ProteomeLab™ PA 800 and P/ACE™ MDQ *plus* Systems

Protein Method Development Guide

SCIEX

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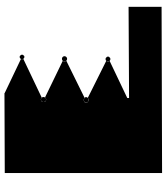

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Introduction

With capillary zone electrophoresis (CZE) proteins are separated by their electrophoretic mobility, a function of the charge and mass of a protein at a given pH. When using electrolytes with pH >4.0, the surface of bare-fused silica becomes negatively charged, inducing both electroosmotic flow (EOF) and the adsorption of proteins that possess a net positive charge. The SCIEX Protein Methods Development kit contains coated capillaries, buffers, standards and EOF markers to allow you to optimize a separation method for the analysis of a broad spectrum of proteins. The use of this neutral surface serves to significantly reduce EOF and to prevent adsorption of proteins to the capillary surface. These Neutral Capillaries have a two-layer coating, with the first layer a bonded phase serving to deactivate the silanol groups and a second hydrophilic layer that protects against hydrophobic interactions, improving the overall efficiency and resolution of proteins separated by CZE.

Note: This chemistry kit is designed to be used with CE systems equipped with a UV detector. Do not use a photodiode array (PDA) detector because its high energy will degrade the capillary coating.

Safety

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

General Precautions

- Before beginning the procedure, review this manual carefully and make sure all required materials and kit components are present.
- Equilibrate all samples and reagents to room temperature (20°C to 25°C) before use.
- Avoid exposing samples and reagents to excessive heat or light during storage.
- Follow the guidelines listed on the labels for proper storage requirements.
- Do not freeze capillary and buffer solutions.
- Do not use the kit after the expiration date.

Principle of the Method

This document is a general guide to help the user develop methods to separate proteins using a neutral coated capillary designed to reduce protein adsorption and minimize electroosmotic flow (EOF). While the suggested procedures are expected to give good results in many applications, variables such as temperature, concentration, time, capillary length, voltage and pH may need to be altered to optimize the separation.

The Neutral Capillary is designed to reduce the electrostatic interactions between proteins and the capillary wall by deactivating the silanol groups on the silica capillary wall. As a result, the EOF of the coated capillary is extremely slow (less than 5% of the EOF determined for bare fused silica). The pH 3 and pH 6 buffers provided in this kit are used for the separation of proteins with isoelectric points greater than pH 4.0 and pH 6.7, respectively, using normal polarity (inlet is on the cathode side). The pH 8 buffer is used for the separation of proteins with isoelectric points less than pH 6.7, using reverse polarity. It is, therefore, necessary to determine the pH of the run buffer and set the polarity before starting a run (Table 1-1).

Table 1-1 pH Range and Marker for the Buffers in the Kit

Buffer	Buffer pH	Marker	Polarity	Applicable pH Range
Citrate	3	Histamine	Normal	> 4.0
Citrate/MES	6	Histamine	Normal	≥ 6.7
Tricine	8	Orange G	Reversed	≤ 6.7

The capillary is designed to be effective between pH 3 and pH 8. Use of buffers outside this pH range may shorten the life of the capillary.

Capillary Zone Electrophoresis generally uses an electric field in the range of 250 V/cm to 500 V/cm. Since the EOF of the Neutral Capillary is negligible, it is recommended that a high voltage be used in order to obtain the greatest peak efficiencies.

Note: The maximum electric field the coated capillary can tolerate is 500 V/cm. The use of field strength higher than this will result in shorter lifetime of the capillary.

Although the Neutral Capillary is coated with a hydrophilic layer that reduces the adsorption of proteins on the capillary wall, sample clean-up may still be necessary prior to injection. Also, if peak broadening begins to occur, or if migration times begin to increase, the capillary surface can be regenerated by rinsing the capillary with 0.1 N hydrochloric acid for 30 to 60 seconds prior to performing a 1.5-minute rinse with buffer.

Materials and Reagents

Note: Performance specifications of this product are based on using the buffers supplied in this kit or replacements supplied by SCIEX.

