

# SCIEX Anion Analysis Kit

For P/ACE™ MDQ and P/ACE™ MDQ *plus* Capillary Electrophoresis Systems

*Instruction Guide*



A49108AE  
May 2015

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## Introduction

The SCIEX Anion Analysis Kit contains the supplies necessary for the separation and quantitation of anions, using the SCIEX P/ACE MDQ and P/ACE MDQ *plus* Capillary Electrophoresis Systems. Each anion kit yields approximately 500 tests.



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**Note:** The system must be equipped with a UV detector and a 230 nm filter to perform this assay.

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This kit permits the analysis of small inorganic anions and organic acids, which are often UV transparent. For this reason, the separation buffer contains a chromophore, and detection is achieved in indirect mode.

The separation method is performed under reverse polarity so that the negatively charged ions migrate toward the anode (the positively-charged electrode). In addition, the capillary is dynamically coated with a polycation, which reverses the electro-osmotic flow (EOF) toward the anode, thus reducing the separation time while maximizing migration time reproducibility.

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**IMPORTANT:** The main focus of this application is in the biopharmaceutical market. This product can also be used for environmental testing and food and beverage markets. This product is **for research use only**. It is not for use in diagnostic procedures. No clinical decision or patient notification may be made based on results using this research assay.

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## Safety

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

## Materials and Reagents

**Table 1-1 Kit Contents (PN A53537)**

Component	Quantity
Anion Coating	1
Anion Separation Buffer	1
Conditioner — Na	1
Anion Acid Rinse	1
Anion Internal Standard	1
Anion Organic Test Mix	1
Anion Inorganic Test Mix	1
Capillary, 50 cm, 75 µm I.D.	3 pieces
Rinse Solution	1
Ion Analysis Insert	1

**Table 1-2 Materials Required but Not Included in This Kit**

Description	Part Number	P/ACE System	
		MDQ	MDQ <i>plus</i>
230 nm filter (see note below)	144433	✓	✓
Adequate pipettes and pipette tips		✓	✓
PCR vials (100-pack)	144709	✓	✓
2 mL glass vials (100-pack)	144980	✓	
Red caps for 2 mL glass vials (100-pack)	144648	✓	
PCR vial holders (50-pack)	144657	✓	
PCR vial springs (10-pack)	358821	✓	
Gray caps for PCR vials (50-pack)	144656	✓	
Universal plastic vials (100-pack)	A62251		✓
Blue rubber caps for universal vials (100-pack)	A62250		✓



**Note:** A 230 nm filter is required for the analysis. This filter is included with the P/ACE MDQ *plus* system and is installed at position 4. However, it is not provided for the P/ACE MDQ system and must be purchased separately.

## Storing Kit Components

Upon receipt, store all components at room temperature and away from direct sunlight.

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## Cleaning Vial Caps



**Note:** The vial caps may contain impurities that can be detected with the Anion Analysis Kit, therefore wash the caps before use.

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1. Using a clean beaker, rinse the vial caps twice with double-deionized (DDI) water. Do not use soap.
2. Let the caps soak in DDI water for at least one hour, making sure the caps are completely submerged.
3. Remove the caps from the water.
4. To dry the caps, either place them in an oven set at 55°C for two hours, or allow them to dry overnight at room temperature covered by clean, lint-free laboratory tissue.

The vial caps become compressed and lose elasticity during use, which can lead to pressure failures and current leakage errors. Therefore, reusing caps is not recommended.

## Turning on the UV Lamp

Turn on the UV lamp and allow the system to warm up for at least 30 minutes prior to experimentation.

## 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

1. Install a 75  $\mu\text{m}$  I.D., 60.2 cm long (50 cm from injection site to detector) fused-silica capillary into a capillary cartridge using the *Capillary Cartridge Rebuild Instructions* (PN 144655).
2. Use an 800  $\mu\text{m}$  aperture in the cartridge. This aperture is labeled with an “8.”
3. After the capillary has been installed in the cartridge, insert the cartridge in the instrument.
4. Close the cartridge cover and tray cover.

## Conditioning a New Capillary

After installing a new capillary, rinse the capillary for one minute with Conditioner — Na. Wait four minutes, then rinse for 30 seconds with Conditioner — Na. Rinse for one minute with Rinse Solution. Use 20 psi of pressure for all rinses.

## Storing the Capillary

After use, store the capillary on the instrument or in the original capillary storage box, with both ends submerged in Rinse Solution. Do not allow the capillary ends to dry, because the capillary may become plugged.

