

---

# SDS-MW Analysis Kit

## For the PA 800 Plus Pharmaceutical Analysis System

### Application Guide

---

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

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

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

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

(GEN-IDV-09-10816-C)

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

Trademarks and/or registered trademarks mentioned herein, including associated logos, are the property of AB Sciex Pte. Ltd., or their respective owners, in the United States and/or certain other countries.

AB SCIEX™ is being used under license.

© 2020 DH Tech. Dev. Pte. Ltd.



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

# Contents

---

<b>SDS-MW Analysis Kit</b> .....	<b>5</b>
Safety .....	5
Intended Use .....	5
Introduction .....	5
Protein Size Standard .....	6
Internal Standard .....	6
Sample Buffers .....	6
Equipment and Materials Required .....	7
Storage Conditions .....	8
Customer-Supplied Equipment and Supplies .....	8
Required Detector .....	8
Required Cartridge or Capillary .....	8
Methods and Sequences .....	9
Prepare the Samples .....	9
Prepare the SDS-MW Size Standard .....	9
Perform a Buffer Exchange for the Protein Sample .....	10
Determine the Protein Sample Concentration .....	10
Prepare the Reduced Protein Sample .....	11
Prepare the Non-reduced Protein Sample .....	11
Prepare the Alkylating Agent (250 mM IAM Solution) .....	11
Prepare Non-reduced Samples Using an Alkylating Agent .....	12
Prepare the PA 800 Plus System .....	13
Install the PDA Detector .....	13
Clean the Interface Block .....	13
Insert the Cartridge and Calibrate the Detector .....	13
Load the Buffer Trays .....	14
Load the Sample Tray .....	17
Run the Samples .....	19
Create the Sequence and Start the Run .....	19
Waste Disposal .....	23
Store the Cartridge .....	23
Store the Cartridge for Less Than 10 Days .....	23
Store the Cartridge for More Than 10 Days .....	24
Prepare the Cartridge After Storage .....	24
Analyze the Results .....	25
Estimate the Protein Molecular Weights .....	25
Troubleshooting .....	27
<b>A Hazardous Substance Information</b> .....	<b>29</b>
Acid Wash/Regenerating Solution (0.1 M HCl) .....	29
Basic Wash Solution (0.1 M NaOH) .....	29
Low pH SDS Sample Buffer (100 mM Tris-HCl, pH 6.8, 1% SDS) .....	29
Low pH Phosphate SDS Sample Buffer (40 mM Phosphate, pH 6.5, 1% SDS) .....	30

## Contents

---

SDS-MW Sample Buffer (100 mM Tris-HCl, pH 9.0, 1% SDS) . . . . .	30
SDS-MW Gel Buffer, proprietary formulation (pH 8.0, 0.2% SDS) . . . . .	30
Other Reagents . . . . .	30
<b>B Methods . . . . .</b>	<b>31</b>
Initial Conditions . . . . .	31
Detector Initial Conditions . . . . .	32
Time Programs . . . . .	32
<b>C Using the Low pH SDS Sample Buffer . . . . .</b>	<b>35</b>
Add a Pressure Injection to the SDS MW Separation Method. . . . .	35
Results Obtained Using the Low pH SDS Sample Buffer . . . . .	37
<b>D Using the Low pH Phosphate SDS Sample Buffer . . . . .</b>	<b>39</b>
About the Low pH Phosphate SDS Sample Buffer . . . . .	39
Results Obtained Using the Low pH Phosphate SDS Sample Buffer . . . . .	40
Typical Results Using Non-reducing Conditions . . . . .	40
Typical Results Using Reducing Conditions . . . . .	41
<b>E Run the Samples with the Waters Empower™ Software . . . . .</b>	<b>43</b>
Create the Instrument Methods . . . . .	43
Create the Method Sets . . . . .	47
Configure the Software to Use Multiple Plates . . . . .	49
Create the Sample Set Method and Run the Samples . . . . .	52
<b>Revision History . . . . .</b>	<b>57</b>
<b>Contact Us . . . . .</b>	<b>59</b>
Customer Training . . . . .	59
Online Learning Center . . . . .	59
Purchase Consumables . . . . .	59
SCIEX Support . . . . .	59
CyberSecurity . . . . .	60
Documentation . . . . .	60

# SDS-MW Analysis Kit

The SCIEX SDS-MW Analysis Kit provides reagents and supplies for sample preparation and methods to resolve both reduced and non-reduced proteins using the PA 800 Plus Pharmaceutical Analysis System.

This document provides instructions for sample preparation and methods to resolve both reduced and non-reduced proteins by size using the PA 800 Plus Pharmaceutical Analysis System.

---

**Note:** Refer to the *System Overview Guide* for instructions for safe use of the system.

---

## Safety

---

Refer to the Safety Data Sheets (SDS), available at [sciex.com/tech-regulatory](http://sciex.com/tech-regulatory), for information about the proper handling of materials and reagents. Always follow standard laboratory safety guidelines. Refer to [Hazardous Substance Information](#) for hazardous substances information.

## Intended Use

---

The SDS-MW Analysis Kit is for laboratory use only.

## Introduction

---

Capillary electrophoresis (CE) has become an effective replacement for manual slab gel electrophoresis processes due to its automation, quantitation, fast speed, and high efficiency. Many biomolecules, such as proteins, carbohydrates, and nucleic acids are separated by molecular sieving electrophoresis using gel matrices, a technique referred to as capillary gel electrophoresis (CGE). The separation results from the differential migration of the analyte through a gel matrix. In this case, smaller molecules will move faster than large molecules through the separation gel. For polypeptides and proteins, it is necessary to denature the sample in the presence of SDS, an anionic detergent that binds the proteins in a constant ratio of 1:1.4 of protein. The constant mass-to-charge property of the SDS-bound proteins allows separation in a constant ratio of 1 gram of protein : 1.4 grams of SDS.

## SDS-MW Analysis Kit

---

The methodology involves the separation of protein-SDS complexes using a replaceable gel matrix. The gel is formulated to provide an effective sieving range of approximately 10 kDa to 225 kDa. Within this size range, the logarithm of protein molecular mass is linear with its reciprocal electrophoretic mobility. Therefore, the molecular weight of an unknown protein may be estimated from a standard curve of known protein sizes. This kit can also be used to quantify the amount of protein and to determine the purity of a protein product.

---

**Note:** This application guide has been validated with the PA 800 Plus Pharmaceutical Analysis System.

---

### Protein Size Standard

The SDS-MW Size Standard contains 10 kDa, 20 kDa, 35 kDa, 50 kDa, 100 kDa, 150 kDa, and 225 kDa proteins. The SDS-MW Size Standard is used to calibrate the gel to estimate the protein molecular weight of the sample. It also provides confirmation of the resolving power of your experiment.

### Internal Standard

A 10 kDa protein Internal Standard is used as a mobility marker. The mobility of all protein samples are calculated relative to this mobility marker allowing for more accurate size estimation and analyte identification.

### Sample Buffers

- **SDS-MW Sample Buffer:** The SDS-MW Sample Buffer is provided as part of the SDS-MW Analysis Kit. This buffer consists of 100 mM Tris-HCl at pH 9.0 with 1% SDS.
- **Low pH SDS sample buffers:** In some cases, a sample buffer with a lower pH (than that of the SDS-MW Sample Buffer) might improve sample stability by minimizing protein degradation. For these samples, the SCIEX low pH SDS sample buffers are available separately:
  - Low pH SDS Sample Buffer: This buffer consists of 100 mM Tris-HCl at pH 6.8 with 1% SDS.
  - Low pH Phosphate SDS Sample Buffer: This buffer consists of 40 mM Phosphate at pH 6.5 with 1% SDS. This buffer meets the *China Pharmacopeia* specification.

## Equipment and Materials Required

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

**Table 1** Kit Contents (PN 390953)

Component	Quantity	Reorder Part Number
Capillary, 50 $\mu\text{m}$ i.d. bare-fused silica	2	338451
SDS-MW Gel Buffer, proprietary formulation, pH 8.0, 0.2% SDS	140 mL, 4-pack	A30341
SDS-MW Sample Buffer, 100 mM Tris-HCl, pH 9.0, 1% SDS	50 mL	N/A
SDS-MW Size Standard, 10 kDa to 225 kDa, 16 mg/mL	100 $\mu\text{L}$	A22196
Internal Standard, 10 kDa protein, 5 mg/mL	0.4 mL	A26487
Acid Wash/Regenerating Solution, 0.1 M HCl	100 mL	N/A
Basic Wash Solution, 0.1 M NaOH	100 mL	338424

**Table 2** Additional Supplies from SCIEX

Component	Quantity	Part Number
(Optional) Low pH SDS Sample Buffer, 100 mM Tris-HCl, pH 6.8, 1% SDS	140 mL	C44807
(Optional) Low pH Phosphate SDS Sample Buffer, 40 mM Phosphate, pH 6.5, 1% SDS	140 mL	C57805
Micro Vials, 200 $\mu\text{L}$	100	144709
Universal Vial caps, blue	100	A62250
Universal Vials	100	A62251

**Table 3** Additional Required Reagents and Equipment

Component	Quantity	Part Number
2-mercaptoethanol	Sigma-Aldrich	M7154
Iodoacetamide	Sigma-Aldrich	I-1149
(Optional) Amicon Ultra-4 Centrifugal Filter Unit with Ultracel-10 membrane	Millipore	UFC801024

### Storage Conditions

- Upon receipt, store the SDS-MW Size Standard and 10 kDa Internal Standard at 2 °C to 8 °C.
- Store the capillary, SDS-MW Sample Buffer, SDS-MW Gel Buffer, Acid Wash/Regenerating Solution, and Basic Wash Solution at room temperature.
- Store the Low pH SDS Sample Buffer and Low pH Phosphate SDS Sample Buffer at room temperature.

---

**Note:** If the gel buffer or sample buffers are refrigerated, then precipitates might form. If precipitates are present, stir the buffer until the precipitate is fully dissolved before using.

---

### Customer-Supplied Equipment and Supplies

- Powder-free gloves, neoprene or nitrile recommended
- Safety glasses
- Laboratory coat
- Table-top mini centrifuge
- Microcentrifuge, or equivalent, and microcentrifuge tubes
- Water bath or heat block, 37 °C to 100 °C
- Vortex mixer
- Analytical balance
- Pipettes and appropriate tips
- Parafilm
- Spatula
- Double-deionized (DDI) water (MS-grade water filtered through a 0.2 µm filter and with resistance greater than 18 MΩ)

### Required Detector

A photodiode array (PDA) detector is required.

### Required Cartridge or Capillary

One of the following:

- Pre-assembled cartridge (PN A55625)
- Capillary cartridge (PN 144738) and capillaries, bare-fused silica, 50 µm i.d. (PN 338451)
- Cartridge Rebuild Kit (PN 144645)



---

## Methods and Sequences

---

**Note:** The following information applies to users using the PA 800 Plus System with the PA 800 Plus and 32 Karat™ Software. If the system will be used with the Empower™ Software, then the methods are different. Refer to [Run the Samples with the Waters Empower™ Software](#).

---

The methods and data files are installed on the PA 800 Plus controller. They are not available for download. The methods and sequence can also be created manually with the 32 Karat™ Software. Refer to [Methods](#).

The methods are in the PA 800 Plus controller at C:\32Karat\projects\SDS-MW\Method.

- **SDS MW Conditioning - PA 800 plus.met:** Conditions the capillary at the start of each day.
- **SDS MW Separation - PA 800 plus.met:** Performs an SDS-MW separation.
- **SDS MW Shutdown - PA 800 plus.met:** Shuts down and cleans the capillary at the end of a sequence, to rinse the capillary for storage, and to turn off the UV lamp (turns off the laser in the PDA detector).

The sequence file is in the PA 800 Plus controller at C:\32Karat\projects\SDS-MW\Sequence.

- **SDS MW - 24 samples - PA 800 plus.seq**

---

## Prepare the Samples

---

### Prepare the SDS-MW Size Standard

---

- 1 Thaw the SDS-MW Size Standard at room temperature for 15 minutes.
  - 2 Mix the SDS-MW Size Standard thoroughly and using a standard microcentrifuge, spin the tube briefly.
  - 3 Pipette 10 µL of the SDS-MW Size Standard into a 0.5 mL microcentrifuge tube.
  - 4 Add 85 µL of the SDS-MW Sample Buffer to the microcentrifuge tube.
  - 5 Add 2 µL of the 10 kDa Internal Standard to the microcentrifuge tube.
  - 6 Inside a fume hood, add 5 µL of 2-mercaptoethanol to the microcentrifuge tube.
  - 7 Seal the vial cap with Parafilm, mix thoroughly, and heat in a water bath at 100 °C for three minutes.
-

- 8 Using a centrifuge, spin the tube for 1 minute at 300 *g*.
  - 9 Put the tube in a room-temperature water bath to cool for five minutes before injection. The sample will remain stable for approximately 24 hours.
  - 10 Transfer 70  $\mu\text{L}$  to 90  $\mu\text{L}$  of the prepared sample to a micro vial, put the micro vial inside a universal vial, then cap the universal vial.
- 

### Perform a Buffer Exchange for the Protein Sample

---

**Note:** The signal intensity and resolution of this assay are sensitive to the salt concentration in the protein sample. If the sample concentration is too high, then low signal and peak tailing is likely to occur. Exchange the SDS-MW Sample Buffer with an Amicon Ultra-4 centrifugal filter unit using the following procedure.

---

**Note:** For desalting/buffer exchange procedures using a device from another vendor, read the user guide from the vendor before use.

---

- 1 Add 1 mL of protein sample to filter unit.
  - 2 Using a centrifuge, spin the sample for 15 minutes at 4,000 *g*.
  - 3 Add 2 mL of SDS-MW Sample Buffer, then use a centrifuge and spin for 25 minutes at 4,000 *g*.
  - 4 Put the filter unit into a new vial in an inverted position and then use a centrifuge to spin the vial for 3 minutes at 1,000 *g*. The protein solution will collect in the vial.
  - 5 Transfer the collected protein to an appropriate sterile tube. Add SDS-MW Sample Buffer to give a final volume of 1 mL.
- 

### Determine the Protein Sample Concentration

After addition of the SDS-MW Sample Buffer, the total protein concentration should be between 0.2 mg/mL to 2 mg/mL. For best results, the recommended protein concentration is 1 mg/mL. If the protein concentration is too high, it can result in insufficient SDS binding, giving broad peaks and poor resolution. If the protein concentration is too low, a low signal is likely to occur.

---

## Prepare the Reduced Protein Sample

Reduction of the disulfide bonds will provide a more accurate assessment of the molecular weight of a protein, and will allow you to gain additional structural information on a given protein.