Table 1-2 Kit Contents (PN 477455)

Component	Quantity	Reorder PN
Neutral Capillary, 50 µm ID	1	477441
Orange G Reference Marker, 0.1% aqueous solution	1 mL	241524
Histamine Reference Marker, 1% aqueous solution	1 mL	477446
Citrate Buffer, pH 3, 50 mM	100 mL	477442
Citrate/MES Buffer, pH 6, 50 mM	100 mL	477443
Tricine Buffer, pH 8, 50 mM	100 mL	477444
Protein Test Mix (1 mg each of lysozyme, ribonuclease A, and cytochrome C)	1	477436

Table 1-3 Materials Needed but Not Provided in This Kit

Description	Part Number	System	
		PA 800	P/ACE MDQ *plus*
From SCIEX			
PCR vials (100-pack)	144709	✓	✓
2 mL glass vials (100-pack)	144980	✓	
Red caps for 2mL glass vials (100-pack)	144648	✓	
PCR vial holders (50-pack)	144657	✓	
PCR vial springs (10-pack)	358821	✓	
Gray PCR vial caps (50-pack)	144656	✓	
Universal plastic vials (100-pack)	A62251		✓
Blue rubber caps for universal vials (100-pack)	A62250		✓
From Other Laboratory Suppliers			
Double-deionized (DDI) water with 16 to 18 megaohm resistance, filtered with 0.2 µm pore filter	Various	✓	✓
0.1 N HCl	Various	✓	✓
0.2 µm nylon syringe filter	Various	✓	✓
Adequate pipettes and pipette tips	Various	✓	✓

Storing Kit Components

Upon receipt, store all components at 2°C to 8°C.

Preparing the Test Mix

Note: The Test Mix is intended to be used to check the performance of the capillary at pH 3 or pH 6 using normal polarity. No test mix is supplied to test the separation using reverse polarity at pH 8, but an injection of 0.01% Orange G Reference Marker should give a sharp peak at 3 to 4 minutes under these separation conditions.

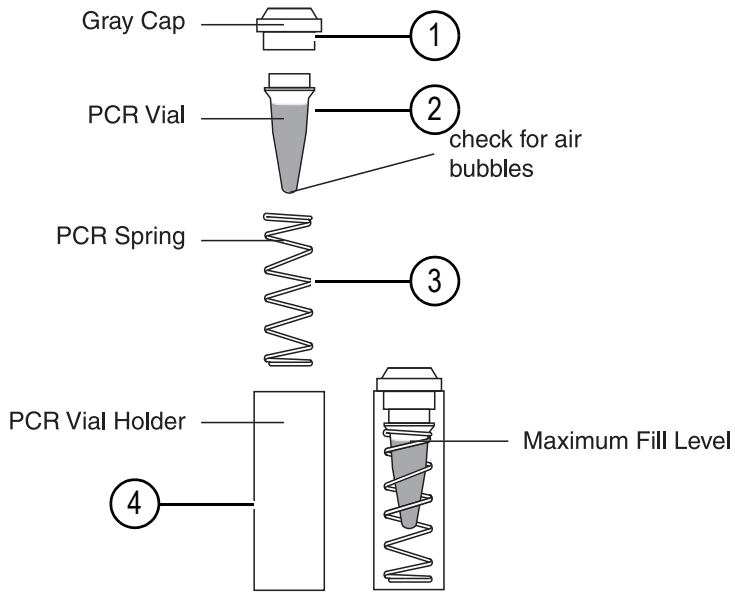
1. Add 1 mL of DDI water to the dry Test Mix and mix well until dissolved.
2. Set aside 100 µL in a 200 µL PCR vial to use in step 4 below.
3. Pipette 100 µL aliquots of the remaining solution and store promptly between -35°C and -15°C. Avoid repeated freezing and thawing.
4. Add 10 µL Histamine Reference Marker to the Test Mix sample and mix well.
5. Pipette 100 µL of test or sample mix into a PCR vial.
6. Make sure there are no air bubbles at the bottom of the vial. Air bubbles can affect the sample injection.

   If bubbles exist, centrifuge the vials for 2 minutes at 1000 x g and repeat if necessary.
7. Follow the appropriate instructions for your system to set up the vials:
   - PCR Vial Setup–PA 800 System
   - Universal Vial Setup–P/ACE MDQ plus System

PCR Vial Setup–PA 800 System

Place the PCR vial in a PCR vial holder equipped with a vial spring and seal with a clean gray cap (Figure 1-1).

