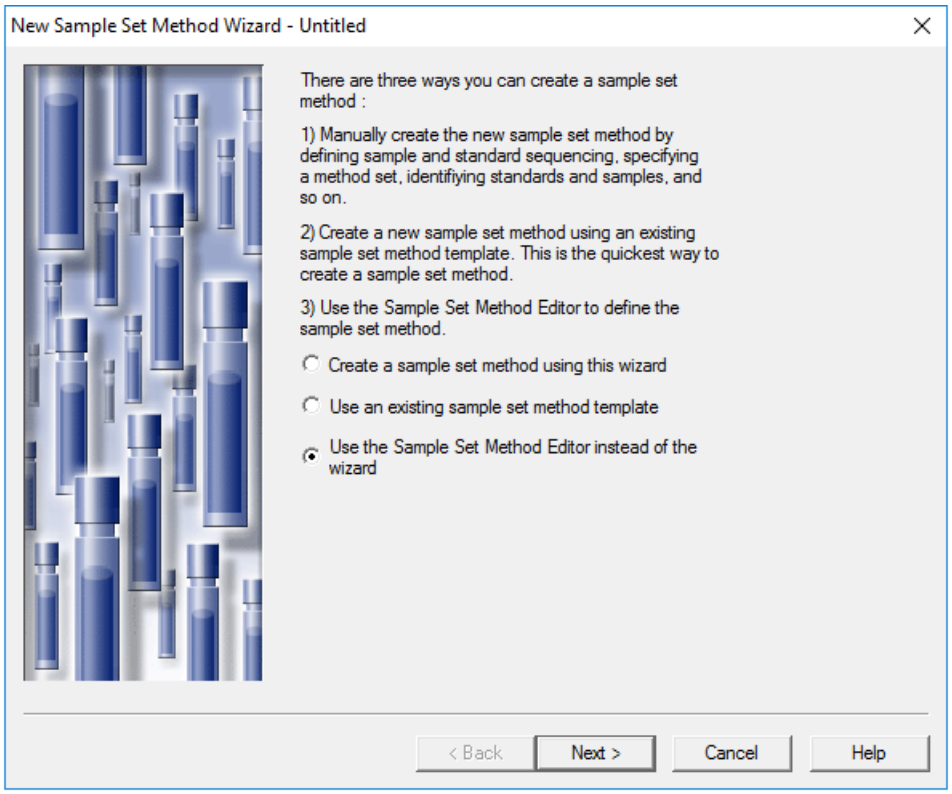


6. To save the changes and close the dialog, click **OK**.

Create the Sample Set Method and Run the Samples

1. In the Empower™ CDS software Project window, click **File** > **New Method** > **Sample Set Method**.
   The New Sample Set Method Wizard opens.
2. Click **Use the Sample Set Method Editor instead of the wizard**, and then click **Next**.

Figure 8-5 New Sample Set Method Wizard



The Sample Set Method Editor opens.

3. Set up the sample set method:
   a. In the first row, in the **Method Set/Report or Export Method** cell, select **OLIGO 200 SEPARATION**.
   b. For row 18, in the **Method Set/Report or Export Method** cell, select **OLIGO 200 SHUTDOWN 1**.
   c. For row 19, in the **Method Set/Report or Export Method** cell, select **OLIGO 200 SHUTDOWN 2**.
   d. Use the table that follows to add the required information for the samples.

Table 8-1 Required Fields for a Sample Set Method

Name	Description
Plate/Well	The position of the sample in the sample tray.
# of Injs	The number of times the sample is to be injected.
SampleName	The name of the sample.

Table 8-1 Required Fields for a Sample Set Method (continued)

Name	Description
Run Time (Minutes)	The duration of the run. **CAUTION: Potential Incorrect Result. Make sure that the Run Time is more than or equal to the duration of the time program in the instrument method. If the Run Time is less than the duration of the time program in the instrument method, then the system stops the run before the time program is completed.**

The completed sample set is shown.