- 1 Dilute the sample with the SDS-MW Sample Buffer to a total volume of 95  $\mu\text{L}$  to give a final protein concentration between 0.2 mg/mL to 2 mg/mL.
- 2 Add 2  $\mu\text{L}$  of the 10 kDa Internal Standard to the protein sample tube.
- 3 Inside a fume hood, add 5  $\mu\text{L}$  of 2-mercaptoethanol to the protein sample tube.
- 4 Cap the tube tightly, seal with Parafilm, and mix thoroughly.
- 5 Heat the tube in a water bath at 100  $^{\circ}\text{C}$  for 3 minutes.
- 6 Using a centrifuge, spin the tube for 1 minute at 300  $g$ .
- 7 Put the tube in a room-temperature water bath to cool for 5 minutes before injection.
- 8 Transfer 70  $\mu\text{L}$  to 90  $\mu\text{L}$  of the prepared sample to a micro vial. Put the micro vial inside a universal vial, then cap the universal vial. Make sure there are no bubbles in the micro vial.

---

**Tip!** To remove bubbles from the micro vial, use a pipette to gently aspirate any bubbles.

---

## Prepare the Non-reduced Protein Sample

Comparison of the reduced versus the non-reduced state of a protein can yield important structural information.

Before preparing the non-reduced sample, prepare a 250 mM iodoacetamide (IAM) solution. The IAM solution acts as the alkylating agent during preparation of the sample to minimize any heterogeneity created from partial auto-reduction of the protein.

### Prepare the Alkylating Agent (250 mM IAM Solution)

- 1 Weigh 46 mg of iodoacetamide (IAM).

## SDS-MW Analysis Kit

---

- 2 Transfer the IAM to a 1.5 mL centrifuge tube.
  - 3 Add 1 mL of DDI water to the 1.5 mL centrifuge tube.
  - 4 Cap the tube tightly, mix thoroughly until dissolved, and then store in the dark at room temperature. The solution is stable for approximately 24 hours at room temperature.
- 

### Prepare Non-reduced Samples Using an Alkylating Agent

---

**Note:** Under non-reducing conditions, heating the sample solution at high temperature is required to accelerate SDS binding. However, heating a protein sample at high temperature may introduce fragmentation and aggregation, and introduce artifacts to the sample analysis.

We recommend this alkylation step to minimize temperature-induced artifacts in the protein sample. We also recommend using one of the low pH SDS sample buffers for non-reduced samples. The Low pH SDS Sample Buffer has been proven to further reduce method-induced artifacts.

---

- 1 Dilute the sample with the SDS-MW Sample Buffer to a total volume of 95  $\mu\text{L}$  to give a final protein concentration between 0.2 mg/mL to 2 mg/mL.
  - 2 Add 2  $\mu\text{L}$  of the 10 kDa Internal Standard to the protein sample tube.
  - 3 Inside a fume hood, add 5  $\mu\text{L}$  of the 250 mM IAM solution to the sample protein tube.
  - 4 Cap the tube tightly, seal with Parafilm, and mix thoroughly.
  - 5 Using a centrifuge, spin the tube for 1 minute at 300  $g$ .
  - 6 Heat the vial in a water bath at 70  $^{\circ}\text{C}$  for three minutes.
  - 7 Using a centrifuge, spin the tube for 1 minute at 300  $g$ .
  - 8 Put the tube in a room-temperature water bath to cool for 5 minutes before injection.
  - 9 Transfer 70  $\mu\text{L}$  to 90  $\mu\text{L}$  of the prepared sample to a micro vial. Put the micro vial inside a universal vial, then cap the universal vial. Make sure there are no bubbles in the micro vial.
-

---

**Tip!** To remove bubbles from the micro vial, use a pipette to gently aspirate any bubbles.

---

## Prepare the PA 800 Plus System

---

This section describes the steps to prepare the PA 800 Plus System to acquire data.

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

### Install the PDA Detector

---

- 1 Turn off the PA 800 Plus System and then install the PDA detector. Refer to the *System Maintenance Guide*.
  - 2 Turn on the system and permit the lamp to warm up for at least 30 minutes.
- 

### Clean the Interface Block

---

**CAUTION: Potential System Damage. Do not allow the gel to accumulate on the electrodes, opening levers, capillary ends, and interface block. Gel accumulation might cause broken capillaries, bent electrodes, jammed vials, or missed injections.**

---

Clean the electrodes, opening levers, capillary tips, and interface block weekly or when changing chemistries. Refer to the *System Maintenance Guide* for detailed instructions.

The SDS-MW Gel Buffer is very viscous and can accumulate in the system unless regular and thorough cleaning is performed. Gel accumulation might cause broken capillaries, bent electrodes, vial jams, and missed injections.

### Insert the Cartridge and Calibrate the Detector

---

**Note:** To make sure that the analysis results are consistent over time, we strongly recommend calibrating the detector each time it is installed in the PA 800 Plus System. Also calibrate the detector after replacing the capillary in the cartridge or installing a different cartridge.

---

**Note:** For Empower™ Software users, calibration instructions are located in the *PA 800 Plus Empower™ Driver User Guide*.

---

## SDS-MW Analysis Kit

---

- 1 Remove the cartridge from the box and, if necessary, install the capillary.
  - 2 Install the cartridge in the PA 800 Plus System. Refer to the *System Maintenance Guide* for detailed instructions.
  - 3 Close the front panel.
  - 4 Calibrate the detector.  
Use the Calibration wizard, available from the Instrument Configuration dialog in the 32 Karat™ Software.
- 

## Load the Buffer Trays



**DANGER! Toxic Chemical Hazard. Read the *Safety Data Sheets* for Acid Wash/Regenerating Solution (0.1 M HCl), Basic Wash Solution (0.1 M NaOH), and SDS-MW Gel Buffer before use.**

---

Refer to [Hazardous Substance Information](#) for additional information.

---

**Note:** Before starting the separation, bring the SDS-MW Gel Buffer and the SDS-MW Sample Buffer to room temperature.

---

The number of reagent vials depends on upon the number of method cycles. The methods have been developed to automatically advance the reagent vials after eight cycles, providing a fresh set of buffers every eight cycles.

---

- 1 Fill the gel rinse (Gel-R) vials with 1.2 mL of SDS-MW Gel Buffer.

**Note:** Make sure to fill the buffer vials with SDS-MW Gel Buffer without producing bubbles and use the recommended volume. If the volume is too low (less than half the volume of the vial), the capillary and electrode might not be able to dip into the SDS-MW Gel Buffer during the separation. If the volume is too high, the SDS-MW Gel Buffer may accumulate on the capillary ends and electrodes, resulting in system failure.

---

- 2 Fill the gel separation (Gel-S) vials with 1.1 mL of SDS-MW Gel Buffer.
  - 3 Fill the water (H<sub>2</sub>O) vials with 1.5 mL of DDI water.
  - 4 Fill the NaOH and HCl vials with 1.5 mL of 0.1 M NaOH and 0.1 M HCl solutions, respectively.
-

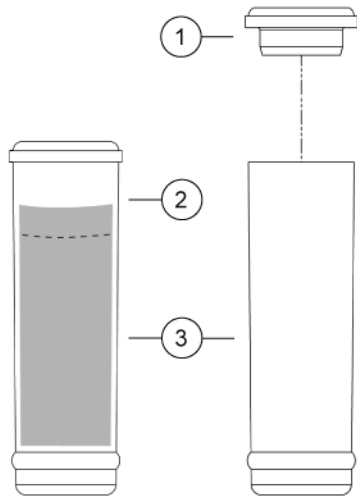
- 5 Fill the waste vials with 1.0 mL of DDI water.

---

**CAUTION: Potential System Damage. Do not fill the waste vial with more than 1.8 mL. If the vial has more than 1.8 mL, the pressure system might be damaged.**

---

Figure 1 Universal Vial and Cap Setup



1. Universal vial cap
2. Maximum fill line
3. Universal vial

- 6 Cap the universal vials with the blue caps.

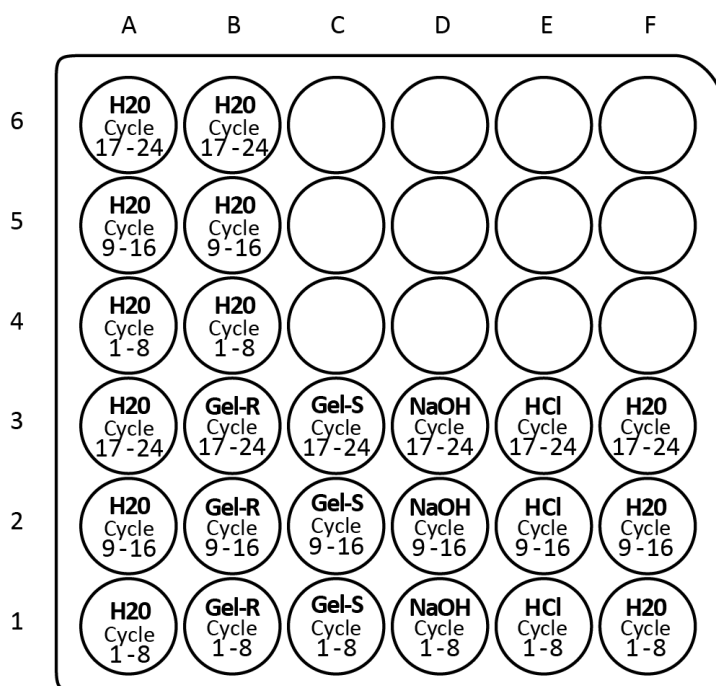
- 7 Load the reagent vials into the buffer trays as shown in [Figure 2](#) and [Figure 3](#).

---

**IMPORTANT** For this application, all vials and caps are designed for a maximum of eight runs. Do not reuse the caps because they might be contaminated with dried gel and other chemicals.

---

Figure 2 Inlet Buffer Tray Layout



A1 to A6: 1.5 mL DDI H<sub>2</sub>O, used in dip step to clean capillary tip

B4 to B6: 1.5 mL, DDI H<sub>2</sub>O, used in dip step to clean capillary tip

B1 to B3: 1.2 mL SDS-MW Gel Buffer, used to rinse/fill capillary prior each cycle (Gel-R)

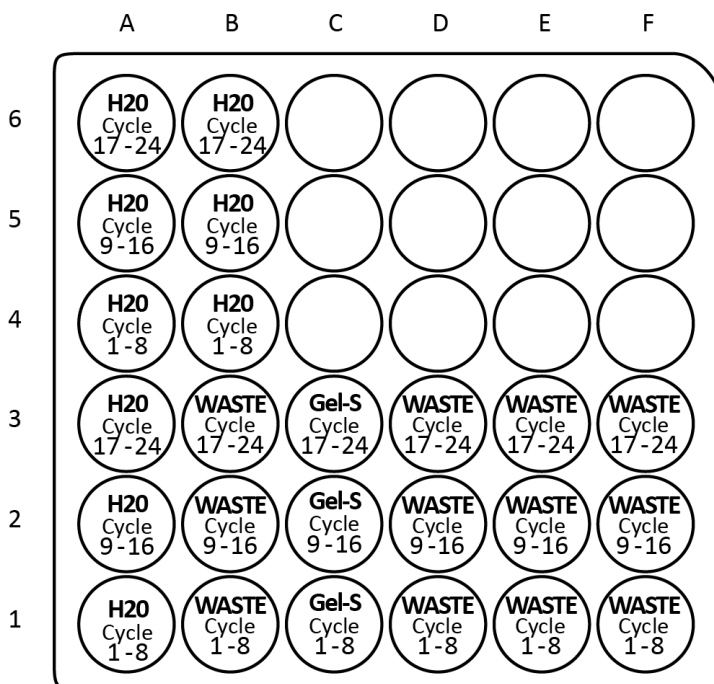
C1 to C3: 1.1 mL SDS-MW Gel Buffer, used for separation (Gel-S)

D1 to D3: 1.5 mL 0.1 M NaOH solution, used to precondition capillary

E1 to E3: 1.5 mL 0.1 M HCl solution, used to precondition capillary

F1 to F3: 1.5 mL DDI H<sub>2</sub>O, used to precondition capillary



**Figure 3** Outlet Buffer Tray Layout

A1 to A6: 1.5 mL DDI H<sub>2</sub>O, used in dip step to clean capillary tip

B4 to B6: 1.5 mL DDI H<sub>2</sub>O, used in dip step to clean capillary tip

B1 to B3: 1.0 mL DDI H<sub>2</sub>O, waste for SDS-MW Gel Buffer rinse

C1 to C3: 1.1 mL SDS-MW Gel Buffer, used for separation

D1 to D3: 1.0 mL DDI H<sub>2</sub>O, waste for 0.1 M NaOH solution rinse

E1 to E3: 1.0 mL DDI H<sub>2</sub>O, waste for 0.1 M HCl solution rinse

F1 to F3: 1.0 mL DDI H<sub>2</sub>O, waste for DDI H<sub>2</sub>O rinse

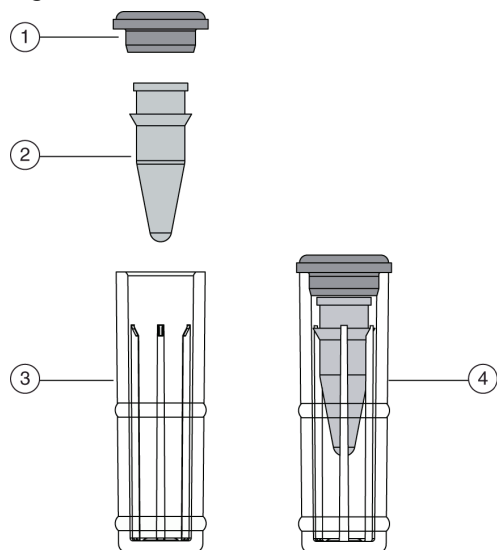
**Note:** During electrophoresis the ionic strength of the buffer will change. The separation method is programmed to increment the buffer vials after eight runs to avoid ionic depletion.

## Load the Sample Tray

- 1 Prepare the samples. For each sample:
  - a. Make sure the prepared samples are at room temperature.
  - b. Put 70 µL to 90 µL of the sample in a micro vial.
  - c. Make sure that no bubbles are present at the bottom of the vials. If bubbles are present, then use a centrifuge to spin the micro vials for 2 minutes at 1,000 *g*. Repeat if necessary.