Figure 1-1 PCR Vial Setup–PA800 System

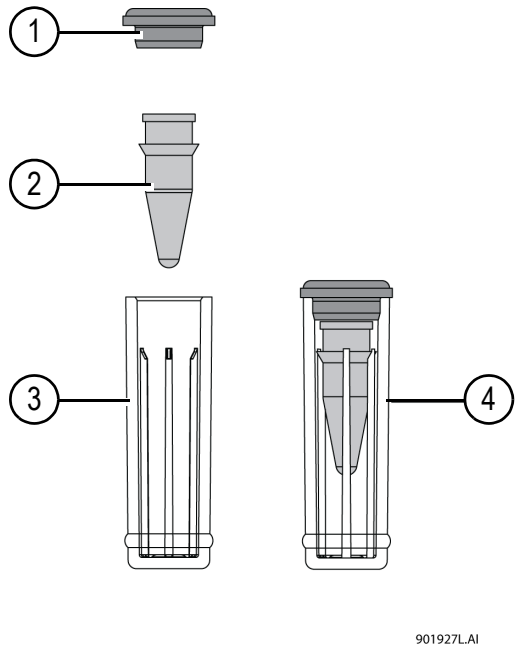


Item	Description
1	Vial cap (PN 144656)
2	PCR vial (PN 144709)
3	PCR vial spring (PN 358821)
4	PCR vial holder (PN 144657)

Universal Vial Setup–P/ACE MDQ *plus* System

Place the PCR vial into the universal vial and seal with a clean blue cap (Figure 1-2).

Figure 1-2 Universal Vial Setup–P/ACE MDQ *plus* System



Item	Description
1	Universal vial cap (PN A62250)
2	PCR vial (PN 144709)
3	Universal vial (PN A62251)
4	PCR vial inside of universal vial

Preparing the Sample

The suggested protein sample concentration is between 50 µg/mL and 1000 µg/mL for optimum separations. This range may vary depending upon the wavelength and buffers used.

1. Add 10 µL of Reference Marker to 100 µL of sample solution. Use the appropriate marker for the buffer:
   - For Citrate buffer (pH 3) or Citrate/MES Buffer (pH 6)–use the Histamine Reference Marker
   - For Tricine buffer (pH 8)–use the Orange G Reference Marker

Preparing the Buffer Vials

Use the correct vials and caps for your system:

- For the P/A 800 system–use glass vials and red caps, and fill with 1.8 mL of buffer
- For the P/ACE MDQ *plus* system–use universal vials and blue caps, and fill with 1.4 mL of buffer

Fill three vials with the selected buffer. Use the Citrate/MES Buffer (pH 6) to separate the Test Mix. Sonicate or degas the buffer to remove small bubbles that might interfere with the separation.

Note: Buffer solutions may develop microbiological growth. If signs of cloudiness appear in the buffer, filter the buffer through a 0.2 µm filter prior to use.

Cleaning the Capillary Interface

Carefully clean the system electrodes and interface block as described in the “Maintenance Procedure” section of the instrument manual. Repeat this procedure after every 24 hours of operation.

Installing the Capillary

Install the Neutral Capillary into a capillary cartridge using the *Capillary Cartridge Rebuild Instructions* (PN 144655). Use a 100 x 200 µm aperture (labeled “2”).

The length of the capillary depends on the separation requirements and nature of the samples. In general, samples are well separated on a 30.2 cm capillary. A 40.2 cm capillary may have better resolution but at the expense of a longer separation time.

Note: After the cartridge is installed, it is critical to the performance of the coated capillary that the ends are not exposed to air for longer than 20 minutes to avoid drying. Be sure to immerse the ends of the capillary in vials of DDI water.

Capillary Equilibration

After installing a new capillary, perform a rinse for 1 minute (30 psi) with 0.1N HCl. Follow this with a 10-minute rinse (30 psi) with the selected pH buffer. Equilibrate the capillary with the buffer by applying 500 V/cm for 10 minutes. Follow this equilibration with a 10-minute buffer rinse (30 psi).

Capillary Reconditioning

When changing to a different pH buffer, recondition the capillary by performing a 10-minute rinse (30 psi) with the new buffer. Allow the new buffer to remain in the capillary, without voltage, for 10 minutes prior to performing a separation.

