SCIEX Cation Analysis Kit

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

Instruction Guide

SCIEX

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

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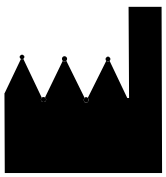

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Introduction

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

Note: The system must be equipped with a UV detector and a 200 nm filter to perform this assay.

This kit permits the analysis of small inorganic cations and aliphatic amines, 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 normal polarity so that the positively charged ions migrate toward the cathode (the negatively-charged electrode). In addition, the capillary is dynamically coated first with a polycation and later with a polyanion, which directs the electro-osmotic flow (EOF) toward the cathode, thus reducing the separation time while maximizing migration time reproducibility.

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.

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.

Materials and Reagents

Table 1-1 Kit Contents (PN A53540)

Component	Quantity
Cation Coating A	1
Cation Coating B	1
Cation Separation Buffer	1
Conditioner—Na	1
Conditioner—Li	1
Cation Internal Standard	1
Cation Test Mix	1
Capillary, 50 cm, 75 µm I.D.	3 pieces
Rinse Solution	2
Ion Analysis Insert	1

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

Description	Part Number	P/ACE System	
		MDQ	MDQ *plus*
200 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		✓

Storing Kit Components

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

Cleaning Vial Caps

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

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 µm 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 µm 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.

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

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

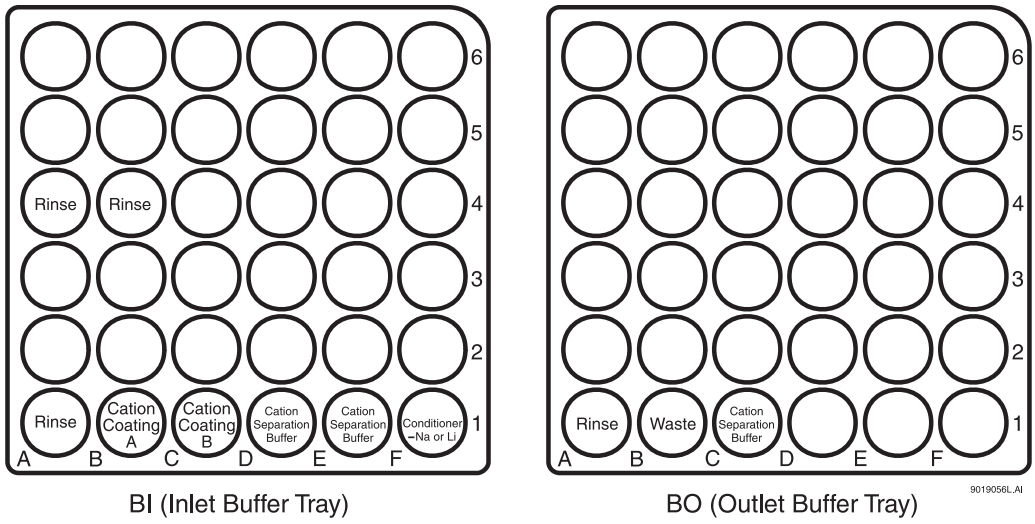
2. In the **Waste** position, place a vial partially filled with Rinse Solution.

   Use the correct volume for your system:

   - For the P/ACE MDQ system–700 µL
   - For the P/ACE MDQ *plus* system–600 µL

3. Close each vial with a clean cap.

Figure 1.1 Buffer Tray Configuration for Cation Analysis



BI (Inlet Buffer Tray) BO (Outlet Buffer Tray)

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

Note: A small amount of sodium can be detected when using Conditioner—Na (0.1 M NaOH). If you are analyzing for sodium, fill the buffer inlet vial at position F1 with Conditioner—Li (0.1 M LiOH) to minimize sodium carryover. However, a small amount of lithium may then be detected.