After a long storage period, or at the start of each day, condition the capillary using the Capillary Conditioning method described in [Running Methods on page 11](#).

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**Caution: Do not share capillaries between applications. If the capillary has been used for cation analysis, do not use it for anion analysis.**

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## Preparing the Buffer Trays

Use the correct vials and caps for your system:

- For the P/ACE MDQ system—use glass vials and red caps
- For the P/ACE MDQ *plus* system—use universal vials and blue caps

Replace all vials after twenty runs or after 24 hours inside the instrument. The increment option in the method can be used to automatically increment the vials every twenty runs on both buffer trays.

1. Fill the vials with equal volumes of each reagent in [Table 1-1](#) and position them in the buffer trays (refer to [Figure 1.1](#)).

Use the correct volume for your system:

- For the P/ACE MDQ system—1.4 mL
- For the P/ACE MDQ *plus* system—1.5 mL

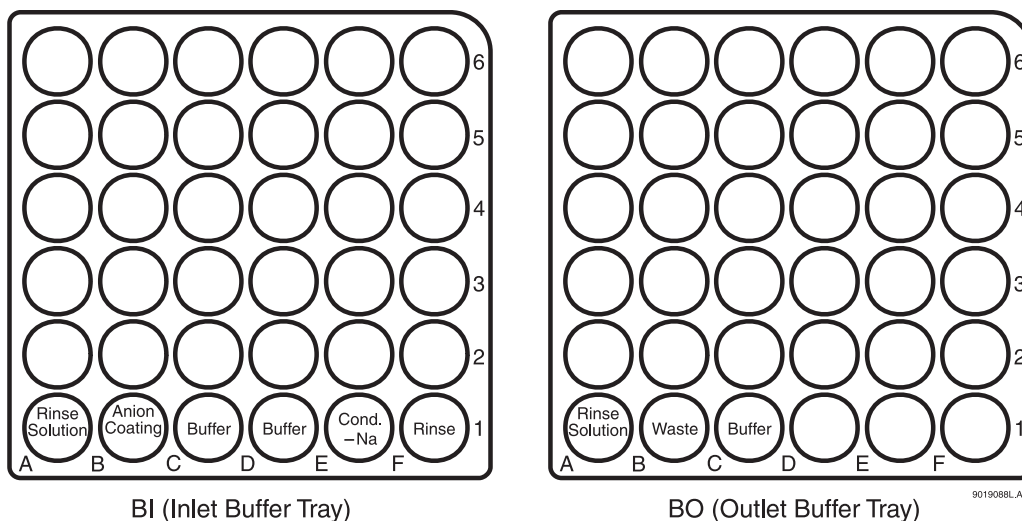
2. In the **Waste** position, place a vial partially filled with Rinse Solution.

Use the correct volume for your system:

- For the P/ACE MDQ system—700  $\mu$ L
- For the P/ACE MDQ *plus* system—600  $\mu$ L

3. Close each vial with a clean cap.



**Figure 1.1 Buffer Tray Configuration for Anion Analysis**

4. Load the Inlet Buffer and Outlet Buffer trays in the instrument.



**Note:** When analyzing fluoride and/or phosphate ions, fill a vial with Anion Acid Rinse and place it in position F1 of the Inlet Buffer Tray. The use of the Anion Acid Rinse is required when running the Anion Inorganic Test Mix to avoid peak tailing.

**Note:** The rinse solution used in this kit is ultra-purified water specifically for capillary electrophoresis analysis of ions.

## Preparing the Sample

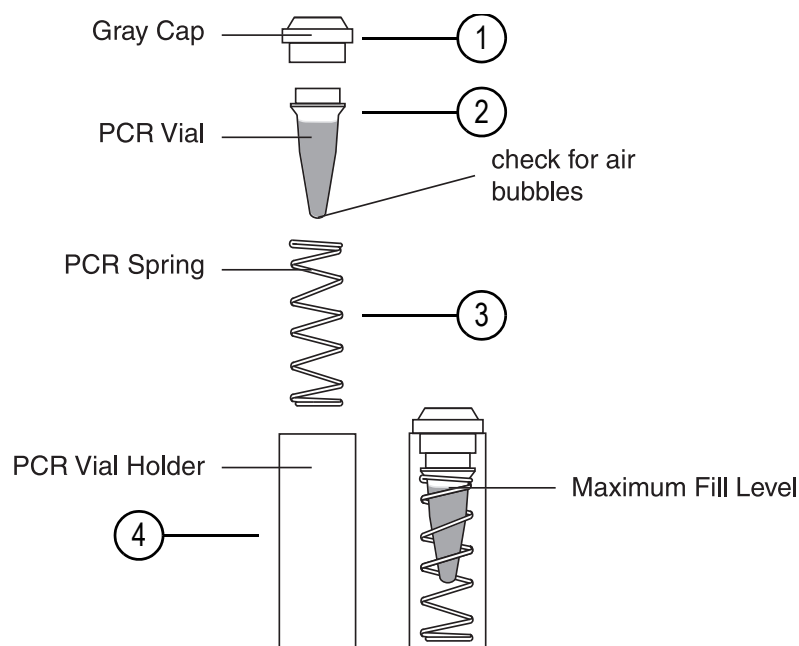
Depending on the concentration of the analytes, the sample should be injected as is or diluted. Dilution should be done so that the final concentration of the sample anions is between 5 ppm and 200 ppm. Special care should be taken to verify the pH of the sample, which should be above pH 5. A 50 mM NaOH solution can be used to dilute the sample and adjust its pH value.