Note: In the figure that follows, the **Level** and **Label Reference** columns are not shown.

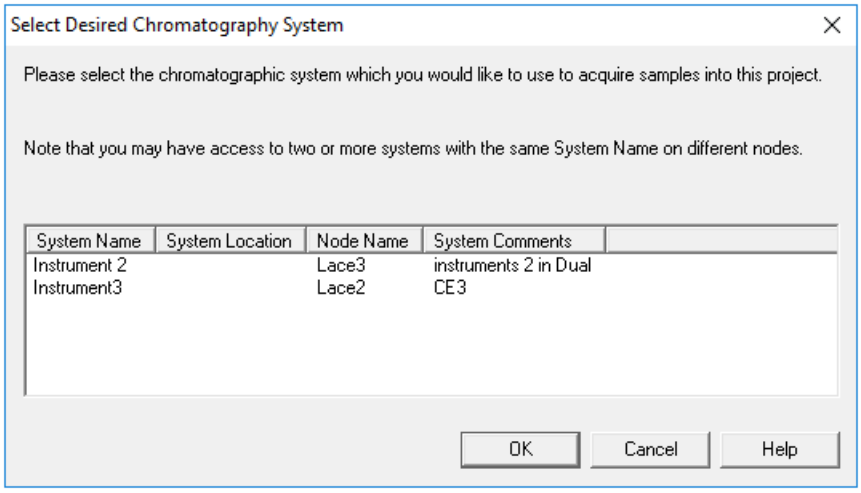
Figure 8-6 Sample Set Method

	Plate/Well	Inj Vol (uL)	# of Injs	Label	SampleName	Function	Method Set / Report or Export Method	Processing	Run Time (Minutes)
1	SI:A,1	10.0	1		Separate	Inject Samples	OLIGO 200 SEPARATION	Normal	40.10
2	SI:A,1	10.0	1		Separate	Inject Samples	OLIGO 200 SEPARATION	Normal	40.10
3	SI:A,2	10.0	1		Separate	Inject Samples	OLIGO 200 SEPARATION	Normal	40.10
4	SI:A,2	10.0	1		Separate	Inject Samples	OLIGO 200 SEPARATION	Normal	40.10
5	SI:A,3	10.0	1		Separate	Inject Samples	OLIGO 200 SEPARATION	Normal	40.10
6	SI:A,3	10.0	1		Separate	Inject Samples	OLIGO 200 SEPARATION	Normal	40.10
7	SI:A,1	10.0	1		Separate	Inject Samples	OLIGO 200 SEPARATION	Normal	40.10
8	SI:E,1	10.0	1		shutdown 1	Inject Samples	OLIGO 200 SHUTDOWN 1	Normal	20.10
9	SI:E,1	10.0	1		shutdown 2	Inject Samples	OLIGO 200 SHUTDOWN 2	Normal	10.10

4. Save the sample set method:
   a. Click **File** > **Save**.
      The Save current sample set method dialog opens.
   b. In the **Name** field, type `Oligo 200 nt Sample Set Method`.
   c. (Optional) Type information in the **Method Comments** field.
   d. If a prompt is shown, then type the user password for the Empower™ CDS software in the **Password** field, and then click **Save**.

   The method set is saved to the selected project.
5. Click **Tools** > **Run Samples**.

