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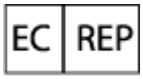
CE-SDS Protein Analysis Kit

For the BioPhase 8800 System

Application Guide

RUO-IDV-05-8662-D

April 2023



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Contents

CE-SDS Protein Analysis Kit 1

The CE-SDS Protein Analysis Kit provides the reagents and supplies to resolve both reduced and non-reduced proteins by size, and to quantify the heterogeneity and impurities that might exist in a protein preparation.

This document provides instructions for sample preparation with the CE-SDS Protein Analysis Kit. It also provides instructions for data acquisition with the BioPhase software and the Waters Empower™ software with the BioPhase 8800 driver for Empower™. This document also provides instructions for analysis of data acquired with BioPhase software. Analysis of data acquired with the Waters Empower™ software must be done using the Waters Empower™ software.

Use the information in this application guide as a starting point. As required, change injection time, voltage, injection type, or other parameters to find the best conditions for your needs.

Note: For instructions for safe use of the system, refer to the document: *Operator Guide*.

Safety

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

Intended Use

The CE-SDS Protein Analysis kit is for laboratory use only.

Introduction

The CE-SDS Protein Analysis Kit includes reagents to resolve both reduced and non-reduced proteins by size, and to quantify the heterogeneity and impurities that might exist in the protein. The methodology involves heat denaturation of a specified concentration of protein in the presence of sodium dodecyl sulfate (SDS). Once denatured, the sample is separated by size in a capillary containing a replaceable SDS polymer matrix, which provides the sieving selectivity for the separation.

Workflow

The workflow consists of the following steps:

1. Determine the number of samples to be analyzed and the number of replicates.

2. For BioPhase software users:

   a. Create or modify the methods. Refer to the section: Methods.

   b. Create the sequence and the sample and reagent plate layouts. Refer to the section: Create the Sequence.

3. For Waters Empower™ software users:

   a. Import BioPhase software methods. Refer to the section: Import the BioPhase Software Methods to Create the Instrument Methods.

   b. Create the sample set methods and the sample and reagent plate layouts. Refer to the section: Create the Sample Set Method.

4. Prepare the samples. Refer to the section: Prepare the Samples.

   There are two separate workflows, reduced and non-reduced.

5. Use the sample and reagent plate layouts to prepare the plates.

6. Put the plates in the BioPhase 8800 system. Refer to the sections: Load the Sample Inlet and Outlet Plates and Load the Reagent Inlet and Outlet Plates.

7. Start data acquisition.

   - (BioPhase software) Start the sequence from the front panel. Refer to the section: Run the Samples.
   - (Waters Empower™ software) Start the sample set method from the Waters Empower™ software with the BioPhase 8800 driver for Empower™. Refer to the section: Start the Sample Set Method.

8. Analyze the data.

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

Required Equipment and Materials 2

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

Table 2-1 CE-SDS Protein Analysis Kit (PN C30085)

Component	Quantity	Reorder Part Number
10 kDa Internal Standard	2	A26487
Acid Wash/Regenerating Solution (100 mL)	1	N/A
Capillary Regenerator Solution A Basic Wash (100 mL)	1	N/A
CE Grade Water (140 mL)	3	C48034
CE-SDS Gel Buffer (140 mL)	2	A30341
Low pH SDS Sample Buffer (55 mL)	1	C44807
SDS-MW Sample Buffer	1	N/A

Table 2-2 Additional Supplies from SCIEX

Component	Quantity	Part Number
(Optional) Low pH Phosphate SDS Sample Buffer (40 mM Phosphate, pH 6.5, 1% SDS) (140 mL)	1	C57805
Capillary cartridge coolant (450 mL)	1	359976
IgG Control Standard (1 mg/mL) (1 mL)	3	391734
MW Size Standard (sizing ladder containing 10 kDa, 20 kDa, 35 kDa, 50 kDa, 100 kDa, 150 kDa, and 225 kDa proteins) (100 µL)	3	A22196
BioPhase 8800 bare fused-silica capillary cartridge (50 µm i.d. × 30 cm capillaries)	1	5080121
BioPhase 8800 outlet plates	20	5080315
BioPhase 8800 reagent plates	20	5080314
BioPhase 8800 sample plates	20	5080313

Table 2-2 Additional Supplies from SCIEX (continued)

Component	Quantity	Part Number
BioPhase 8800 Starter Plate Pack (4 sample plates, 4 reagent plates, 8 outlet plates)	1	5080311

Table 2-3 Additional Required Reagents or Supplies

Description	Vendor	Part Number
(Optional) Amicon Ultra-4 Centrifugal Filter Unit with Ultracel-10 membrane	MilliporeSigma	UFC801024
(Optional) MicroCon-10 kDa Centrifugal Filter Unit with Ultracel-10 membrane	MilliporeSigma	MRCPRT010
(Optional) MicroCon-30 kDa Centrifugal Filter Unit with Ultracel-30 membrane	MilliporeSigma	MRCF0R030
2-mercaptoethanol	MilliporeSigma	M7154
Iodoacetamide	MilliporeSigma	I-1149
X-Pierce Film	USA Scientific	2997-0100

Storage Conditions

Note: For storage conditions for prepared reagents, refer to the preparation instructions.

- Upon receipt, store the 10 kDa Internal Standard at 2 °C to 8 °C.
- Store the remainder of the kit contents at room temperature.

Customer-Supplied Equipment and Supplies

- Powder-free gloves, neoprene or nitrile recommended
- Safety glasses
- Laboratory coat
- Appropriate centrifuge
- Microcentrifuge, or equivalent, and microcentrifuge tubes
- Vortex mixer
- Pipettes and appropriate tips

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

- Parafilm
- Centrifuge with swinging-bucket rotor to hold plates
- Water bath or heat block capable of 37 °C to 100 °C temperature
- Analytical balance
- Spatula

Required Detector

A UV detector equipped with a 220 nm filter is required.

Required Cartridge

CAUTION: Potential Wrong Result. If a cartridge is used with the CE-SDS Protein Analysis kit, then do not use the same cartridge for another application. If the same cartridge with different buffers and sample types is being used, then sample carryover, nonspecific binding, and poor separation can occur.

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

Refer to the table: Table 2-2.

Methods and Sequences 3

For Systems That Use the BioPhase Software

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

The following methods and sequence are required.

- CE-SDS Conditioning: Conditions the capillaries.
- Separation methods:
  - Reduced CE-SDS Separation: For reduced samples.
  - Non Reduced CE-SDS Separation: For non-reduced samples.
  - Low pH Sample Buffer Separation: For samples prepared with a low pH sample buffer.
- CE-SDS Shutdown: Cleans the capillaries at the end of a sequence, rinses the capillaries for storage, and then turns off the lamp.
- Sequence templates:
  - Reduced CE-SDS Separation: For reduced samples.
  - Non Reduced CE-SDS Separation: For non-reduced samples.
  - Low pH Sample Buffer Separation: For samples prepared with a low pH sample buffer.

For Systems That Use the Waters Empower™ Software

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

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

Create the Sequence

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

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

This procedure gives instructions for creating a sequence with a template supplied with the BioPhase software. The template is set up for eight samples in the first column, and uses the validated methods supplied with the software.

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

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

1. On the Home page of the BioPhase software, click **Sequence Editor**.
2. Click **Open Sequence**.
   The Open a Sequence dialog opens.
3. Search for, and then select the appropriate sequence:
   a. (Optional) Type a **Start Date** and **End Date** for the search or click the calendar icons, select the dates, and then click **Search**.
      The available project folders are shown in the Folder Name pane.
   b. Click the **CE-SDS Project** project folder.
      The available sequences in the project are shown in the table to the right.
   c. In the table, click the appropriate sequence template and then click **Open**.
      - For reduced samples, click **CE-SDS Test Sequence**.
      - For non-reduced samples, click **Non Reduced CE-SDS Sequence**.
      - For samples prepared with a low pH sample buffer, click **Low pH Sample Buffer CE-SDS Sequence**.

      The Open a Sequence dialog closes, and then the Sequence Summary tab opens.
4. Above the Sequence Summary pane, click **Edit**.
   The Sample Plate Setup tab opens.
5. If required, do any of the following:
   - Add or remove samples.
   - Clear a method assigned to a sample well.
   - Assign a different method to a sample well.
   - In the Sequence Summary table, assign the recovery method to a method in the sequence. Typically, the shutdown method should be assigned as the recovery method.

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

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

Note: The method names are different for each sequence.

Figure 3-1 Sequence Summary Table: CE-SDS Test Sequence

	Run #	Column	Method Name	Rep. #	Error Recovery
	1	0	CE-SDS Conditioning	1	☐
⊞	2	1	Reduced CE-SDS Separation	1	☐
	3	0	CE-SDS Shutdown	1	☑

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

Figure 3-2 Sequence Summary Table Expanded

	Run #	Column	Method Name	Rep. #	Error Recovery
	1	0	CE-SDS Conditioning	1	☐
⊟	2	1	Reduced CE-SDS Separation	1	☐

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

	3	0	CE-SDS Shutdown	1	☑

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

10. To save the sequence, click **SAVE**, and then add the required information.

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

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

    a. Click **PRINT**.
    The Print Preview dialog opens.

    b. Click **Plate Layout Report**, and then click **Print**.

    c. Select the printing options, and then click **OK**.
    The report is printed.

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

Prepare the Samples 4

The comparison of the reduced and the non-reduced states of a protein can supply important structural information. This section includes instructions to prepare reduced and non-reduced standards and samples.

Prepare a Standard

Depending on the goal of the analysis, different standards might be appropriate. Use a standard that is appropriate for the analysis.

Prepare the Reduced MW Size Standard

This standard is prepared under reducing conditions to break or prevent any protein aggregation formed by disulfide bridges.

Note: The following instructions give quantities for one sample.

1. Mix the MW Size Standard thoroughly, and then spin the vial for a few seconds in a standard microcentrifuge.
2. Add 10 µL of the MW Size Standard to a 0.5 mL microcentrifuge tube.
3. Add 85 µL of the SDS-MW Sample Buffer to the microcentrifuge tube.
4. Add 2 µL of the 10 kDa Internal Standard to the microcentrifuge tube.
5. Add 5 µL of 2-mercaptoethanol to the microcentrifuge tube inside a fume hood.
6. Secure the vial cap with Parafilm, mix thoroughly using a vortex mixer, and then heat the mixture in a water bath at 70 °C for 10 min.
7. Spin the tube in a centrifuge for 1 min at 300 *g*.
8. Remove the vial from the water bath and let it sit for a minimum of 3 min to cool the solution to room temperature.

   The standard stays stable for approximately 24 hours.
9. Add 100 µL of the prepared standard to the sample plate well.

   Make sure that, for any column on the sample plate with sample, the corresponding column on the outlet plate has 2.0 mL of CE-SDS Gel Buffer.
10. Put a film cover on the plate, and then use a centrifuge to spin the sample plate for 4 min at 30 *g* to remove any bubbles at the bottom of the wells.

Prepare the IgG Control Standard

For analysis of immunoglobulin preparations, the SCIEX IgG Control Standard can be used as a standard. The standard can be prepared under reducing or non-reducing conditions.

Prepare the Reduced IgG Control Standard

Note: The following instructions give quantities for one sample.

1. Prepare the IgG Control Standard.
    a. For the initial run, remove one vial of IgG Control Standard from the freezer and let it thaw completely at room temperature.
    b. Mix briefly for a few seconds with a vortex mixer and then aliquot the solution in 95 µL portions.
    c. Reserve one aliquot and then store the remaining aliquots at –35 °C to –15 °C.
2. Use one of the 95 µL aliquots of the IgG Control Standard. If it is frozen, then thaw it at room temperature before use.
3. Add 2 µL of the 10 kDa Internal Standard to the IgG vial.
4. Add 5 µL of 2-mercaptoethanol to the IgG vial inside a fume hood.
5. Secure the vial with the vial cap and then mix it thoroughly using a vortex mixer.
6. Spin the vial using a centrifuge for 1 min at 300 *g*.
7. Secure the vial cap with Parafilm and then heat the mixture in a water bath at 70 °C for 10 min.
8. Remove the vial from the water bath, and then let it sit for a minimum of 3 min to cool the solution to room temperature.
9. Add 100 µL of the prepared standard to the sample plate well.
10. Put a film cover on the plate, and then use a centrifuge to spin the sample plate for 4 min at 30 *g* to remove any bubbles at the bottom of the wells.

Prepare the Non-Reduced IgG Control Standard

Before preparing the non-reduced IgG Control Standard, prepare a 250 mM iodoacetamide (IAM) solution. Refer to the section: Prepare the 250 mM Iodoacetamide Solution.

Prepare the 250 mM Iodoacetamide Solution

1. Weigh 46 mg of iodoacetamide (IAM) and add it to a 1.5 mL microcentrifuge vial.
2. Add 1 mL of CE Grade Water to make a solution that is 46 mg/mL.
3. Seal the vial with a cap and then mix the solution until the solids dissolve fully.

4. When it is not in use, keep the solution in the dark.
   The iodacetamide solution is stable for approximately 24 hours at room temperature.

Prepare the Non-reduced IgG Control Standard

Note: The following instructions give quantities for one sample.

1. Prepare the IgG Control Standard.
   a. For the initial run, remove one vial of IgG Control Standard from the freezer and let it thaw completely at room temperature.
   b. Mix briefly for a few seconds with a vortex mixer and then aliquot the solution in 95 µL portions.
   c. Reserve one aliquot and then store the remaining aliquots at –35 °C to –15 °C.
2. Use one of the 95 µL aliquots of the IgG Control Standard. If it is frozen, then thaw it at room temperature before use.
3. Add 2 µL of 10 kDa Internal Standard to the IgG Control Standard tube.
4. Add 5 µL of 250 mM IAM solution.
5. Secure the vial with a cap and then mix thoroughly using a vortex mixer.
6. Spin the vial in a centrifuge for 1 min at 300 *g*.
7. Secure the vial cap with Parafilm and then heat the mixture in a water bath at 70 °C for 10 min.
8. Remove the vial from the water bath, and then let it sit for a minimum of 3 min to cool the solution to room temperature.

The standard remains stable for approximately 24 hours.

9. Mix the solution in the vial and then spin the vial in a centrifuge for 1 min at 300 *g*.
10. Add 100 µL of the prepared standard to the sample plate.
    Make sure that, for any column on the sample plate with sample, the corresponding column on the outlet plate has 2.0 mL of CE-SDS Gel Buffer.
11. Put a film cover on the plate, and then use a centrifuge to spin the sample plate for 4 min at 30 *g* to remove any bubbles at the bottom of the wells.

Prepare the Samples

Use the following procedure to prepare one sample.

Recommended Protein Concentration

After the SDS-MW Sample Buffer is added, the total protein concentration must be between 0.2 mg/mL and 2 mg/mL. For best results, the recommended protein concentration is 1 mg/mL.

If the protein concentration is too high, then insufficient SDS binding might result, causing broad peaks and poor resolution. If the protein concentration is too low, a low signal may occur.

The signal intensity and resolution of this assay are also sensitive to the salt concentration in the protein sample. If the salt concentration is too high, then low signal and peak tailing is likely to occur. Refer to the section: Do a Buffer Exchange for the Protein Sample.

Prepare the Reduced Samples

Note: The following instructions give quantities for one sample.

1. Dilute the sample with the SDS-MW Sample Buffer to a volume of 95 µL to give a final protein concentration between 0.2 mg/mL to 2 mg/mL.

   Note: Make sure that no more than 45 µL of the sample is added to the SDS-MW sample buffer. If more than 45 µL is required, then concentrate the sample before diluting it with buffer.

2. Add 2 µL of 10 kDa Internal Standard to the sample microcentrifuge tube.
3. Inside the fume hood, add 5 µL of 2-mercaptoethanol to the microcentrifuge tube.
4. Secure the microcentrifuge tube cap and then mix it thoroughly using a vortex mixer.
5. Use a centrifuge to spin the tube for 1 min at 300 *g*.
6. Seal the vial cap with Parafilm, and then heat at 70 °C for 10 min.

   Note: If the protein is stable at high temperatures, then it can be heated at 100 °C for 3 min instead.

7. Remove the vial from the water bath, and then let it sit for a minimum of 3 min to cool the solution to room temperature.
8. Mix the sample tube, and then spin using a centrifuge for 1 min at 300 *g*.
9. Add 100 µL of the prepared sample to the sample plate.

