

# **SDS-MW Analysis Kit**

For the PA 800 Plus Pharmaceutical Analysis System

*Application Guide*



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# SDS-MW Analysis Kit

## CAUTION

**Prior to using the system, refer to the *PA 800 Plus Pharmaceutical Analysis System Overview Guide* for detailed information on the safe use and operation of the system.**

## Safety

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Refer to the Safety Data Sheets (SDS), available at [sciex.com/tech-regulatory](https://sciex.com/tech-regulatory), regarding the proper handling of materials and reagents. Always follow standard laboratory safety guidelines.

## Introduction

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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 according to differences in protein molecular weight.

The SDS-MW Analysis Kit is designed for 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.

**NOTE** The PA 800 Plus series systems must be equipped with a photodiode array (PDA) detector to perform this assay.

## 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.

## Intended Use

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

## Equipment and Materials Required

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

Component	Quantity	Reorder Part Number
Capillary, 50 $\mu$ m I.D. bare-fused silica	2	N/A
SDS-MW Gel Buffer - proprietary formulation, pH 8, 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 $\mu$ L	N/A
Internal Standard, 10 kDa protein, 5 mg/mL	0.4 mL	A26487
Acidic Wash Solution, 0.1 M HCl	100 mL	N/A
Basic Wash Solution, 0.1 M NaOH	100 mL	N/A

**Table 2** Additional Supplies from SCIEX

Component	Quantity	Part Number
Capillary, 50 $\mu$ m I.D. bare-fused silica	1 box	N/A
SDS-MW Gel Buffer - proprietary formulation, pH 8, 0.2% SDS	140 mL, 4-pack	A30341
SDS-MW Size Standard, 10 kDa to 225 kDa, 16 mg/mL	3	A22196

**Table 2** Additional Supplies from SCIEX (*Continued*)

Component	Quantity	Part Number
Internal Standard, 10 kDa protein, 5 mg/mL	1	A26487
Universal vials	100	A62251
Universal vial caps - blue	100	A62250
Micro vials, 200 $\mu$ L	50	144709

**Table 3** Additional Required Reagents

Component	Vendor	Part Number
2-mercaptoethanol	Sigma-Aldrich	M7154, M6250
Iodoacetamide	Sigma-Aldrich	I-1149
Microcon-10 kDa centrifugal filter unit with Ultracel-10 membrane	Millipore	MRCF0R010

## Customer-Supplied Equipment and Supplies

- Pipettors and appropriate tips
- Vortex mixer
- Microcentrifuge
- Double-deionized (DDI) water (MS-grade water filtered through a 0.2  $\mu$ m filter and with resistance greater than 18 M $\Omega$ )
- Water bath (37 °C to 100 °C) or heat block

## Storage Conditions

- Upon receipt, store the SDS-MW size standard and internal standard at 2 °C to 8 °C.
- Store the capillary, SDS-MW sample buffer, and SDS-MW gel buffer at room temperature.

If precipitation is present in the SDS-MW gel buffer or the SDS-MW sample buffer, bring buffer to room temperature overnight.

## Prepare the PA 800 Plus System

Before proceeding, you must understand the following procedures as described in the *PA 800 Plus System Maintenance Guide*:

- How to replace the capillary cartridge
- How to install the PDA detector
- How to calibrate the PDA detector

For instructions on loading and unloading trays, refer to the *PA 800 Plus System Overview Guide*.

## Install the PDA Detection Module

- 1 Turn off the PA 800 Plus instrument and install the PDA detection module.
  - 2 Turn on the instrument and permit the UV lamp to warm up for at least 30 minutes.
- 

## Clean the Interface

Carefully clean the system electrodes, capillary ends, and interface block as described in the *PA 800 Plus System Maintenance Guide*, either every other day or after the finish of the sequence. The SDS-MW gel buffer is very viscous and will accumulate on the capillary ends, electrodes, interface block, and opening levers if regular and thorough cleaning is not performed. Gel accumulation might cause broken capillaries bent electrodes, vial jams, and missed injections.

## Insert the Cartridge and Calibrate the PDA

Insert the cartridge into the system. Close the front panel and calibrate the PDA detector. This procedure should be employed daily or any time the cartridge is replaced.