2 Put the micro vial in a universal vial, and then cap the universal vial.

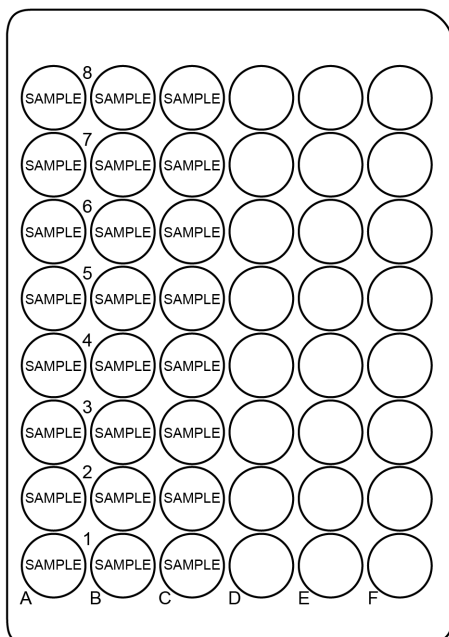
**Figure 4** Sample Vial Setup



1. Universal vial cap	3. Universal vial
2. Micro vial	4. Micro vial inside universal vial

3 Put the universal vials in the 48-position inlet sample tray from positions A1 through C8.


**Figure 5** Sample Tray Layout



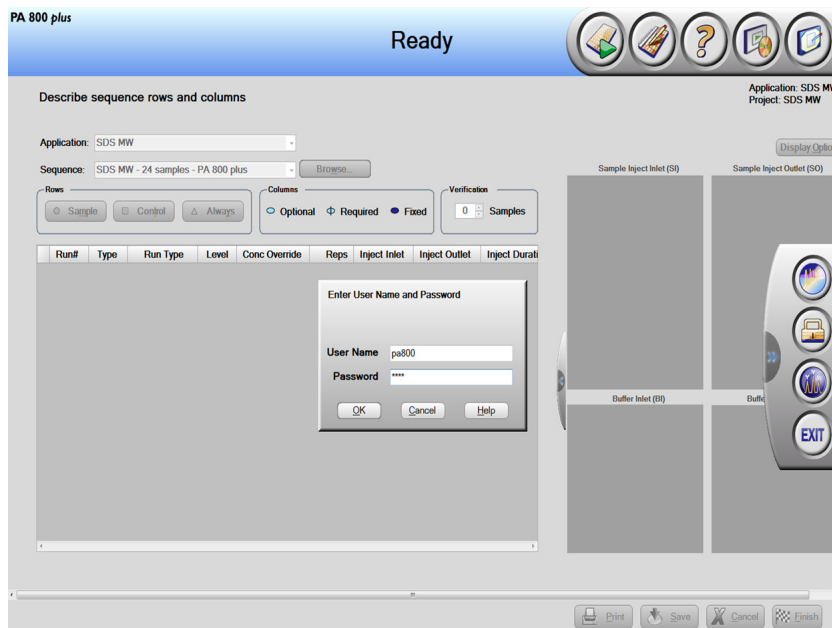
## Run the Samples

### Create the Sequence and Start the Run

**Note:** For Empower™ Software users, refer to [Run the Samples with the Waters Empower™ Software](#).

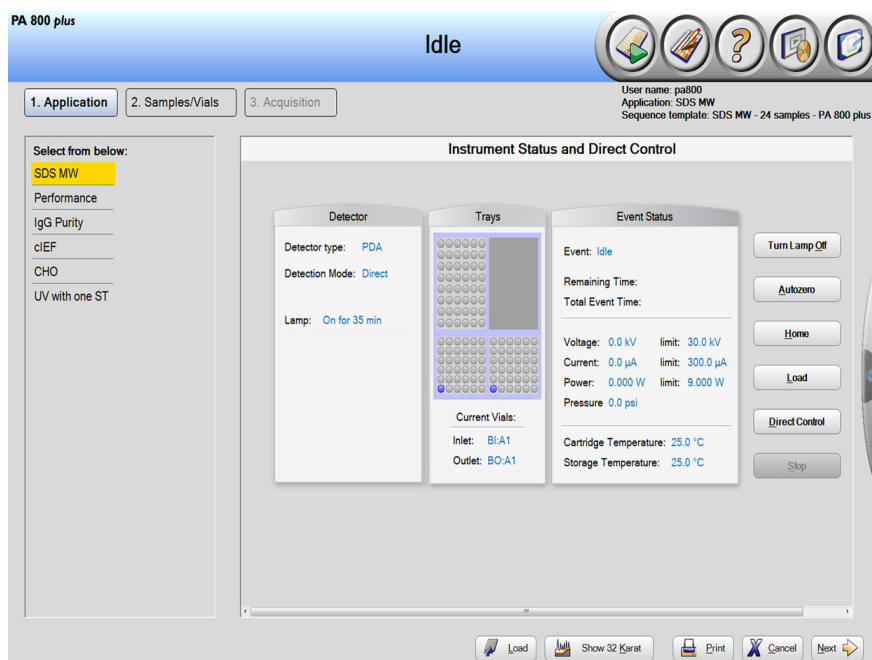
- 1 Double-click the PA 800 Plus Software icon on the desktop.
- 2 In the PA 800 Plus window, click  (Run) in the upper right corner of the window.
- 3 In the **Application** list, click **SDS MW**. In the **Sequence** list, click **Browse** and select **SDS MW - 24 samples - PA 800 plus**.  
If system administration is enabled, type the user name and password when prompted, and then click **OK**. Refer to [Figure 6](#). The default user name is **pa800**, and the default password is **plus**.


**Figure 6** User Name and Password Entry



The **Instrument Status and Direct Control** window opens. Refer to [Figure 7](#).

Figure 7 Instrument Status and Direct Control Window: Idle



4 In the **Instrument Status and Direct Control** window, click  (**Next**) in the bottom right corner of the window.  
The sequence opens.

5 Select **SDS MW - 24 samples - PA 800 plus** to open the sequence. This sequence will run a maximum of 24 samples where sample 1 is (always) the control standard.

6 Click  (**Describe**) in the upper right hand corner of the window to edit the sequence.

**Note:** The Describe function can be accessed from the PA 800 Plus window, the application window, or the Samples/Vials window by clicking the Describe icon.

Use the Describe function to customize the sequence table and edit the number of samples that can be run in the sequence. The Describe function can set the row types as controls for system suitability standards and blanks, samples for unknowns, and when preparing for capillary conditioning and shutdown runs.

7 In the **Application** list, click **SDS MW**. In the **Sequence** list, click **Browse**, and then select **SDS MW - 24 samples - PA 800 plus**. If prompted, type a user name and password.  
The page updates to show the selected sequence, and all rows in the sequence are designated as samples.

8 (Optional) Edit the **Sample ID** and the **Data File Name** as desired.

Editable fields such as **Sample ID** and **Data File Name** can be set as Mandatory, Optional, or Fixed.

- After the sequence is loaded, set rows to **Sample**, **Control**, or **Always**. Click a row to select it and then click the button in the **Rows** area.

**Capillary Conditioning** and **Shutdown** runs are set as **Always**. Refer to In [Figure 8](#). **Sample ID** is set as **Optional**. Reps are set as **Required**.

**Figure 8** Describe sequence rows and columns Window – Conditioning Method Set to “Always”

**Describe sequence rows and columns**

Application: SDS MW

Sequence: SDS MW - 24 samples - PA 800 plus Browse...

Rows:  Sample  Control  Always

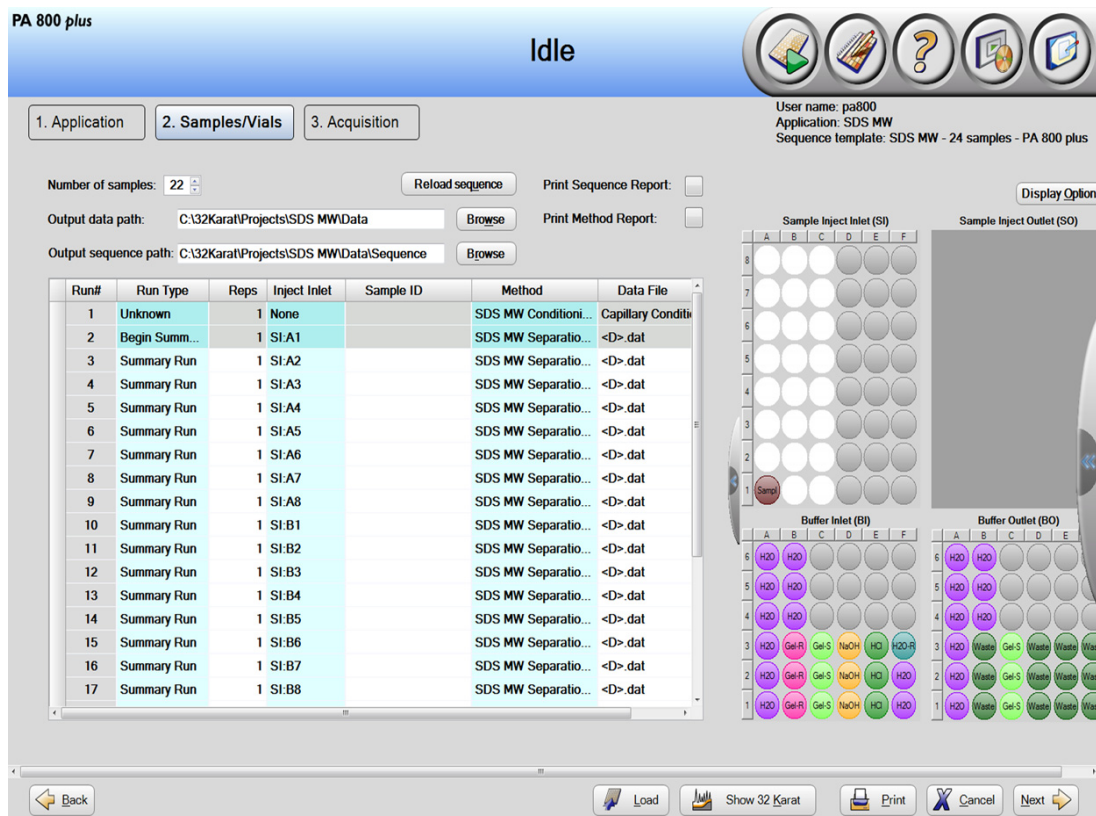
Columns:  Optional  Required  Fixed

Verification: 22 Samples


Run#	Type	Run Type	Reps	Inject Inlet	Sample ID	Method	Data
1	Always	Unknown	1	None		SDS MW Condition...	Capillar
2	Control	Begin Summ...	1	SI:A1		SDS MW Separati...	<D>.da
3	Sample	Summary Run	1	SI:A2		SDS MW Separati...	<D>.da
4	Sample	Summary Run	1	SI:A3		SDS MW Separati...	<D>.da
5	Sample	Summary Run	1	SI:A4		SDS MW Separati...	<D>.da
6	Sample	Summary Run	1	SI:A5		SDS MW Separati...	<D>.da
7	Sample	Summary Run	1	SI:A6		SDS MW Separati...	<D>.da
8	Sample	Summary Run	1	SI:A7		SDS MW Separati...	<D>.da
9	Sample	Summary Run	1	SI:A8		SDS MW Separati...	<D>.da
10	Sample	Summary Run	1	SI:B1		SDS MW Separati...	<D>.da
11	Sample	Summary Run	1	SI:B2		SDS MW Separati...	<D>.da
12	Sample	Summary Run	1	SI:B3		SDS MW Separati...	<D>.da
13	Sample	Summary Run	1	SI:B4		SDS MW Separati...	<D>.da
14	Sample	Summary Run	1	SI:B5		SDS MW Separati...	<D>.da
15	Sample	Summary Run	1	SI:B6		SDS MW Separati...	<D>.da
16	Sample	Summary Run	1	SI:B7		SDS MW Separati...	<D>.da
17	Sample	Summary Run	1	SI:B8		SDS MW Separati...	<D>.da
18	Sample	Summary Run	1	SI:C1		SDS MW Separati...	<D>.da
19	Sample	Summary Run	1	SI:C2		SDS MW Separati...	<D>.da
20	Sample	Summary Run	1	SI:C3		SDS MW Separati...	<D>.da
21	Sample	Summary Run	1	SI:C4		SDS MW Separati...	<D>.da

- 10 In the lower right corner of the window, click  (Save) and then click  (Finish). The Run Sequence window opens. Refer to [Figure 9](#).

**Figure 9** Describe sequence rows and columns Window – Reload Sequence



**Note:** The Describe function can be accessed from the PA 800 Plus window, the application window, or the Samples/Vials window by clicking the Describe icon.

**Note:** In the upper left corner next to Run #1 of the table in [Figure 9](#), a blinking red exclamation mark (not shown) indicates that the sequence has changed and that the software expects an action from the user. Move the cursor over the exclamation point to view a tooltip with the required action. In this example, the user is prompted to click  (**Reload sequence**) to update the sequence.

A required action is also used if in the data file name is a required field and the data file does not contain any information. In this case, the user is required to enter the appropriate data file name.

The number of samples shown for this sequence is 22 instead of 23 because the first run is the control. If required, the user can reduce the number the samples to be run in the sequence in the **Run Sequence** window by editing the **Number of samples** list.

- 11 Click **Load** to load the sample and reagent vials as shown in [Figure 9](#) and then close the door.


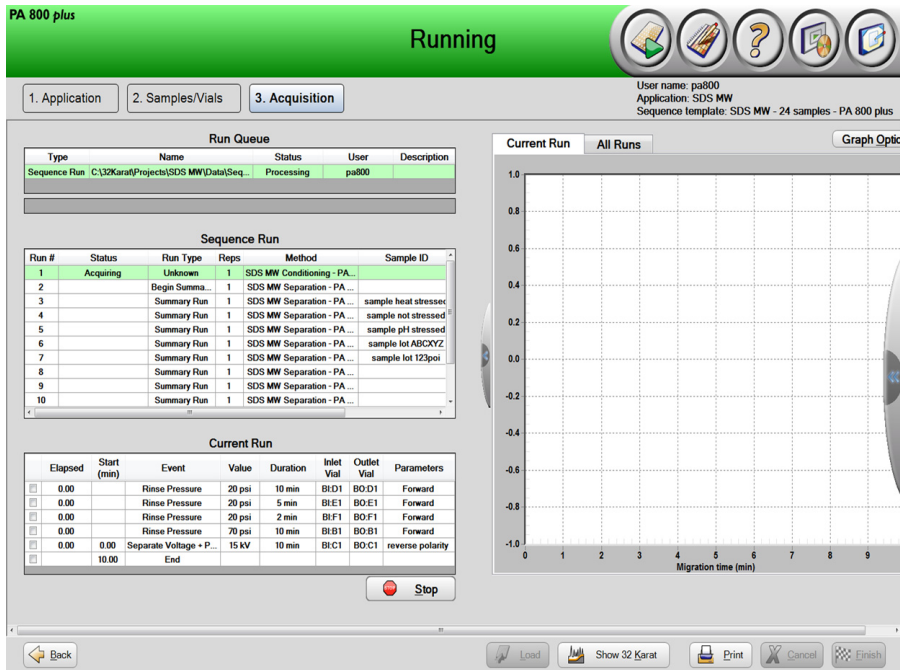
12 Click  (Next) and then click **Yes - run now**.

Figure 10 PA 800 Plus System During Data Acquisition



## Waste Disposal



**WARNING! Biohazard or Toxic Chemical Hazard.** Follow local directives when disposing of chemicals, vials and caps, and the remains of the prepared samples, if applicable. They might contain regulated compounds and biohazardous agents.