The Anion Internal Standard (I.S.) consists of 30 mM sodium octanoate (sodium caprylate), which is equivalent to 4296 ppm of octanoate ion. The I.S. can be used in the quantitation of the sample anions. To use it, dilute the I. S. by a factor of 50 with the sample. For example, mix 4  $\mu$ L of I.S. with 200  $\mu$ L of sample to yield 86 ppm of octanoate ion.

## PCR Vial Setup–P/ACE MDQ System

Fill a PCR vial with 200  $\mu\text{L}$  of test or sample mix. Make sure there are no air bubbles at the bottom of the PCR 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. Place the PCR vial in a PCR holder equipped with a vial spring (Figure 1.2). Seal the PCR vial with a clean gray cap and place it in the inlet sample tray.

**Figure 1.2 PCR Vial Setup–P/ACE MDQ System**

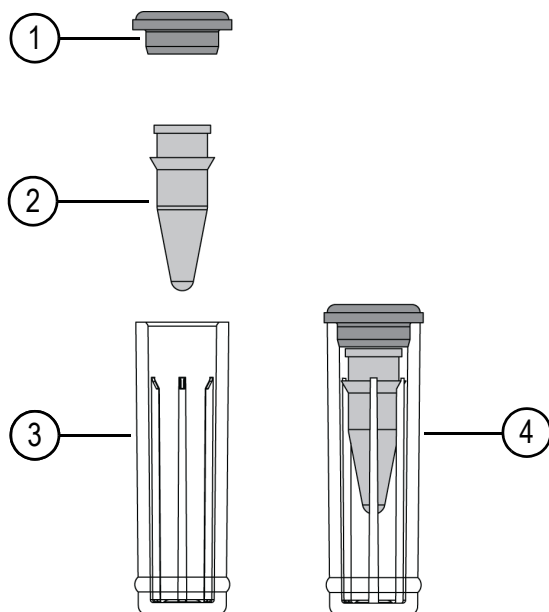


Item	Description
1	Vial cap (PN144656)
2	PCR vial (PN 144709)
3	PCR vial spring (PN 358821)
4	PCR vial holder (PN 144657)

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

Fill a PCR vial or micro vial with 200  $\mu\text{L}$  of test or sample mix. 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. Place the vial into the universal vial and seal with a blue cap (Figure 1.3).

**Figure 1.3 Universal Vial Setup–P/ACE MDQ *plus* System**



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Item	Description
1	Universal vial cap (PNA62250)
2	PCR vial (PN 144709)
3	Universal vial (PN A62251)
4	Micro vial inside of universal vial

## Running Methods

Three methods are required for performing anion analysis:

- Anion Capillary Conditioning
- Anion Separation
- Anion Shutdown

Save all three methods, with their respective names, in the 32 Karat folder.



**Note:** These methods can be downloaded from [sciex.com/products/capillary-electrophoresis-instruments/p/ace-mdq-plus](https://sciex.com/products/capillary-electrophoresis-instruments/p/ace-mdq-plus) (click **Resources**).

## Initial Conditions for All Methods

All three methods utilize the same **Initial Conditions** (Figure 1.4) and **UV Detector Settings** (Figure 1.5).



**Note:** When analyzing samples containing inorganic anions or organic acids that absorb in the low UV (such as nitrate and azide), the detection wavelength (Figure 1.5) should be set at 254 nm for optimal detection. Save this method with a different name, such as “Anion Separation Method at 254 nm”.