Storing the Capillary

Short Term Storage (<24 hours)

1. Perform a 5-minute rinse (30 psi) with DDI water.
2. The capillary may be stored on the instrument with capillary ends immersed in DDI water.
3. Perform a 5-minute rinse (30 psi) with DDI water before performing a separation, whenever the capillary has not been used for 3 hours or longer.

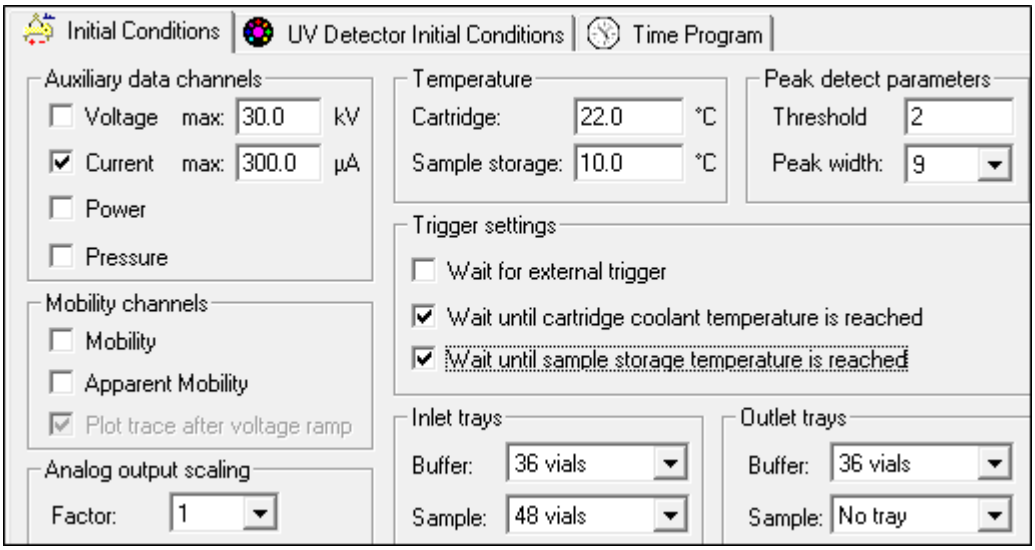
Long Term Storage (>24 hours)

1. Perform a 1-minute (30 psi) rinse with 0.1 N HCl. Follow this with a 3-minute (30 psi) rinse of unused Citrate/MES Buffer, pH 6.0.
2. Remove the capillary from the instrument and place in the capillary storage box with the ends submerged in vials of unused Citrate/MES Buffer, pH 6.0.
3. Store the capillary storage box at 2°C to 8°C in an upright position.
4. Perform a 5-minute rinse (30 psi) with DDI water before performing a separation.

Performing a Test Run

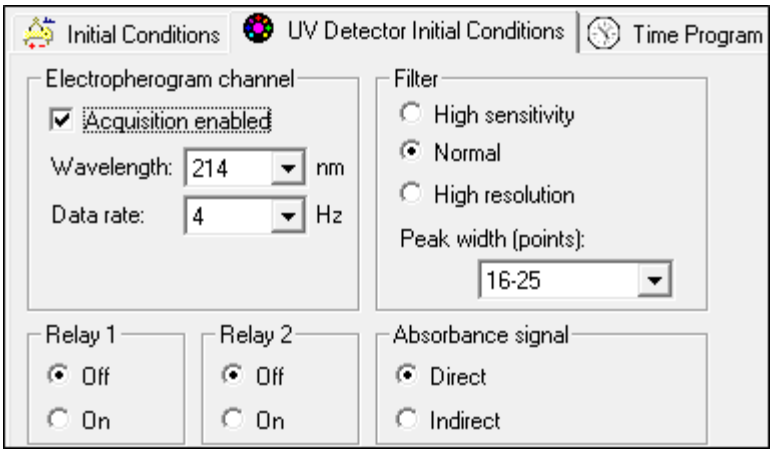
1. Clean the electrodes, injection ports and capillary ends to ensure reproducibility of migration time.
2. Program a test method using the following separation conditions.
   a. Set the parameters in the **Initial Conditions** tab as shown in Figure 1-3.

Figure 1-3 Initial Conditions Tab



b. Set the parameters in the **UV Detector Initial Conditions** tab as shown in Figure 1-4.