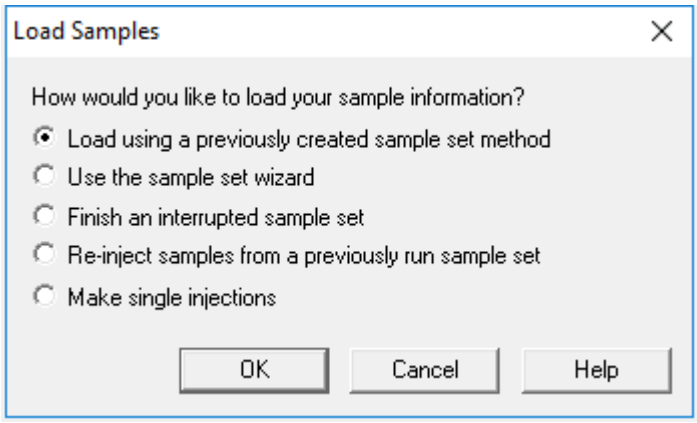
Figure 8-7 Select Desired Chromatography System Dialog



6. Click the system to be used, and then click **OK**.
   Make sure that the instrument is configured with a UV detector.
   The Run Samples window opens.

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

Figure 8-8 Load Samples Dialog



8. Click **Load using a previously created sample set method**, and then click **OK**.
   The Open an existing sample set method dialog opens.

9. Click **Oligo 200 nt Sample Set Method**, and then click **Open**.
   The sample set method opens in the Samples tab.

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

Data acquisition starts. During the run, the sample row for which acquisition is in progess shows in red text in the Sample Set Method window.

Note: To stop data acquisition, click (**Stop**).

11. Monitor the voltage and current data.
    When the run is completed, the text in all of the rows in the Sample Set Method window is red.

Stow the Cartridge After the Run 9

Stow the Cartridge for Less than 2 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 Oligo 200 nt Purity and Integrity Gel.
2. Keep the cartridge in the system for a maximum of 2 days with the capillary tips put fully in vials of CE Grade Water.

Stow the Cartridge for More than 2 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 Oligo 200 nt Purity and Integrity Gel.
2. Remove the cartridge from the system.
3. Stow the cartridge upright in the cartridge storage box at 2 °C to 8 °C.
4. 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: *Methods Development Guide*.