**Figure 1.4 Initial Conditions for Anion Capillary Conditioning, Anion Separation, and Anion Shutdown Methods**

**Figure 1.5 UV Detector Initial Conditions for Anion Capillary Conditioning, Anion Separation, and Anion Shutdown Methods**

Figure 1.6 Time Program for Anion Capillary Conditioning Method

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:E1	BO:B1	forward	Rinse with Conditioner-Na.
2		Rinse - Pressure	20.0 psi	1.00 min	BI:F1	BO:B1	forward	Rinse with Rinse Solution.
3		Rinse - Pressure	20.0 psi	0.50 min	BI:B1	BO:B1	forward	Rinse with Anion Coating.
4		Rinse - Pressure	20.0 psi	0.50 min	BI:C1	BO:B1	forward	Rinse with Anion Separation Buffer.
5	0.00	Separate - Voltage	30.0 KV	10.00 min	BI:D1	BO:C1	1.00 Min ramp, reverse polarity	Separation with Anion Separation Buffer.
6	10.00	Stop data						
7	10.10	Rinse - Pressure	20.0 psi	0.50 min	BI:E1	BO:B1	forward	Rinse with Conditioner-Na.
8	10.60	Rinse - Pressure	20.0 psi	0.50 min	BI:F1	BO:B1	forward	Rinse with Rinse Solution.
9	11.10	End						
10								

## Time Programs for Anion Separation Methods

There are two Anion Separation methods: one for the P/ACE MDQ system and the other for the P/ACE MDQ *plus* system. The difference between the two methods is the duration of the Anion Coating rinse (step 1 in the Time Program):

- For the P/ACE MDQ system—0.5 min (Figure 1.7)
- For the P/ACE MDQ *plus* system—1.0 min (Figure 1.8)

Figure 1.7 Time Program for Anion Separation Method—P/ACE MDQ System

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	0.50 min	BI:B1	BO:B1	forward	Rinse with Anion Coating .
2		Rinse - Pressure	20.0 psi	0.50 min	BI:C1	BO:B1	forward	Rinse with Anion Separation Buffer.
3		Inject - Pressure	0.5 psi	8.0 sec	SI:A1	BO:A1	Override, forward	Sample Injection
4		Inject - Pressure	0.1 psi	10.0 sec	BI:A1	BO:A1	No override, forward	Water Injection
5	0.00	Separate - Voltage	30.0 KV	8.00 min	BI:D1	BO:C1	1.00 Min ramp, reverse polarity	Separation
6	1.25	Autozero						
7	8.00	Stop data						
8	8.10	Rinse - Pressure	20.0 psi	0.50 min	BI:E1	BO:B1	forward	Rinse with Conditioner - Na.
9	8.60	Rinse - Pressure	20.0 psi	0.50 min	BI:F1	BO:B1	forward	Rinse with Rinse Solution or Anion Acid Rinse.
10	9.10	End						
11								

Figure 1.8 Time Program for Anion Separation Method—P/ACE MDQ *plus* System

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:B1	BO:B1	forward	Rinse with Anion Coating.
2		Rinse - Pressure	20.0 psi	0.50 min	BI:C1	BO:B1	forward	Rinse with Anion Separation Buffer
3		Inject - Pressure	0.5 psi	8.0 sec	SI:A1	BO:A1	Override, forward	Sample Injection
4		Inject - Pressure	0.1 psi	10.0 sec	BI:A1	BO:A1	No override, forward	Water Injection
5	0.00	Separate - Voltage	30.0 KV	8.00 min	BI:D1	BO:C1	1.00 Min ramp, reverse polarity	Separation with Anion Separation Buffer
6	1.25	Autozero						
7	8.00	Stop data						
8	8.10	Rinse - Pressure	20.0 psi	0.50 min	BI:E1	BO:B1	forward	Rinse with Conditioner-Na.
9	8.60	Rinse - Pressure	20.0 psi	0.50 min	BI:F2	BO:B1	forward	Rinse with Rinse Solution.
10	9.10	End						