## Store the Cartridge

### Store the Cartridge for Less Than 10 Days

- 1 Perform a shutdown method to clean the capillary.  
The shutdown method fills the capillaries with water.

- 2 Store the cartridge for up to 10 days in the system with the capillary ends immersed in vials of DDI water.
- 

### Store the Cartridge for More Than 10 Days

---

- 1 Perform a shutdown method to clean the capillary.
  - 2 Rinse the capillary with DDI water for 10 minutes at 100 psi.
  - 3 Remove the cartridge from the system.
  - 4 Store the cartridge upright in the cartridge box at room temperature, with the capillary ends immersed in vials of DDI water.
- 

### Prepare the Cartridge After Storage

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

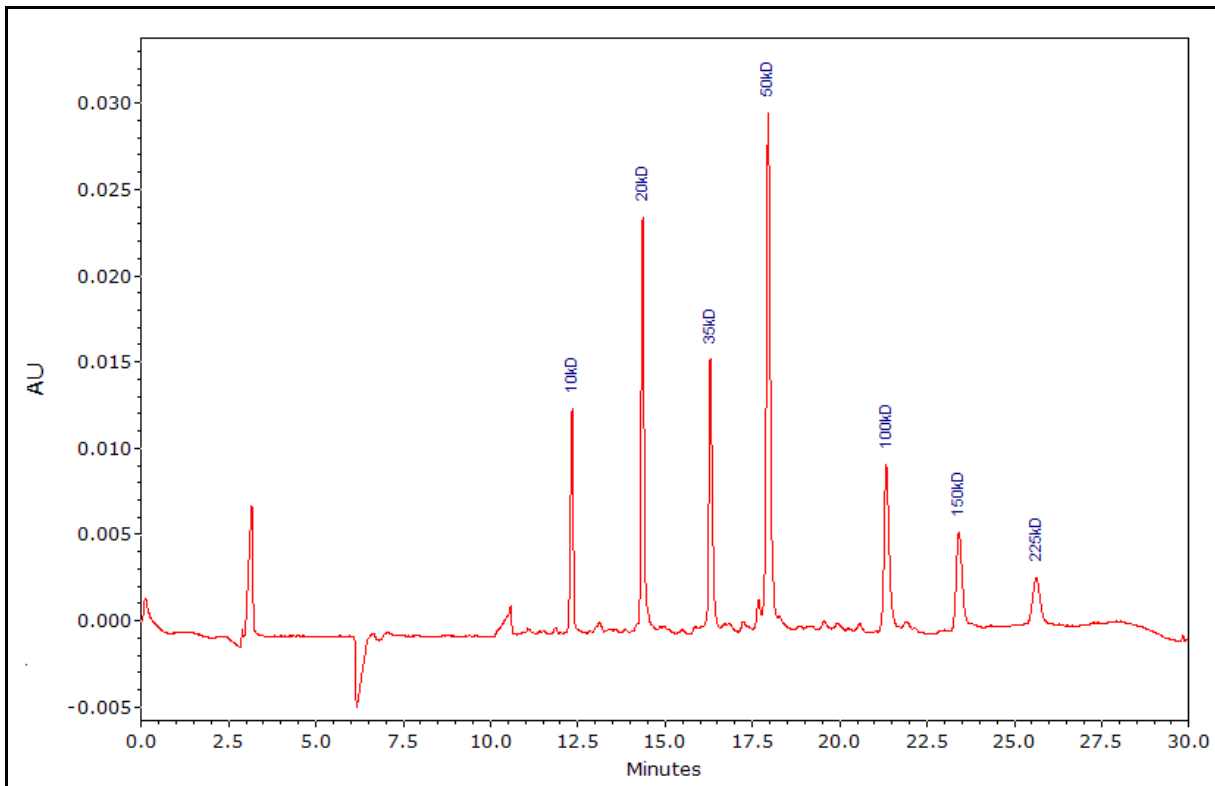


## Analyze the Results

The SDS-MW Size Standard contains seven proteins (10 kDa, 20 kDa, 35 kDa, 50 kDa, 100 kDa, 150 kDa, and 225 kDa). All proteins should be completely separated within 30 minutes using our recommended method. Refer to [Figure 11](#) for a typical separation of the SDS-MW Size Standard.

**Note:** The figure below is based on ideal results. Peak heights may vary depending on sample preparation and UV lamp life.

**Figure 11** Separation of SDS-MW Size Standard using SDS-MW Sample Buffer

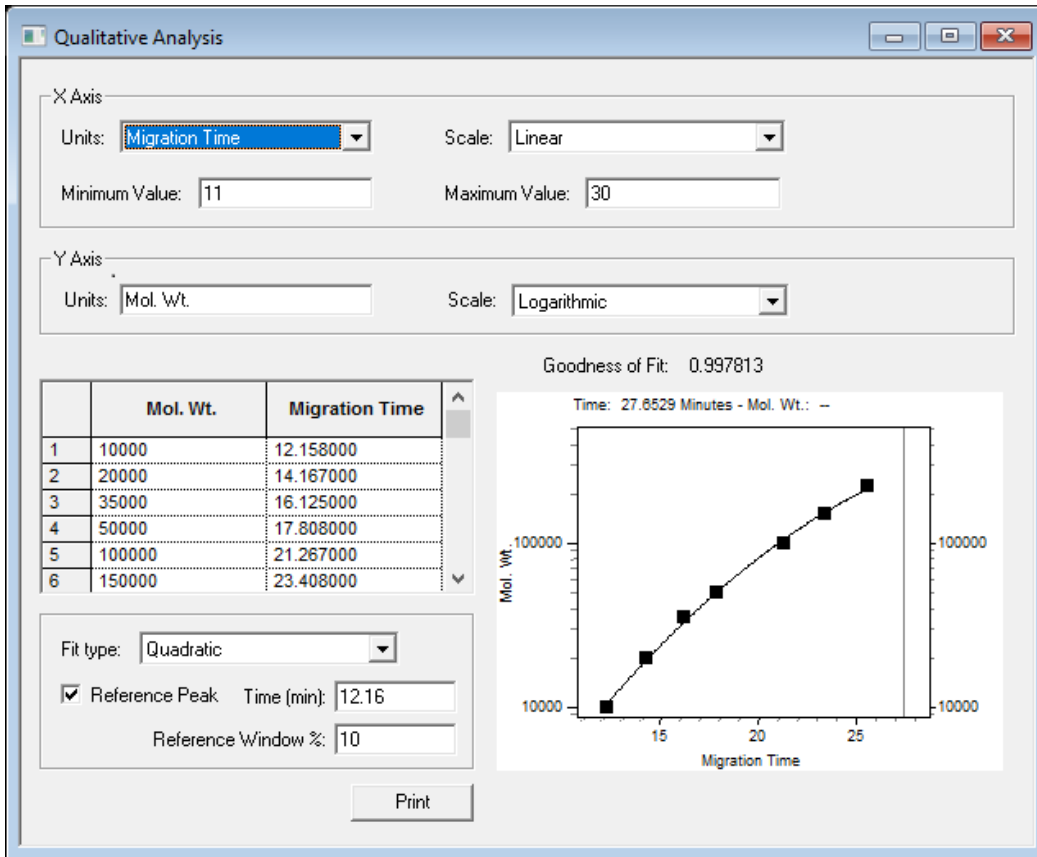


## Estimate the Protein Molecular Weights

Refer to [Figure 12](#) for a typical calibration curve obtained by plotting the known molecular weight vs. migration time of each protein in the SDS-MW Size Standard. The molecular weight of an unknown protein can be estimated by using this calibration curve. The calculated molecular weights are displayed in the electropherogram by selecting **Quality** as an annotation.

Re-calibrate this curve every 24 cycles. This is done by running the SDS-MW Size Standard and updating the migration time values for each standard to reflect the new run. This update is performed in the qualitative analysis of the 32 Karat™ Software. Refer to [Figure 12](#).

Figure 12 Qualitative Analysis Dialog



## Troubleshooting

**Table 4** Troubleshooting

Problem	Possible Cause	Corrective Action
Low or unsteady current resulting in slow migration and poor resolution resulting in failed system suitability test; current should be close to $-25 \mu\text{A}$	Capillary plugged	1) Rinse the capillary with DDI water at 100 psi for 10 minutes and then perform the capillary conditioning method. 2) If low or unsteady current continues, replace the capillary.
	Air bubbles in the gel	Degas SDS-MW Gel Buffer under 5 Hg to 15 Hg vacuum for 5 minutes.
High current	Contaminated gel buffer	Replace the SDS-MW Gel Buffer as needed.
	Contamination of the electrode	Clean the electrodes. Refer to the <i>System Maintenance Guide</i> .
Spikes in electropherogram	Air bubbles in gel buffer	Degas SDS-MW Gel Buffer under 5 Hg to 15 Hg vacuum for 5 minutes.
Broad peaks, poor resolution	Poor capillary end cut	Inspect the capillary end under magnification. If the cut is jagged, then cut the end again or replace the capillary.
	Improper reduction of sample	Reduce the sample using recommended procedure. Use fresh 2-mercaptoethanol for sample reduction. Refer to <a href="#">Prepare the Samples</a> .
	Deteriorated capillary	Replace the capillary when other attempts to reduce peak broadening fail. 1) Rinse the capillary with DDI water for 10 minutes at 100 psi and then perform the capillary conditioning method. 2) Install a new capillary if the same problem is observed.
	Dust or gel build up on capillary end	Clean the capillary tip using DDI water. Refer to <a href="#">Clean the Interface Block</a> .

Table 4 Troubleshooting (Continued)

Problem	Possible Cause	Corrective Action
No peaks or low signal	Capillary inlet longer than the inlet electrode	Push the capillary up inside the cartridge or cut the capillary inlet to make sure it is the same length as the electrode.
	Dirty or plugged capillary tip	1) Clean the capillary tip using DDI water. Refer to <a href="#">Clean the Interface Block</a> . 2) Replace the capillary if the plug cannot be removed.
	Not enough sample	Make sure there is a minimum of 20 µL of sample in the sample vial.
	Slow sample migration	Increase the separation time in the method and repeat the analysis.
	High salt in protein sample	Perform a buffer exchange to remove salt from the sample. Refer to <a href="#">Perform a Buffer Exchange for the Protein Sample</a> .

# Hazardous Substance Information

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

Hazard classification is according to HCS 2012.

## Acid Wash/Regenerating Solution (0.1 M HCl)

---



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

---

## Basic Wash Solution (0.1 M NaOH)

---



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

---

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

---

**WARNING!** Causes mild skin irritation.

---

## Hazardous Substance Information

---

### Low pH Phosphate SDS Sample Buffer (40 mM Phosphate, pH 6.5, 1% SDS)

---

**WARNING!** Causes mild skin irritation.

---

### SDS-MW Sample Buffer (100 mM Tris-HCl, pH 9.0, 1% SDS)

---

**WARNING!** Causes mild skin irritation.

---

### SDS-MW Gel Buffer, proprietary formulation (pH 8.0, 0.2% SDS)

---



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

---

## Other Reagents

---

These components are not classified as hazardous:

- SDS-MW Size Standard
- Internal Standard, 10 kDa protein, 5 mg/mL

For reagents from other vendors, read the Safety Data Sheet from the vendor before use.

The SDS-MW Analysis Kit application requires three methods. The methods are installed with the software and are not available for download. The following figures are provided for reference.

**Note:** The values on the Initial Conditions and PDA Detector Initial Conditions tabs are the same for all of the methods.

## Initial Conditions

**Note:** The values on the Initial Conditions and PDA Detector Initial Conditions tabs are the same for all of the methods.

**Figure B.1** Initial Conditions for All Methods

The screenshot shows the 'Initial Conditions' configuration window. It features three tabs: 'Initial Conditions', 'PDA Detector Initial Conditions', and 'Time Program'. The 'Initial Conditions' tab is selected. The window is organized into several sections:

- Auxiliary data channels:** Includes checkboxes for Voltage (unchecked), Current (checked), Power (unchecked), and Pressure (unchecked). The 'Current' section shows a maximum value of 300.0  $\mu$ A.
- Temperature:** Includes input fields for Cartridge (25.0  $^{\circ}$ C) and Sample storage (25.0  $^{\circ}$ C).
- Peak detect parameters:** Includes a Threshold of 2 and a Peak width of 9.
- Trigger settings:** Includes checkboxes for 'Wait for external trigger' (unchecked), 'Wait until cartridge coolant temperature is reached' (checked), and 'Wait until sample storage temperature is reached' (checked).
- Inlet trays:** Includes dropdown menus for Buffer (36 vials) and Sample (48 vials).
- Outlet trays:** Includes dropdown menus for Buffer (36 vials) and Sample (No tray).
- Mobility channels:** Includes checkboxes for Mobility (unchecked), Apparent Mobility (unchecked), and Plot trace after voltage ramp (unchecked).
- Analog output scaling:** Includes a Factor of 1.

## Detector Initial Conditions

Figure B.2 PDA Detector Initial Conditions for All Methods

Initial Conditions | PDA Detector Initial Conditions | Time Program

Electropherogram scan data

Acquisition enabled

Data rate: 2 Hz

Scan range from 190 to 400 nm

Electropherogram channel data

Data Rate: 2 Hz

	Acquisition enabled	Reference channel	Wavelength (nm)	Bandwidth (nm)
Channel 1:	<input checked="" type="checkbox"/>	<input type="checkbox"/>	220	10
Channel 2:	<input type="checkbox"/>	<input type="checkbox"/>	254	10
Channel 3:	<input type="checkbox"/>	<input type="checkbox"/>	280	10

Peak detect:   250 120

Filter

High sensitivity

Normal

High resolution

Peak width (points): 16-25

Relay 1:  Off  On

Relay 2:  Off  On

Reference channel

Wavelength: 350 nm

Bandwidth: 10 nm

Absorbance signal

Direct  Indirect

## Time Programs

The time programs are different for each method.

