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 SCIEX’s sole and exclusive representations, warranties, and obligations. 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.

**For research use only. Not for use in diagnostic procedures.**

AB Sciex is doing business as SCIEX.

The trademarks mentioned herein are the property of AB Sciex Pte. Ltd. or their respective owners.

AB SCIEX™ is being used under license.

© 2018 AB Sciex
Contents

SDS-MW Analysis Kit, 5
Safety, 5
Introduction, 5
  Protein Size Standard, 6
  Internal Standard, 6
  Intended Use, 6
Equipment and Materials Required, 6
  Customer-Supplied Equipment and Supplies, 7
  Storage Conditions, 7
Prepare the PA 800 Plus System, 7
  Install the PDA Detection Module, 8
  Clean the Interface, 8
  Insert the Cartridge and Calibrate the PDA, 8
Prepare the Samples, 8
  Prepare the SDS-MW Size Standard, 8
  Prepare the Protein Sample, 9
    Desalt the Protein Sample, 9
    Determine the Protein Sample Concentration, 9
    Prepare the Reduced Protein Sample, 10
    Perform a Buffer Exchange for the Protein Sample, 10
    Prepare the Non-reduced Protein Sample, 11
    Prepare the Alkylation Reagent (250 mM IAM Solution), 11
    Prepare the Sample, 11
Set Up the System, 12
  Methods, 12
    Prepare the Sample Vials, 12
    Prepare and Load the Buffer Vials, 13
      Prepare the Reagent Vials, 13
Run the Assay, 16
  Create the Sequence and Start the Run, 16
Capillary Cleaning and Storage, 21
Evaluate the Results, 22
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

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

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 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)

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Reorder Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary, 50 μm I.D. bare-fused silica</td>
<td>2</td>
<td>N/A</td>
</tr>
<tr>
<td>SDS-MW Gel Buffer - proprietary formulation, pH 8, 0.2% SDS</td>
<td>140 mL, 4-pack</td>
<td>A30341</td>
</tr>
<tr>
<td>SDS-MW Sample Buffer - 100 mM Tris-HCl, pH 9.0, 1% SDS</td>
<td>50 mL</td>
<td>N/A</td>
</tr>
<tr>
<td>SDS-MW Size Standard, 10 kDa to 225 kDa, 16 mg/mL</td>
<td>100 μL</td>
<td>N/A</td>
</tr>
<tr>
<td>Internal Standard, 10 kDa protein, 5 mg/mL</td>
<td>0.4 mL</td>
<td>A26487</td>
</tr>
<tr>
<td>Acidic Wash Solution, 0.1 M HCl</td>
<td>100 mL</td>
<td>N/A</td>
</tr>
<tr>
<td>Basic Wash Solution, 0.1 M NaOH</td>
<td>100 mL</td>
<td>N/A</td>
</tr>
</tbody>
</table>

**Table 2** Additional Supplies from SCIEX

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary, 50 μm I.D. bare-fused silica</td>
<td>1 box</td>
<td>N/A</td>
</tr>
<tr>
<td>SDS-MW Gel Buffer - proprietary formulation, pH 8, 0.2% SDS</td>
<td>140 mL, 4-pack</td>
<td>A30341</td>
</tr>
<tr>
<td>SDS-MW Size Standard, 10 kDa to 225 kDa, 16 mg/mL</td>
<td>3</td>
<td>A22196</td>
</tr>
</tbody>
</table>
Customer-Supplied Equipment and Supplies

- Pipettors and appropriate tips
- Vortex mixer
- Microcentrifuge
- Double-deionized (DDI) water (MS-grade water filtered through a 0.2 μm filter and with resistance greater than 18 MΩ)
- 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.

---

Table 2  Additional Supplies from SCIEX (Continued)

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal Standard, 10 kDa protein, 5 mg/mL</td>
<td>1</td>
<td>A26487</td>
</tr>
<tr>
<td>Universal vials</td>
<td>100</td>
<td>A62251</td>
</tr>
<tr>
<td>Universal vial caps - blue</td>
<td>100</td>
<td>A62250</td>
</tr>
<tr>
<td>Micro vials, 200 μL</td>
<td>50</td>
<td>144709</td>
</tr>
</tbody>
</table>

Table 3  Additional Required Reagents

<table>
<thead>
<tr>
<th>Component</th>
<th>Vendor</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-mercaptoethanol</td>
<td>Sigma-Aldrich</td>
<td>M7154, M6250</td>
</tr>
<tr>
<td>Iodoacetamide</td>
<td>Sigma-Aldrich</td>
<td>I-1149</td>
</tr>
<tr>
<td>Microcon-10 kDa centrifugal filter unit with Ultrace-10 membrane</td>
<td>Millipore</td>
<td>MRCF0R010</td>
</tr>
</tbody>
</table>
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

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 μL of the SDS-MW size standard into a 0.5 mL microcentrifuge vial.
4. Add 85 μL of the SDS-MW sample buffer to the microcentrifuge vial.
5. Add 2 μL of internal standard to the microcentrifuge vial.
6. Inside a fume hood, add 5 μL of 2-mercaptoethanol.
Cap the vial tightly, seal with parafilm, mix thoroughly, then heat in a water bath at 100 °C for 3 minutes.