## Time Program for Anion Shutdown Method

Figure 1.9 Time Program for Anion Shutdown Method

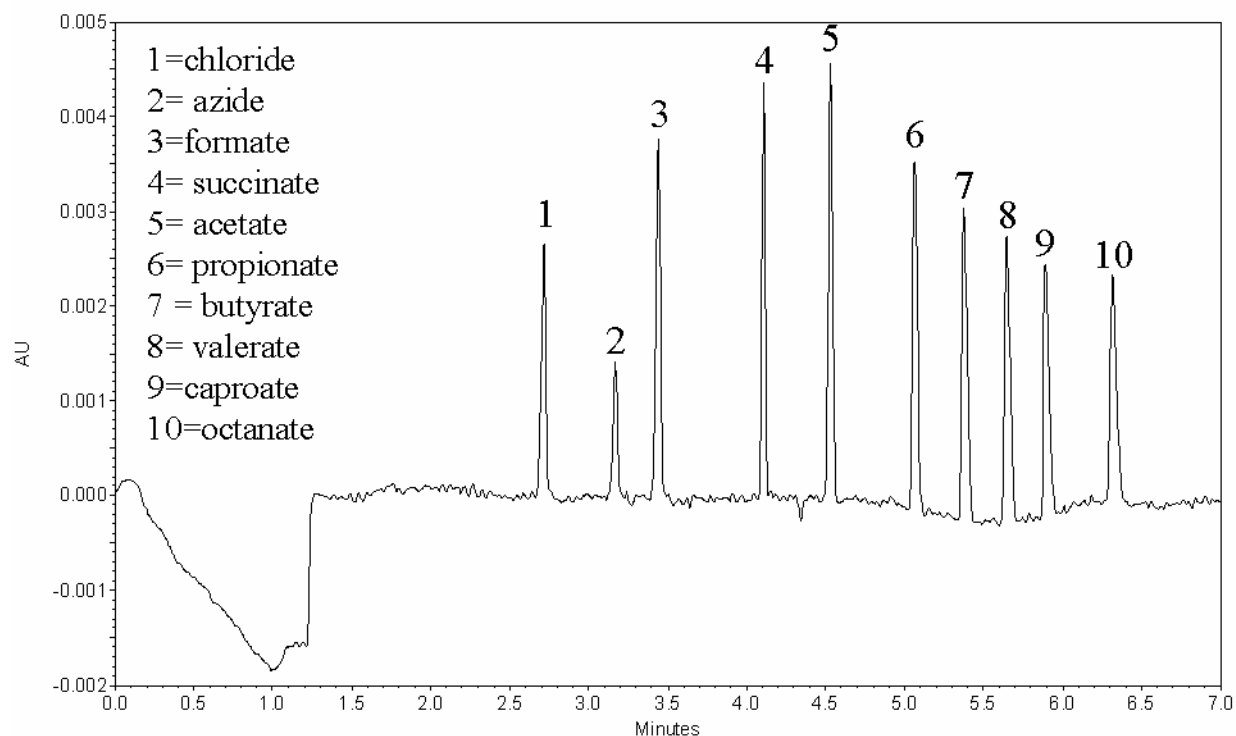
Initial Conditions   UV Detector Initial Conditions   Time Program								
	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	20.0 psi	1.00 min	Bl:A1	BD:A1	forward	Rinse with Rinse Solution.
2	0.00	Separate - Pressure	0.1 psi	1.00 min	Bl:A1	BD:A1	forward	Rinse with Rinse Solution.
3	1.00	Lamp - Off						
4	1.20	End						
5								

## Checking System Performance

### Using the Anion Organic Test Mix

To check system performance, run the Anion Organic Test Mix after performing the Capillary Conditioning method. Compare the electropherogram obtained with the one shown in [Figure 1.10](#). The electrical current during the separation should be stable around  $-53 \mu\text{A}$ . A negative value indicates that reverse polarity was used in the separation.