## Prepare the Samples

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### 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 centrifuge briefly in a standard microcentrifuge.
  - 3 Pipette 10  $\mu$ L of the SDS-MW size standard into a 0.5 mL microcentrifuge vial.
  - 4 Add 85  $\mu$ L of the SDS-MW sample buffer to the microcentrifuge vial.
  - 5 Add 2  $\mu$ L of internal standard to the microcentrifuge vial.
  - 6 Inside a fume hood, add 5  $\mu$ L of 2-mercaptoethanol.
-

- 7 Cap the vial tightly, seal with parafilm, mix thoroughly, then heat in a water bath at 100 °C for 3 minutes.
- 8 Put the vial in a room-temperature water bath to cool for five minutes before injection. The sample will remain stable for approximately 24 hours.

## Prepare the Protein Sample

### Desalt the Protein Sample

The signal intensity and resolution of this kit are sensitive to the salt concentration in the protein sample. Generally, if the final salt concentration is above 50 mM, the sample loading efficiency will be reduced. The sample should be desalted with a Microcon-10 kDa centrifugal filter unit using the following procedure:

- 1 Add 1 mL of protein sample to a Microcon-10 kDa centrifugal filter unit, then add 1 mL of SDS-MW sample buffer.
- 2 Centrifuge for 20 minutes at 7000 *g*.
- 3 Add 2 mL of SDS-MW sample buffer, then centrifuge for 20 minutes at 7000 *g*.
- 4 Insert the Microcon-10 kDa centrifugal filter unit into a new vial and then centrifuge for 3 minutes at 5000 *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 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 of 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 internal standard.
- 3 Inside a fume hood, add 5  $\mu\text{L}$  of 2-mercaptoethanol.
- 4 Cap the vial tightly, seal with parafilm, and mix thoroughly.
- 5 Heat in a water bath at 100  $^{\circ}\text{C}$  for 3 minutes.
- 6 Put the vial in a room temperature water bath to cool for 5 minutes before injection.

### Perform 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 too high, low signal and peak tailing is likely to occur. Exchange the sample buffer with a Microcon-10 kDa centrifugal filter unit using the following procedure:

- 1 Add 1 mL of protein sample to a Microcon-10 kDa centrifugal filter unit.
- 2 Centrifuge for 15 minutes at 4000  $g$ .
- 3 Add 2 mL of SDS-MW sample buffer, then centrifuge for 25 minutes at 4000  $g$ .
- 4 Insert the Microcon-10 kDa centrifugal filter unit into a new vial and then centrifuge for 3 minutes at 1000  $g$ . 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.

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## Prepare the Non-reduced Protein Sample

Comparison of the reduced versus 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 alkylation reagent during preparation of the sample to minimize any heterogeneity created from partial auto-reduction of the protein. The IAM solution is stable for approximately 24 hours at room temperature.

## Prepare the Alkylation Reagent (250 mM IAM Solution)

- 1 Weigh 46 mg of iodoacetamide (IAM).
- 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 vial tightly, mix thoroughly until dissolved, and then store in the dark.

## Prepare the Sample

- 1 Dilute the sample with the SDS-MW sample buffer to a total volume of 95  $\mu$ L to give a final protein concentration between 0.2 mg/mL to 2 mg/mL.
- 2 Add 2  $\mu$ L of the 10 kDa internal standard to the sample tube.
- 3 Inside a fume hood, add 5  $\mu$ L of the IAM solution.
- 4 Cap the vial tightly, seal with parafilm, and mix thoroughly.
- 5 Heat the vial in a water bath at 70 °C for 3 minutes.
- 6 Put the vial in a room temperature water bath to cool for 5 minutes before injection.

## Set Up the System

### Methods

The methods and data files are installed on the PA 800 Plus controller. They are not available for download.

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

- **SDS MW Conditioning - PA 800 plus.met** — To condition the capillary at the start of each day.
- **SDS MW Separation - PA 800 plus.met** — To perform an SDS-MW separation.
- **SDS MW Shutdown - PA 800 plus.met** — For shutting down at the end of a sequence: to rinse the capillary for storage and to turn off the UV lamp.