   Make sure that, for any column on the sample plate with sample, the corresponding column on the outlet plate has 2.0 mL of CE-SDS Gel Buffer.

10. Put a film cover on the plate, and then use a centrifuge to spin the sample plate for 4 min at 30 *g* to remove any bubbles at the bottom of the wells.

Prepare the Non-reduced Samples

Before the non-reduced sample is prepared, prepare a 250 mM iodoacetamide (IAM) solution. Refer to the section: Prepare the 250 mM Iodoacetamide Solution. The IAM solution operates as the alkylating agent during preparation of the sample to decrease any heterogeneity that is created from the partial auto-reduction of the protein.

For non-reducing conditions, the sample solution must be heated at a high temperature to increase SDS binding. However, high temperatures might cause fragmentation and aggregation, which can result in artifacts in the sample analysis. SCIEX recommends that this alkylation step be done to minimize temperature-induced artifacts in the protein sample. SCIEX also recommends the use of one of the low pH SDS sample buffers for non-reduced samples. The Low pH Phosphate SDS Sample Buffer has been proven to decrease heat-induced artifacts more than the SDS-MW Sample Buffer.

Note: The following instructions give quantities for one sample.

1. Dilute the sample with the SDS-MW Sample Buffer to a volume of 95 µL to give a final protein concentration between 0.2 mg/mL to 2 mg/mL.

   Note: Make sure that no more than 45 µL of the sample is added to the SDS-MW sample buffer. If more than 45 µL is required, then concentrate the sample before diluting it with buffer.

2. Add 2 µL of the 10 kDa Internal Standard to the sample microcentrifuge tube.
3. Inside a fume hood, add 5 µL of the 250 mM IAM solution to the sample tube.
4. Use a centrifuge to spin the vial for 1 min at 300 *g*.
5. Seal the vial with Parafilm.
6. Heat the mixture in a water bath at 70 °C for 10 min.
7. Remove the vial from the water bath, and then let it sit for a minimum of 3 min to cool the solution to room temperature.
8. Add 100 µL of the prepared sample to the sample plate.

   Make sure that, for any column on the sample plate with sample, the corresponding column on the outlet plate has 2.0 mL of CE-SDS Gel Buffer.
9. Put a film cover on the plate, and then use a centrifuge to spin the sample plate for 4 min at 30 *g* to remove any bubbles at the bottom of the wells.

Do a Buffer Exchange for the Protein Sample

The signal intensity and resolution of this assay is sensitive to the salt concentration in the protein sample. If the salt concentration is above 1× phosphate buffered saline (PBS), then low signal and peak tailing might occur. Use the steps in this procedure to do a buffer exchange.

Note: For desalting or buffer exchange procedures that use a device from another manufacturer, refer to the manufacturer *User Guide*.

Note: For IgG samples, do not use a MicroCon-30kDa filter because it can filter out the free light chain (25 kDa) in the IgG sample, resulting in biased or inaccurate purity results.

1. Add 1 mL of protein sample to the appropriate centrifugal filter unit.
   - For IgG samples, use an Microcon-10kDa centrifugal filter unit.
   - For other proteins, use an Amicon Ultra-4 centrifugal filter unit.
2. Use a centrifuge to spin the sample for 15 min at 4,000 *g*.
3. Add 2 mL of SDS-MW Sample Buffer, and then use a centrifuge to spin the sample for 25 min at 4,000 *g*.
4. Carefully put the centrifugal filter unit in a new vial in an inverted position, and then use a centrifuge to spin the vial for 3 min at 1,000 *g*.

   The protein solution is collected in the vial.
5. Transfer the collected protein solution to an appropriate tube.
6. Determine the protein concentration.
7. Add the SDS-MW Sample Buffer to a final concentration of 1 mL.

Using a Low pH Sample Buffer

Note: SCIEX carries two different low pH sample buffers, the Low pH SDS Sample Buffer (Tris, pH 6.8) (included in the CE-SDS Protein Analysis Kit) and the optional Low pH Phosphate SDS Sample Buffer (pH 6.5).

Some samples are more stable in a sample buffer with a lower pH. If the separation profile changes with each repetition, then the protein may not be stable in SDS-MW Sample Buffer, which is pH 9. Prepare the sample again using a low pH buffer.

To use either of the low pH sample buffers, prepare the samples as described previously, but replace the SDS-MW Sample Buffer with either the Low pH SDS Sample Buffer (pH 6.8) or Low pH Phosphate SDS Sample Buffer (pH 6.5).

Because the low pH buffers have increased ionic strength, we recommend changing the separation method by increasing the injection voltage or duration to prevent any signal loss. Adjust the separation time based on the samples to be analyzed. Alternatively, use a pressure injection in the separation method.

Prepare the BioPhase 8800 System 5

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

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

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

Load the Reagent Inlet and Outlet Plates

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

1. Add the reagents to the reagent inlet and outlet plates according to the reagent plate layout. Refer to the figure: Figure D-4.

   Use the volumes in the following table.

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

   Table 5-1 Reagents for the Reagent Inlet and Outlet Plates

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

2. Put a film cover on the plates.

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

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

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

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

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

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

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

Figure 5-1 Eject Reagent Button

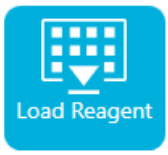
The plate compartment opens.

6. Remove the film cover from the plates.

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

7. If the plate compartment already contains reagent plates, then remove the reagent plates.
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**.

Figure 5-2 Load Reagent Button



The plate compartment closes.

Load the Sample Inlet and Outlet Plates

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

   The recommended sample volume is 100 µL.

   The minimum sample volume is 50 µL. The maximum sample volume is 200 µL.

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

   If a column has no samples, then the wells can be left empty.

3. Add the reagents to the sample outlet plate according to the sample plate layout. Refer to the figure: Figure D-3.

   The maximum volume is 2.0 mL.

   Use the volume in the following table.

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

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

   Table 5-2 Reagents for the Sample Outlet Plate

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

4. Put a film cover on the plates.

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

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

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

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

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

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

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

Figure 5-3 Eject Sample Button

The plate compartment opens.

8. Remove the film cover from the plates.

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

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

Figure 5-4 Load Sample Button

The plate compartment closes.

Examine the Capillary Cartridge

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

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

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

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

3. If the capillary tips are blocked, then do this:
   a. Use CE Grade Water to clean the capillary inlets.
   b. Use a lint-free laboratory wipe to wipe the capillary inlets carefully in an outward direction.
4. Use a magnifying glass to examine both sides of the capillary window. If lint or other particles are present, then use short bursts of electronics-grade compressed air to remove them. Do not use water or other liquids to clean the capillary window.

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

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

Install the Cartridge

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

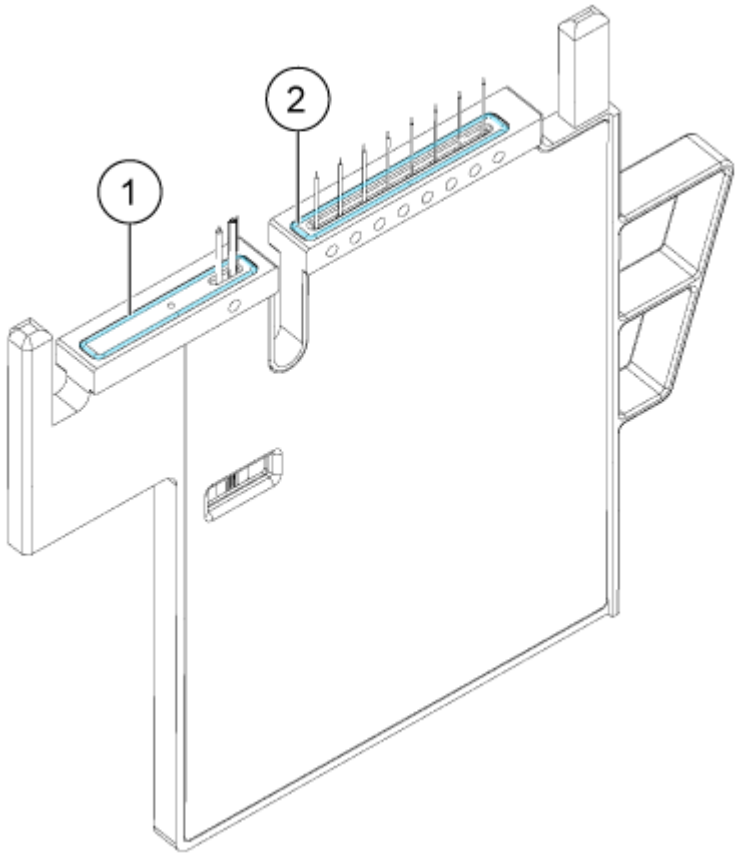
WARNING! Pinching Hazard. When opening the front panel, be careful not to put fingers to the left of the front panel.

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

1. If the cartridge was stored in the refrigerator, then let the cartridge equilibrate to room temperature for approximately 30 min to prevent condensation in the system.
2. Remove the cartridge from the wetting tray.

3. Use a disposable laboratory wipe to dry the cartridge body to prevent arcing.
4. Turn the bottom of the cartridge up.
5. Use a disposable lint-free laboratory wipe to very gently dry the area where the capillaries and electrodes emerge from the cartridge. Do not disturb the seals.

Figure 5-5 Bottom of the Cartridge



Item	Description
1	Outlet plate seal
2	Inlet plate seal

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

Figure 5-6 EJECTED Button

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

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

9. Examine the coolant level on the front panel. If required, add coolant into the fill port on the system.

   Refer to the section: "Add Capillary Cartridge Coolant" in the document: *Operator Guide*.

Run the Samples 6

Start the Sequence from the Front Panel

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

1. If required, load the cartridge, reagent plates, and sample plates.
2. On the front panel, touch **RUN SEQUENCE**.

Figure 6-1 RUN SEQUENCE Button

3. In the Projects pane, touch **CE-SDS Project**.
4. In the Available Sequences pane, touch the appropriate sequence in the list.
   - For reduced samples, click **CE-SDS Test Sequence**.
   - For non-reduced samples, click **Non Reduced CE-SDS Sequence**.
   - For samples prepared with a low pH sample buffer, click **Low pH Sample Buffer CE-SDS Sequence**.
5. (Optional) To view the details of the method, sample plates, or reagent plates, touch anywhere in the **Method** column.
   To hide the details, touch the column or box again.
6. Touch **Run Sequence**.

Figure 6-2 Run Sequence Button

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

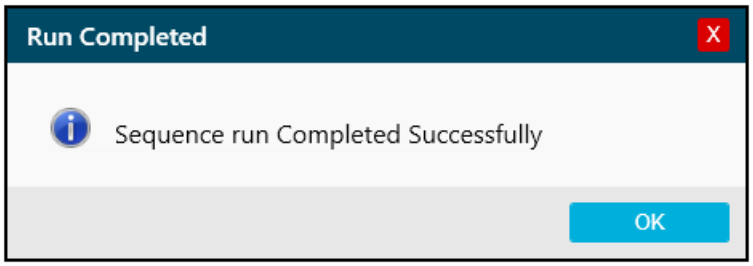
The data files are saved at the location specified in the sequence.

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

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

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

Figure 6-3 Run Completed Dialog



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

Monitor the Run on the BioPhase 8800 Front Panel

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

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

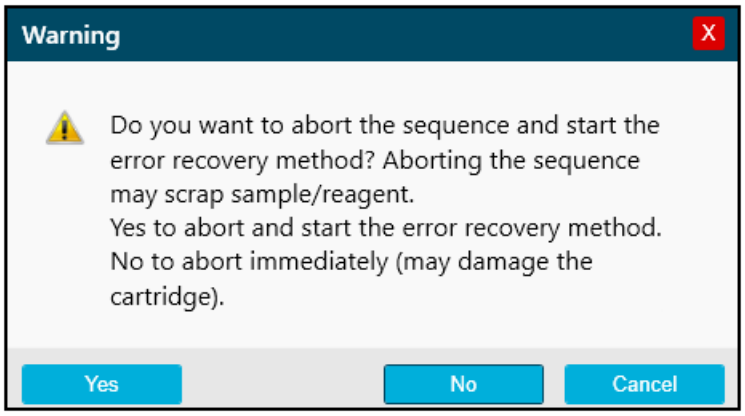
Note: The sequence shown in the following figures is for the purpose of illustration. It does not show a sequence for the CE-SDS Protein Analysis Kit.

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

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

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

Figure 6-4 Warning Dialog



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

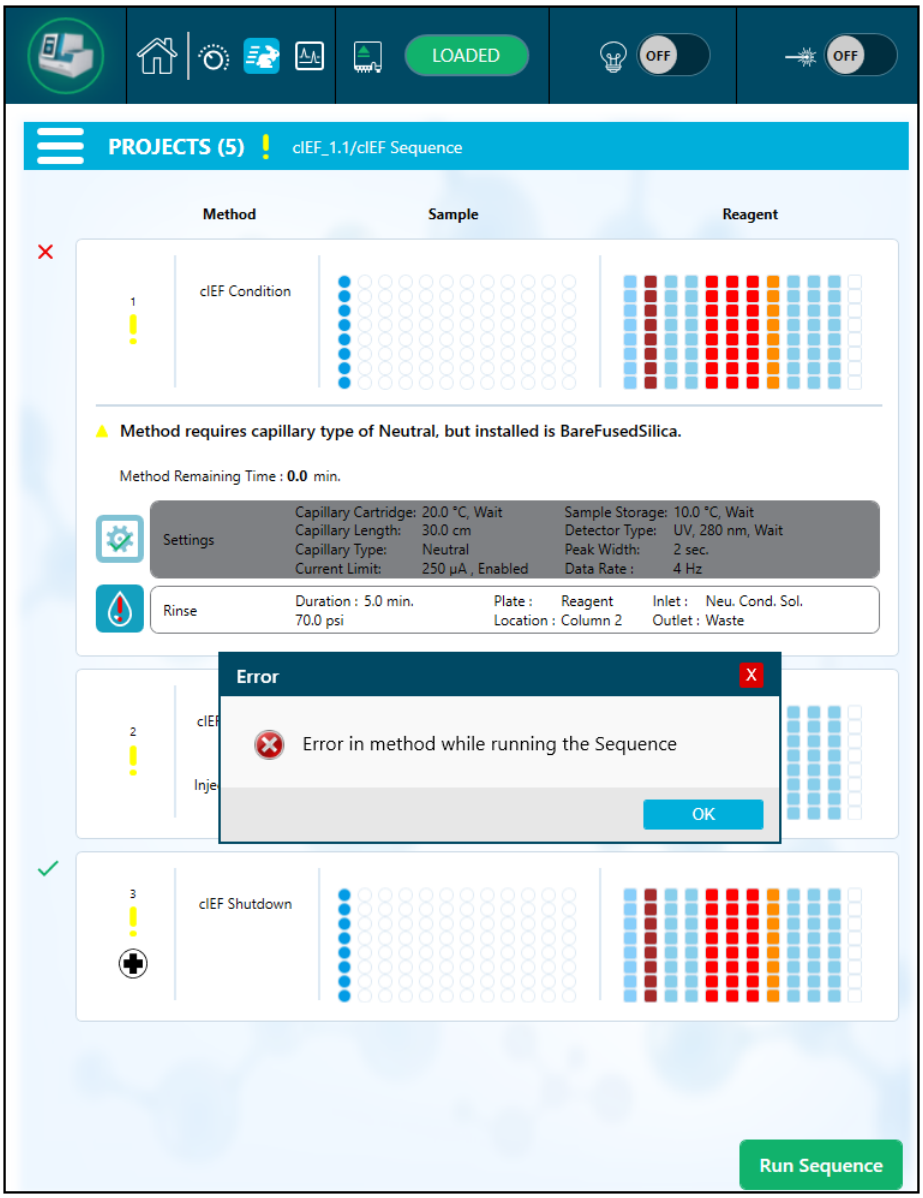
CAUTION: Potential System Damage. Before starting the run again, make sure to empty or replace the outlet plate to prevent overflow of reagent and possible damage to the instrument.