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 μL to give a final protein concentration between 0.2 mg/mL to 2 mg/mL.

2. Add 2 μL of internal standard.

3. Inside a fume hood, add 5 μL of 2-mercaptoethanol.

4. Cap the vial tightly, seal with parafilm, and mix thoroughly.

5. Heat in a water bath at 100 °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.
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 μL to give a final protein concentration between 0.2 mg/mL to 2 mg/mL.
2. Add 2 μL of the 10 kDa internal standard to the sample tube.
3. Inside a fume hood, add 5 μL 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 μL 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]

1. Universal Cap  
2. Micro Vial  
3. Universal Vial  
4. Micro Vial inside Universal Vial
Prepare and Load the Buffer Vials

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.

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

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 (H₂O) 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.
**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 (< ½ 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

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>H2O Cycle 17-24</td>
<td>H2O Cycle 17-24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>H2O Cycle 9-16</td>
<td>H2O Cycle 9-16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>H2O Cycle 1-8</td>
<td>H2O Cycle 1-8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>H20 Cycle 17-24</td>
<td>Gel-R Cycle 17-24</td>
<td>Gel-S Cycle 17-24</td>
<td>NaOH Cycle 17-24</td>
<td>HCl Cycle 17-24</td>
</tr>
<tr>
<td>2</td>
<td>H20 Cycle 9-16</td>
<td>Gel-R Cycle 9-16</td>
<td>Gel-S Cycle 9-16</td>
<td>NaOH Cycle 9-16</td>
<td>HCl Cycle 9-16</td>
</tr>
<tr>
<td>1</td>
<td>H20 Cycle 1-8</td>
<td>Gel-R Cycle 1-8</td>
<td>Gel-S Cycle 1-8</td>
<td>NaOH Cycle 1-8</td>
<td>HCl Cycle 1-8</td>
</tr>
</tbody>
</table>

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

A1 to A6: DDI H2O, use in dip step to clean capillary tip, 1.5 mL
B4 to B6: DDI H2O, 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 H2O, use to precondition capillary, 1.5 mL.
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).
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.
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”

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

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.
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

<table>
<thead>
<tr>
<th>Mol. Wt.</th>
<th>Migration Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.1530000</td>
</tr>
<tr>
<td>2</td>
<td>14.167000</td>
</tr>
<tr>
<td>3</td>
<td>16.125000</td>
</tr>
<tr>
<td>4</td>
<td>17.808000</td>
</tr>
<tr>
<td>5</td>
<td>21.267000</td>
</tr>
<tr>
<td>6</td>
<td>23.403000</td>
</tr>
</tbody>
</table>

Fit type: Quadratic
Reference Peak Time (min): 12.15
Reference Window %: 10

Goodness of Fit: 0.988127
## Troubleshooting

### Table 4  Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Corrective Action</th>
</tr>
</thead>
</table>
| Low or unsteady current resulting in slow migration and poor resolution resulting in failed system suitability test; current should be close to −25 µA | 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 *PA 800 Plus System Maintenance Guide*.        |
| 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 *Prepare the Samples*. |
|                                                                        | Deteriorated capillary          | Replace the capillary when other attempts to reduce peak broadening fail.         |
|                                                                        | Dust or gel build up on capillary end | Clean the capillary tip using DDI water. Refer to *Clean the Interface*.        |
Table 4 Troubleshooting (Continued)

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Corrective Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>No peaks or low signal</td>
<td>Capillary inlet longer than the inlet electrode</td>
<td>Push the capillary up or cut the capillary inlet to make sure it is the same length as the electrode.</td>
</tr>
</tbody>
</table>
| Dirty or plugged capillary tip | 1) Clean the capillary tip using DDI water. Refer to Clean the Interface.  
2) Replace 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 Perform a Buffer Exchange for the Protein Sample. |                                                                                   |
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.

Hazard classification according to HCS 2012.

Hazardous Substances

Acidic Wash 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.

SDS-MW Gel Buffer

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

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 and download the latest version of the guide.