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

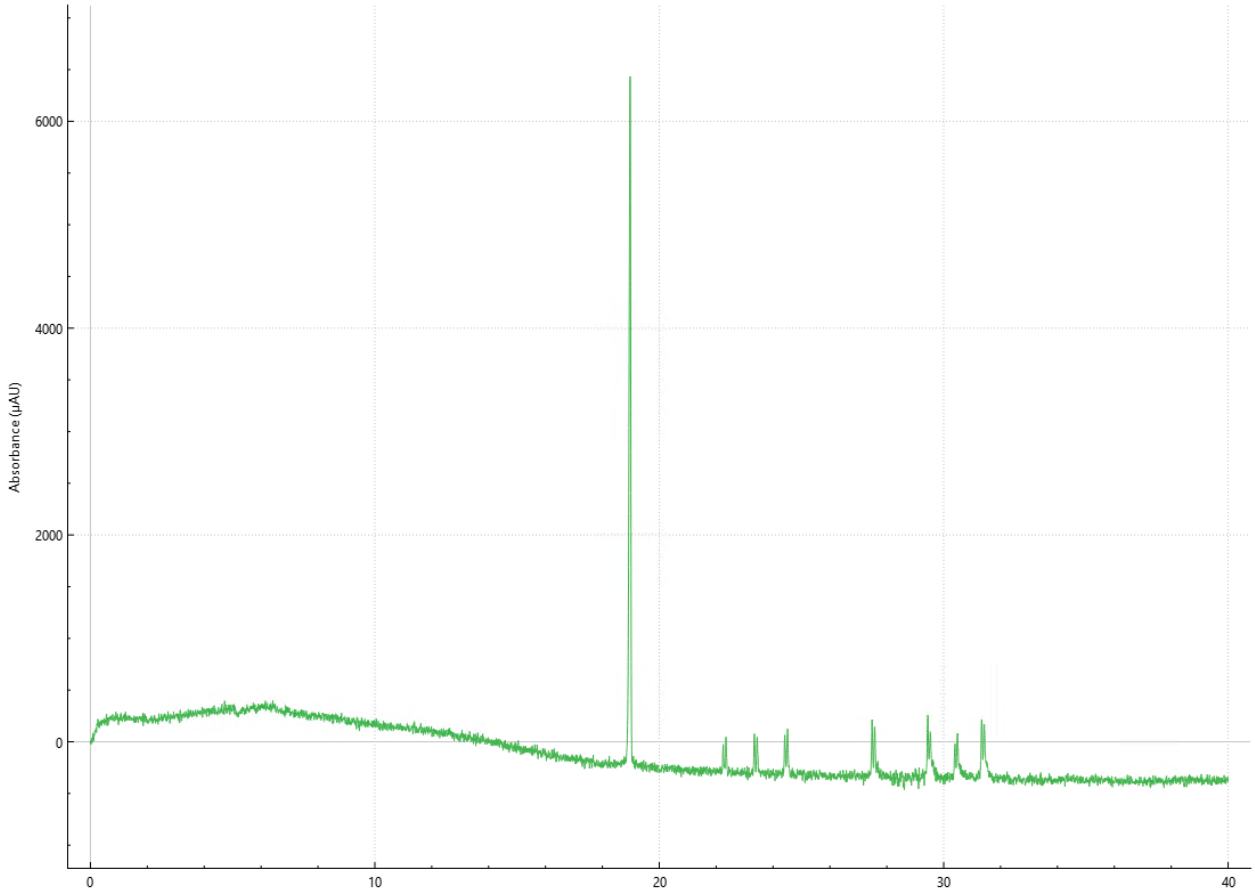
Analyze the Data 10

Analyze the Data for the Oligo 200 nt Test Mix (50 nt to 141 nt) and Single-Stranded Oligo Standard (15 nt)

The Oligo 200 nt Test Mix (50 nt to 141 nt) should give a single base resolution to a maximum of 141 bases when a 30 cm capillary is used at a field strength of 277 V/cm. As a result of batch-to-batch variation, peak intensity might be irregular. Refer to the electropherogram that is supplied with the Oligo 200 nt Test Mix (50 nt to 141 nt) to make sure that the results are correct. The electrical current should stay stable between 9 µA and 11 µA.

The high resolving power of the kit can not only get single-base resolution of each oligonucleotide, but also show the appearance of shoulders on the major peaks that might be the dephosphorylated form of each oligonucleotide or other contamination.

Figure 10-1 Example Electropherogram for the Oligo 200 nt Test Mix (50 nt to 141 nt) and Single-Stranded Oligo Standard (15 nt)



Tips for Best Results

- If resolution decreases over time, then use the gel-filling method to replace the Oligo 200 nt Purity and Integrity Gel. Do a test run to make sure that the resolution increases.
- If baseline separation cannot be done, then use the same field strength (V/cm) and increase the capillary length as required.
- Monitor the electrical current at all times. Changes in the average current or fluctuations in the current can identify changes in ionic strength, gel buffer degradation, or the formation of bubbles.

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.

Waste Disposal 11

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

Troubleshooting 12

Symptom	Possible Cause	Corrective Action
Decreased resolution	1. The quality of the ladder standard is decreased. 2. The quality of the gel inside the capillary is decreased. 3. The capillary coating is bad.	1. Change to a new ladder standard. 2. Rinse the capillary with CE Grade Water for 10 minutes at 20 psi to remove the gel, and then condition the capillary with the conditioning method. 3. Replace the capillary. Refer to the section: Install the Capillary.
Low or unsteady current	1. The capillary is blocked. 2. The gel has air bubbles. 3. The capillary window or tip is broken.	1. Do one of these steps: • Rinse the capillary with CE Grade Water for 10 minutes at 20 psi to remove the gel, and then condition the capillary with the conditioning method. • Replace the capillary. Refer to the section: Install the Capillary. 2. Rinse the capillary with CE Grade Water for 10 minutes at 20 psi to remove the gel, and then condition the capillary with the conditioning method. 3. Replace the capillary. Refer to the section: Install the Capillary.