Figure 1.10 Anion Organic Test Mix—Typical Electropherogram

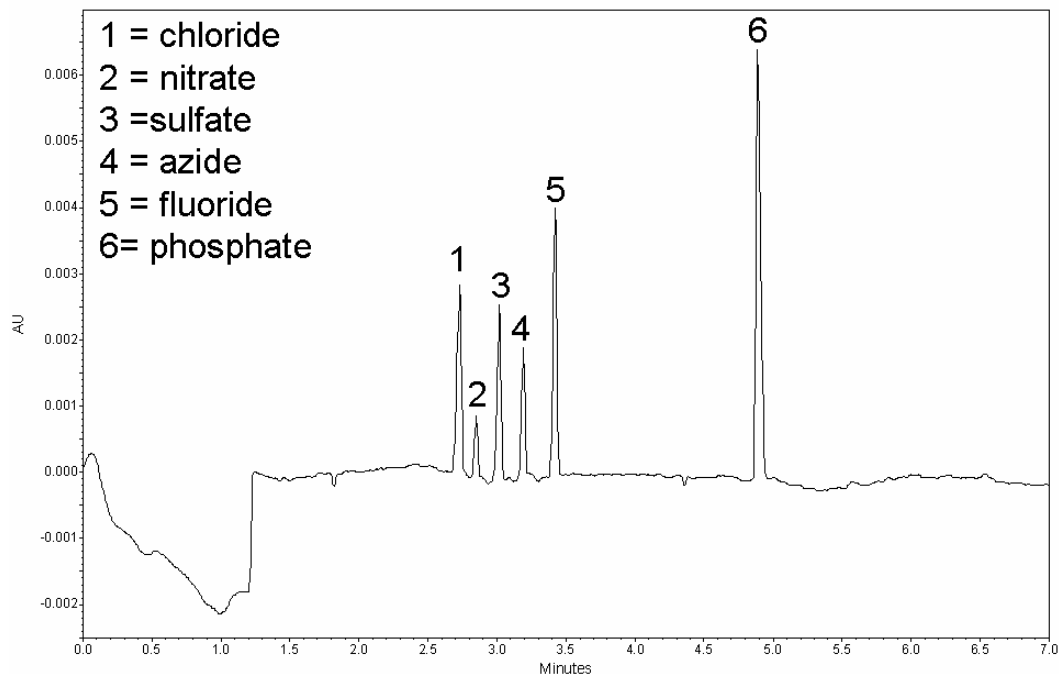


In [Figure 1.10](#), the concentration of each ion in the test mix is approximately 20 ppm, with the exception of azide, which is about 10 ppm.

## Using the Anion Inorganic Test Mix

To check system performance, run the Anion Inorganic Test Mix after performing the Capillary Conditioning method. Compare the electropherogram obtained with the one shown in [Figure 1.11](#). The electrical current during the separation should be stable around  $-53 \mu\text{A}$ . A negative electrical value indicates that reverse polarity was used in the separation. Make sure to use the Anion Acid Rinse in the separation to prevent the tailing of the fluoride and phosphate peaks.

**Figure 1.11 Anion Inorganic Test Mix–Typical Electropherogram**



In [Figure 1.11](#), the concentration of each ion in the test mix is approximately 20 ppm, with the exception of azide, which is about 10 ppm.

## Integration Parameters

The integration parameters in the analysis method should be optimized for each sample. As a starting point, use the values in [Figure 1.12](#). These values will successfully integrate both Anion Test Mixes.

**Figure 1.12 Recommended Integration Parameters and Initial Values**

#	Event	Start Time	Stop Time	Value
1	<input checked="" type="checkbox"/> Integration Off	0.000	2.000	0
2	<input checked="" type="checkbox"/> Width	0.000	8.000	0.05
3	<input checked="" type="checkbox"/> Threshold	0.000	8.000	350
4	<input checked="" type="checkbox"/> Shoulder Sensitivity	0.000	0.000	500
5	<input checked="" type="checkbox"/>			

The parameters have the following effects on the integration:

- **Integration off** sets time intervals in the electropherogram that are not integrated.
- **Width** sets the sensitivity of the peak detection regarding changes in the baseline.
- **Threshold** determines how high a peak must rise above the baseline noise before it is recognized as a peak.
- **Shoulder sensitivity** enables the detection of shoulders in large peaks. Its value specifies the slope value for splitting a peak.
- (Optional, not shown) **Minimum Cluster Distance** can be used to split peaks when shoulder sensitivity does not provide proper integration. It specifies the distance between non-baseline separated peaks so that they are not identified as one peak.

Additional help is available from the *32 Karat Software Online Help*.

## Troubleshooting

<b>Problem</b>	<b>Possible Cause</b>	<b>Corrective Action</b>
Unstable current	Problem with capillary	Replace capillary with new one
No peaks	Wrong polarity in method	Use reverse polarity in method
	No sample vial or sample at wrong location	Check sample vial position
No stable migration time	Buffer depletion	Replace all buffer vials after every 20 runs
Presence of ghost peaks	Contaminated buffer vials	Replace all buffer vials after every 20 runs
	Vial caps are wet	Replace caps with clean, dry caps
	Vial caps are dirty	Always use clean caps
Fluoride and phosphate peaks are tailing	Using Rinse Solution instead of Anion Acid Rinse.	Make sure that a vial filled with Anion Acid Rinse is placed at position F1 and successive incremental positions in the Inlet Buffer Tray
Anion peaks have longer migration times.	Rinse time with Anion Coating is too short.	Increase Anion Coating rinse time from 0.5 min to 1.0 min in the separation method when using the P/ACE MDQ <i>plus</i> system.