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

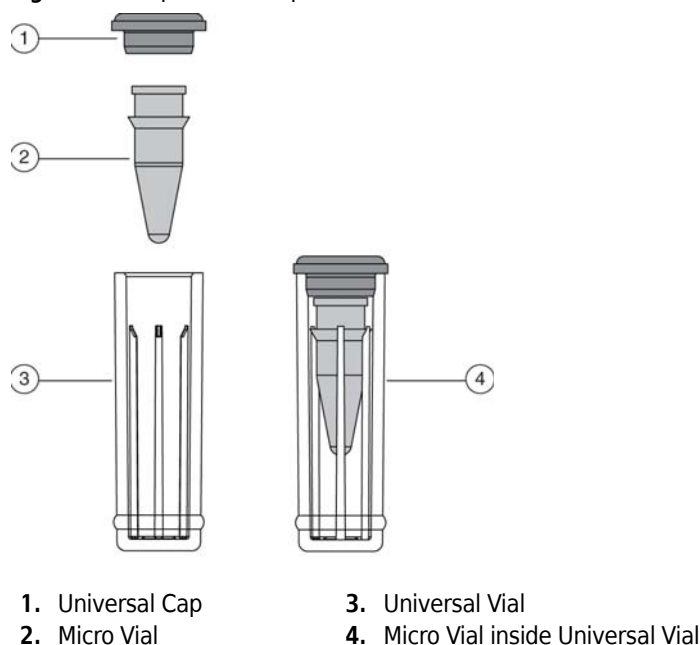
- **SDS MW - 24 samples - PA 800 plus.seq**

### Prepare the Sample Vials

Before placing the 200  $\mu$ L sample vials (or micro vials) into the universal vials, make sure there are no bubbles at the bottom of the vials. If bubbles exist, centrifuge the micro vials for 2 minutes at 1000 *g* and repeat if necessary. Put a blue cap on the universal vial and make sure it is secure. Refer to [Figure 1](#).

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

**Figure 1** Sample Vial Setup



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## Prepare and Load the Buffer Vials

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**DANGER! Toxic Chemical Hazard. Read the *Safety Data Sheets* for 0.1 M NaOH Solution, 0.1 M NaOH, and SDS-MW Gel Buffer before use.**

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Refer to [APPENDIX A](#) for additional information.

Fill the appropriate number of reagent vials with the SDS-MW Gel Buffer, 0.1 M NaOH solution, 0.1 M HCl solution, and DDI water according to the buffer tray maps in [Figure 3](#) and [Figure 4](#).

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. The buffer tray maps are designed for use with high-resolution methods, which introduce the sample from the left side tray.

### Prepare the Reagent Vials

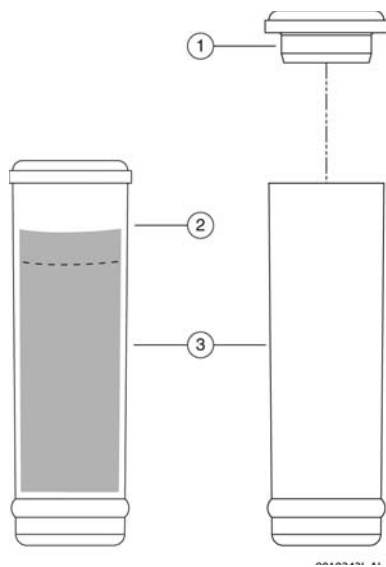
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- 1 Fill the gel rinse (Gel-R) vials with 1.2 mL of SDS-MW gel buffer.
  - 2 Fill the gel separation (Gel-S) vials with 1.1 mL of SDS-MW gel buffer.
  - 3 Fill the water (H<sub>2</sub>O) vials with 1.5 mL of DDI water.
  - 4 Fill the NaOH and HCl vials with 1.5 mL of NaOH and HCl, respectively.
  - 5 Fill the waste vials with 1.0 mL of DDI water.
- 