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

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

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

Figure 6-5 Run Sequence Error



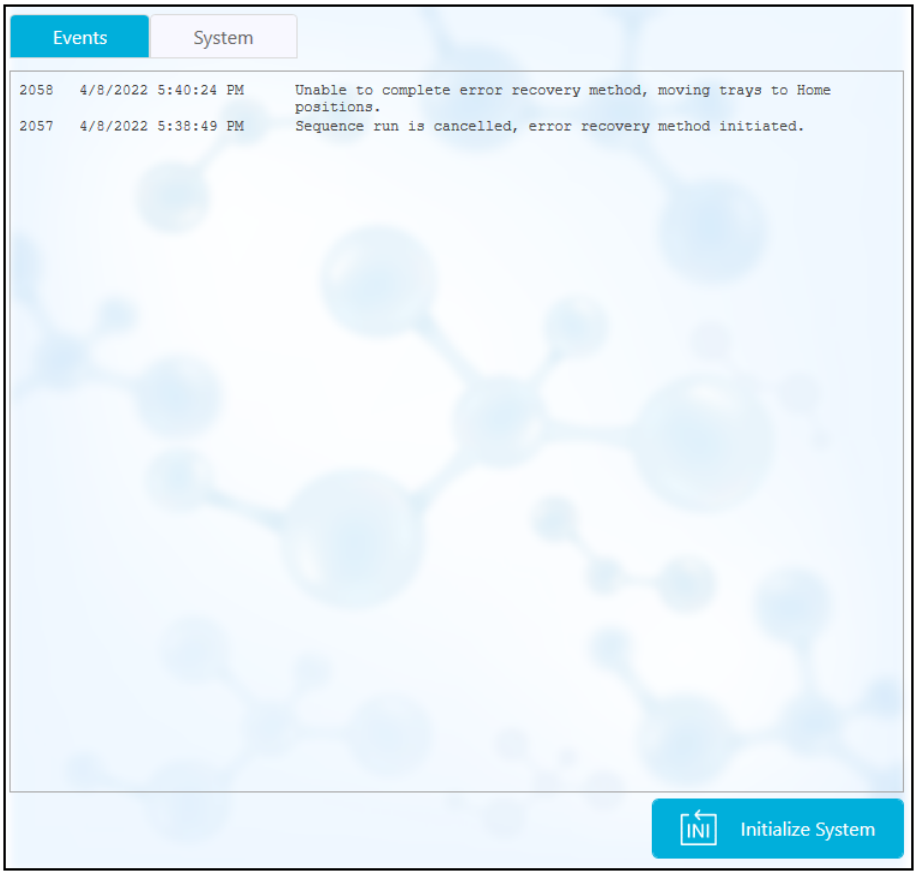
Note: The  shows an error at the **Rinse** action. The grey shading in the row above the **Rinse** action indicates that the action is in progress or completed.

4. Review the error:

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

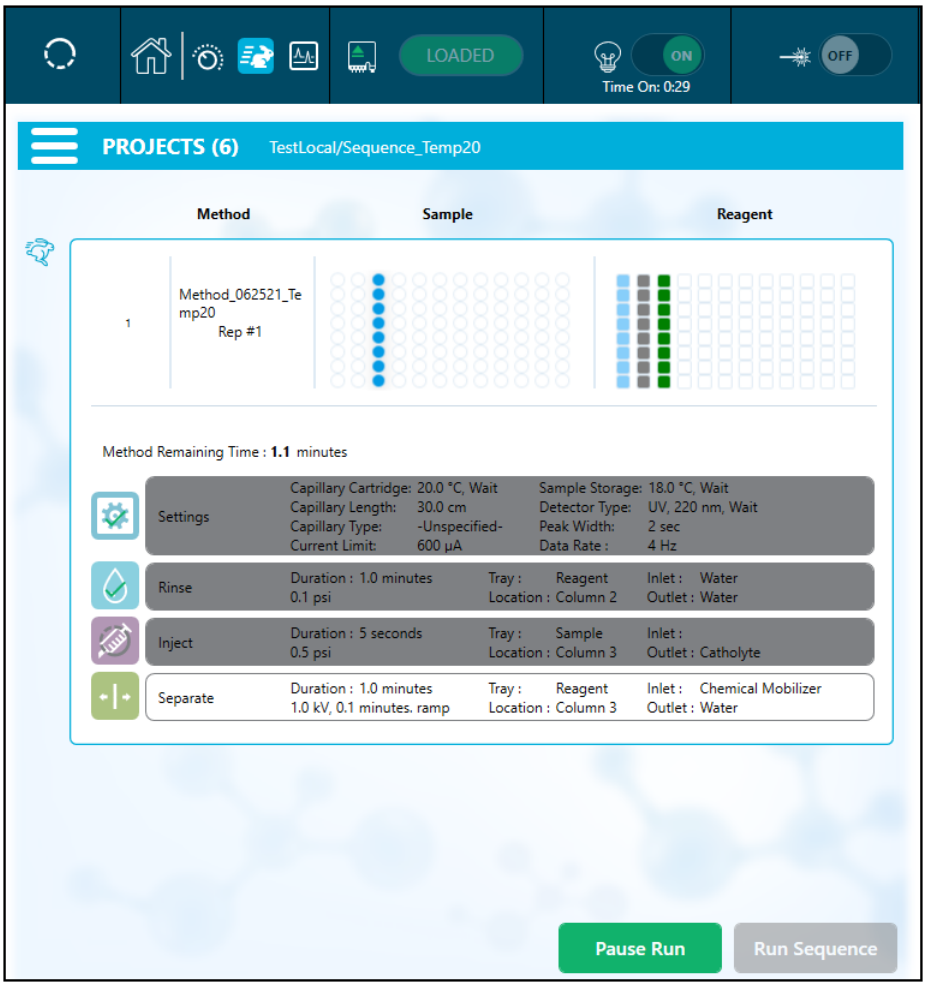
b. Touch **Initialize System** to reinitialize the system, and then change the system status to idle.

Figure 6-6 Sequence Error Events Log



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

Figure 6-7 Run Sequence in Progress



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

Figure 6-8 Restart the Run Sequence

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

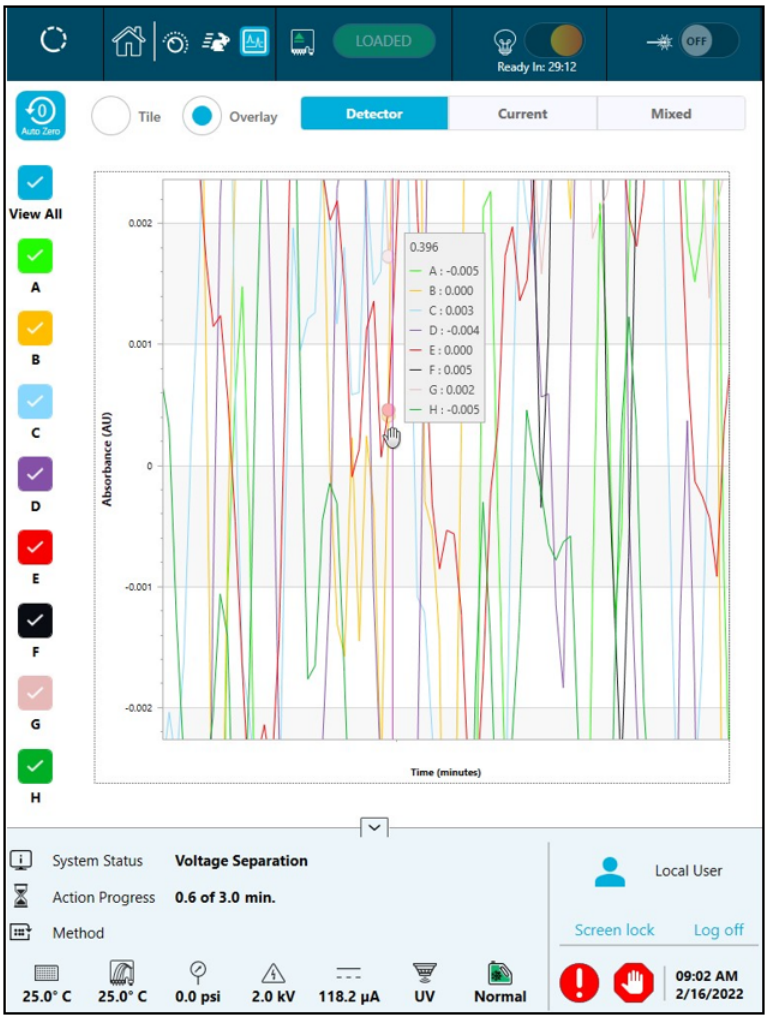
Note: The data in the following figure is for the purposes of illustration. It does not show results for samples prepared with the CE-SDS Protein Analysis Kit.

Figure 6-9 Capillary View

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

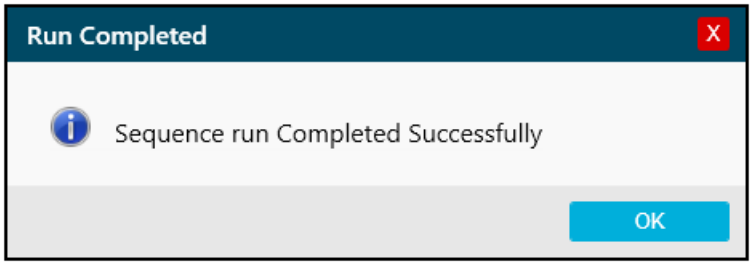
Note: The zoom feature only works with the overlay view for detector and current.

Figure 6-10 Zoom In or Out



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

Figure 6-11 Run Completed



Waste Disposal

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

Store the Cartridge After the Run

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

Store the Cartridge for Less than Three Days

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

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

Store the Cartridge for More than Three Days

1. If the sequence or sample set method does not include the shutdown method, then use the shutdown method to clean the capillary.
   The shutdown method fills the capillaries with CE-SDS Gel Buffer.
2. Rinse the capillary for 10 min at 80 psi with CE Grade Water to clean it.
3. On the ribbon on the BioPhase 8800 system front panel, touch 

(**Loaded**) and then wait for about one minute.
   Waiting lets the coolant return to the coolant reservoir before the cartridge is removed.
4. Remove the cartridge from the system, and then store it upright in the cartridge box at 2 °C to 8 °C with the capillary ends immersed in CE Grade Water.

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

Prepare the Cartridge After Storage

- If the cartridge has not been used for more than a day, or if it has been stored for an extended time, then use the CE-SDS Conditioning method to condition the capillary.

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

Analyze the Data 7

Analyze the Data with an Analysis Parameters File

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

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

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

Note: The analysis parameters 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 main window opens.
2. Click **File** > **Open**, select the data files to analyze, and then click **Open**.
3. In the **Project** toolbar, click  , navigate to CE-SDS Reduced IgG Analysis, and then click **Open**.
   The CE-SDS Reduced IgG Analysis file is a starting point for the analysis.
4. Right-click  , and then select **Apply & Analyze (all)**.

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

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

   In the Data pane, the analysis results are shown in the table below the graph. At the top of the table, **RMS Noise**, **P-P Noise**, and **Drift** are shown. In the graph, the baseline is shown in red and the threshold is shown as a grey horizontal line. Any peaks identified in the analysis have a blue marker at the peak start, a red marker at the peal apex, and a green marker at the peak end.

   Peaks in the graph are shaded as follows:

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

Refer to the section: Examine the Results.

5. Show the peak names on the graph.

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

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

   c. Click  .

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

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

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

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

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

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

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

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

      - **Tol** is the tolerance for matching a peak in the graph to a peak in the Marker Table or the Peak Table. Type % to use the tolerance as a percentage.
      - **Crit** is the peak characteristic to be matched.
        - **Ctr**: The peak closest to the center of the range is matched.
        - **Ht**: The tallest peak in the range is matched.

- **Area**: The largest peak in the range is matched.