## Installing the 230 nm Filter



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**Note:** A 230 nm filter is required for the analysis. This filter is included in the P/ACE MDQ *plus* system and is installed at position 4. However, it is not provided for the P/ACE MDQ system and must be purchased separately.

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1. Before installing the 230 nm filter, check the condition of the filter as instructed in the appropriate guide for your system.
  - For the P/ACE MDQ system—“Installation UV detector wavelength filters” in the *P/ACE MDQ Installation and Maintenance Guide* (PN A36419).
  - For the P/ACE MDQ *plus* system—“Install Wavelength Filters for the UV Detector” in the *P/ACE MDQ plus System Maintenance Guide* (PN B54955).
2. Set the buffer trays to the **load** position in the **Direct Control** window.
3. Lift the cartridge cover door and allow the coolant to drain from the capillary cartridge.
4. Turn off the instrument.
5. Loosen the two thumb screws and lift the insertion bar.
6. Remove the capillary cartridge.
7. Loosen the thumb screws and remove the optics source assembly.
8. Wearing clean gloves, remove the filter wheel access cover and rotate the filter wheel to the correct position for your system:
  - For the P/ACE MDQ system—position 6
  - For the P/ACE MDQ *plus* system—position 4
9. Place the 230 nm filter at the appropriate position with the reflective side facing inward (toward the back of the instrument). Do not touch the filter with your hands.
10. Reinstall the filter wheel cover on the optics source assembly.
11. Replace the optics source assembly and tighten the two thumb screws.
12. Place the cartridge inside the instrument, lower the insertion bar, and tighten the two thumb screws.
13. Close the cartridge cover door.
14. Turn on the instrument.
15. Follow the instructions in [Configuring the P/ACE™ MDQ or P/ACE™ MDQ plus System on page 19](#) to configure the 32 Karat software for performing anion analysis.

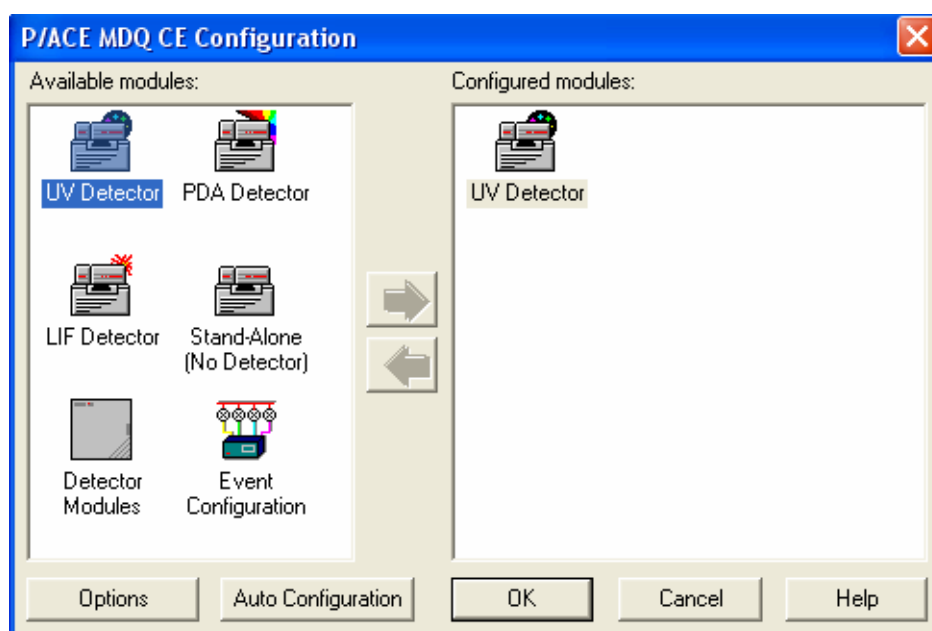


## Configuring the P/ACE™ MDQ or P/ACE™ MDQ *plus* System

**IMPORTANT:** Make sure that the system is turned on, and that the UV detector has been installed.

1. Open the 32 Karat software.
2. Right-click in the right pane of the **Enterprise** window.
3. Select **New > Instrument**.  
A new icon that looks like a question mark appears.
4. Right-click the question mark icon and select **Rename**.
5. Rename this icon **Anion**.
6. Right-click the **Anion** icon and select **Configure**.
7. Select **P/ACE MDQ CE** as the instrument type and click **Configure**.  
A new window opens.
8. Click the **UV detector** icon on the left.
9. Click the **Green arrow**. The UV detector icon should now be on the right side under **Configured Modules**.