#### **WARNING**

**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 2** Universal Vials and Caps

1. Universal Vial Cap
2. Maximum Fill Level
3. Universal Vials

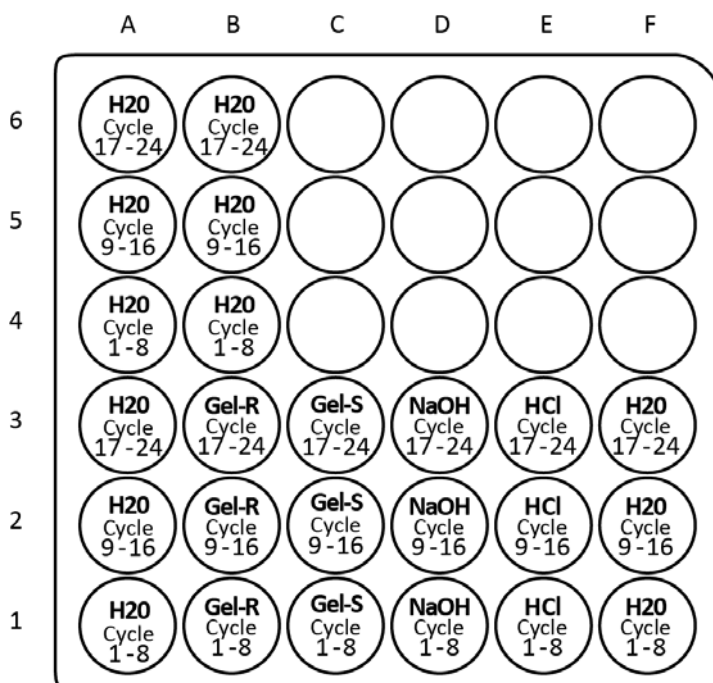
**NOTE** Carefully fill the buffer vials with SDS-MW gel buffer without producing bubbles and use the recommended volume. If the volume is too low ( $< \frac{1}{2}$  of vial volume), the capillary and electrode might not be able to dip into the SDS-MW gel buffer during the separation. If the volume is too high, the SDS-MW gel buffer may accumulate on the capillary ends and electrodes, causing various modes of system failure.

## 6 Cap the universal vials with the blue caps.

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

- 7 Load the reagent vials into the inlet (Figure 3) and outlet (Figure 4) 6x6 buffer trays.

**Figure 3** Inlet Buffer Tray Map



- |                                  |         |
|----------------------------------|---------|
| 1. H2O - DDI Water               | 4. NaOH |
| 2. Gel-R - SDS-MW Gel Rinse      | 5. HCl  |
| 3. Gel-S - SDS-MW Gel Separation |         |



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

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

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

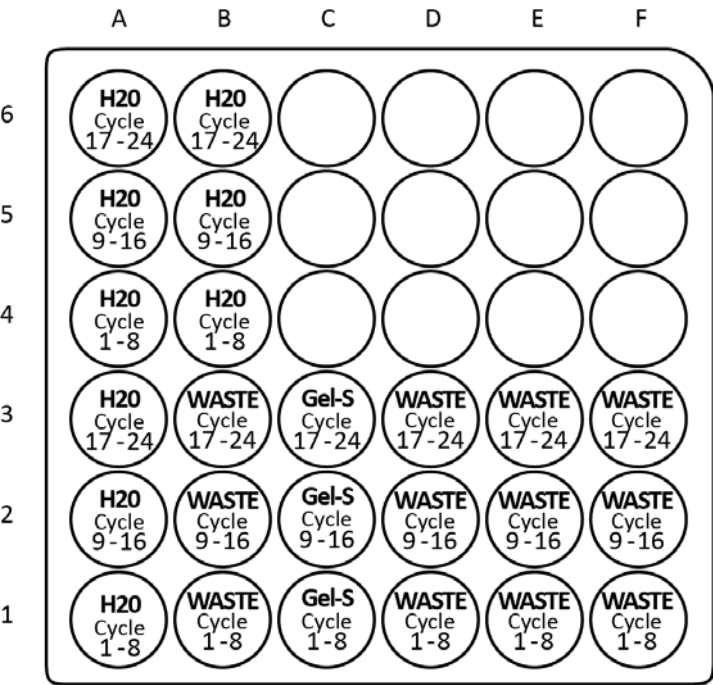
C1 to C3: SDS-MW gel buffer for separation (Gel-S), 1.1 mL

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

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

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

Figure 4 Outlet Buffer Tray Map



1. H2O - DDI Water	3. Gel-S - SDS-MW Gel Separation
2. Waste	


- A1 to A6: DDI H<sub>2</sub>O, use in dip step to clean capillary tip, 1.5 mL
- B4 to B6: DDI H<sub>2</sub>O, use in dip step to clean capillary tip, 1.5 mL
- B1 to B3: Waste vial for SDS-MW gel buffer rinse, 1.0 mL of DDI water
- C1 to C3: SDS-MW gel buffer for separation, 1.1 mL
- D1 to D3: Waste vial for 0.1 M NaOH rinse, 1.0 ml of DDI water
- E1 to E3: Waste vial for 0.1 M HCl rinse, 1.0 ml of DDI water
- F1 to F3: Waste vial for DDI H<sub>2</sub>O rinse, 1.0 ml of DDI water