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

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

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

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

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

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

Examine the Results

Figure 7-1 Non-reduced IgG Sample in SDS-MW Sample Buffer

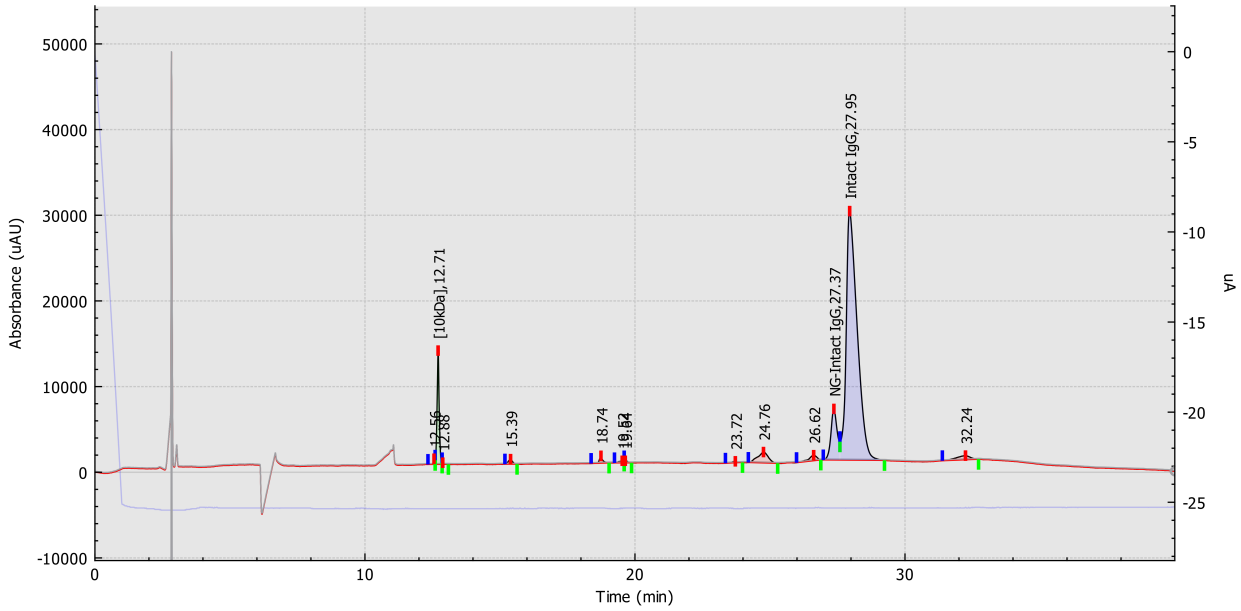


Figure 7-2 Reduced IgG Sample in SDS-MW Sample Buffer

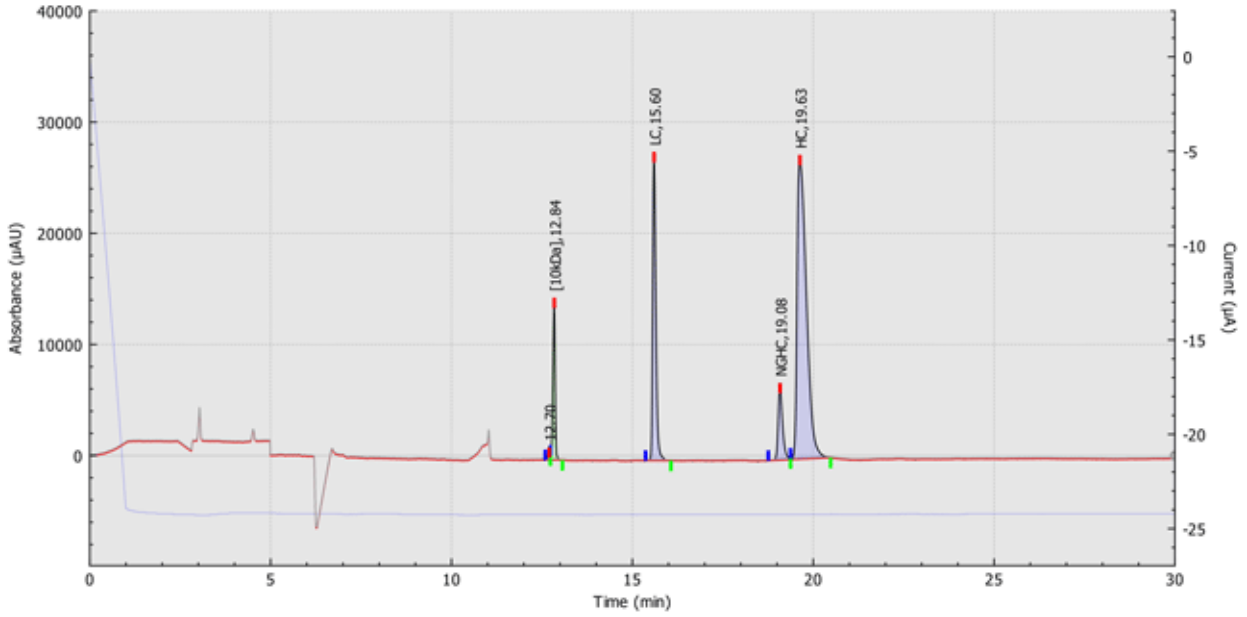


Figure 7-3 NIST IgG Sample in Low pH SDS Sample Buffer

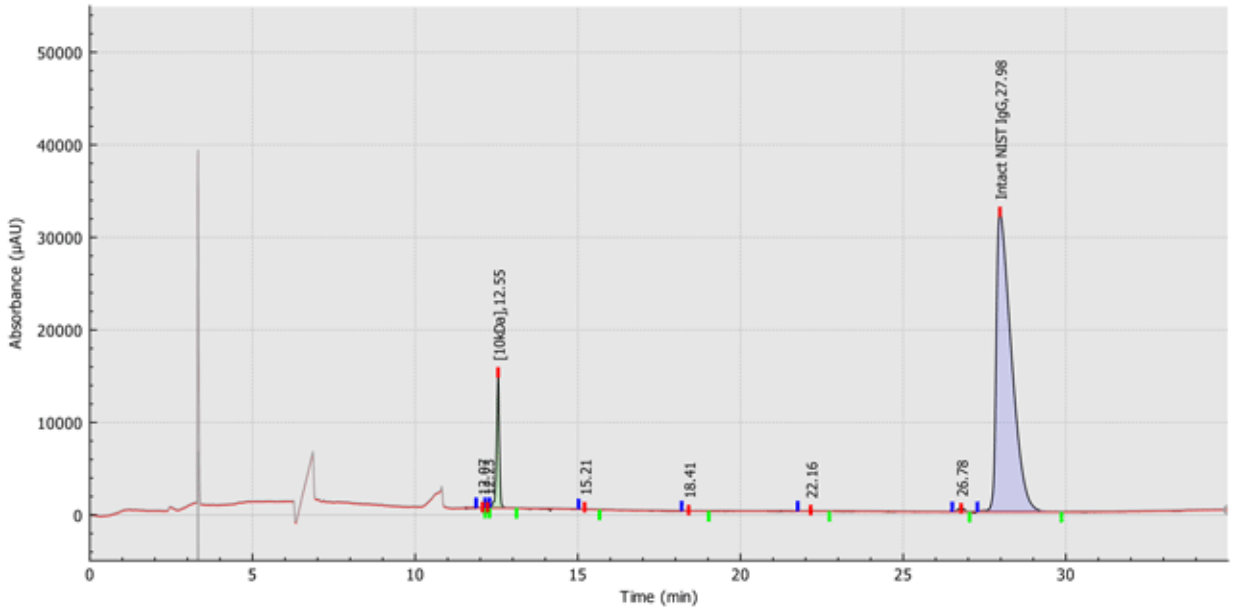
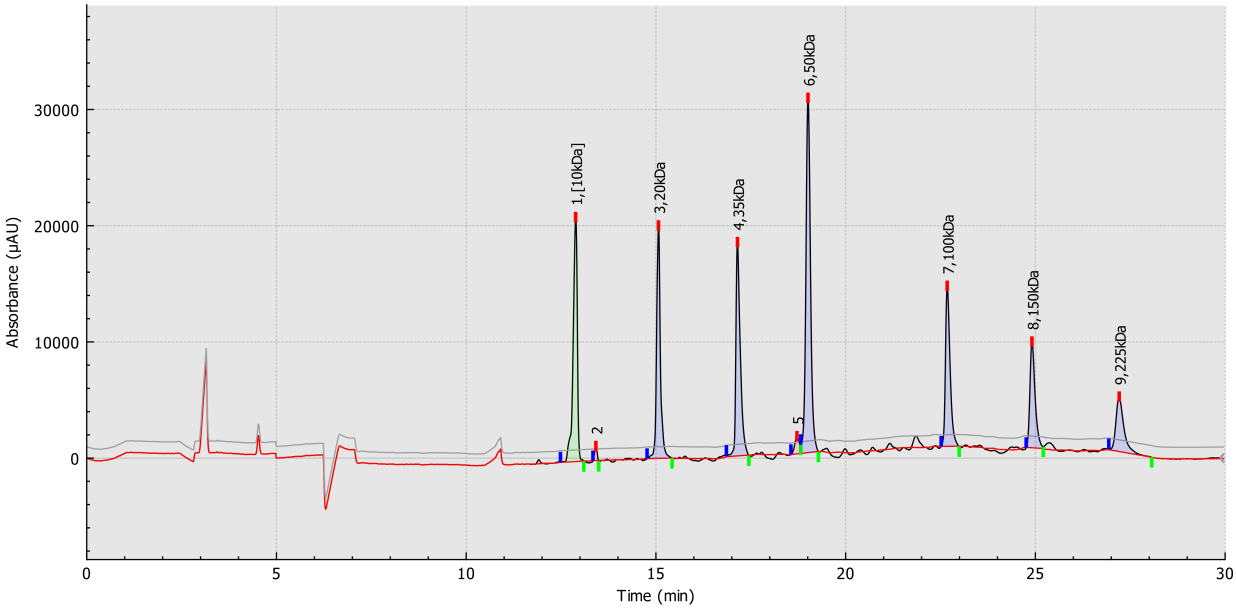


Figure 7-4 MW Size Standard in SDS-MW Sample Buffer



Review the Results on the Overlay Tab

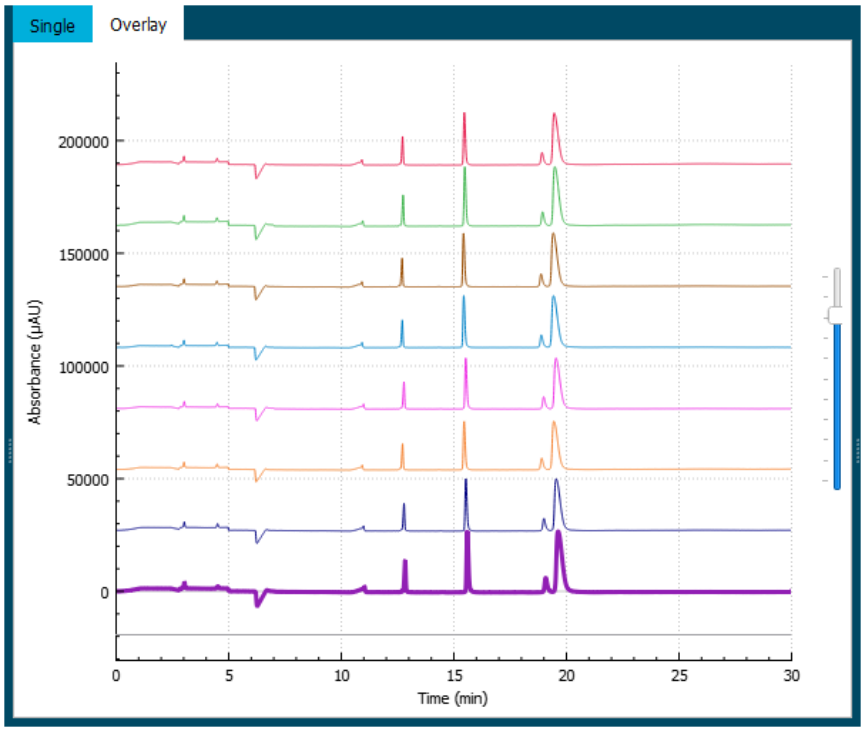
The Overlay tab shows the graphs for the selected data files. This tab contains the statistics for the selected data files, as well as the system suitability report.

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

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

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

Figure 7-5 Overlay Tab



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

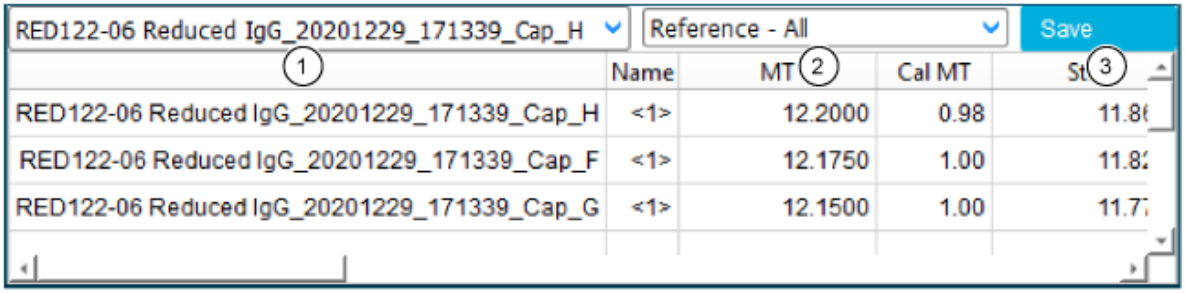
The thicker line is the trace that corresponds to the file selected in the Files pane.

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

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

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

Figure 7-6 Results Table



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

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

   These options are available:

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

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

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

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

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

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

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

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

   Note: To save the system suitability results, click **File** > **Save Report**. The results are saved as a PDF.

7. (Optional) Click **File** > **Print**.

The contents of the Overlay tab are printed in the current report template.

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

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

Guidance for Developing Acceptance Criteria

Acceptance criteria that are created for use with this kit for SOPs or other purposes should be based on parameters inherent to the quality of the separation and attributes that reflect critical sample qualities. Differences between gel and capillary lots and different systems might lead to variation in absolute migration times.

Peak purity (as corrected area % of peak), peak resolution, and relative migration time (with the 10 kDa Internal Standard from SCIEX or the main peak in the sample as a reference marker) are commonly accepted system suitability criteria for CE-SDS analysis. SCIEX strongly discourages the use of absolute migration time as an acceptance criterion.

Use the MW Size Standard to Estimate Molecular Weight

To estimate the molecular weight of an unknown protein, use the X-axis external marker calibration feature. Refer to the section: Create a Calibration Curve Using External Markers.

Note: If the 10 kDa Internal Standard is present in the unknown sample, then use the 10 kDa Internal Standard as a reference peak to correct for variations in the migration time and to increase the accuracy of the estimated molecular weight.

1. Do a run with the MW Size Standard.
2. In the BioPhase Analysis software, use the migration times and molecular weights of the MW Size Standard to create a calibration curve.
3. Apply the calibration curve to the data. The estimated molecular weight of the unknown protein is shown as **Cal MT** in the Results Table and in the annotations on the graph, if shown.

SCIEX recommends that the external calibration curve be calibrated again after 24 runs. To calibrate the curve again, do another run with the MW Size Standard, and then update the migration time for each standard with the results of the run.

Create a Calibration Curve Using External Markers

Use this function to generate the calibration curve for the X-axis from a set of external markers. If a reference marker is present in both the external marker set and the unknown sample, then the reference marker can be used to correct for variation in migration time.

1. Set the parameters for the calibration curve.
   a. Open the data file that contains the results from the separation of the standards.
   b. Integrate the data.
   c. In the Marker Table, assign the peaks for the MW Size Standard as markers.
   d. For each marker, type the molecular weight in the corresponding **Cal MT** cell.
   e. Select **External markers**, above the Marker Table on the right side of the tab.
   f. Type the **X-axis Name** and the **Units**.
   g. In the **Fit Type** list, select the type of calibration curve.
2. (Optional) Set the parameters for matching a reference peak to a peak in the data for the sample containing the unknown.
   a. Select **Ref** in the Marker Table for the reference peak.
   b. Click ... .
   c. In the **Ref MT** field, type the expected migration time of the reference peak in the data for the sample containing the unknown.
   d. In the **Tol** field, type the tolerance.
   e. In the **Crit** list, select the criteria.
   f. Click **OK**.

   If a reference peak is selected, then the migration times in the calibration curve are corrected by multiplying the migration time by the ratio of the **Ref Peak MT** (unknown) and **Ref Peak MT** (external marker set). This results in a more accurate determination of **Cal MT**, despite variations in migration time.
3. Click  , and then examine the graph and the Results Table to make sure that the **Cal MT** values are correct.
4. Click **Show Graph**, and then make sure that the data points from the Marker Table fit the calibration curve.
   The calibration curve is based on the **MT** and **Cal MT** values in the Marker Table.
5. Click  , and then save the parameters to an analysis parameters (dana) file.
6. Click **File** > **Open**, and then select the files to be analyzed.

7. Open the analysis parameters file created in step 5.

8. Right-click  , and then select **Apply & Analyze (all)** or **Apply & Analyze (checked)**.
   If the reference peak is present in both the external marker set and the unknown data, then examine the results. If required, click , and then adjust the **Ref MT**, **Tol** and **Crit** values in the dialog so that the reference peak in the unknown is found in the migration time window.

Run the Samples with the Waters Empower™ Software 8

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

Import the BioPhase Software Methods to Create the Instrument Methods

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

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

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

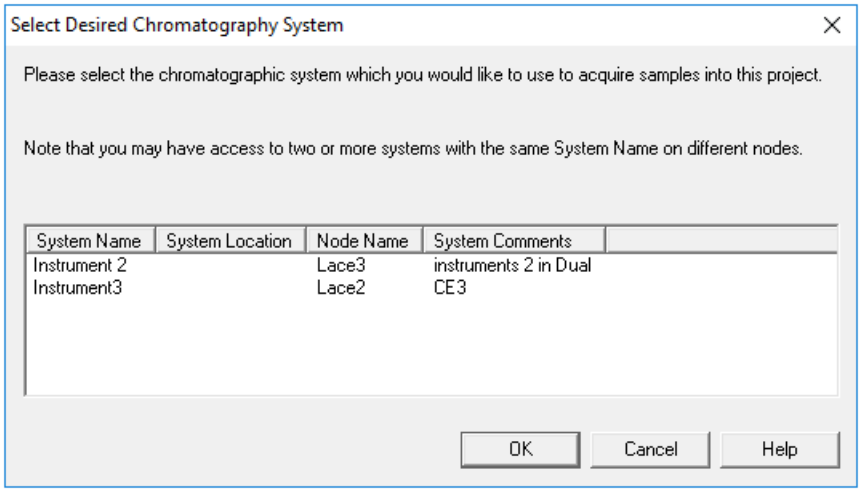
The following methods are available.

- CE-SDS Conditioning: Conditions the capillaries.
- Separation methods:
  - Reduced CE-SDS Separation: For reduced samples.
  - Non Reduced CE-SDS Separation: For non-reduced samples.
  - Low pH Sample Buffer Separation: For samples prepared with a low pH sample buffer.
- CE-SDS Shutdown: Cleans the capillaries at the end of a sequence, rinses the capillaries for storage, and then turns off the lamp.

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

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

Figure 8-1 Select Desired Chromatography System Dialog



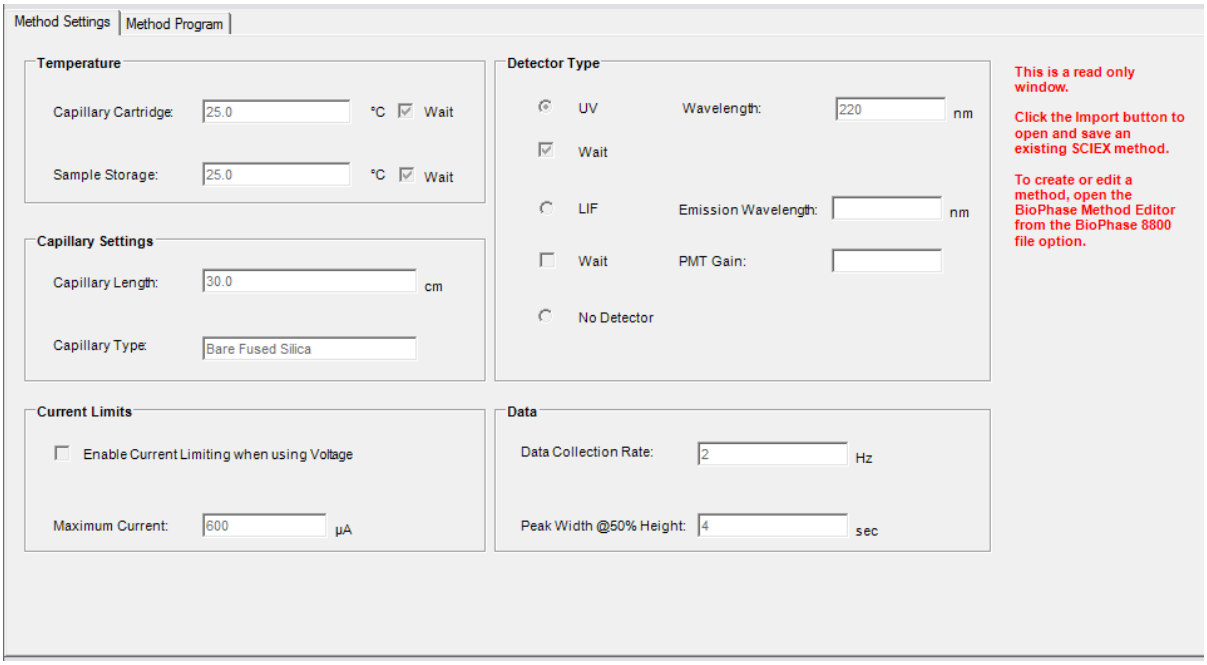
2. Click the system to be used, and then click **OK**.