Figure 1-4 UV Detector Initial Conditions Tab



c. Set the parameters in the **Time Program** tab as shown in Figure 1-5.

Figure 1-5 Time Program Tab

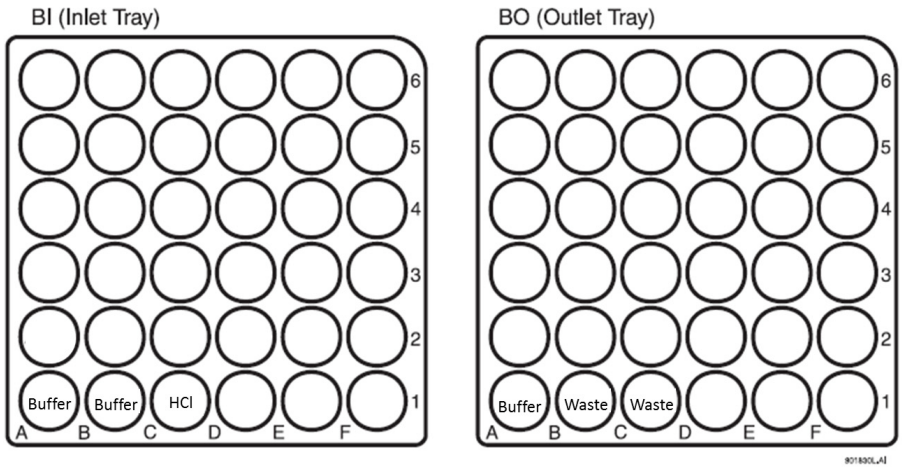
Initial Conditions | UV Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	20.0 psi	1.00 min	BI:C1	BO:C1	forward, In / Out vial inc 15	0.1N HCL rinse
2		Rinse - Pressure	20.0 psi	2.00 min	BI:B1	BO:B1	forward, In / Out vial inc 15	Buffer rinse
3		Inject - Pressure	0.5 psi	3.0 sec	SI:A1	BO:B1	Override, forward	Injection
4	0.00	Separate - Voltage	15.5 KV	20.00 min	BI:A1	BO:A1	0.17 Min ramp, normal polarity, In / Out vial inc 15	Separation
5	1.00	Autozero						
6								

3. Prepare the Test Mix, Citrate/MES Buffer (pH 6) vials, the 0.1 N HCl rinse, and DDI water vials.
   - For the P/A 800 system–use glass vials and red caps, and fill with 1.8 mL
   - For the P/ACE MDQ *plus* system–use universal vials and blue caps, and fill with 1.4 mL

4. Place the vials in the positions shown in Figure 1-6.

Figure 1-6 Buffer Tray Configuration



5. Run the test method.

Checking the Results

A typical result for the test run is shown in Figure 1-7 with the corresponding current shown in Figure 1-8. The first peak, Histamine Reference Marker, normally migrates at 2.5 min to 3.5 min. For best migration time reproducibility, the run buffer vials should be replaced every 15 runs and the rinse buffer vial refilled as needed. The reproductivity of an analytes mobility for separations made from this kit is typically less than 2% relative standard deviation, run-to-run, day-to-day.

Note: When using the Citrate Buffer, pH 3.0, with a 30.2 cm capillary and an electric field of 500 V/cm, the migration time of the Histamine Reference Marker should be 2 to 3 minutes.