8 Load the trays into the PA 800 Plus system.

Run the Assay

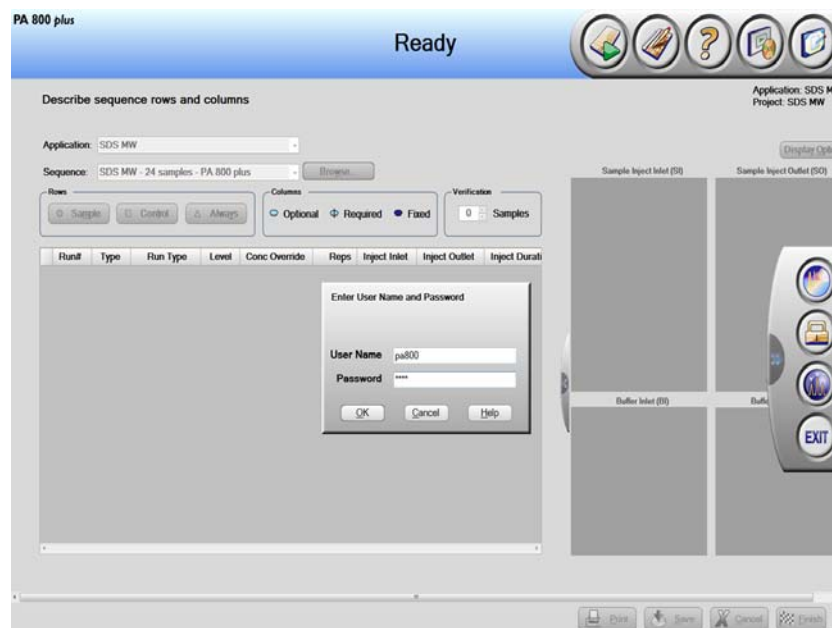
Create the Sequence and Start the Run

- 1 Double-click the PA 800 Plus software icon on the desktop.

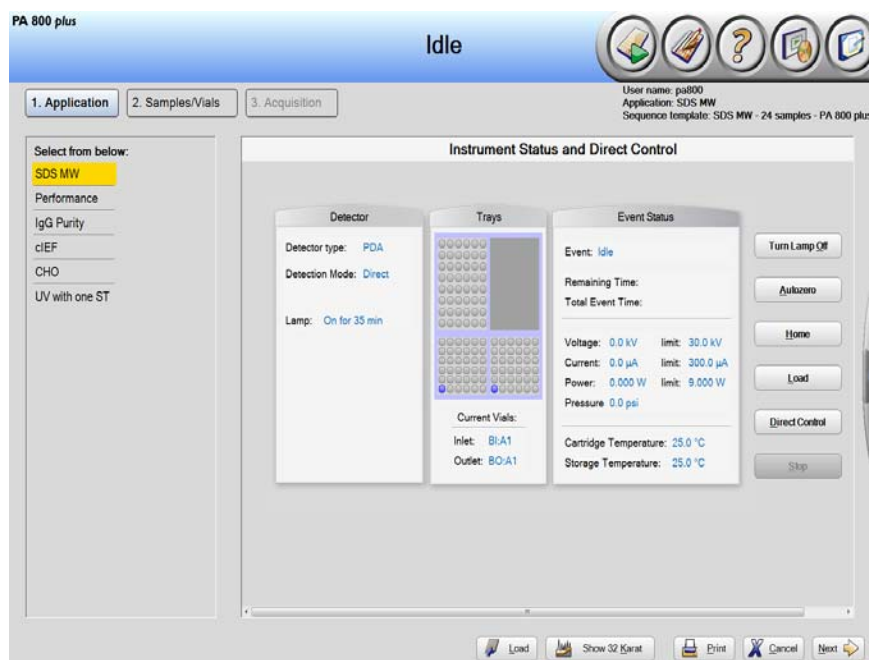
- 2 In the PA 800 Plus window, click  (Run) in the upper right corner of the window.
- 3 In the **Application** list, click **SDS MW**. In the **Sequence** list, click **Browse** and select **SDS MW - 24 samples - PA 800 plus.seq**.


If system administration is enabled, type the user name and the password when prompted, and then click **OK** (Figure 5). The default user name is **pa800**, and the default password is **plus**.

**Figure 5** User Name and Password Entry




The Instrument Status and Direct Control window opens (Figure 6).

**Figure 6** Instrument Status and Direct Control Window

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

5 Select **SDS MW - 24 samples - PA 800 plus.seq** 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 from the Samples/Vials window by clicking the Describe icon.