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

Preparing the Sample

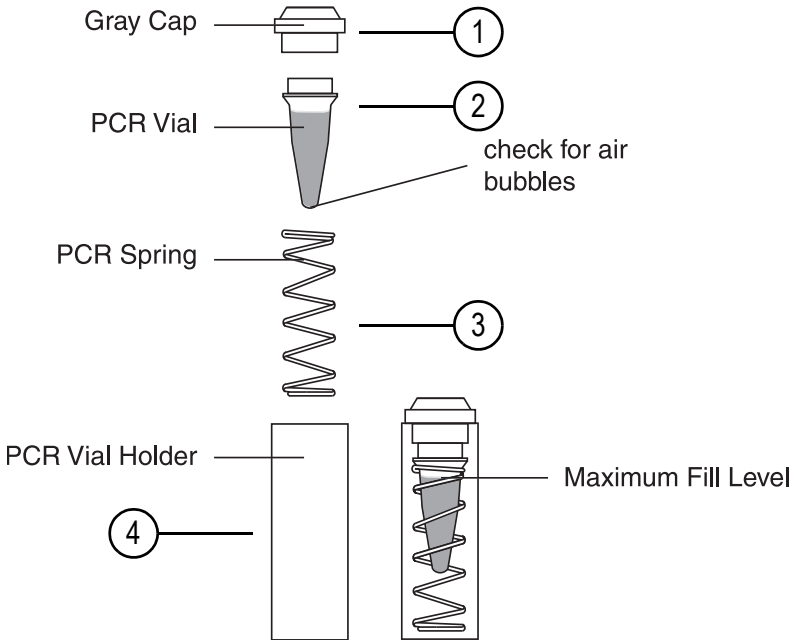
Depending on the concentration of the analytes, the sample should be injected as is or diluted. Dilution should be done so that the final concentration of the sample cations is between 1 ppm and 50 ppm. Special care should be taken to verify the pH of the sample, which should be slightly acidic, by adding 3 mM HCl or nitric acid.

The Cation Internal Standard (I.S.) consists of 0.20 M lithium chloride (LiCl), which is equivalent to 1388 ppm of lithium ion. The I.S. can be used in the quantitation of the sample cations. To use it, dilute the I. S. by a factor of 50 with the sample. For example, mix 4 µL of I.S. with 200 µL of sample to yield 28 ppm of lithium ion.

PCR Vial Setup–P/ACE MDQ System

Fill a PCR vial with 200 µL 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

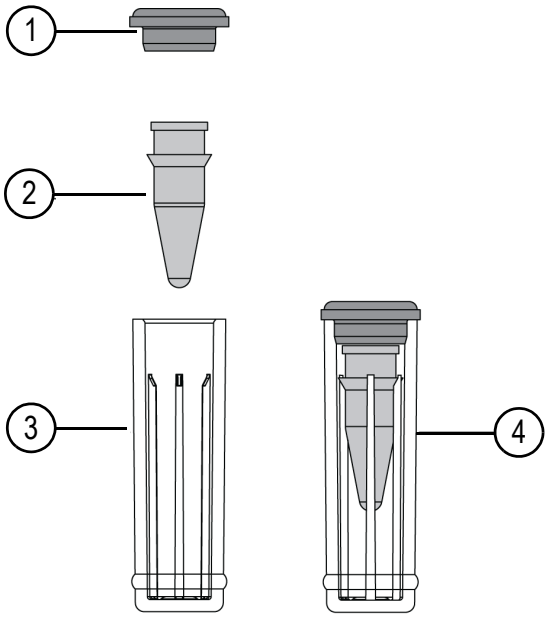


Item	Description
1	Vial cap (PN144656)
2	PCR vial (PN 144709)
3	PCR vial spring (PN 358821)
4	PCR vial holder (PN 144657)

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

Fill a PCR vial or micro vial with 200 µL 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



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 cation analysis:

- Cation Capillary Conditioning
- Cation Separation
- Cation 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 (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).