Symptom	Possible Cause	Corrective Action
Inconsistent migration time from run to run	1. The capillary is not equilibrated. 2. The quality of the gel in the universal vials is decreased. 3. The electrodes are contaminated.	1. Rinse the capillary with CE Grade Water for 10 minutes at 20 psi to remove the gel, and then condition the capillary with the conditioning method. 2. Replace the gel vials. 3. Clean the electrodes and then the capillary ends.
No peaks or low UV absorption	1. The capillary is blocked. 2. The capillary window or tip is broken. 3. The capillary is not equilibrated.	1. Do one of these steps: • Rinse the capillary with CE Grade Water for 10 minutes at 20 psi to remove the gel, and then condition the capillary with the conditioning method. • Replace the capillary. Refer to the section: Install the Capillary. 2. Replace the capillary. Refer to the section: Install the Capillary. 3. Rinse the capillary with CE Grade Water for 10 minutes at 20 psi to remove the gel, and then condition the capillary with the conditioning method.
Spikes in the electropherogram	1. The gel has air bubbles. 2. The gel or buffer have been contaminated or contain microparticles.	1. Make sure that the gel is at room temperature and that the air bubbles are removed. 2. Replace the used vials with clean vials. Put the gel and buffer solutions through a filter.

Symptom	Possible Cause	Corrective Action
Unstable or shifting baseline	1. The capillary is not equilibrated. 2. The quality of the gel in the universal vials is decreased.	1. Rinse the capillary with CE Grade Water for 10 minutes at 20 psi to remove the gel, and then condition the capillary with the conditioning method. 2. Replace the gel vials.

Create the Instrument Methods A

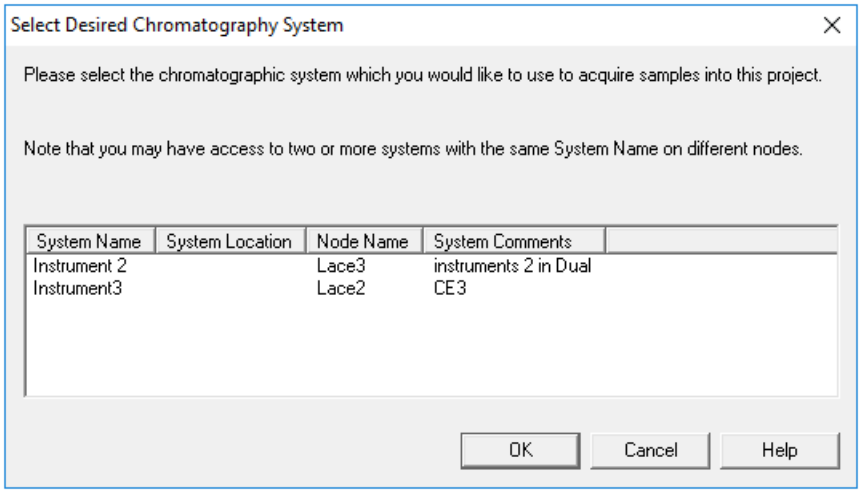
If required, then create these instrument methods for use with the Empower™ CDS software:

- OLIGO 200 SEPARATION
- OLIGO 200 SHUTDOWN 1
- OLIGO 200 SHUTDOWN 2

Note: Pressure values can be shown in millibar (mbar) or pounds per square inch (psi). The registry setting for the Empower™ CDS software controls which unit of measurement is used. The default unit is millibar. To change the unit of measurement, refer to the documentation that is supplied with the Empower™ CDS software.