Figure 1-7 Electropherogram of Test Run

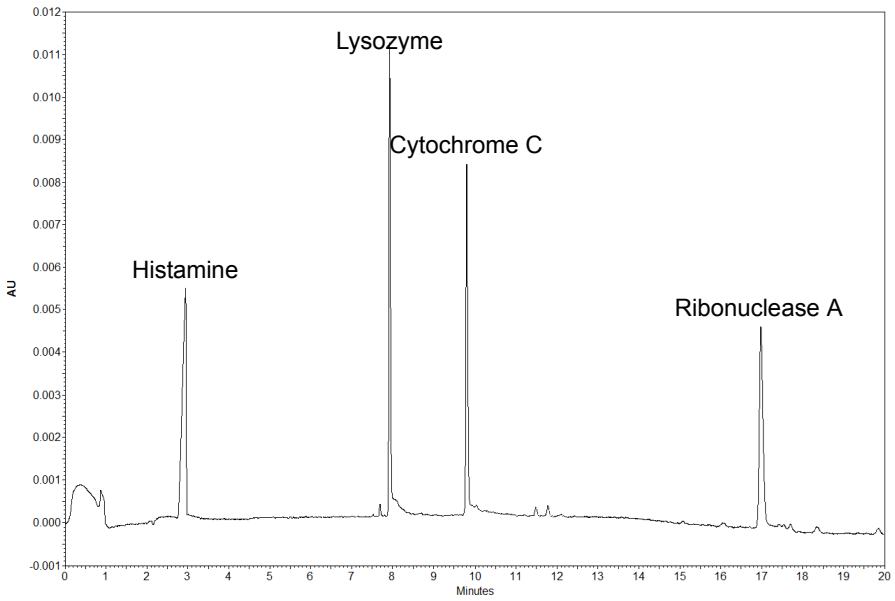
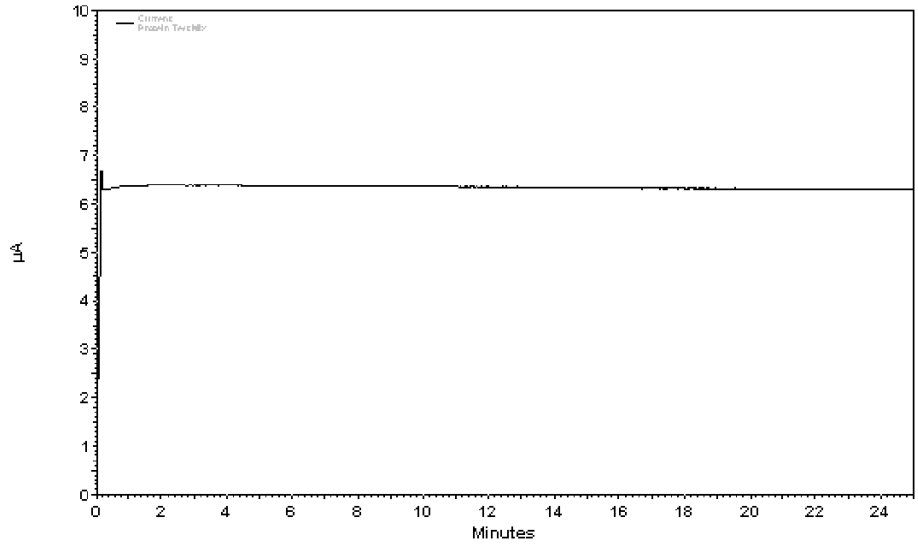


Figure 1-8 Current During the Test Run



Running a Sample

Samples are run as described in Performing a Test Run on page 12 with the following differences:

1. Set the polarity in the **Time Program** tab as required.

   If the Tricine Buffer, pH 8, is used to separate a negatively charged sample, reverse the polarity (inlet on the cathode side).

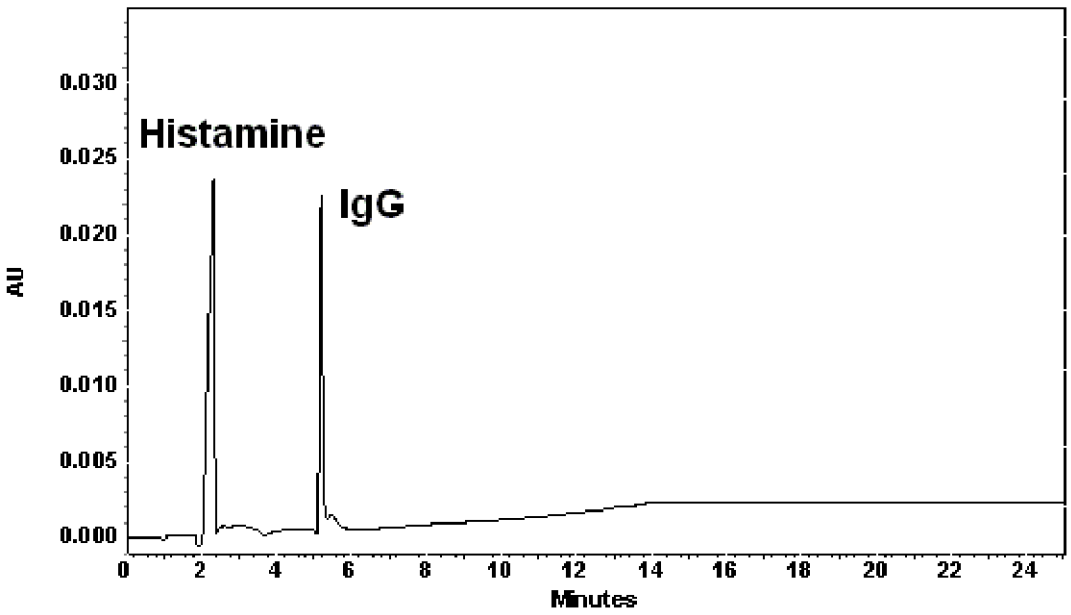
2. Pipette 100 µL of sample into a PCR vial. Follow the appropriate instructions for your system to set up the vials:
   - For the P/A 800 system–use glass vials and red caps (Figure 1-1)
   - For the P/ACE MDQ *plus* system–use universal vials and blue caps (Figure 1-2)
3. Place the sample vial in the inlet sample tray.