Figure B.3 Time Program for SDS MW Conditioning Method

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	20.0 psi	10.00 min	BI:D1	BO:D1	forward	0.1 N NaOH rinse to clean capillary surface
2		Rinse - Pressure	20.0 psi	5.00 min	BI:E1	BO:E1	forward	0.1 N HCl rinse to neutralize capillary surface silanol group
3		Rinse - Pressure	20.0 psi	2.00 min	BI:F1	BO:F1	forward	ddH2O rinse to remove the acid residue
4		Rinse - Pressure	70.0 psi	10.00 min	BI:B1	BO:B1	forward	SDS Gel rinse to fill the capillary
5	0.00	Separate - Voltage	15.0 KV	10.00 min	BI:C1	BO:C1	5.00 Min ramp, reverse polarity, both	SDS Gel for voltage equilibration
6								

Apply



Figure B.4 Time Program for SDS MW Separation Method

Initial Conditions   PDA Detector Initial Conditions   Time Program									
Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments		
1	Rinse - Pressure	70.0 psi	3.00 min	BI:D1	BO:D1	forward, In / Out vial inc 8	0.1 N NaOH rinse to clean capillary surface - Automatic increment every 8 runs		
2	Rinse - Pressure	70.0 psi	1.00 min	BI:E1	BO:E1	forward, In / Out vial inc 8	0.1 N HCl rinse to neutralize capillary surface silanol group - Automatic increment every 8 runs		
3	Rinse - Pressure	70.0 psi	1.00 min	BI:F1	BO:F1	forward, In / Out vial inc 8	Water rinse to remove the acid residue - Automatic increment every 8 runs		
4	Rinse - Pressure	70.0 psi	10.00 min	BI:B1	BO:B1	forward, In / Out vial inc 8	SDS Gel rinse to fill the capillary with SDS gel - Automatic increment every 8 runs		
5	Wait		0.00 min	BI:A1	BO:A1	In / Out vial inc 8	ddH2O, use for dipping to clean capillary tip - Automatic increment every 8 runs		
6	Wait		0.00 min	BI:A4	BO:A4	In / Out vial inc 8	ddH2O, use for dipping to clean capillary tip - Automatic increment every 8 runs		
7	Inject - Voltage	5.0 KV	20.0 sec	SI:A1	BO:C1	Override, reverse polarity	Sample injection		
8	Wait		0.00 min	BI:B4	BO:B4	In / Out vial inc 8	ddH2O, use for dipping to avoid sample carry over - Automatic increment every 8 runs		
9	Separate - Voltage	15.0 KV	30.00 min	BI:C1	BO:C1	1.00 Min ramp, reverse polarity, both, In / Out vial inc 8	SDS Gel for separation - Automatic increment every 8 runs		
10	5.00 Autozero								
11									

Apply

Figure B.5 Time Program for SDS MW Shutdown Method

Initial Conditions   PDA Detector Initial Conditions   Time Program									
Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments		
1	Rinse - Pressure	70.0 psi	10.00 min	BI:D1	BO:D1	forward	0.1 N NaOH rinse to clean capillary surface		
2	Rinse - Pressure	50.0 psi	5.00 min	BI:E1	BO:E1	forward	0.1 N HCl rinse to neutralize capillary surface silanol group		
3	Rinse - Pressure	50.0 psi	2.00 min	BI:F1	BO:F1	forward	Water rinse to remove the acid residue		
4	Rinse - Pressure	70.0 psi	10.00 min	BI:B1	BO:B1	forward	SDS Gel rinse to fill the capillary with SDS gel		
5	0.00 Separate - Voltage	15.0 KV	10.00 min	BI:C1	BO:C1	5.00 Min ramp, reverse polarity, both	SDS Gel for separation		
6	10.00 Wait		0.00 min	BI:A1	BO:A1		ddH2O use for capillary dip to prevent capillary from drying		
7	10.00 Lamp - Off								
8									

Apply



# Using the Low pH SDS Sample Buffer

---

**Note:** SCIEX carries two different low pH sample buffers, the Low pH SDS Sample Buffer (Tris; pH 6.8) and the Low pH Phosphate SDS Sample Buffer (pH 6.5).

---

Some samples may be more stable in a sample buffer with a lower pH. To use the Low pH SDS Sample Buffer (pH 6.8), prepare the samples as described previously, but replace the SDS-MW Sample Buffer with the Low pH SDS Sample Buffer (pH 6.8).

Due to the increased ionic strength of low pH sample buffers, we recommend modifying the SDS-MW separation method by increasing the injection voltage or duration to avoid any signal loss. Adjust the separation time based on the samples to be analyzed. For example, for analysis of a Rituxan (rituximab) sample, change the separation time to 35 minutes.

Alternatively, use a pressure injection in the separation method. Start with the same SDS-MW separation method as for samples prepared with the SDS-MW Sample Buffer and edit the method as described in the following section.

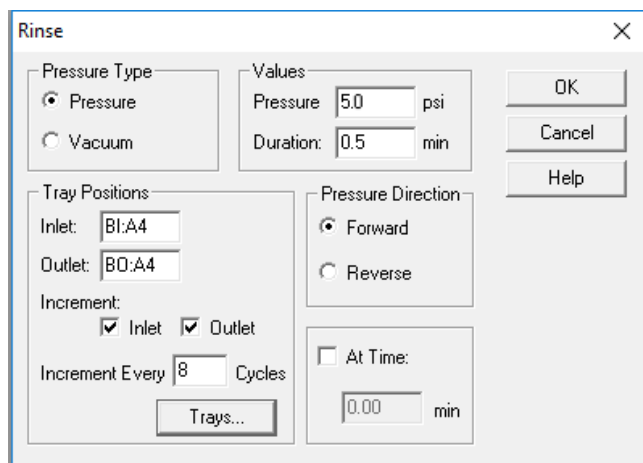
## Add a Pressure Injection to the SDS MW Separation Method

---

Use the following instructions to add a pressure injection and make other necessary changes to the separation method.

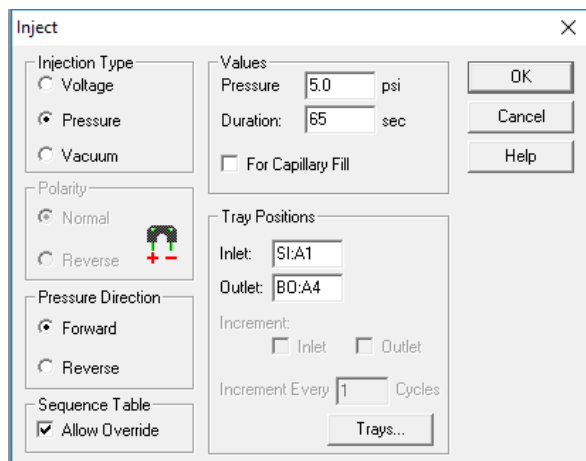
- 1 Open the SDS MW separation method in the 32 Karat™ Software.  
No changes to the Initial Conditions or the PDA Detector Initial Conditions are required.
- 2 Click the **Time Program** tab.
- 3 Add a **Rinse** event after row 5. Set the parameters as shown.

Figure C.1 Rinse Dialog



- 4 Edit the **Inject-Voltage** event to match the following figure.

Figure C.2 Inject Dialog



- 5 Adjust the duration of the **Separation-Voltage** event based on samples to be analyzed. The time program should match the following figure.

Figure C.3 Separation Method Time Program after Editing (showing Rinse and Injection Pressure Events)

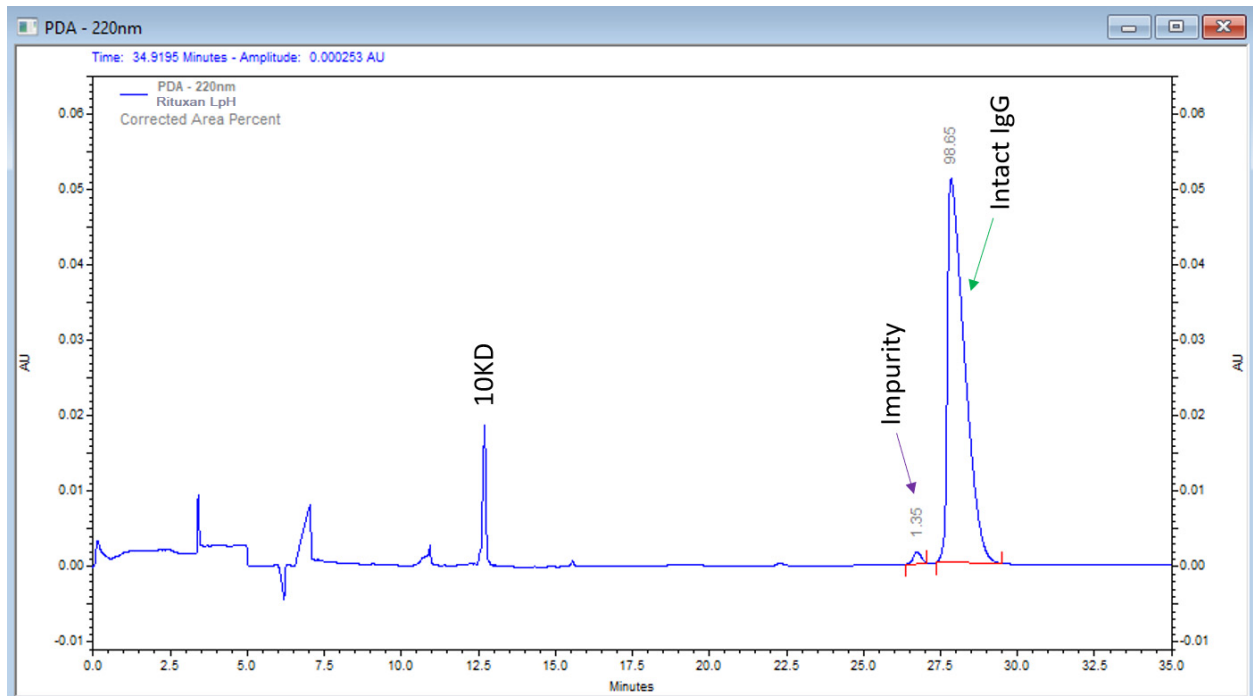
Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1	Rinse - Pressure	70.0 psi	3.00 min	BI:D1	BO:D1	forward, In / Out vial inc 8	0.1 M NaOH rinse to clean capillary surface - Automatic increment every 8 runs
2	Rinse - Pressure	70.0 psi	1.00 min	BI:E1	BO:E1	forward, In / Out vial inc 8	0.1 M HCl rinse to neutralize capillary surface - Automatic increment every 8 runs
3	Rinse - Pressure	70.0 psi	1.00 min	BI:F1	BO:F1	forward, In / Out vial inc 8	Water rinse to remove the residual acid - Automatic increment every 8 runs
4	Rinse - Pressure	70.0 psi	10.00 min	BI:B1	BO:B1	forward, In / Out vial inc 8	SDS Gel rinse to fill the capillary with SDS gel - Automatic increment every 8 runs
5	Wait	0.00 min	0.00 min	BI:A1	BO:A1	In / Out vial inc 8	Water dip to clean capillary tip - Automatic increment every 8 runs
6	Rinse - Pressure	5.0 psi	0.50 min	BI:A4	BO:A4	forward, In / Out vial inc 8	Introduce a water plug
7	Wait	0.00 min	0.00 min	BI:A4	BO:A4	In / Out vial inc 8	Water dip to clean capillary tip - Automatic increment every 8 runs
8	Inject - Pressure	5.0 psi	65.0 sec	SI:A1	BO:A4	Override, forward	Sample injection
9	Wait	0.00 min	0.00 min	BI:B4	BO:B4	In / Out vial inc 8	Water dip to prevent sample carry over - Automatic increment every 8 runs
10	Separate - Voltag	15.0 KV	35.00 min	BI:C1	BO:C1	1.00 Min ramp, reverse polarity, both, In / Out vial inc 8	SDS Gel for separation - Automatic increment every 8 runs
11	5.00	Autozero					
12							

6 Save the method. If the method name is not changed, then no changes to the sequence are required.

## Results Obtained Using the Low pH SDS Sample Buffer

The following figure shows the results obtained with Rituxan (rituximab) and the Low pH SDS Sample Buffer. Refer to Figure C.4. Sample impurity was 1.35%.

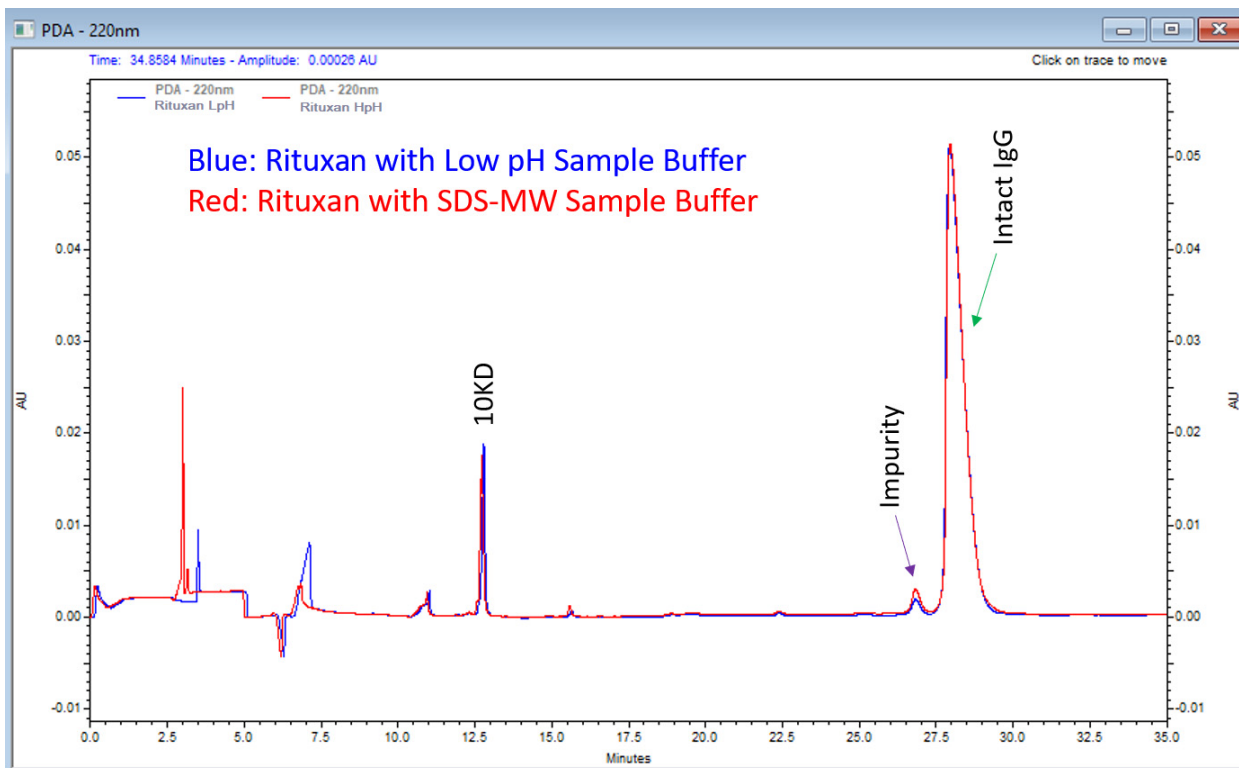
Figure C.4 Rituxan (rituximab) Electropherogram Using the Low pH SDS Sample Buffer (Tris)



## Using the Low pH SDS Sample Buffer

A comparison of results obtained with the SDS-MW Sample Buffer (red trace) and the Low pH SDS Sample Buffer (blue trace) with the Rituxan (rituximab) is shown in the following figure.