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

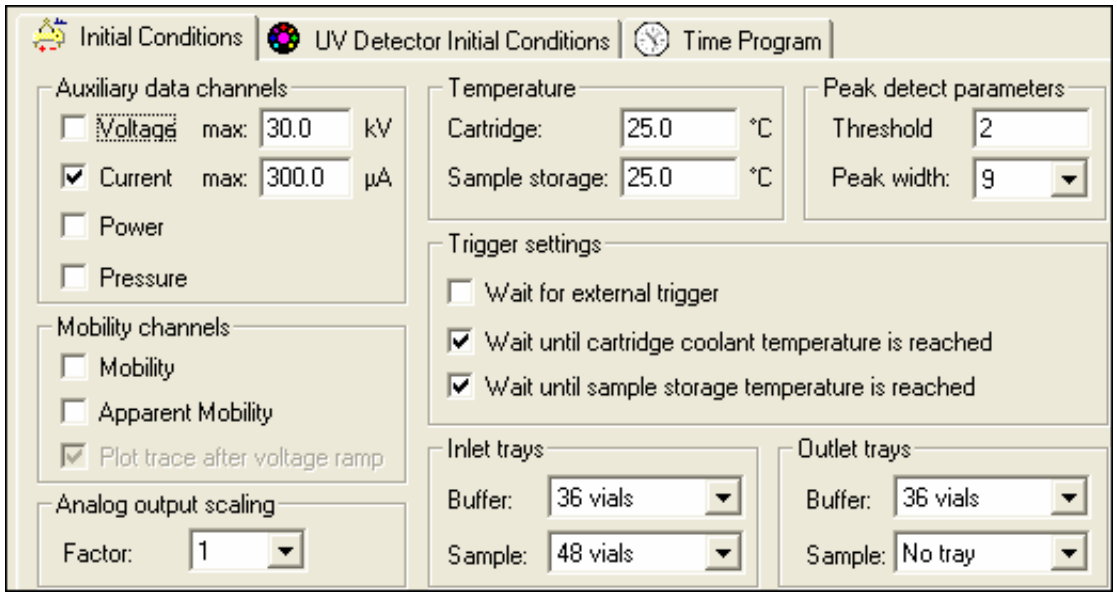


Figure 1.5 UV Detector Initial Conditions for Cation Capillary Conditioning, Cation Separation, and Cation Shutdown Methods

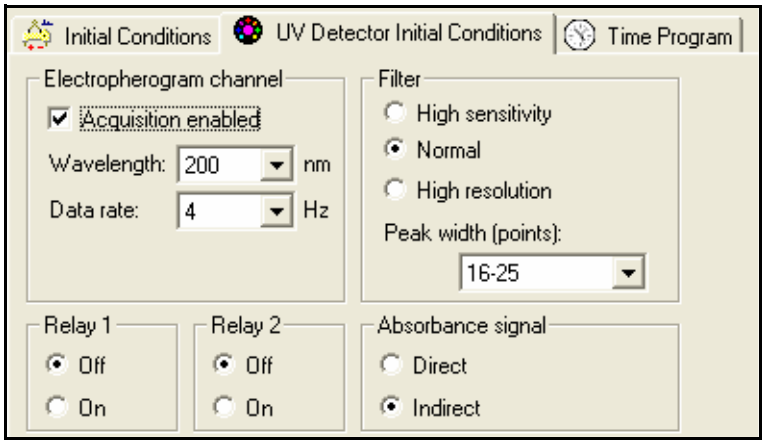


Figure 1.6 Time Program for Cation Capillary Conditioning Method

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:F1	BO:B1	forward	Rinse with Conditioner-Na or Li.
2		Rinse - Pressure	20.0 psi	1.00 min	BI:A4	BO:B1	forward	Rinse with Rinse Solution.
3		Rinse - Pressure	20.0 psi	1.00 min	BI:B1	BO:B1	forward	Rinse with Cation Coating A.
4		Rinse - Pressure	20.0 psi	2.00 min	BI:C1	BO:B1	forward	Rinse with Cation Coating B.
5		Rinse - Pressure	20.0 psi	1.50 min	BI:D1	BO:B1	forward	Rinse with Cation Separation Buffer.
6	0.00	Separate - Voltage	30.0 KV	5.00 min	BI:E1	BO:C1	1.00 Min ramp, normal polarity	Separation.
7	5.00	Stop data						
8	5.10	Rinse - Pressure	20.0 psi	0.50 min	BI:F1	BO:B1	forward	Rinse with Conditioner-Na or Li.
9	5.60	Rinse - Pressure	20.0 psi	0.50 min	BI:B4	BO:B1	forward	Rinse with Rinse Solution.
10	6.10	End						
11								