**Figure B.1** P/ACE MDQ CE Configuration for Anion Analysis



10. Double-click the **UV Detector** icon to display the configuration settings.

If necessary, edit the settings to match the appropriate figure.

- For the P/ACE MDQ system—refer to [Figure B.2](#).
- For the P/ACE MDQ *plus* system—refer to [Figure B.3](#).

**Figure B.2 Anion Analysis Settings—P/ACE MDQ System**

**Figure B.3 Anion Analysis Settings—P/ACE MDQ *plus* System**

11. Click **OK** to accept the detector configuration.

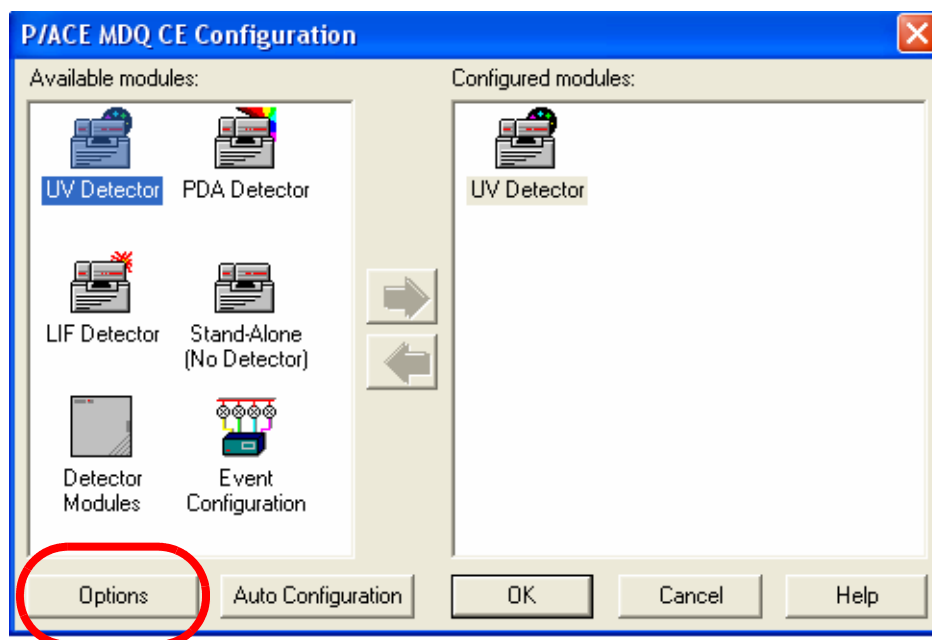
12. Follow the instructions in [Activating Caesar Integration](#).

## Activating Caesar Integration

The Caesar Integration must be activated in the anion configuration to perform peak integration and quantitation.

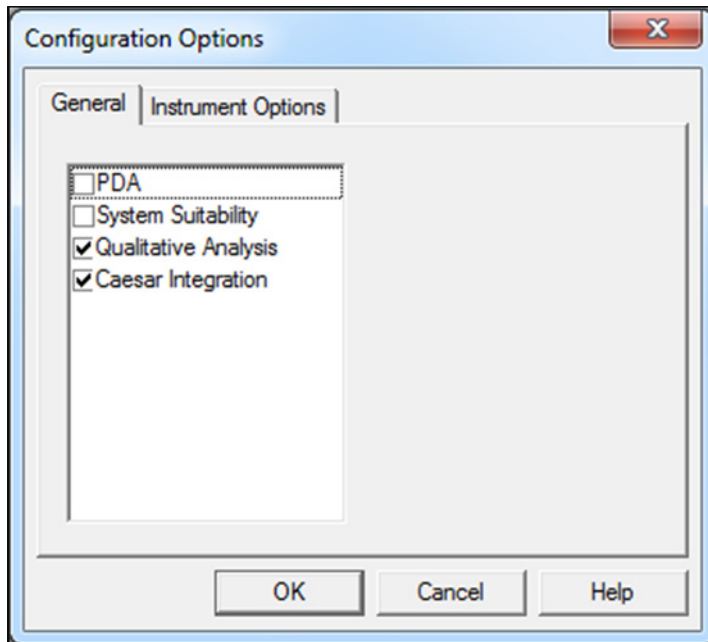
1. In the **CE Configuration** dialog, click **Options** ([Figure B.4](#))

**Figure B.4 P/ACE MDQ CE Configuration for Anion Analysis**



2. Under **General**, make sure that only **Qualitative Analysis** and **Caesar Integration** are selected ([Figure B.5](#)).

**Figure B.5 Configuration Options Dialog**



3. Click **OK** in the next three windows to accept the changes.