**Figure C.5** Comparison of SDS-MW Sample Buffer and Low pH SDS Sample Buffer (Tris) for Rituxan (rituximab)



# Using the Low pH Phosphate SDS Sample Buffer

---

**Note:** SCIEX carries two different low pH sample buffers, the Low pH SDS Sample Buffer (Tris; pH 6.8) and the Low pH Phosphate SDS Sample Buffer (pH 6.5).

---

## About the Low pH Phosphate SDS Sample Buffer

---

The Low pH Phosphate SDS Sample Buffer is designed to meet specifications provided in the *Chinese Pharmacopeia* set forth by the Pharmacopoeia Commission of the Ministry of Health of the People's Republic of China for capillary electrophoresis SDS separations.

In addition to specifying the buffer, the *Chinese Pharmacopeia* (2019-06-27) has recommendations for the separation method. Refer to *Chinese Pharmacopeia* 2020 Edition, Chapter 3127, 3127 单抗分子大小变异体测定法 (CE-SDS法) or <https://www.chp.org.cn/gjydw/swzp/5032.jhtml>. The link was current at the time of publication.

---

**Note:** The *Chinese Pharmacopeia* specifies a range for some assay parameters instead of a single value. For the results in the following figures, the median was used. Specifically, the sample incubation temperature was 70 °C and the sample storage and capillary temperatures were 20 °C.

---

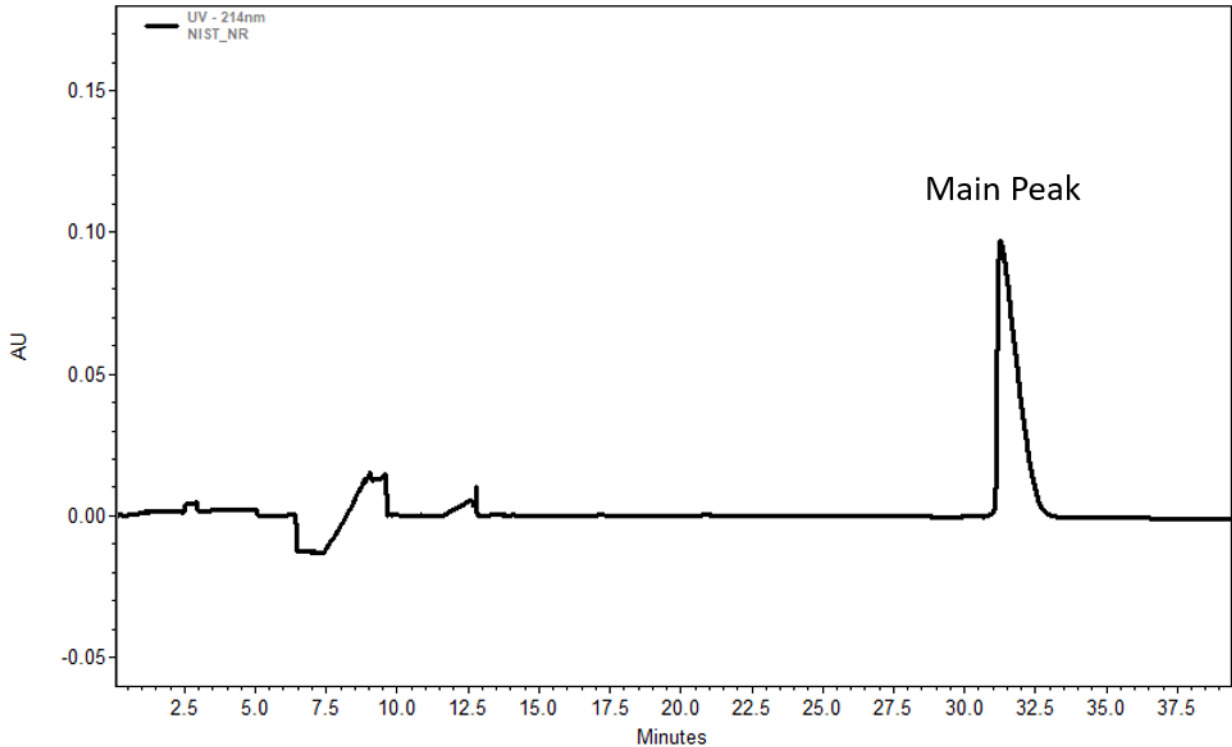
## Results Obtained Using the Low pH Phosphate SDS Sample Buffer

---

### Typical Results Using Non-reducing Conditions

The following figure shows the results obtained with NIST mAb and the Low pH Phosphate SDS Sample Buffer following the *Chinese Pharmacopeia* method under non-reducing conditions.

**Figure D.1** Electropherogram of Non-reduced NIST mAb Using the Low pH Phosphate SDS Sample Buffer with the *Chinese Pharmacopeia* Method

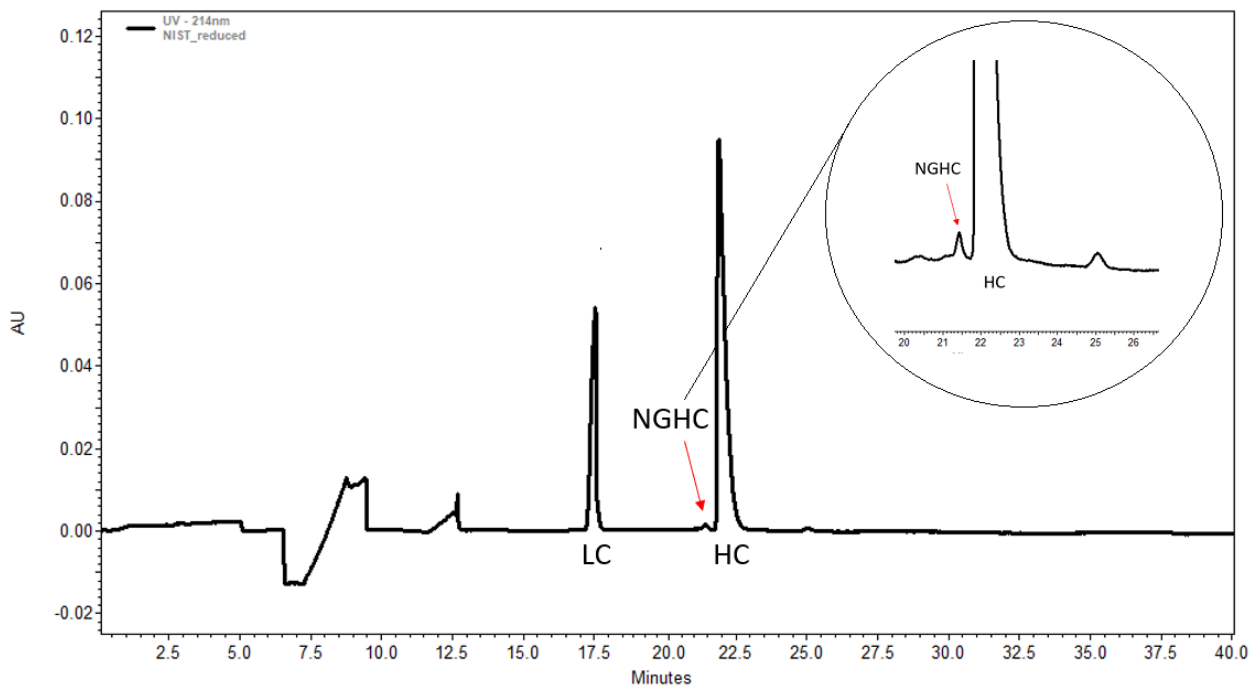




## Typical Results Using Reducing Conditions

The following figure shows the results obtained with NIST mAb and the Low pH Phosphate SDS Sample Buffer following the *Chinese Pharmacopeia* method under reducing conditions.

**Figure D.2** Electropherogram of Reduced NIST mAb Using the Low pH Phosphate SDS Sample Buffer with the *Chinese Pharmacopeia* Method



- LC: light chain
- NGHC: non-glycosylated heavy chain
- HC: heavy chain



# Run the Samples with the Waters Empower™ Software

This section gives instructions on data acquisition using the Empower™ Software. Refer to the Empower™ Software guides and help file for data analysis instructions.

---

**Note:** Calibrate the PDA detector before acquiring data. Refer to the *PA 800 Plus Empower™ Driver User Guide* for instructions.

---

## Create the Instrument Methods

---

---

**Note:** If a low pH SDS sample buffer is used, the instrument methods might need adjustments to accommodate the increased ionic strength of the buffer. Refer to [Using the Low pH SDS Sample Buffer](#).

---

Three instrument methods are required:

- SDS MW\_CONDITIONING
- SDS MW\_SEPARATION
- SDS MW\_SHUTDOWN

---

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

---

---

**Note:** Pressure values can be shown in millibar (mbar) or pounds per square inch (psi), depending on a registry setting for the Empower™ Software. The default unit is millibar. To change the units, refer to the *PA 800 Plus Empower™ Driver Release Notes*.

---

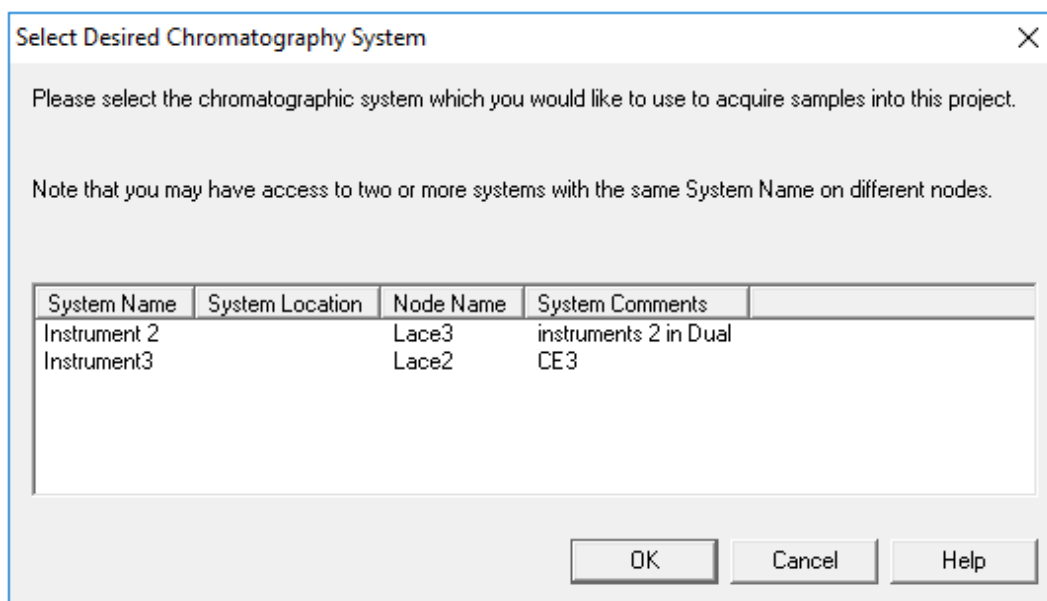
---

**Note:** The instrument methods that follow were validated using the SDS-MW Sample Buffer.

---

- 1 In the Empower™ Software Project window, click **File > New Method > Instrument Method**.  
The Select Desired Chromatography System dialog opens.

**Figure E.1** Select Desired Chromatography System Dialog



- Click the system to be used and then click **OK**. Make sure that the instrument is configured with a PDA detector.

The Instrument Method Editor opens.

- Set the parameters in the **General** tab.

**Figure E.2** General Parameters for the SDS MW\_CONDITIONING Instrument Method

General | Detector | Time Program

**Auxiliary Data Channels**

- Voltage Max: 30.0 kV
- Current Max: 300.0  $\mu$ A
- Power Max: 9.000 W
- Pressure
- Cartridge Temperature

**Peak Detect Parameters**

- Peak Noise Multiplier: 2
- Peak Filter Width: 9

**Capillary Settings**

- Capillary Total Length: 30.2 cm
- Capillary Length: 20.0 cm

**Temperature**

- Cartridge: 25.0  $^{\circ}$ C
- Sample Storage: 25.0  $^{\circ}$ C

**Trigger Settings**

- Wait For External Trigger
- Wait for Temperature: Wait for Cartridge and Storage Temperature

**Inlet Trays**

- Buffer: 36 vials
- Sample: 48 vials

**Outlet Trays**

- Buffer: 36 vials
- Sample: 48 vials

- 4 Click the **Detector** tab, select **PDA** in the **Detector Type** list, and then set the parameters.

**Note:** For 3D data, in **Electropherogram Scan Data**, select **On** for **Data Rate**.

**Figure E.3** Detector Parameters for the SDS MW\_CONDITIONING Instrument Method

General | Detector | Time Program

Detector Type: PDA

Electropherogram Scan Data

Data Rate: 2 Hz  
Scan Range from: 190 to 400 nm

Filter: General Purpose 16-25

Electropherogram Channel Data

Data Rate: 2 Hz

	Acquire	Ref	Wl [nm]	Bw [nm]
Channel 1	<input checked="" type="checkbox"/>	<input type="checkbox"/>	220	10
Channel 2	<input type="checkbox"/>	<input type="checkbox"/>	254	10
Channel 3	<input type="checkbox"/>	<input type="checkbox"/>	280	10
Peak Detect.	<input type="checkbox"/>	<input type="checkbox"/>	250	120

Relays

Relay 1: Closed  
Relay 2: Closed

Reference Channel

Wavelength: 350 nm  
Bandwidth: 10 nm

Absorbance Signal

Signal: Direct

- 5 Add the events in the following figure to the time program.

**Note:** For the pressure in the **Separate Voltage Pressure** event (step 5), type **20**.

**Figure E.4** Time Program for the SDS MW\_CONDITIONING Instrument Method

Time (min)	Event	Value	Duration	Inlet vial	Inlet tray	Outlet vial	Outlet tray	Summary	Comments
1	Rinse Pressure	20.0 psi	10.00 min	D1	Buffer	D1	Buffer	Forward:0.0	0.1 N NaOH rinse to clean capillary surface
2	Rinse Pressure	20.0 psi	5.00 min	E1	Buffer	E1	Buffer	Forward:0.0	0.1 N HCl rinse to neutralize capillary surface silanol group
3	Rinse Pressure	20.0 psi	2.00 min	F1	Buffer	F1	Buffer	Forward:0.0	Water rinse to remove the acid residue
4	Rinse Pressure	70.0 psi	10.00 min	B1	Buffer	B1	Buffer	Forward:0.0	SDS Gel buffer rinse to fill the capillary
5	0.00 Separate Voltage Pressure	15.0 kV	10.00 min	C1	Buffer	C1	Buffer	Reverse (-):5:Simultaneous:0.0	SDS Gel Buffer Voltage Separation
6	10.00 End								
7									

**Note:** If the system is using mbar as the units for pressure, then type the following:

- For the pressure in the **Rinse Pressure** events (steps 1, 2, and 3), type **1379.0**.
- For the pressure in the **Rinse Pressure** event (step 4), type **4826.3**.
- For the pressure in the **Separate Voltage Pressure** event (step 5), type **1379.0**.