Time Program for the Cation Separation Method

Figure 1.7 Time Program for Cation Separation 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	0.50 min	BI:B1	BO:B1	forward	Rinse with Cation Coating A.
2		Rinse - Pressure	20.0 psi	0.50 min	BI:C1	BO:B1	forward	Rinse with Cation Coating B.
3		Rinse - Pressure	20.0 psi	1.50 min	BI:D1	BO:B1	forward	Rinse with Cation Separation Buffer.
4		Wait		0.20 min	BI:A1	BO:A1		Water dip.
5		Inject - Pressure	0.5 psi	5.0 sec	SI:A1	BO:A1	Override, forward	Sample injection.
6		Inject - Pressure	0.1 psi	10.0 sec	BI:A4	BO:A1	No override, forward	Water injection.
7	0.00	Separate - Voltage	30.0 KV	5.00 min	BI:E1	BO:C1	1.00 Min ramp, normal polarity	Voltage Separation.
8	2.00	Autozero						
9	5.00	Stop data						
10	5.10	Rinse - Pressure	20.0 psi	0.50 min	BI:F1	BO:B1	forward	Rinse with Conditioner-Na or Conditioner-Li.
11	5.60	Rinse - Pressure	20.0 psi	0.50 min	BI:B4	BO:B1	forward	Rinse with Rinse Solution.
12	6.10	End						
13								

Time Program for Cation Shutdown Method

Figure 1.8 Time Program for Cation Shutdown Method

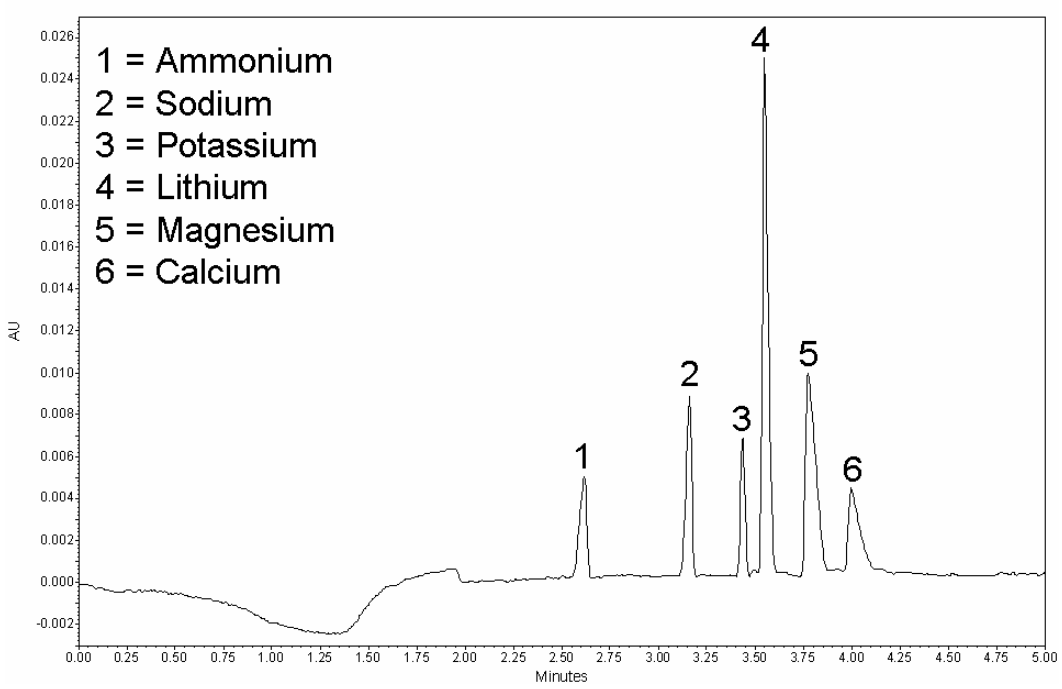
Initial Conditions | UV Detector Initial Conditions | Time Program