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

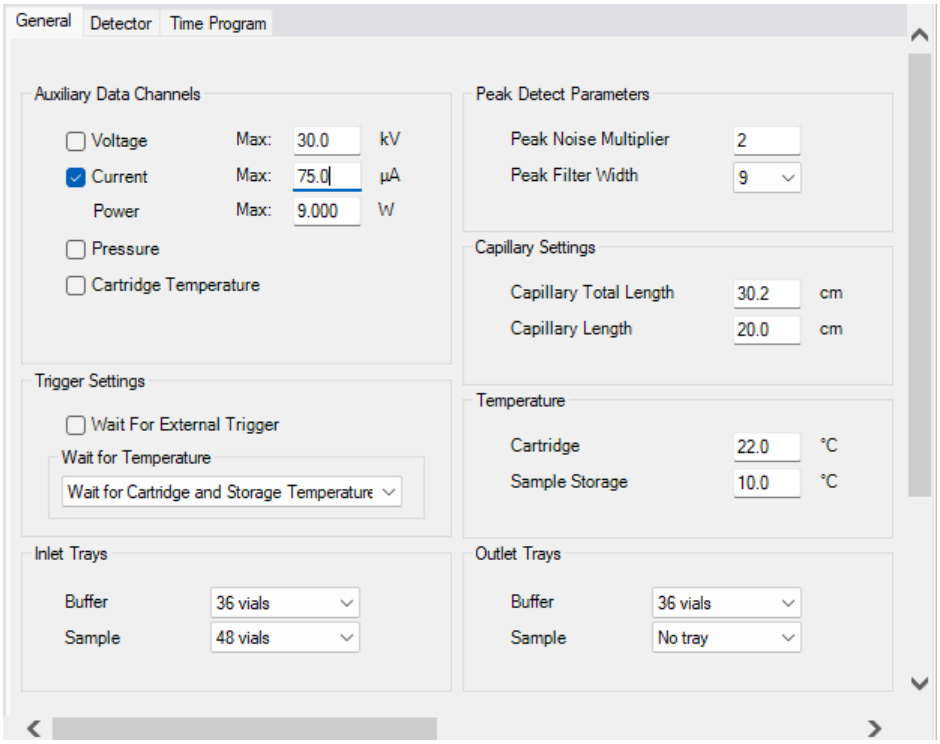
   Figure A-1 Select Desired Chromatography System Dialog



2. Click the system to be used, and then click **OK**.
   Make sure that the instrument is configured with a UV detector.
   The Instrument Method Editor opens.
3. Set the parameters on the General tab.

   Note: The values on the General and Detector tabs are the same for all of the methods.

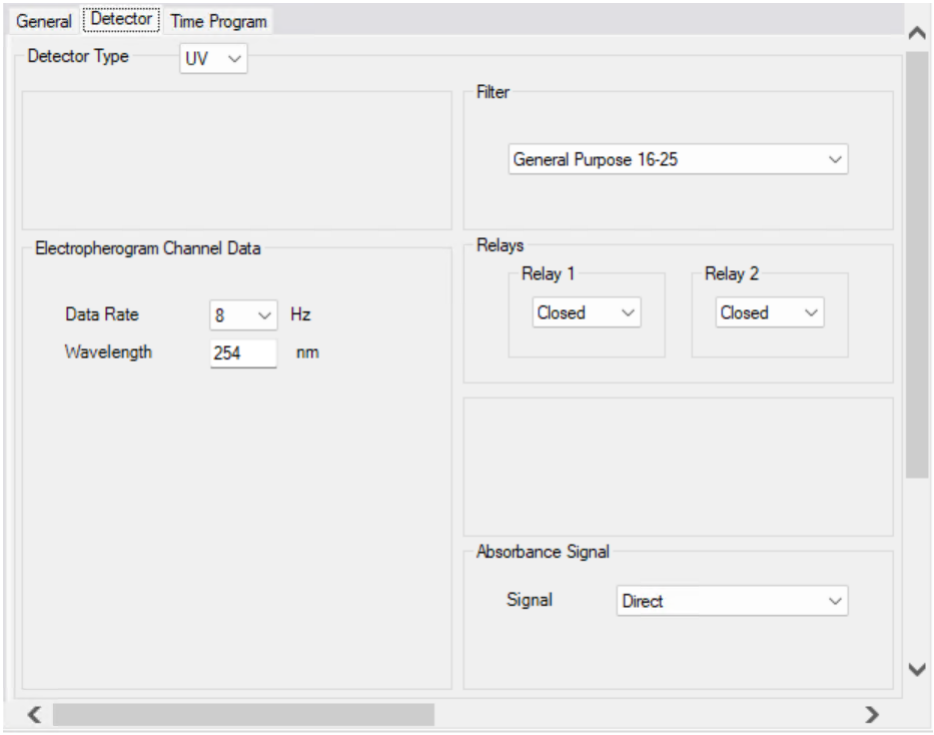
Figure A-2 General Parameters for the OLIGO 200 SEPARATION Instrument Method