### 6 Save the instrument method.

- a. Click **File > Save**. The Save current Instrument Method dialog opens.
- b. Type **SDS MW\_CONDITIONING** in the **Name** field.
- c. (Optional) Type information in the **Method Comments** field.
- d. If prompted, type the Empower™ Software password for the current user in the **Password** field and then click **Save**.

The instrument method is saved to the current project.

### 7 Create the separation instrument method.

- a. Set the parameters on the **General** tab. Refer to [Figure E.2](#).
- b. Set the parameters on the **Detector** tab. Refer to [Figure E.3](#).
- c. Add the events in the following figure to the time program.

**Note:** For the pressure in the **Separate Voltage Pressure** event (step 9), type **20**.

**Figure E.5** Time Program for the SDS MW\_SEPARATION Instrument Method

Time (min)	Event	Value	Duration	Inlet vial	Inlet tray	Outlet vial	Outlet tray	Summary	Comments
1	Rinse Pressure	70.0 psi	3.00 min	D1	Buffer	D1	Buffer	Forward:8:8	0.1 N NaOH rinse to clean capillary surface
2	Rinse Pressure	70.0 psi	1.00 min	E1	Buffer	E1	Buffer	Forward:8:8	0.1 N HCl rinse to neutralize capillary surface silanol group
3	Rinse Pressure	70.0 psi	1.00 min	F1	Buffer	F1	Buffer	Forward:8:8	Water rinse to remove the acid residue
4	Rinse Pressure	70.0 psi	10.00 min	B1	Buffer	B1	Buffer	Forward:8:8	SDS Gel rinse to fill the capillary with SDS gel
5	Wait		0.00	A1	Buffer	A1	Buffer	8:8	Water clean capillary tip
6	Wait		0.00	A4	Buffer	A4	Buffer	0:0	water clean capillary tip
7	Inject Voltage	5.0 kV	20.0 s	A0	Sample List	C1	Buffer	Reverse (:):0:0	Inject sample
8	Wait		0.00	B4	Buffer	B4	Buffer	0:0	Water use for dipping to avoid sample carry over
9	Separate Voltage Pressure	15.0 kV	30.00 min	C1	Buffer	C1	Buffer	Reverse (:):1:Simultaneous:0:0	SDS MW Gel Separation
10	Autozero								
11	Stop Data								
12	End								
13									

**Note:** If the system is using mbar as the units for pressure, then type the following:

- For the pressure in the **Rinse Pressure** events (steps 1 through 4), type **4826.3**.
- For the pressure in the **Separate Voltage Pressure** event (step 9), type **1379.0**.

- d. Save the method as “SDS MW\_SEPARATION”.

- 8 Create the shutdown instrument method.
  - a. Set the parameters on the **General** tab. Refer to [Figure E.2](#).
  - b. Set the parameters on the **Detector** tab. Refer to [Figure E.3](#).
  - c. Add the events in the following figure to the time program.

**Note:** For the pressure in the **Separate Voltage Pressure** event (step 5), type **20**.

**Figure E.6** Time Program for the SDS MW\_SHUTDOWN Instrument Method

Time (min)	Event	Value	Duration	Inlet vial	Inlet tray	Outlet vial	Outlet tray	Summary	Comments
1	Rinse Pressure	70.0 psi	10.00 min	D1	Buffer	D1	Buffer	Forward:0.0	0.1 N NaOH rinse to clean capillary surface
2	Rinse Pressure	50.0 psi	5.00 min	E1	Buffer	E1	Buffer	Forward:0.0	0.1 N HCl rinse to neutralize capillary surface silanol group
3	Rinse Pressure	50.0 psi	2.00 min	F1	Buffer	F1	Buffer	Forward:0.0	Water rinse to remove the acid residue
4	Rinse Pressure	70.0 psi	10.00 min	B1	Buffer	B1	Buffer	Forward:0.0	SDS Gel buffer rinse to fill the capillary
5	Separate Voltage Pressure	15.0 kV	10.00 min	C1	Buffer	C1	Buffer	Reverse (-):5.Simultaneous:0.0	SDS Gel Buffer Voltage Separation
6	Wait		0.00	A1	Buffer	A1	Buffer	0.0	Store tips in water
7	Lamp Off								
8	End								
** 9									

**Note:** If the system is using mbar as the units for pressure, then type the following:

- For the pressure in the **Rinse Pressure** events (steps 1 and 4), type **4826.3**.
  - For the pressure in the **Rinse Pressure** events (steps 2 and 3), type **3447.4**.
  - For the pressure in the **Separate Voltage Pressure** event (step 5), type **1379.0**.
- d. Save the method as “SDS MW\_SHUTDOWN”.

## Create the Method Sets

Three method sets are required:

- SDS MW Conditioning Method Set
- SDS MW Separation Method Set
- SDS MW Shutdown Method Set

**Note:** A method set can also include processing and report methods. To create those methods, refer to the documentation supplied with the Empower™ Software.

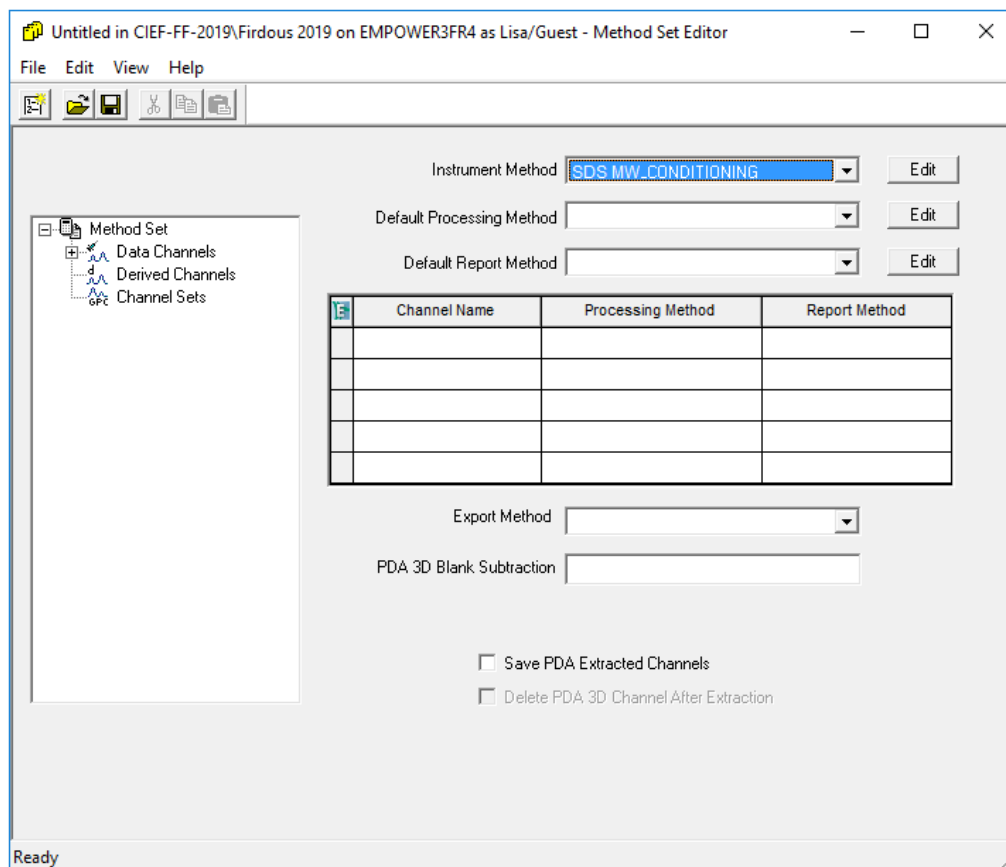
- 1 In the Empower™ Software Project window, click **File > New Method > Method Set**.

- 2 Click **No** in the message.

The Method Set Editor window opens.

- 3 In the **Instrument Method** list, click **SDS MW\_CONDITIONING**. Do not make any other changes.

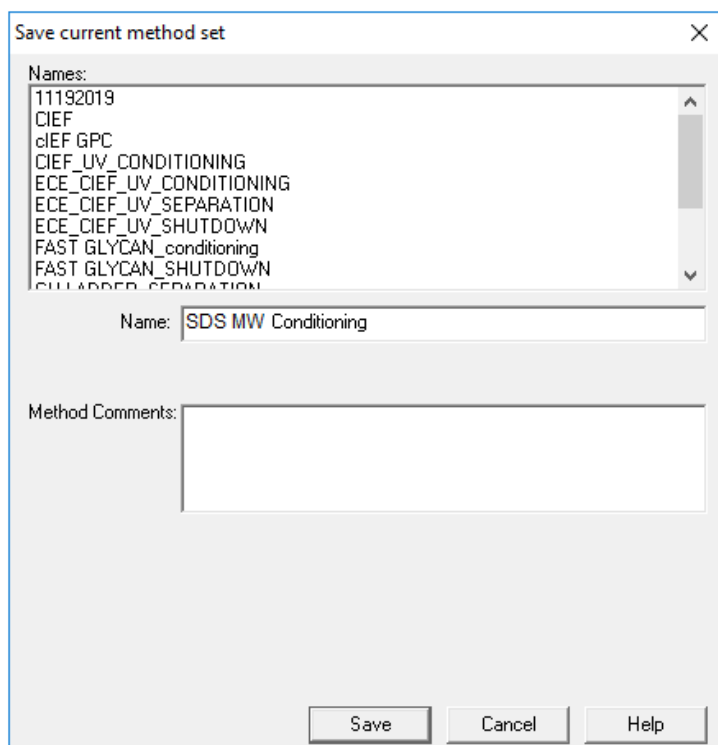
**Figure E.7** Method Set Editor Window



- 4 Save the method set.
  - a. Click **File > Save** to open the Save current method set dialog.
  - b. Type **SDS MW Conditioning** in the **Name** field.
  - c. (Optional) Type information in the **Method Comments** field.
  - d. If prompted, type the Empower™ Software password for the current user in the **Password** field and then click **Save**.



Figure E.8 Save current method set Dialog



The method set is saved to the current project.

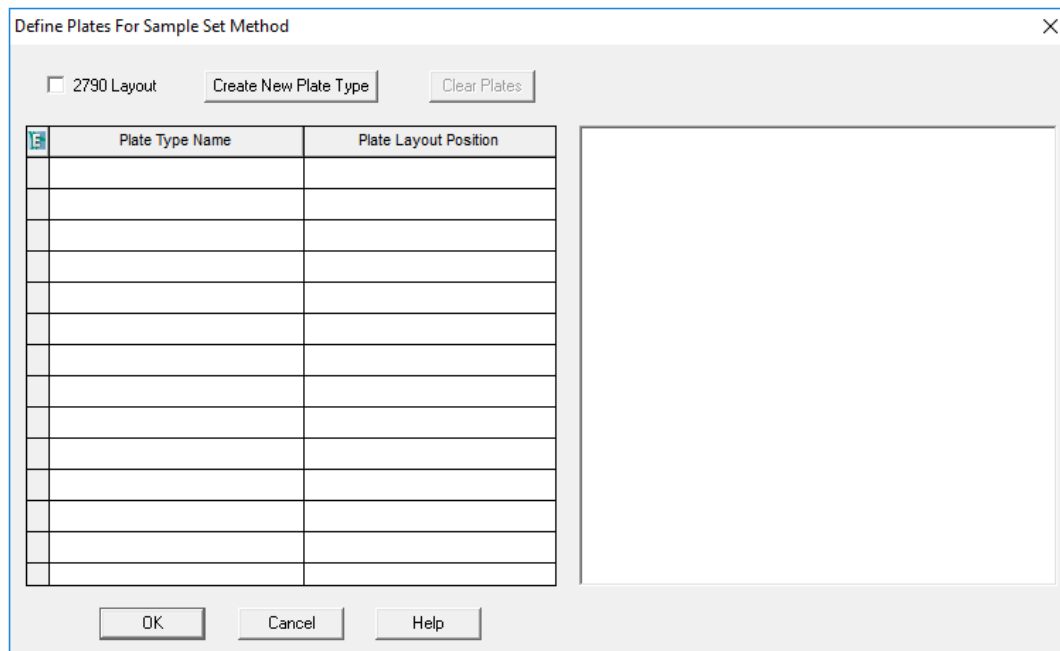
- 5 Repeat the previous steps to create two more method sets.
  - a. Create the separation method set by selecting **SDS MW\_SEPARATION** in the **Instrument Method** list. Save the method set as “SDS MW Separation”.
  - b. Create the shutdown method set by selecting **SDS MW\_SHUTDOWN** in the **Instrument Method** list. Save the method set as “SDS MW Shutdown”.

## Configure the Software to Use Multiple Plates

The Empower™ Software is designed for chromatography systems that do not have buffer trays. To use the buffer trays, configure the Empower™ Software as follows.

- 1 In the Empower™ Software Run Samples window, click **Edit > Plates**.  
The Define Plates for Sample Set Method dialog opens.

**Figure E.9** Define Plates for Sample Set Method Dialog



---

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

---

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

---

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

---

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


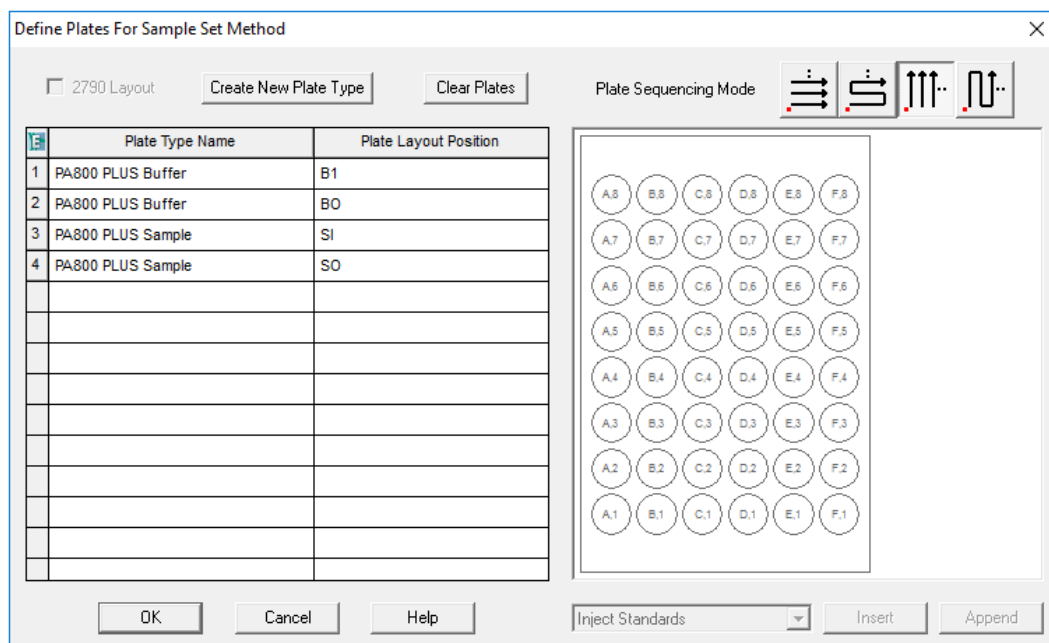
- b. Click the **Plate Layout Position** cell and then type **BI**.
- c. Click  (**Vertical Discontinuous Plate Sequencing Mode**) to indicate the order in which the vials are accessed during the run.