   The Instrument Method Editor opens.

3. Click **Import**, and then browse to the conditioning method.
   The method opens in the Instrument Method Editor window with the Method Settings tab in front.

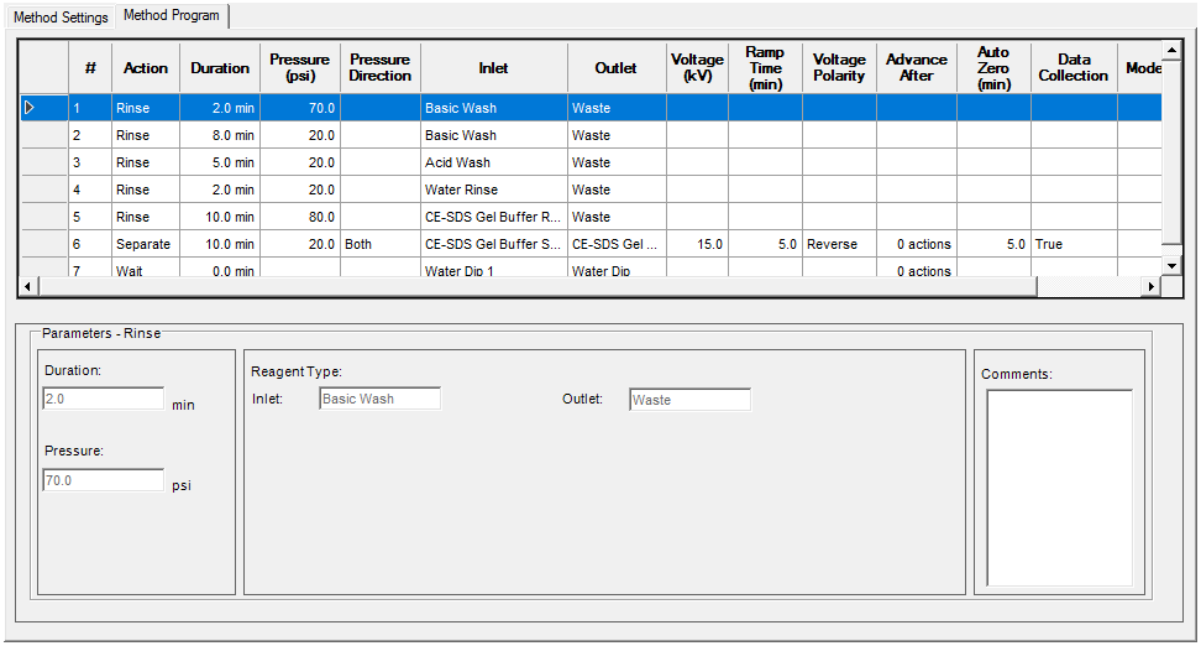
Note: This window is read-only. If changes to the method are required, then save the instrument method, and then edit the method in the Method Editors for BioPhase System software. Refer to the section: "Edit an Existing Instrument Method" in the document: *Software Help*.

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



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

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



6. Save the conditioning instrument method.
   a. Click **File** > **Save with Method Set**.
   The Save current Instrument Method dialog opens.
   b. In the **Name** field, type a name.

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

   c. (Optional) In the **Method Comments** field, type the information.
   d. If prompted, in the **Password** field, type the Waters Empower™ software password for the current user, and then click **Save**.

   The instrument method and method set are saved to the current project.
7. Click **File** > **Exit**.

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

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

Create the Sample Set Method

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

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

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

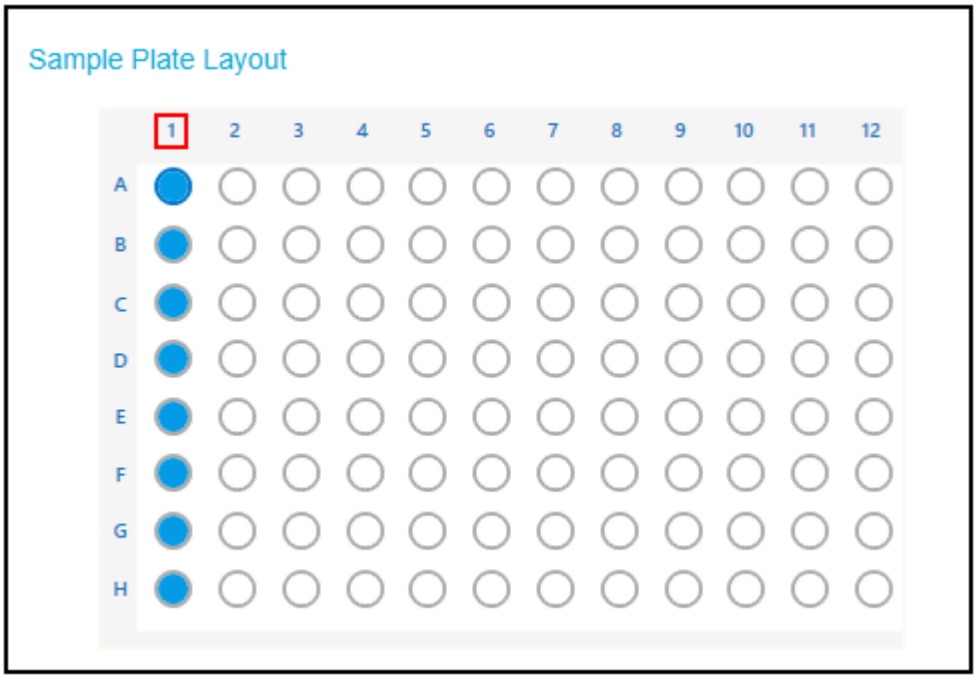
   The Method Editors for BioPhase System software opens, with the Sample Set Method Editor workspace shown.

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

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

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

Figure 8-4 Sample Plate Layout Pane



5. Add the required sample information to the Sample Set Summary table. In rows 2 through 9, do the following:
   a. In the **Sample Name** cell, type a name for the sample.
   b. Click the **Method Set Name** cell, and then select **Reduced CE-SDS Separation** or the appropriate separation method from the list.

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

6. In the last row, click the **Method Set Name** cell, and then select **CE-SDS Shutdown**.

Figure 8-5 Sample Set Summary Table

Sample Set Summary

Column	# of Injs	Plate/Well	Sample Name	Method Name	Run Time (Minutes)
				CE SDS Conditioning	37.0
1	1	1:A,1	Washington	Low pH Sample Buffer	61.5
1	1	1:B,1	Hoover	Low pH Sample Buffer	61.5
1	1	1:C,1	Polk	Low pH Sample Buffer	61.5
1	1	1:D,1	Coolidge	Low pH Sample Buffer	61.5
1	1	1:E,1	Jackson	Low pH Sample Buffer	61.5
1	1	1:F,1	Eisenhower	Low pH Sample Buffer	61.5
1	1	1:G,1	Kennedy	Low pH Sample Buffer	61.5
1	1	1:H,1	Truman	Low pH Sample Buffer	61.5
				CD SDS Shutdown	27.0

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

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

8. Save the sample set method.
   a. Click **SAVE AS**.

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

   The Save Sample Set dialog opens.
   b. Type a name in the **Sample Set Name** field.

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

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

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

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

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

   a. Open the Plate Layouts tab.

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

   c. As required, click the buttons to print or save the plate layouts.
   Refer to the section: "Print Preview Dialog" in the document: *Software Help System*.

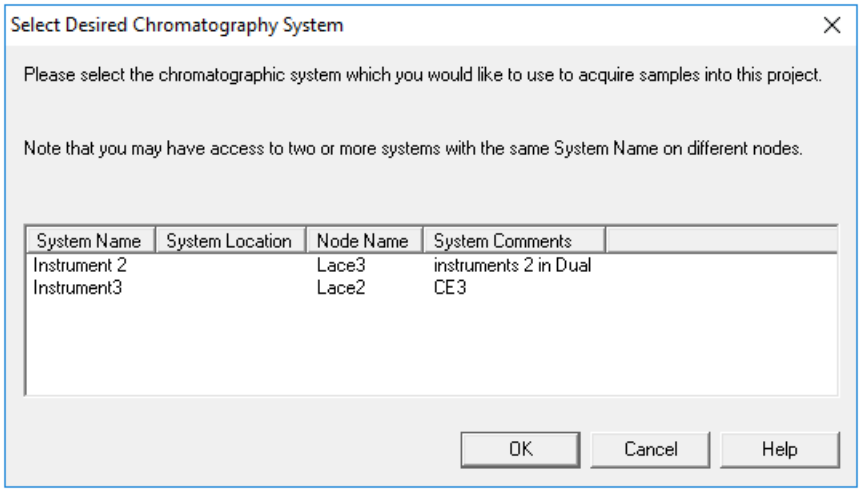
   d. Click the close box, the × in the top right corner.
   The Print Preview dialog closes.

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

Start the Sample Set Method

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

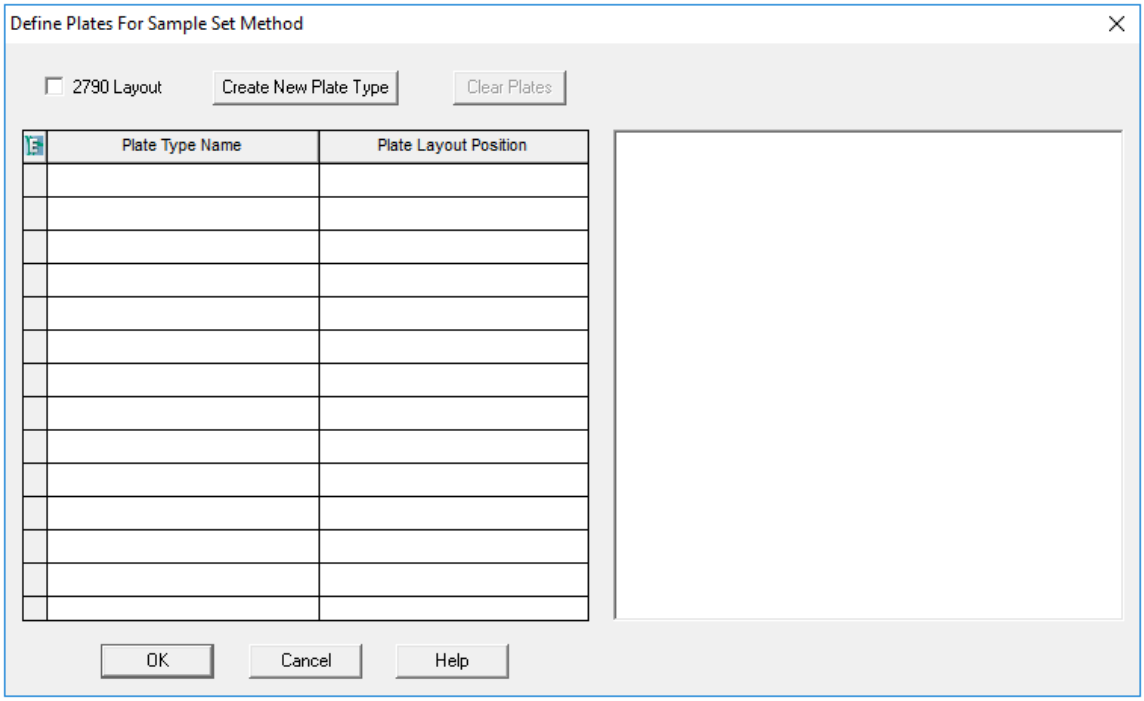
Figure 8-6 Select Desired Chromatography System Dialog



3. Click the system to be used, and then click **OK**.
   The Run Samples window opens.

4. Configure the plate type.
   a. Click **Edit** > **Plates**.

Figure 8-7 Define Plates for Sample Set Method Dialog



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

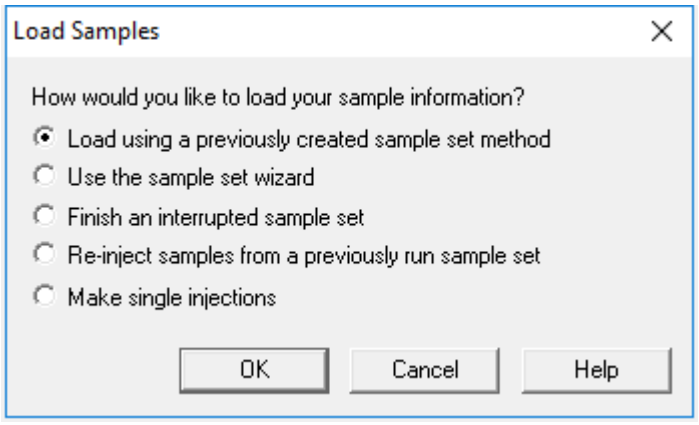
   b. Click the **Plate Type Name** cell, and then select **ANSI-96well2mL**.
   The dialog updates with an image of the plate and buttons for the plate sequencing mode.
   c. Click the **Plate Layout Position** cell, and then type `1`.
   d. Click  to indicate the order that the wells are accessed during the run.
   e. Click **OK** to save the changes, and then close the dialog.

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

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

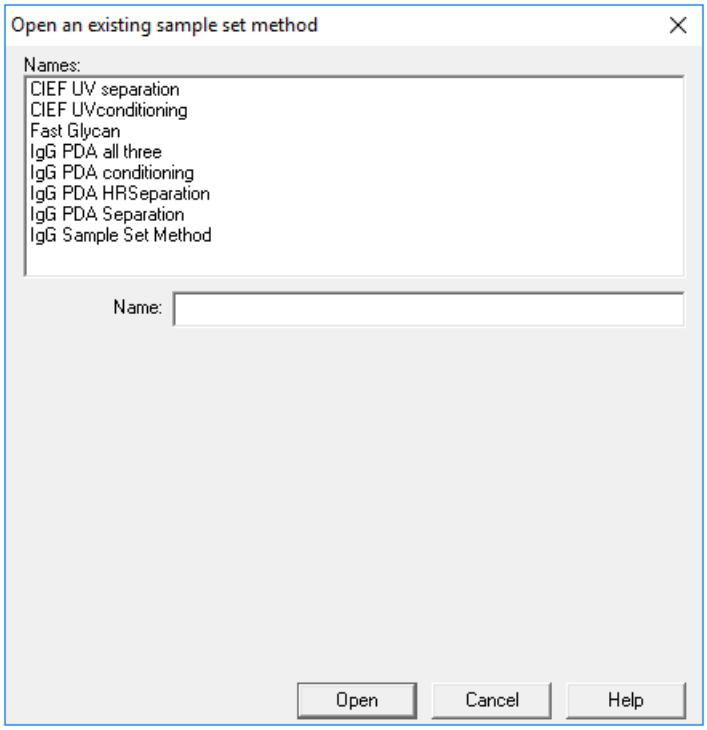
5. Click  (**Load Sample Set**).

Figure 8-8 Load Samples Dialog



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

Figure 8-9 Open an existing sample set method Dialog



7. Click **CE-SDS Kit Sample Set Method** in the list, and then click **Open**.
   The sample set method opens in the Samples tab.
8. (Optional) Configure the table to show only the columns that are relevant for the BioPhase 8800 system.

a. Right-click, and then select **Table Properties**.
The Table Properties dialog opens.

b. Click **Hide All**, and then clear the check boxes for **Plate/Well**, **# of Injs**, **SampleName**, **Function**, and **Method Set / Report or Export Method**.

c. Click **OK**.

The table updates to show the selected columns.