4. Open the Detector tab, click **UV** in the **Detector Type** list, and then set the parameters.

Note: For 3D data, in the Electropherogram Channel Data section, set the **Data Rate** to **On**.

Figure A-3 Detector Parameters for the OLIGO 200 SEPARATION Instrument Method



5. Add the events in the figure that follows to the time program.

Figure A-4 Time Program for the OLIGO 200 SEPARATION Instrument Method

	Time (min)	Event	Value	Duration	Inlet vial	Inlet tray	Outlet vial	Outlet tray	Summary	Comments
1		Rinse Pressure	70.0 psi	2.00 min	E1	Buffer	E1	Buffer	Forward;12;12	Acid Rinse
2		Rinse Pressure	70.0 psi	2.00 min	F1	Buffer	F1	Buffer	Forward;12;12	Water Rinse
3		Rinse Pressure	50.0 psi	15.00 min	B1	Buffer	B1	Buffer	Forward;12;12	Gel Rinse
4		Wait		0.50	A4	Buffer	A4	Buffer	12;12	Water Dip 1
5		Wait		0.50	B4	Buffer	B4	Buffer	12;12	Water Dip 2
6		Wait		0.00	C4	Buffer	C4	Buffer	12;12	Water Dip 3
7		Inject Voltage	8.0 kV	4.0 s	A0	Sample List	C1	Buffer	Reverse (-);0;12	Inject
8		Wait		0.00	D1	Buffer	D1	Buffer	12;12	Water Dip 4
9	0.00	Separate Voltage	8.3 kV	40.00 min	C1	Buffer	C1	Buffer	Reverse (-);0.17;12;12	Separate
10	40.00	Stop Data								Stop Data
11	40.10	End								End
12										

Note: If the system uses mbar as the units for pressure, then do this:

- For the **Rinse Pressure** events in step 1 and step 2, in the **Value** column, type `4826.3`.
- For the **Rinse Pressure** event in step 3, in the **Value** column, type `3447.4`.

6. Save the instrument method:

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

b. In the **Name** field, type the applicable method name:

- For the separation instrument method, type `OLIGO 200 SEPARATION`.
- For the shutdown instrument methods, type `OLIGO 200 SHUTDOWN 1` or `OLIGO 200 SHUTDOWN 2`.

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

d. If a prompt is shown, then type the user password for the Empower™ CDS software, and then click **Save**.

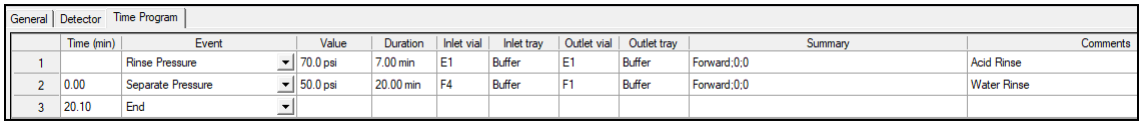
The instrument method is saved to the selected project.