Figure E.11 After Defining All Plate Types

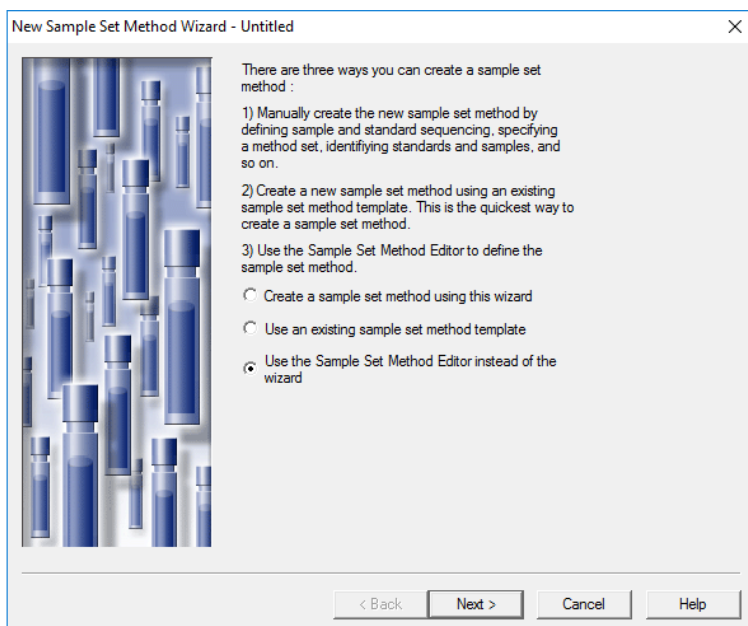


- 6 Click **OK** to save the changes and close the dialog.

## Create the Sample Set Method and Run the Samples

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

Figure E.12 New Sample Set Method Wizard



The Sample Set Method Editor opens.

### 3 Set up the Sample Set Method.

- a. In the first row, select **SDS MW Conditioning** in the **Method Set/Report or Export Method** cell.
- b. For rows 2 through 17, select **SDS MW Separation** in the **Method Set/Report or Export Method** cell.
- c. For row 18, select **SDS MW Shutdown** in the **Method Set/Report or Export Method** cell.
- d. Add the required information for the samples. Refer to [Table E.1](#). Use the default values for the other fields.

**Table E.1** Required Fields for a Sample Set Method

Name	Description
Plate/Well	The position of the sample in the sample tray.
# of Injs	The number of times the sample is to be injected.
SampleName	The name of the sample.
Run Time (Minutes)	The duration of the run.  <b>CAUTION: Possible Wrong Result. Make sure that the Run Time is greater than or equal to the duration of the time program in the instrument method. If the Run Time is shorter, the system stops the run before the time program is complete.</b>

The completed Sample Set Method is shown in the following figure.

**Note:** The **Level** and **Reference Level** columns are hidden in the following figure.

Figure E.13 Sample Set Method

	Plate/Well	Inj Vol (uL)	# of Injs	Label	SampleName	Function	Method Set / Report or Export Method	Processing	Run Time (Minutes)
1	Bl:A,1	10.0	1		Conditioning	Inject Samples	SDS MW CONDITIONING	Normal	10.00
2	Sl:A,1	10.0	1	S0101	SDS MW STD	Inject Standards	SDS MW SEPARATION	Normal	30.00
3	Sl:A,2	10.0	1	U0101	SAMPLE1	Inject Samples	SDS MW SEPARATION	Normal	30.00
4	Sl:A,3	10.0	1	U0102	SAMPLE2	Inject Samples	SDS MW SEPARATION	Normal	30.00
5	Sl:A,4	10.0	1	U0103	SAMPLE3	Inject Samples	SDS MW SEPARATION	Normal	30.00
6	Sl:A,5	10.0	1	U0104	SAMPLE4	Inject Samples	SDS MW SEPARATION	Normal	30.00
7	Sl:A,6	10.0	1	U0105	SAMPLE5	Inject Samples	SDS MW SEPARATION	Normal	30.00
8	Sl:A,7	10.0	1	U0106	SAMPLE6	Inject Samples	SDS MW SEPARATION	Normal	30.00
9	Sl:A,8	10.0	1	U0107	SAMPLE7	Inject Samples	SDS MW SEPARATION	Normal	30.00
10	Sl:B,1	10.0	1	U0108	SAMPLE8	Inject Samples	SDS MW SEPARATION	Normal	30.00
11	Sl:B,2	10.0	1	U0109	SAMPLE9	Inject Samples	SDS MW SEPARATION	Normal	30.00
12	Sl:B,3	10.0	1	U0110	SAMPLE10	Inject Samples	SDS MW SEPARATION	Normal	30.00
13	Sl:B,4	10.0	1	U0111	SAMPLE11	Inject Samples	SDS MW SEPARATION	Normal	30.00
14	Sl:B,4	10.0	1	U0112	SAMPLE11	Inject Samples	SDS MW SEPARATION	Normal	30.00
15	Sl:B,6	10.0	1	U0113	SAMPLE13	Inject Samples	SDS MW SEPARATION	Normal	30.00
16	Sl:B,7	10.0	1	U0114	SAMPLE14	Inject Samples	SDS MW SEPARATION	Normal	30.00
17	Sl:B,8	10.0	1	U0115	SAMPLE15	Inject Samples	SDS MW SEPARATION	Normal	30.00
18	Sl:C,1	10.0	1	U0116	SAMPLE16	Inject Samples	SDS MW SEPARATION	Normal	30.00
19	Sl:C,2	10.0	1	U0117	SAMPLE17	Inject Samples	SDS MW SEPARATION	Normal	30.00
20	Sl:C,3	10.0	1	U0118	SAMPLE18	Inject Samples	SDS MW SEPARATION	Normal	30.00
21	Sl:C,4	10.0	1	U0119	SAMPLE19	Inject Samples	SDS MW SEPARATION	Normal	30.00
22	Sl:C,5	10.0	1	U0120	SAMPLE20	Inject Samples	SDS MW SEPARATION	Normal	30.00
23	Sl:C,6	10.0	1	U0121	SAMPLE21	Inject Samples	SDS MW SEPARATION	Normal	30.00
24	Sl:C,7	10.0	1	U0122	SAMPLE22	Inject Samples	SDS MW SEPARATION	Normal	30.00
25	Sl:C,8	10.0	1	U0123	SAMPLE23	Inject Samples	SDS MW SEPARATION	Normal	30.00
26	Bl:A,1	10.0	1		SAMPLE23	Inject Samples	SDS MW SHUTDOWN	Normal	10.00

## 4 Save the Sample Set Method.

- a. Click **File > Save**.

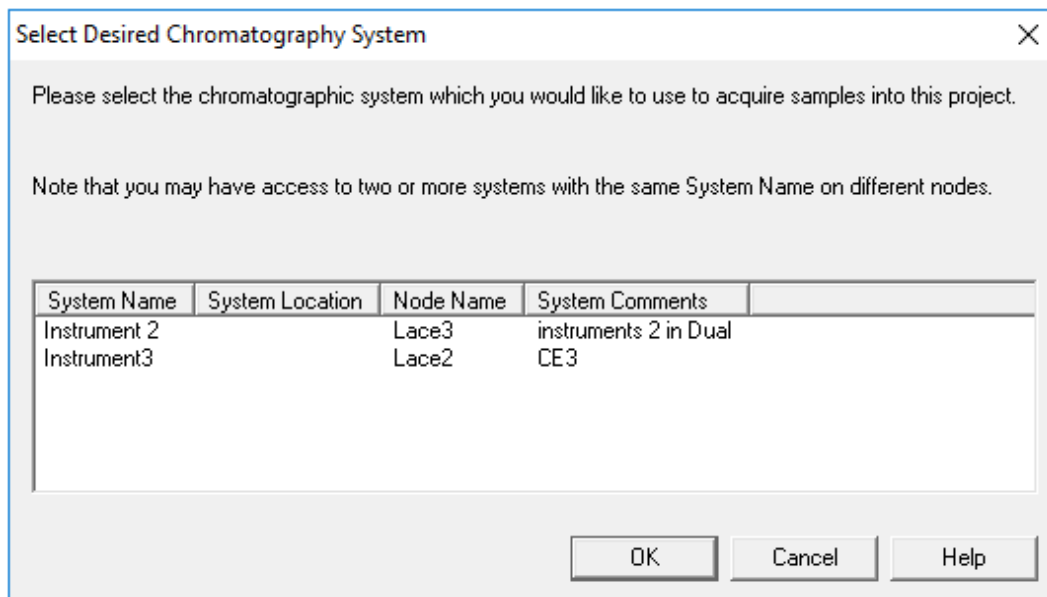
The Save current sample set method dialog opens.

- b. Type **SDS MW Sample Set Method** in the **Name** field.
- c. (Optional) Type information in the **Method Comments** field.
- d. If prompted, type the Empower™ Software password for the current user in the **Password** field and then click **Save**.

The method set is saved to the current project.

- 5 Click **Tools > Run Samples**.

**Figure E.14** Select Desired Chromatography System Dialog

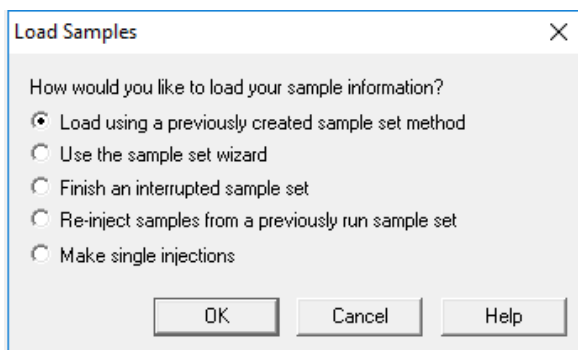


- 6 Click the system to be used and then click **OK**. Make sure that the instrument is configured with a PDA detector.  
The Run Samples window opens.

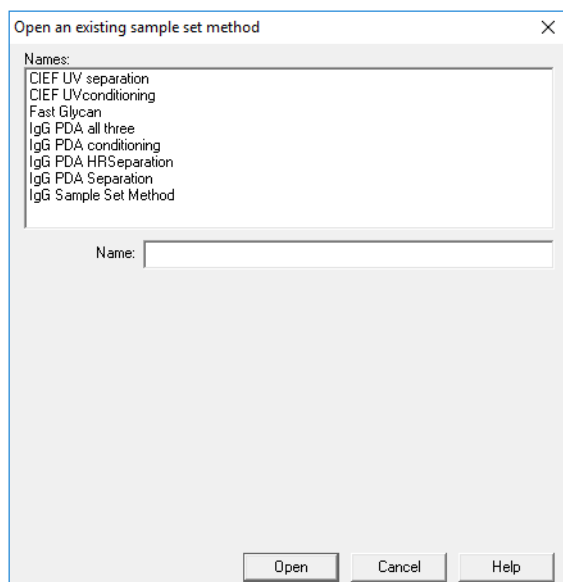
- 7 Click  (**Load Sample Set**).  
The Load Samples dialog opens.

- 8 Click **Load using a previously created sample set method** and then click **OK**.

**Figure E.15** Load Samples Dialog




**Figure E.16** Open an existing sample set method Dialog




- 
- 9** Click **SDS MW Sample Set Method** in the list and then click **Open**.

The sample set method opens in the Samples tab.

- 
- 10** In the Empower™ Software Project window, click  (**Start**). Data acquisition starts.

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

- 
- 11** During the run the following actions are available:

- (Optional) Click  (**Stop**) to halt data acquisition.
- View the voltage and current data.

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

---



---

# Revision History

## **Initial Issue, A51970AA, April 2009**

32 Karat™ Software version 9.1

PA 800 *plus* Software version 1.1

PA 800 *plus* Firmware version 9.0

## **First Revision, A51970AB, December 2009**

Revised corporate address

## **Second Revision, A51970AC, February 2011**

32 Karat™ Software version 9.1 patch

PA 800 *plus* Software version 1.1 patch

PA 800 *plus* Firmware version 9.2

Numerous syntax and grammatical edits

## **Third Revision, A51970AD, January 2014**

Dimension & instruction edit

## **Fourth Revision, A51970AE, RUO-IDV-05-6934-A, April 2018**

Rebranded. Applied new template. Legal content updated. Safety chapter removed and a reference to the safety content found in the System Overview Guide added. Replaced instructions for creating methods with instructions for using the PA 800 Plus Software. Added Hazardous Substance Information appendix.

## **Fifth Revision, RUO-IDV-05-6934-B, November 2019**

Applied new template. Legal content updated. Added low pH SDS sample buffer including sample preparation instructions. Added methods appendix. Added Contact Us.

## **Sixth Revision, RUO-IDV-05-6934-C, July 2020**

Updated Legal Content. Updated Introduction. Added Store the Cartridge for Less Than 10 Days. Updated Appendix A, Hazardous Substance Information. Added Appendix C, Low pH SDS Sample Buffer, including sample preparation instructions. Added Appendix D, Low pH Phosphate SDS Sample Buffer. Added Appendix E, Run the Samples with the Waters Empower™ Software. Updated Contact Us.

*This guide applies to the latest software and firmware listed above, and any higher subsequent versions. When a subsequent software or firmware version affects the information in this guide, a new issue will be released to the SCIEX website. For updates, go to [sciex.com](https://www.sciex.com) and download the latest version of the guide.*



## Customer Training

---

- In North America: [NA.CustomerTraining@sciex.com](mailto:NA.CustomerTraining@sciex.com)
- In Europe: [Europe.CustomerTraining@sciex.com](mailto:Europe.CustomerTraining@sciex.com)
- Outside the EU and North America, visit [sciex.com/education](http://sciex.com/education) for contact information.

## Online Learning Center

---

- [SCIEX University™](#)

## Purchase Consumables

---

Reorder SCIEX consumables online at [store.sciex.com](http://store.sciex.com). To set up an order, use the account number, found on the quote, order confirmation, or shipping documents. The SCIEX online store is currently limited to the US, UK, and Germany but will be expanding to other countries in the future. For customers in other countries, contact the local SCIEX representative.

## SCIEX Support

---

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

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

## CyberSecurity

---

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

## Documentation

---

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

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

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

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

The latest versions of the documentation are available on the SCIEX website, at [sciex.com/customer-documents](https://sciex.com/customer-documents).

---

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

---