Use the Describe function to customize the sequence stable 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, select **SDS MW**, click **Browse** and select **SDS MW - 24 samples - PA 800 plus.seq**. If prompted, type a user name and password.

8 (Optional) Edit the **Sample ID** and **Data** fields as desired.  
Editable fields such as **Sample ID** and **Data File Name** can be set as Mandatory, Optional, or Fixed.

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

In [Figure 7](#), **Capillary Conditioning** and **Shutdown** runs are set as **Always**. **Sample ID** is set as **Optional**. Reps are set as **Required**.

**Figure 7** 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

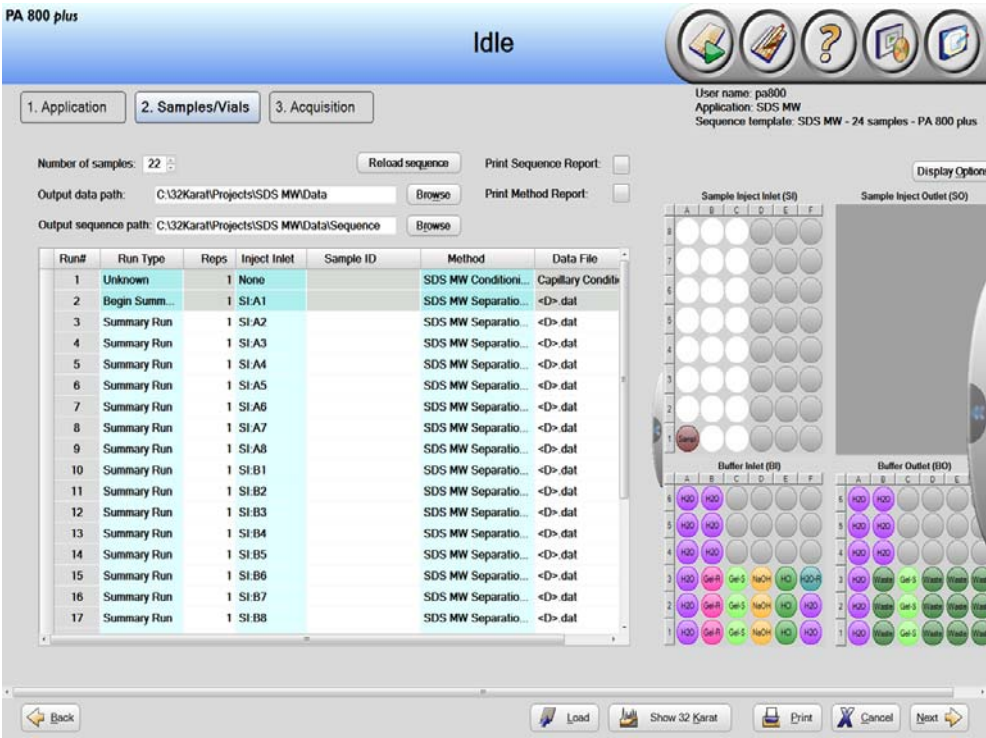
**Verification**

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

- 10 In the lower right corner of the window, click (Save) and then click (Finish). The Run Sequence window opens ([Figure 8](#)).