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

Checking System Performance with the Cation Test Mix

To check system performance, run the Cation Test Mix after performing the Capillary Conditioning method. Compare the electropherogram obtained with the one shown in Figure 1.9. The electrical current during the separation should be stable around +53 µA. A positive value indicates that normal polarity was used in the separation.

Figure 1.9 Cation Organic Test Mix–Typical Electropherogram



In Figure 1.9, the concentration of each ion in the test mix is approximately 20 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 1.10. These values will successfully integrate the Cation Test Mix.

Figure 1.10 Recommended Integration Parameters and Initial Values

#		Event	Start Time	Stop Time	Value
1	✓	Integration Off	0.000	2.200	0
2	✓	Width	0.000	0.000	0.1
3	✓	Threshold	0.000	0.000	500
4	✓	Shoulder Sensitivity	0.000	0.000	9999
5	✓				

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

Problem	Possible Cause	Corrective Action
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
Ammonium (NH_4^+) peak is missing or too small.	Over time, ammonium converts to ammonia (NH_3) and evaporates	Replace samples with fresh ones and analyze immediately.

Installing the 200 nm Filter

1. Before installing the 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 position 2.
9. Place the filter at position 2 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 cation analysis.

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

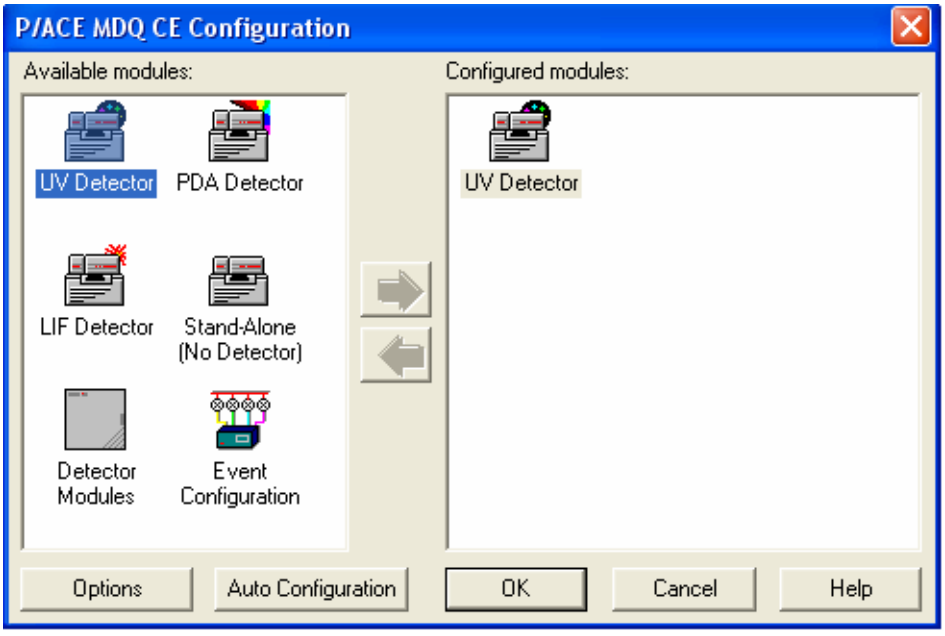
IMPORTANT: Make sure that the system is turned on, and that the UV detector has been installed.