7. Create the shutdown instrument method 1:

a. Do step 3 and step 4 again.

b. Add the events in the figure that follows to the time program.

Figure A-5 Time Program for the OLIGO 200 SHUTDOWN 1 Instrument Method



General | Detector | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Inlet tray	Outlet vial	Outlet tray	Summary	Comments
1		Rinse Pressure	70.0 psi	7.00 min	E1	Buffer	E1	Buffer	Forward;0;0	Acid Rinse
2	0.00	Separate Pressure	50.0 psi	20.00 min	F4	Buffer	F1	Buffer	Forward;0;0	Water Rinse
3	20.10	End								

Note: If the system uses mbar as the units for pressure, then do this:

- For the **Rinse Pressure** events in step 1, in the **Value** column, type `4826.3`.
- For the **Rinse Pressure** event in step 2, in the **Value** column, type `3447.4`.

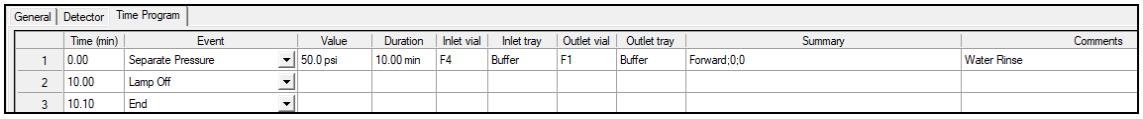
c. Do step 6 again.

8. Create the shutdown instrument method 2:

a. Do step 3 and step 4 again.

b. Add the events in the figure that follows to the time program.

Figure A-6 Time Program for the OLIGO 200 SHUTDOWN 2 Instrument Method



General | Detector | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Inlet tray	Outlet vial	Outlet tray	Summary	Comments
1	0.00	Separate Pressure	50.0 psi	10.00 min	F4	Buffer	F1	Buffer	Forward;0;0	Water Rinse
2	10.00	Lamp Off								
3	10.10	End								

Note: If the system uses mbar as the units for pressure, then do this:

- For the **Rinse Pressure** event in step 1, in the **Value** column, type `3447.4`.

c. Do step 6 again.

Create the Method Sets B

If required, then create these method sets for use with the Empower™ CDS software:

- OLIGO 200 SEPARATION
- OLIGO 200 SHUTDOWN 1
- OLIGO 200 SHUTDOWN 2

Note: A method set can also include processing and report methods. To create these methods, refer to the documentation that is supplied with the Empower™ CDS software.

1. In the Empower™ CDS Software Project window, click **File** > **New Method** > **Method Set**.
2. Click **No** in the message.
   The Method Set Editor window opens.
3. In the **Instrument Method** list, click the applicable instrument method:
   - **OLIGO 200 SEPARATION**
   - **OLIGO 200 SHUTDOWN 1**
   - **OLIGO 200 SHUTDOWN 2**

   Note: Do not make other changes.
4. Save the method set:
   a. Click **File** > **Save**.
   b. In the **Name** field, type the applicable name:
      - `OLIGO 200 SEPARATION`
      - `OLIGO 200 SHUTDOWN 1`
      - `OLIGO 200 SHUTDOWN 2`
   c. If required, then type information in the **Method Comments** field.
   d. If a prompt is shown, then type the user password for the Empower™ CDS software, and then click **Save**.

   The method set is saved to the project.

Hazardous Substance Information C

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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SCIEX Headquarters	AB Sciex LLC 250 Forest Street Marlborough, MA 01752 USA

Customer Training

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Online Learning Center

- SCIEX Now Learning Hub

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SCIEX Support

SCIEX and its representatives have a global staff of fully-trained service and technical specialists. They can supply answers to questions about the system or any technical issues that might occur. For more information, go to the SCIEX website at sciex.com or use one of the links that follow to contact us.

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

Cybersecurity

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

Documentation

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

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

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

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

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