Figure 8-10 Samples Tab

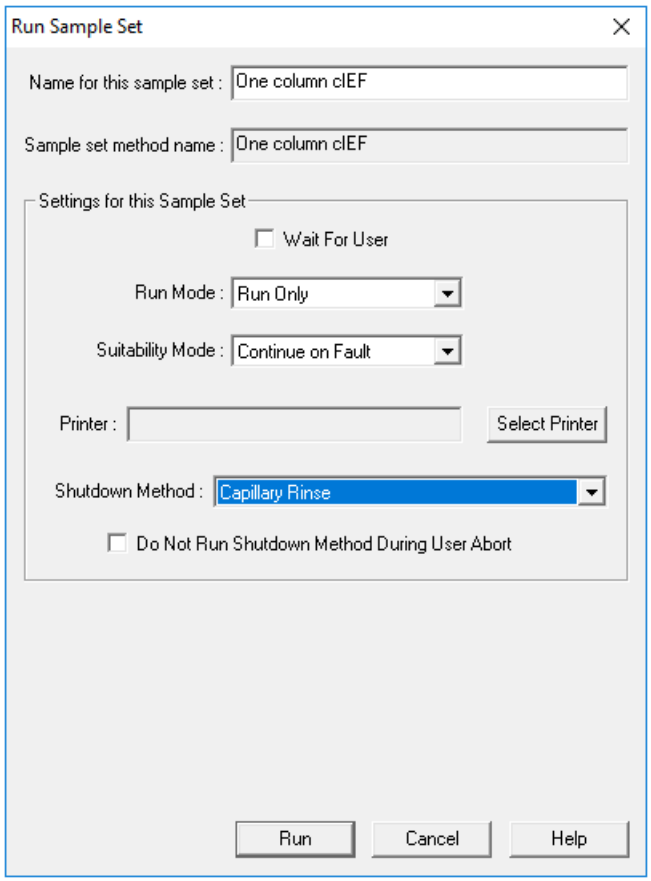
Sample Set Method: CE SDS Kit Sample Set Method

	Plate/Well	# of Injs	SampleName	Function	Method Set / Report or Export Method
1				Condition Column	CE SDS Conditioning
2	1:A,1	1	Washington	Inject Samples	Low pH Sample Buffer
3	1:B,1	1	Hoover	Inject Samples	Low pH Sample Buffer
4	1:C,1	1	Polk	Inject Samples	Low pH Sample Buffer
5	1:D,1	1	Coolidge	Inject Samples	Low pH Sample Buffer
6	1:E,1	1	Jackson	Inject Samples	Low pH Sample Buffer
7	1:F,1	1	Eisenhower	Inject Samples	Low pH Sample Buffer
8	1:G,1	1	Kennedy	Inject Samples	Low pH Sample Buffer
9	1:H,1	1	Truman	Inject Samples	Low pH Sample Buffer
10				Condition Column	CD SDS Shutdown

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

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

Figure 8-11 Run Sample Set Dialog



11. If required, edit the information in the Run Sample Set dialog.
    a. If required, edit the **Name for this sample set** field.
    b. (Optional) Click **Shutdown Method**, and then select an instrument method that rinses the capillaries.

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

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

       If the system encounters an error during a run, then it executes this instrument method, and then stops the run.

c. If required, select **Do Not Run Shutdown Method During User Abort**.

d. Click **Run**.

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

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

Monitor the Run in the Waters Empower™ Software

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

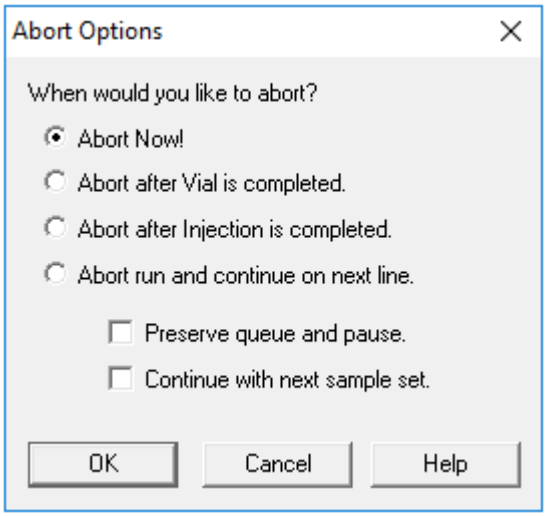
Note: Most of the panes in the Waters Empower™ software are designed for chromatography. Use the following steps to monitor the progress of the capillary electrophoresis separation and disregard information in the Time Remaining and Solvent Required panes.

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

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

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

Figure 8-12 Abort Options Dialog



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

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

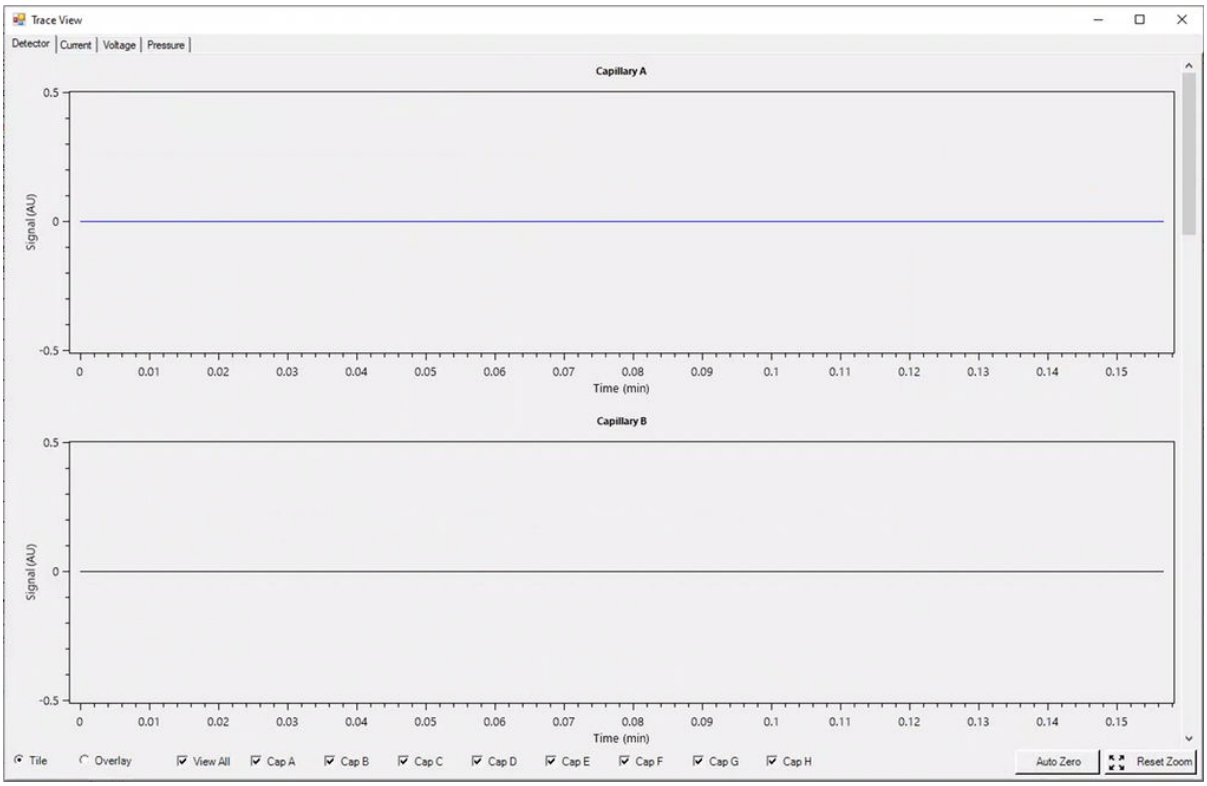
CAUTION: Potential Wrong Result. Before starting the run again, prepare new reagent plates. If the run has been stopped, then there might not be enough reagents available to complete the run.

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

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

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

Figure 8-13 Trace View Window



3. If required, do any of the following:
   - To view current, voltage, or pressure, open the applicable tab in the top left.
   - To view one graph with the data for all of the capillaries, in the bottom left click **Overlay**.
   - To view data for specific capillaries, select or clear the check boxes at the bottom of the window to select the capillaries of interest.
   - To view the time and detector values for any point on a trace, click the trace at the position of interest.
   - To zoom in on the data, make sure that **Overlay** is selected, and then drag to select the area to zoom. The mouse scroll wheel can also be used to zoom.
   - To return the data to the original dimensions, in the bottom right click **Reset Zoom**.
   - To view a different area of a zoomed plot, right-click the X- or Y-axis and then drag.
4. If required, at the bottom right click **Auto Zero**.
   The detector signal is set to zero.
5. Wait until the **Abort** button () changes from red to green ().

There might be a delay between data acquisition and when all of the data is saved. The green button indicates when all of the data is saved.

6. As required, dispose of samples and reagents. Refer to the section: Waste Disposal.
7. As required, store the cartridge. Refer to the section: Store the Cartridge After the Run.

Troubleshooting 9

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

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

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

Symptom	Possible Cause	Corrective Action
Broad peaks, poor resolution	1. The capillary end is damaged. 2. The sample concentration is too high. 3. The capillary is blocked. 4. The internal surface of the capillary is contaminated.	1. To assess the condition of the capillary end: • Use a magnifying lens to examine it. • Use a lint-free laboratory wipe to wipe the capillary inlets carefully in an outward direction. • Make sure that the capillary end extends approximately 2 mm from the gold cannula electrode. • Make sure that the capillary end has a straight cut. If the capillary cannot be used, then refer to the section: Options for a Blocked or Damaged Capillary. 2. Do one or all of the following: • Decrease the **Duration** in the **Inject** action in the separation method to inject less sample. If the results are not satisfactory, then decrease the **Pressure** or **Voltage**. • Dilute the sample again with the sample diluent. 3. Refer to the section: Options for a Blocked or Damaged Capillary. 4. Either edit the sequence to omit the contaminated capillary or replace the cartridge.

Symptom	Possible Cause	Corrective Action
Carryover	1. The sample concentration is too high. 2. The reagent plate is contaminated with sample.	1. Do one or all of the following: • Decrease the **Duration** in the **Inject** action in the separation method to inject less sample. If the results are not satisfactory, then decrease the **Pressure** or **Voltage**. • Dilute the sample again with the sample diluent. 2. In the separation method, add one or more water dip steps after sample injection. With the edited method: a. Create a new sequence that uses this separation method. b. Prepare new reagent plates for the new sequence. Refer to the section: Load the Reagent Inlet and Outlet Plates.
High current	1. The gel buffer is contaminated. 2. The positions of the reagents in the reagent plate do not agree with the plate layouts in the sequence.	1. Prepare the inlet and outlet reagent plates again to replace the gel buffer. 2. Make sure that the positions of the reagents in the reagent plates agree with the plate layouts. If the positions are not correct, then prepare the plates again according to the plate layout. Refer to the section: Plate Layouts.

Symptom	Possible Cause	Corrective Action
Low or unsteady current	1. The capillary end is blocked or contaminated. 2. The gel buffer has air bubbles.	1. Refer to the section: Remove Blockage from a Capillary. If the current is low or unsteady, then replace the cartridge. 2. Do one or all of the following: • Use a centrifuge to spin the plate for 5 min at 30 *g* to remove air bubbles. • De-gas the gel buffer for 5 min with 5 inches Hg to 15 inches Hg vacuum.
Missing peaks in electropherogram sample	1. A pipetting error occurred during preparation of the sample. 2. The method parameters are incorrect. 3. The positions of the reagents in the reagent plate do not agree with the plate layouts in the sequence.	1. Prepare a new sample. 2. Do the following: • In Method Settings, make sure that the value for **Detector Type** is correct. • In the separation method, make sure that the values for **Type of Injection** and **Duration** are correct. • In the separation method, make sure that the value for **Wavelength** is **220 nm**. 3. Make sure that the positions of the samples in the sample plate agree with the plate layouts. If the positions are not correct, then prepare the plates again according to the plate layout. Refer to the section: Reagents, Plate Layouts, and Methods.

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

Symptom	Possible Cause	Corrective Action
No peaks	1. The lifetime of the UV lamp has been exceeded. 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 end 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 plate do not agree with the plate layouts in the sequence.	1. Refer to the section: "Install a UV Lamp" in the document: *Operator Guide*. 2. Do the following: • In **Method Settings**, make sure that the value for **Detector Type** is **UV**. • In the **Inject** and **Separate** actions, make sure that the value for **Polarity** is **Reverse**. • In the separation method, make sure that the value for **Wavelength** is **220 nm**. • Make sure that pressure is applied at the inlet and the outlet during the separation. 3. Use a centrifuge to spin the plate for 5 min at 30 *g* to remove air bubbles. 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. Condition the capillary. Refer to the section: Condition the Capillaries. 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.

Symptom	Possible Cause	Corrective Action
Spikes in electropherogram	1. The gel buffer has air bubbles.	1. Do one or all of the following: • Use a centrifuge to spin the plate for 5 min at 30 *g* to remove air bubbles. • De-gas the gel buffer for 5 min with 5 inches Hg to 15 inches Hg vacuum.

Remove Blockage from a Capillary

1. Rinse the capillary with CE Grade Water for 10 min at 75 psi.
2. Use CE Grade Water to clean the capillary inlets.
3. Use a lint-free laboratory wipe to wipe the capillary inlets carefully in an outward direction.
4. To assess the condition of the capillary, do the following:
    a. Use Direct Control to fill the capillary with separation gel.
    b. Put the inlet and outlet capillaries in the separation buffer in the reagent tray.
    c. Apply the separation voltage and monitor the stability of the current.
5. If the blockage cannot be removed, then either edit the sequence to omit the damaged capillary or replace the cartridge.

Options for a Blocked or Damaged Capillary

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

Condition the Capillaries

- If required, condition the capillaries using the CE-SDS Conditioning method.

Hazardous Substance Information A

The following information must be noted and the relevant safety measures taken. Refer to the respective safety data sheets for more information. The safety data sheets are available upon request or can be downloaded from our website, at sciex.com/tech-regulatory.

Hazard classification according to HCS 2012.

Acid Wash/Regenerating Solution (0.1 M HCl)

DANGER! Causes severe skin burns and eye damage.

Capillary Regenerator Solution A Basic Wash (0.1 M NaOH)

DANGER! Causes severe skin burns and eye damage.

CE-SDS Gel Buffer, pH 8, 0.2% SDS

DANGER! Causes mild skin irritation. May damage fertility of the unborn child.

IgG Control Standard

WARNING! Causes mild skin irritation.

Low pH SDS Sample Buffer (100 mM Tris-HCL, pH 6.8, 1% SDS)

WARNING! Causes mild skin irritation.

Other Reagents

These components are not classified as hazardous:

- CE Grade Water
- MW Size Standard
- SDS-MW Sample Buffer

- 10 kDa Internal Standard

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

References B

1. Liu, L. Y., Ratnayake, C. K., Chapman, J., Dontha, N., Choo, S., and Reddy, M.P., *Assay of IgG Purity and Heterogeneity using High-Resolution Sodium Dodecyl Sulfate Capillary Gel Electrophoresis*, SCIEX 2018
2. Nunnally, B., Park, S.S., Patel, K., Hong, M., et. al., *Chromatographia*, volume 66, pp 955, 2007. "A Series of Collaborations between Various Pharmaceutical Companies and Regulatory Authorities Concerning the Analysis of Biomolecules Using Capillary Electrophoresis: Additional Instruments/Buffer."

Download Required Files C

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

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

Download and Configure the Required Files (BioPhase Software)

Files with methods, sequences, reagents, and analysis parameters for the CE-SDS Protein Analysis Kit are available on sciex.com. Use the following instructions to download the files, and then copy them to the appropriate location.

Note: The following procedure is only required when BioPhase software version 1.1 is being used. The required files for the CE-SDS Protein Analysis Kit are included as part of the BioPhase software version 1.2 or later.

1. Go to sciex.com/software-support/software-downloads, and then click **BioPhase Resources** in the More software downloads section.
2. Click `BioPhase Project Files 1.2`.
3. In File Explorer, right-click the `BioPhase_1.2.zip` file, and then click **Extract All** to extract the installation package.
4. Browse to a location, click **Select Folder**, and then click **Extract**.
   The extracted files are copied to the selected file path.
5. Put the extracted files in the correct locations. Do the following:

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

   a. Drag the `BioPhase_1.2\Projects\CE-SDS` folder to `C:\BioPhase\Projects`.
   b. Drag the `BioPhase_1.2\Reagents\CE-SDS` folder to `C:\BioPhase\Reagents`.
   c. Drag the `BioPhase_1.2\Data Analysis\CE-SDS` folder to `C:\BioPhase\Data Analysis`.