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 **Cation**.
6. Right-click the **Cation** 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 Cation 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 Cation Analysis Settings–P/ACE MDQ System

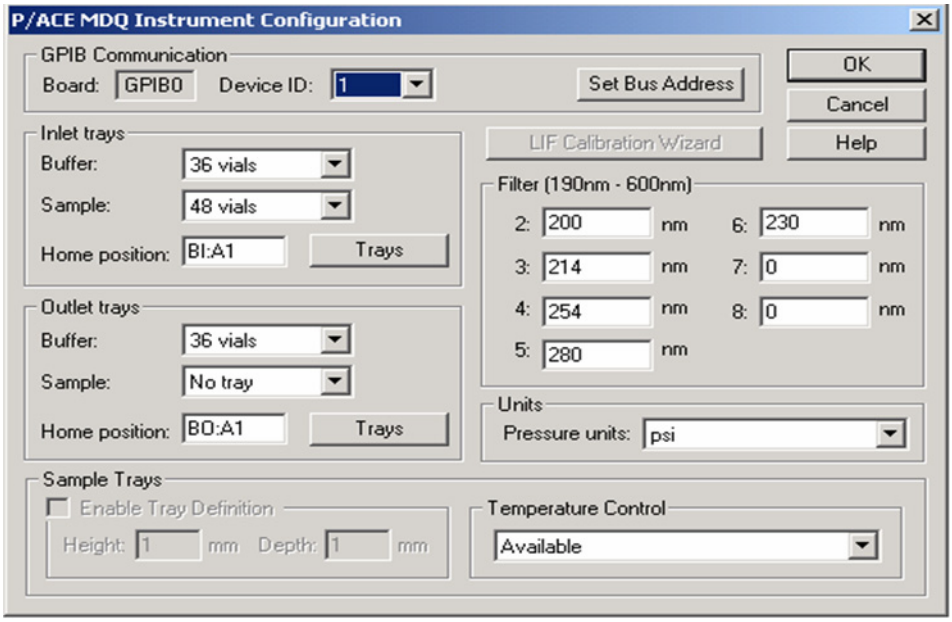
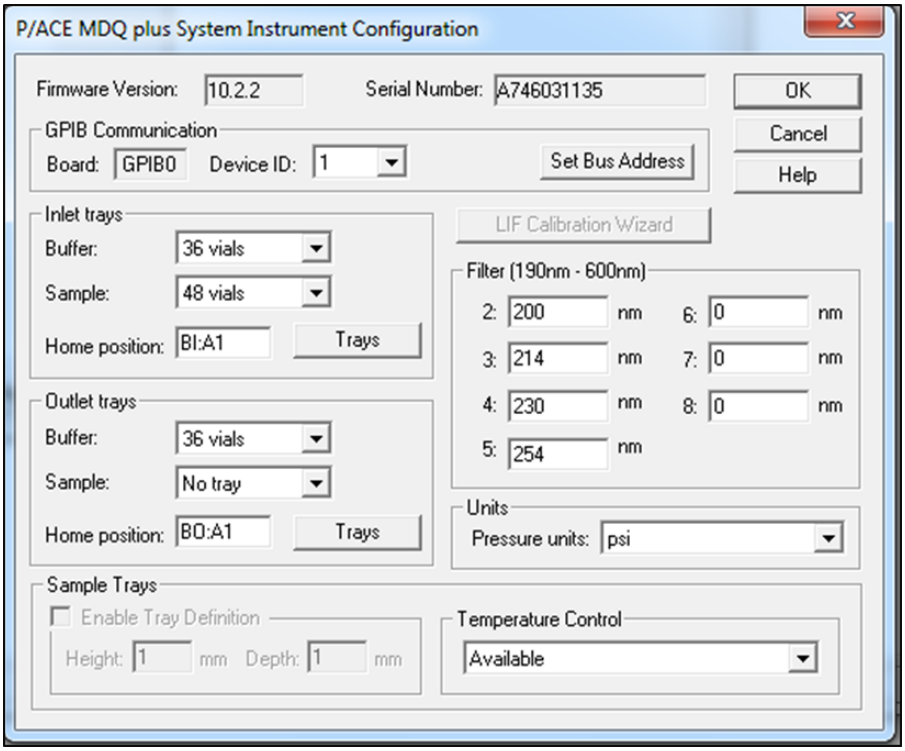