Figure 8 Describe sequence rows and columns Window – Reload Sequence



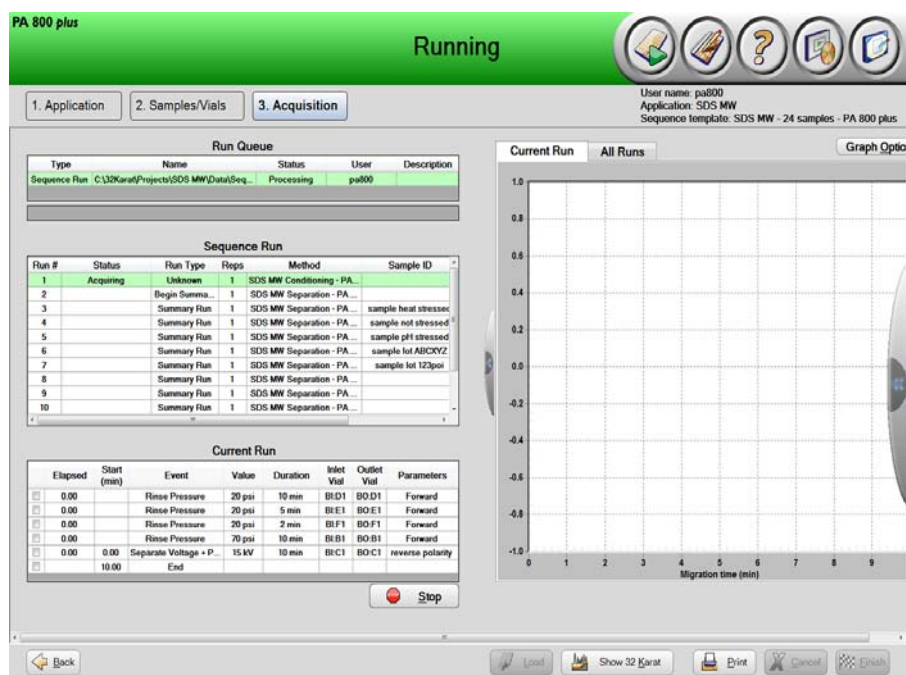
**NOTE** In the upper left corner next to Run #1 of the table in Figure 8, a blinking red exclamation mark (not shown) indicates that the sequence has changed and the software expects an action from the user. Move the cursor over the exclamation point to view a tooltip with the required action. In this example, the user is prompted to click **Reload sequence** (Reload sequence) to update the sequence.

Another example of a required action is in the case of a data file name being 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 8, and then close the door.
- 12 Click **Next** and then click **Yes - run now**.

Figure 9 PA 800 Plus Software: Run Window



## Capillary Cleaning and Storage

**For short-term storage** on the instrument (< 10 days), clean and store the capillary following separation:

Perform a shutdown method to clean the capillary. Leave the capillary ends dipped in the water vials.

**For long-term storage** (> 10 days), clean and store the capillary following separation:

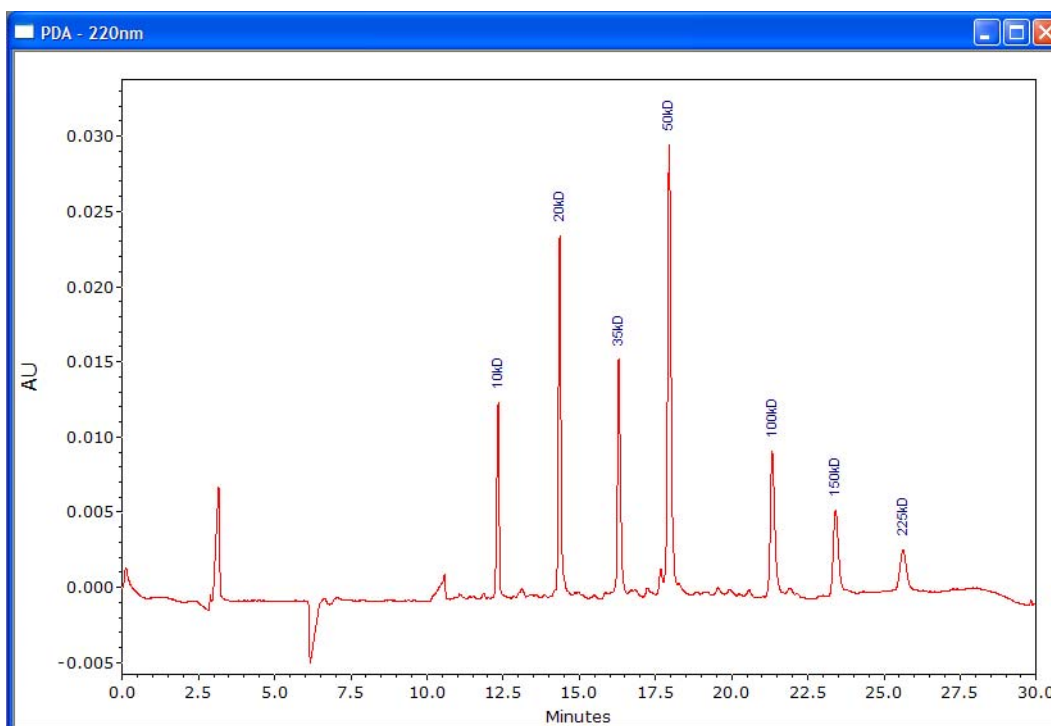
- 1 Perform a shutdown method to clean the capillary.
- 2 Rinse the capillary with DDI water for 10 minutes at 100 psi.
- 3 Remove the cartridge from the instrument.
- 4 Store the cartridge in the cartridge box with the inlet and outlet ends in water vials.