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

Required files for the CE-SDS Protein Analysis Kit are available on sciex.com. Use the following instructions to download the files, and then copy them to the appropriate location.

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

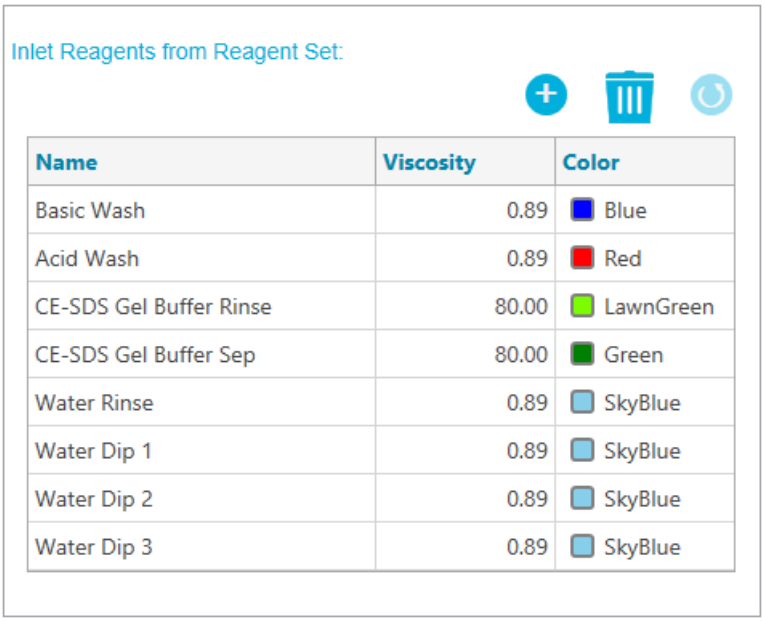
Reagents, Plate Layouts, and Methods

D

Reagent Set

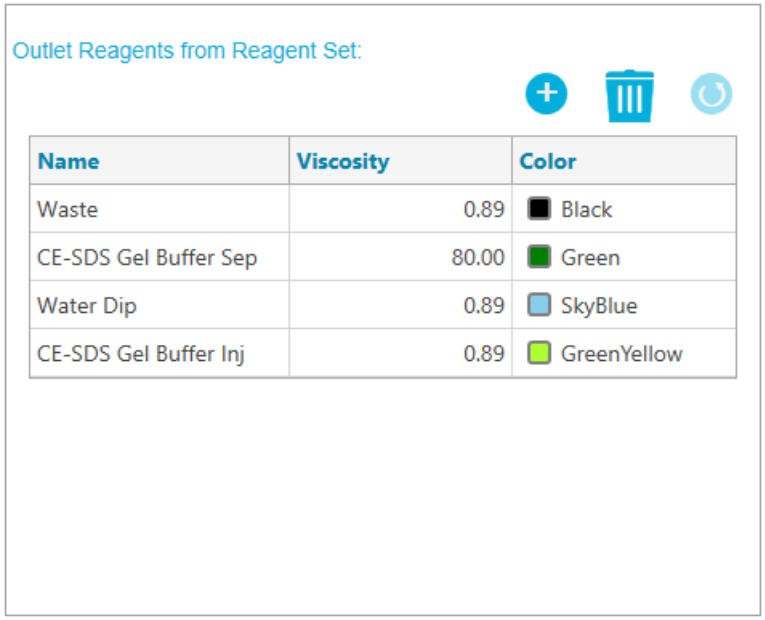
If the reagents are not available, then use the following figures to create a new reagent set. Refer to the figures: Figure D-1 and Figure D-2.

Figure D-1 CE-SDS Protein Analysis Kit Inlet Reagents



Name	Viscosity	Color
Basic Wash	0.89	Blue
Acid Wash	0.89	Red
CE-SDS Gel Buffer Rinse	80.00	LawnGreen
CE-SDS Gel Buffer Sep	80.00	Green
Water Rinse	0.89	SkyBlue
Water Dip 1	0.89	SkyBlue
Water Dip 2	0.89	SkyBlue
Water Dip 3	0.89	SkyBlue

Figure D-2 CE-SDS Protein Analysis Kit Outlet Reagents



Name	Viscosity	Color
Waste	0.89	Black
CE-SDS Gel Buffer Sep	80.00	Green
Water Dip	0.89	SkyBlue
CE-SDS Gel Buffer Inj	0.89	GreenYellow

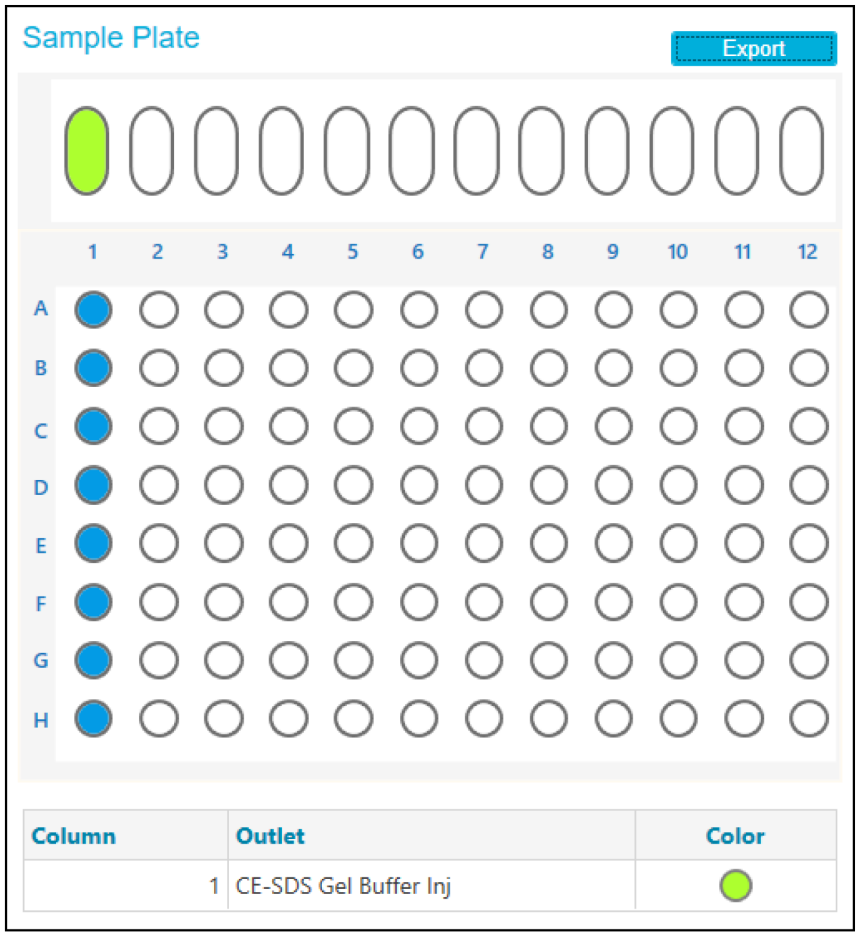
Plate Layouts

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

Sample Plates

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

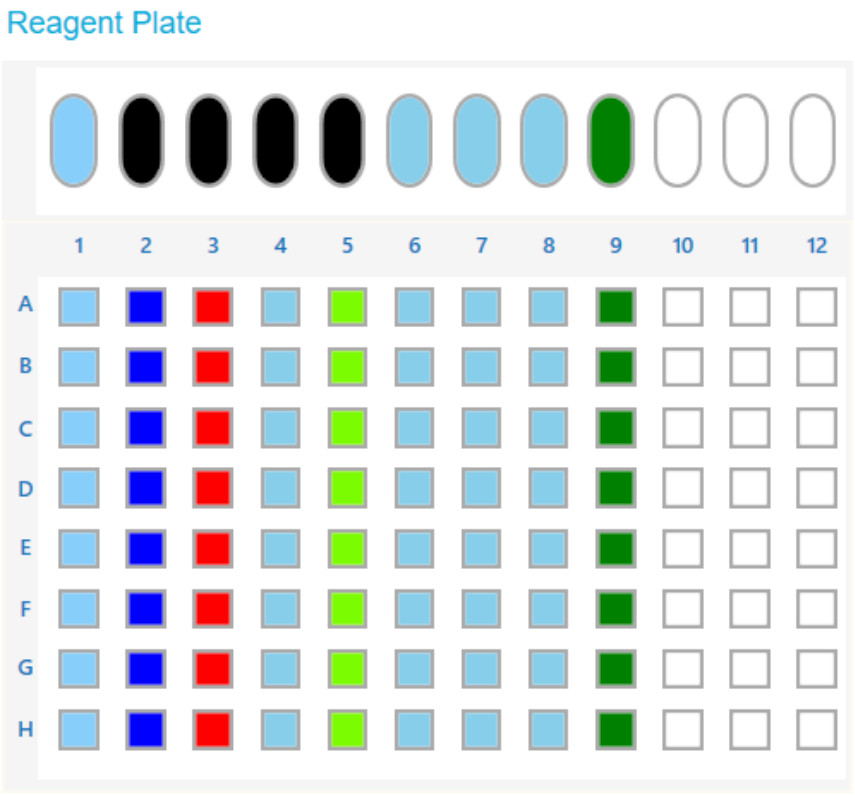
Figure D-3 Layouts for the Sample Inlet Plate and Sample Outlet Plate



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



Column	Inlet	Color	Outlet	Color
1	Capillary Protect		Capillary Protect	
2	Basic Wash		Waste	
3	Acid Wash		Waste	
4	Water Rinse		Waste	
5	CE-SDS Gel Buffer Rinse		Waste	
6	Water Dip 1		Water Dip	
7	Water Dip 2		Water Dip	
8	Water Dip 3		Water Dip	
9	CE-SDS Gel Buffer Sep		CE-SDS Gel Buffer Sep	

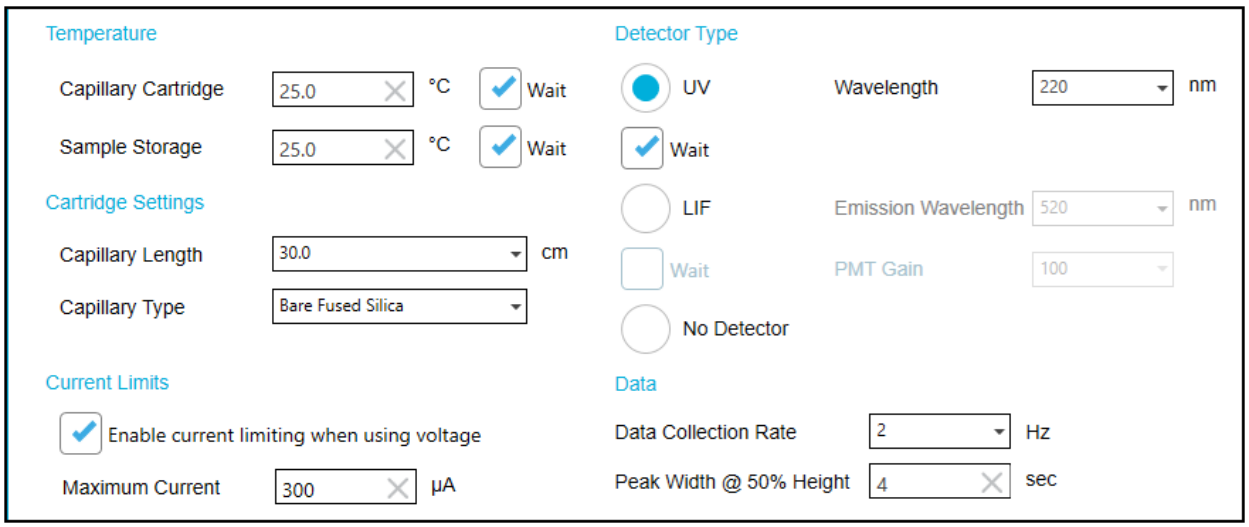
Methods

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

Method Settings

Note: Use these settings for all of the methods.

Figure D-5 Method Settings for CE-SDS Protein Analysis Methods



Conditioning Method

Figure D-6 Actions in the CE-SDS Conditioning Method

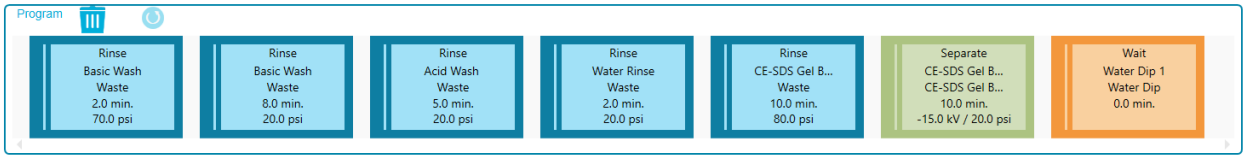


Figure D-7 Summary of Actions in the CE-SDS Conditioning Method

Method Duration: 37.0 min. Number of Actions: 7

Settings	Capillary Cartridge: 25.0 °C, Wait Capillary Length: 30.0 cm Capillary Type: Bare Fused Silica Current Limit: 300 µA , Enabled	Sample Storage: 25.0 °C, Wait Detector Type: UV, 220 nm, Wait Peak Width: 4 sec. Data Rate: 2 Hz	
Rinse	Duration: 2.0 min. 70.0 psi	Inlet: Basic Wash Outlet: Waste	
Rinse	Duration: 8.0 min. 20.0 psi	Inlet: Basic Wash Outlet: Waste	
Rinse	Duration: 5.0 min. 20.0 psi	Inlet: Acid Wash Outlet: Waste	
Rinse	Duration: 2.0 min. 20.0 psi	Inlet: Water Rinse Outlet: Waste	
Rinse	Duration: 10.0 min. 80.0 psi	Inlet: CE-SDS Gel Buffer Rinse Outlet: Waste	
Separate	Duration: 10.0 min. -15.0 kV, 20.0 psi, Both Ramp Time: 5.0 min. Autozero: 5.0 min.	Inlet: CE-SDS Gel Buffer Sep Outlet: CE-SDS Gel Buffer Sep	
Wait	Duration: 0.0 min.	Inlet: Water Dip 1 Outlet: Water Dip	