Figure B.3 Cation Analysis Settings–P/ACE MDQ *plus* System



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

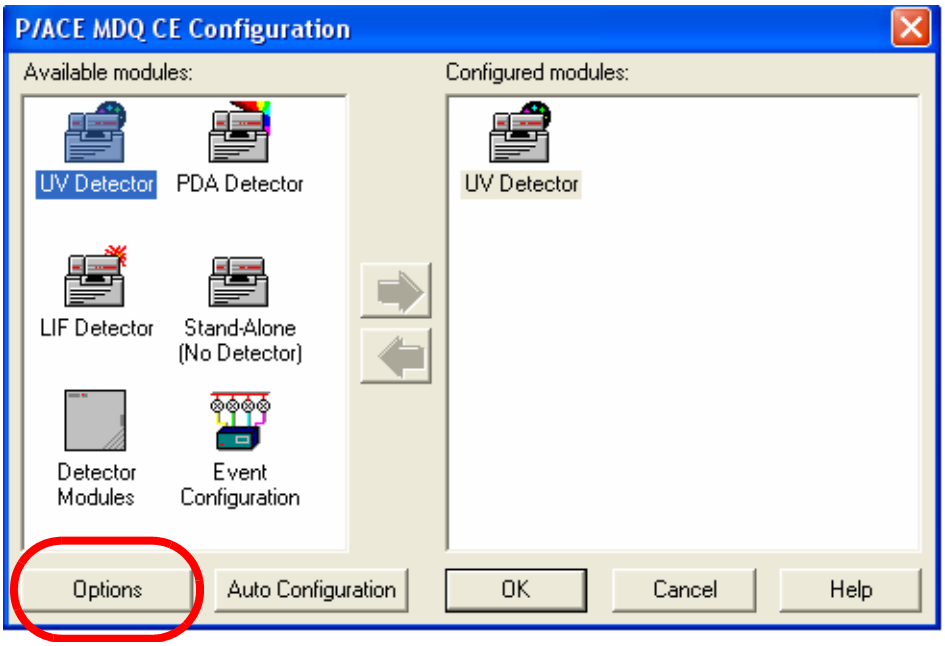
12. Follow the instructions in Activating Caesar Integration.

Activating Caesar Integration

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

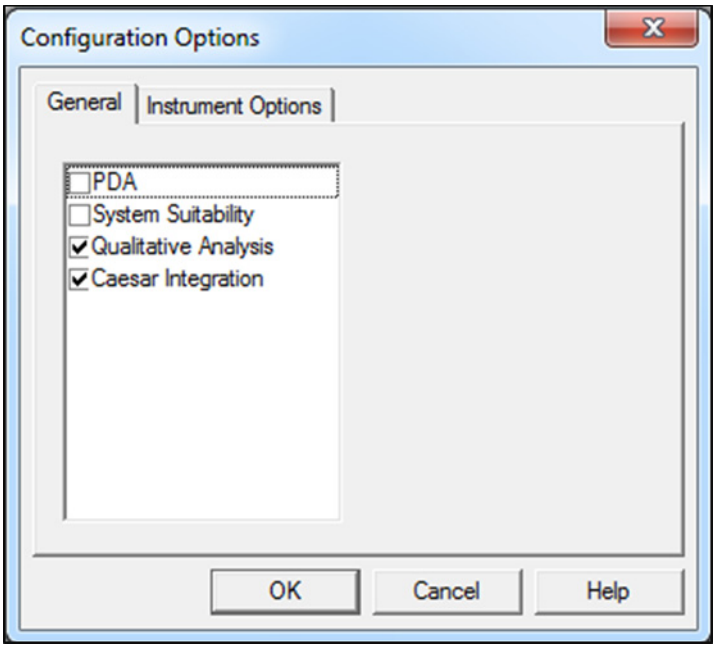
1. In the **CE Configuration** dialog, click **Options** (Figure B.4)

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



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

Figure B.5 Configuration Options



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