## 5 Keep the cartridge upright in the box.

## Evaluate 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 10](#) for a typical separation of the SDS-MW size standard.

**Figure 10** Separation of the SDS-MW Size Standard

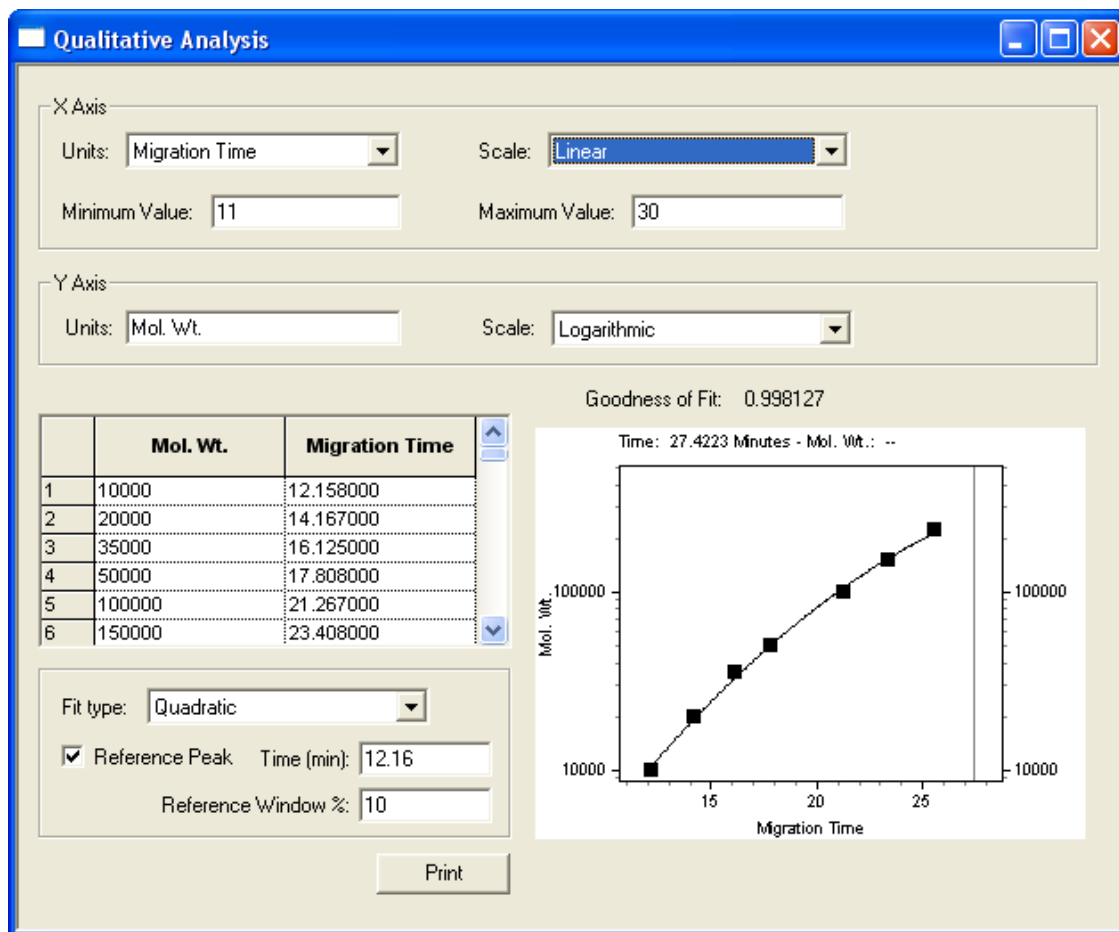


## Estimate the Protein Molecular Weights

Refer to [Figure 11](#) 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 Control and Analysis Software. Refer to [Figure 11](#).

Figure 11 Qualitative Analysis Tab for Updating the Size Calibration Curve



## 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>PA 800 Plus 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</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 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</a> . 2) Replace capillary if the plug cannot be removed.
	Not enough sample	Make sure there is a minimum of 20 $\mu$ L 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](http://sciex.com).

Hazard classification according to HCS 2012.

## Hazardous Substances

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### Acidic Wash Solution (0.1 M HCl)



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

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### Basic Wash Solution (0.1 M NaOH)



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

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### SDS-MW Gel Buffer



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

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# 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, 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.

*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 [www.sciex.com](http://www.sciex.com) and download the latest version of the guide.*