   Make sure its position matches the position in the method or sequence.
4. Adjust the inject time or pressure value in the **Time Program** tab of the separation method to optimize peak height and shape.
5. Recondition the capillary with the selected buffer (refer to Capillary Reconditioning on page 11).
6. Perform the separation.

Note: Some proteins might interact with histamine or orange G, leading to broad and/or split peaks. To confirm this interaction, inject the sample and reference marker separately into the capillary, instead of injecting the mixture of sample and reference marker.

An example separation of immunoglobulin (IgG) is shown in Figure 1-9 with the Citrate Buffer, pH 3.0, using a 30.2 cm capillary and a 500 V/cm electric field.

Figure 1-9 Electropherogram of IgG Sample with Citrate Buffer at pH 3.0



Troubleshooting

Problem	Possible Cause	Corrective Action
Unsteady or low current (<5 µA for Citrate/MES Buffer or Citrate Buffer and <3 µA for Tricine Buffer on 30.2 cm capillary and 500 V/cm).	Capillary is plugged.	Terminate the run, and perform a 5 minute pressure rinse at 40 psi with water to flush the capillary from both ends.
	Broken capillary.	Replace the capillary.
	Air in capillary.	Be sure that buffer vials are adequately filled.
	Contamination on the electrode.	Clean electrodes, ejectors, and interface block.
High current or spikes in current (> 8 µA for Citrate/MES or Citrate Buffer and > 6 µA for Tricine Buffer on 30.2 cm capillary and 500 V/cm).	Contaminated buffer.	Replace buffer as needed.
	Contamination on electrode.	Clean electrodes, ejectors, and interface block.
	HCl still in capillary during the run	Increase buffer rinse time and/ or pressure value in separation method.
	Inadequate conditioning of capillary.	Repeat conditioning with selected buffer, using the recommended method.
Poor migration time reproducibility.	Material adsorbed onto capillary coating.	Rinse the capillary with 0.1 N HCl for 30 to 60 seconds prior to performing a 1.5-minute rinse with buffer
	Aged buffer solution or samples.	Replace buffer or sample vials.
	Plugged capillary.	Terminate the run, and perform 5-minute pressure rinse (40 psi) with water to flush the capillary from both ends.
	Contamination on electrodes and/or build-up of material on the injection port.	Clean electrodes, ejectors, and interface block.
	Deteriorated capillary.	Replace the capillary.
Broad peaks, tailing or low efficiency.	Deteriorating sample or buffer.	Replace buffer or sample as needed
	Material adsorbed onto capillary coating.	Rinse the capillary with 0.1 N HCl for 30 to 60 seconds prior to performing a 1.5-minute rinse with buffer
	Contamination on electrode.	Clean electrodes, ejectors, and interface block.
	Deteriorated capillary.	Replace the capillary.

Problem	Possible Cause	Corrective Action
No peak or low signal intensities.	Polarity of instrument.	Use appropriate polarity
	Capillary is plugged.	Terminate the run, and perform 5-minute pressure rinse (40 psi) with water to flush the capillary from both ends.
Spikes in electropherogram.	Air in the buffer.	Make sure buffer is at room temperature and air is removed by degassing or sonicating.