Separation Method for Non-Reduced Samples

Figure D-8 Actions in the Non Reduced CE-SDS Separation Method

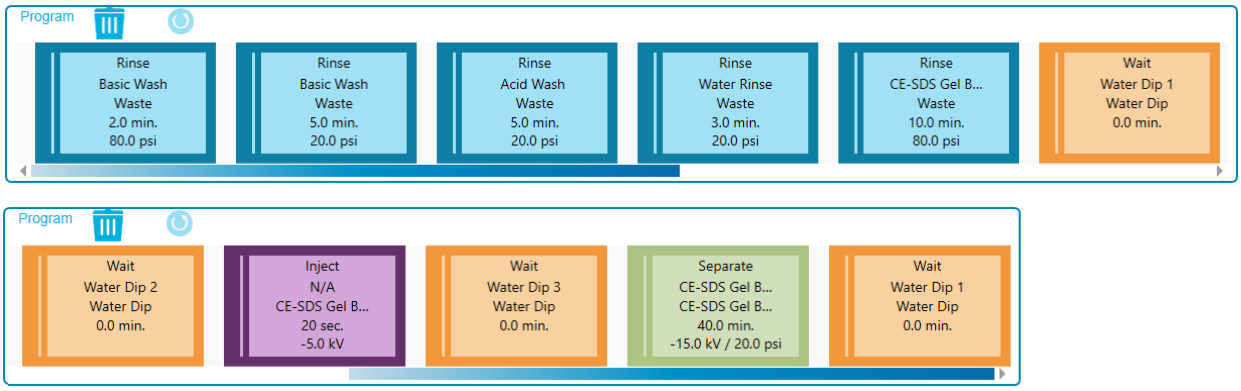
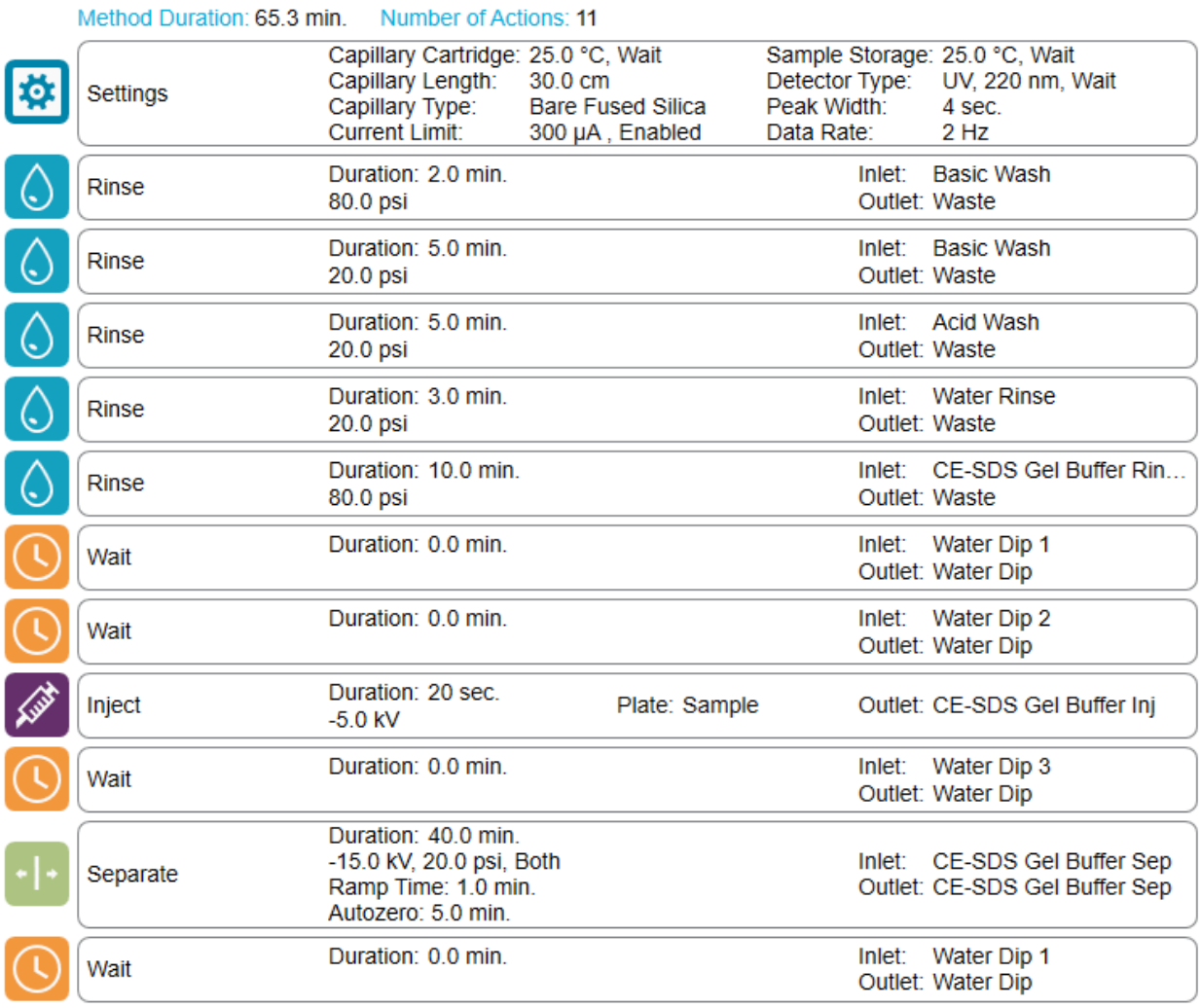


Figure D-9 Summary of Actions in the Non Reduced CE-SDS Separation Method



Separation Method for Reduced Samples

Figure D-10 Actions in the Reduced CE-SDS Separation Method

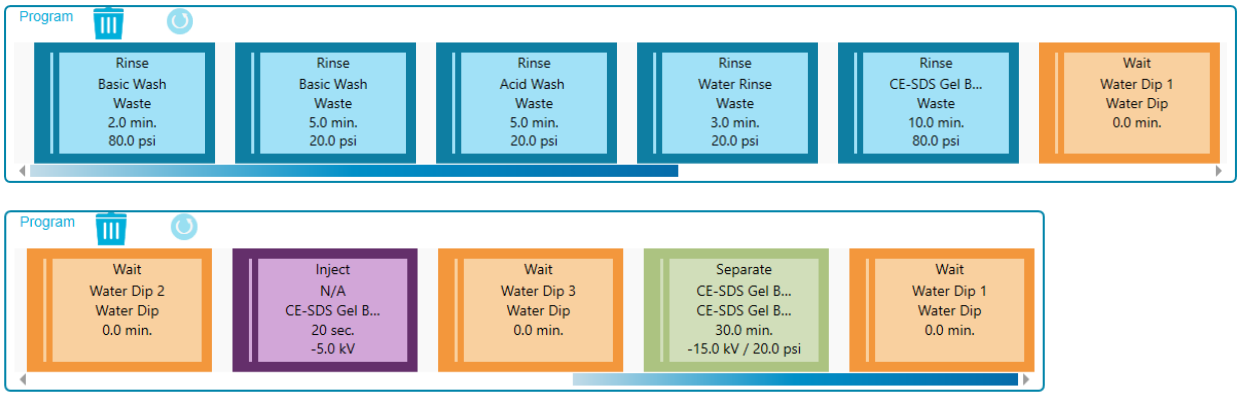
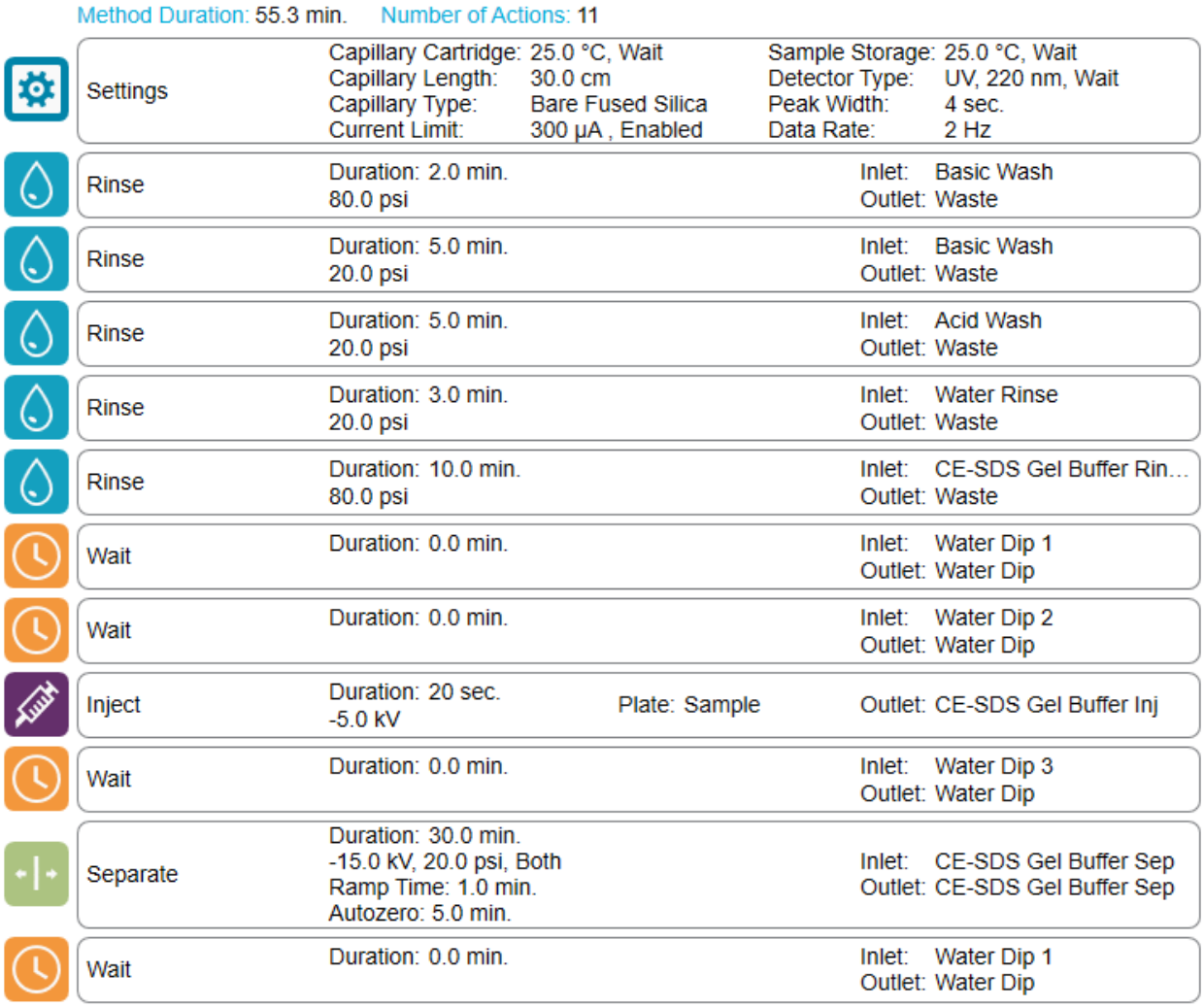


Figure D-11 Summary of Actions in the Reduced CE-SDS Separation Method



Separation Method for Samples Prepared in a Low pH Sample Buffer

Figure D-12 Actions in the Low pH Sample Buffer Separation Method

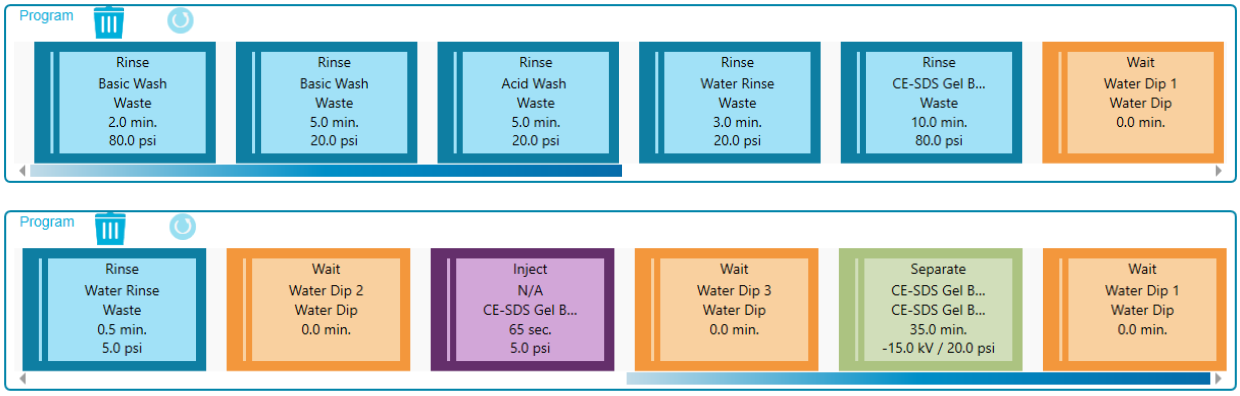


Figure D-13 Summary of Actions in the Low pH Sample Buffer Separation Method

Method Duration: 61.5 min. Number of Actions: 12

Settings	Capillary Cartridge: 25.0 °C, Wait Capillary Length: 30.0 cm Capillary Type: Bare Fused Silica Current Limit: 300 µA , Enabled			Sample Storage: 25.0 °C, Wait Detector Type: UV, 220 nm, Wait Peak Width: 4 sec. Data Rate: 2 Hz	
Rinse	Duration: 2.0 min. 80.0 psi			Inlet: Basic Wash Outlet: Waste	
Rinse	Duration: 5.0 min. 20.0 psi			Inlet: Basic Wash Outlet: Waste	
Rinse	Duration: 5.0 min. 20.0 psi			Inlet: Acid Wash Outlet: Waste	
Rinse	Duration: 3.0 min. 20.0 psi			Inlet: Water Rinse Outlet: Waste	
Rinse	Duration: 10.0 min. 80.0 psi			Inlet: CE-SDS Gel Buffer Rin... Outlet: Waste	
Wait	Duration: 0.0 min.			Inlet: Water Dip 1 Outlet: Water Dip	
Rinse	Duration: 0.5 min. 5.0 psi			Inlet: Water Rinse Outlet: Waste	
Wait	Duration: 0.0 min.			Inlet: Water Dip 2 Outlet: Water Dip	
Inject	Duration: 65 sec. 5.0 psi	Plate: Sample		Outlet: CE-SDS Gel Buffer Inj	
Wait	Duration: 0.0 min.			Inlet: Water Dip 3 Outlet: Water Dip	
Separate	Duration: 35.0 min. -15.0 kV, 20.0 psi, Both Ramp Time: 1.0 min. Autozero: 5.0 min.			Inlet: CE-SDS Gel Buffer Sep Outlet: CE-SDS Gel Buffer Sep	
Wait	Duration: 0.0 min.			Inlet: Water Dip 1 Outlet: Water Dip	

Shutdown Method

Figure D-14 Actions in the CE-SDS Shutdown Method

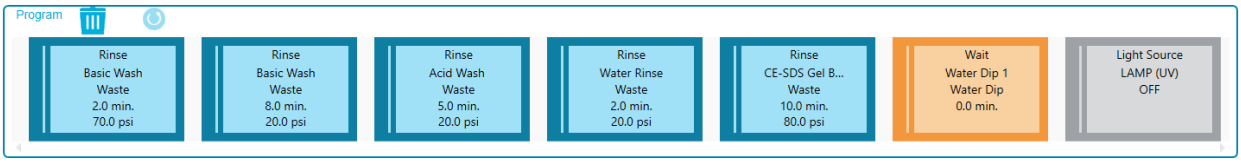
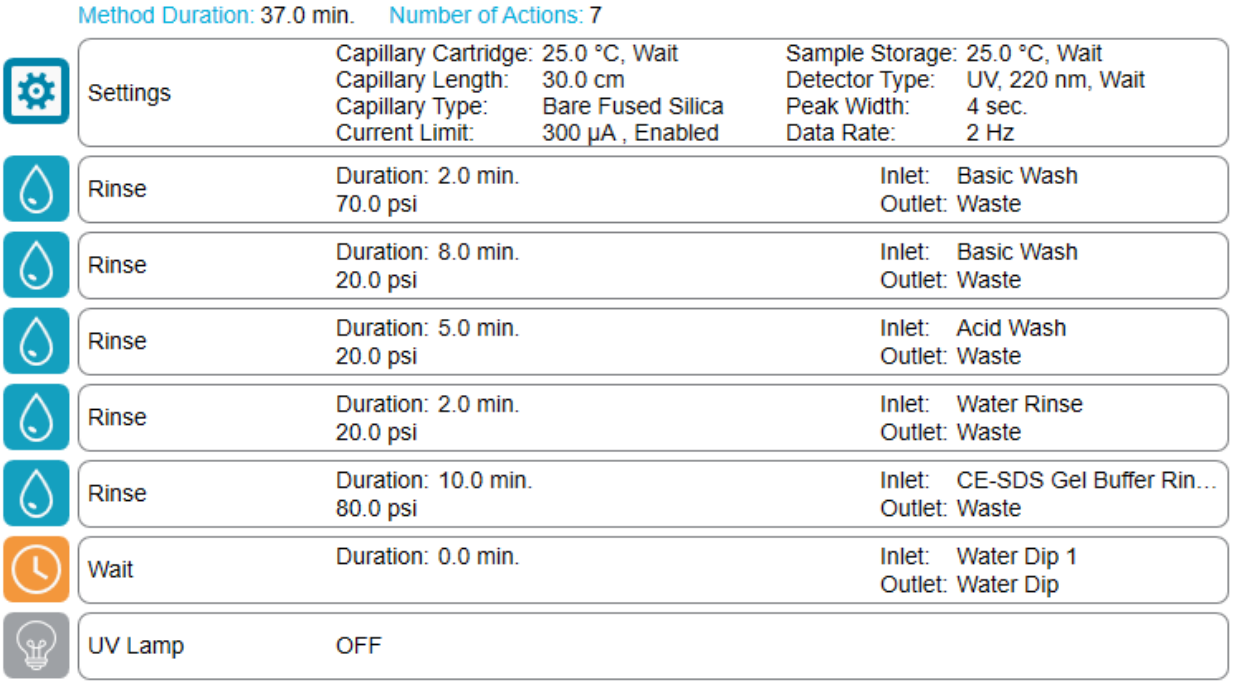


Figure D-15 Summary of Actions in the CE-SDS Shutdown Method



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