

## Anion Analysis

### User's Guide

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# Using the Anion Analysis Kit

## Introduction

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



### NOTE

The P/ACE MDQ must be equipped with a UV detector and a 230 nm filter to perform this assay.

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.



### IMPORTANT

The main focus of this application is in the biopharma market. This product can also be used for environmental testing and food and beverage markets, among other areas. This product is **for laboratory 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 Material Safety Data Sheets (MSDS) information, available at [www.beckmancoulter.com](http://www.beckmancoulter.com), regarding the proper handling of materials and reagents. Always follow standard laboratory safety guidelines.

## Materials and Reagents

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### Contents of this Kit (Reorder # 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 $\mu$ m I.D.	3 pieces
Rinse Solution	1
Ion Analysis Insert	1

### Materials Required but not Included in this Kit

Description	Part Number
2 mL glass vials (pack of 100)	144980
Vial caps for 2 mL glass vials, red caps (pack of 100)	144648
Filter 230 nm (see note below)	144433
PCR vial holders (pack of 50)	144657
PCR vial springs (pack of 10)	358821
PCR vials (pack of 50)	144709
PCR vials gray caps (pack of 50)	144656
Adequate pipettes and pipette tips	



#### NOTE

A 230 nm filter, for use with the UV Detector, is not provided as one of four standard filters on P/ACE MDQ UV Systems. Please check the condition of this filter as instructed in the *P/ACE MDQ Installation and Maintenance Guide* (A36419).

## Storing Kit Components

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

## Cleaning Vial Caps

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### NOTE

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

1. Using a clean beaker, rinse the 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 wipes, such as Kimwipes.



### NOTE

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

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

## Cleaning the Capillary Interface

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

## Installing the Capillary Inside the Cartridge

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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 Procedure* (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 P/ACE MDQ system.
4. Close the cartridge cover and tray cover.

## Conditioning a New Capillary

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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

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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 the **Running Methods** section of this manual.

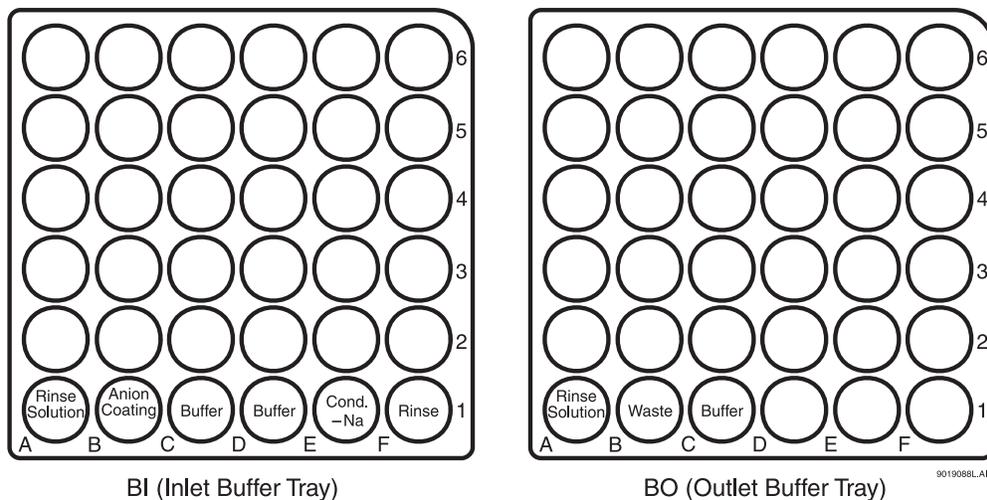


Do not share capillaries between applications. If the capillary has been used for cation analysis, do not use it for anion analysis.

## Preparing the Buffer Trays

1. Fill 2 mL vials with 1.4 mL of each reagent and position in the buffer trays, as shown in Figure 1.
2. In the **Waste** position, place a vial filled with 700  $\mu$ L of Rinse Solution.
3. Close each vial with a clean red cap.

**Figure 1.** Buffer tray configurations for performing anion analysis



4. Load the Inlet Buffer and Outlet Buffer trays inside the P/ACE MDQ system.
5. 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.



### 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.

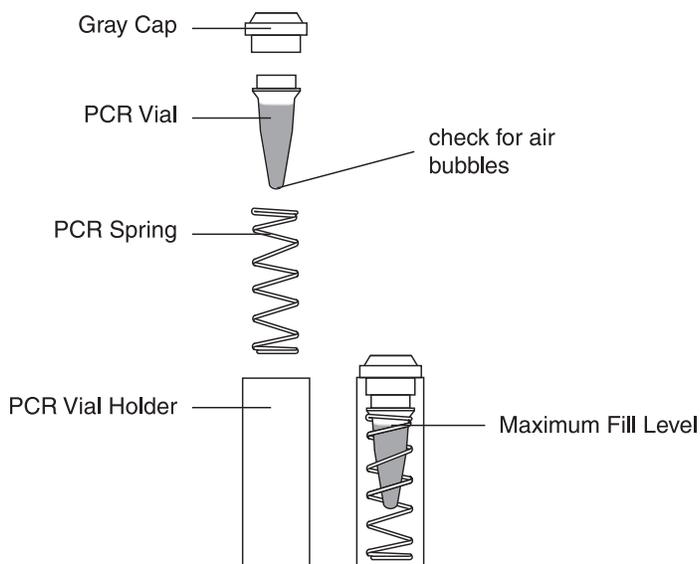
## Preparing the Sample

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 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 4,296 ppm of octanoate ion. The Anion I.S. can be used in the quantitation of the sample anions. To use it, dilute the Anion I. S. by a factor of 50 with the sample. For example, mix 4  $\mu\text{L}$  of I.S. with 200  $\mu\text{L}$  of sample to yield 86 ppm of octanoate ion.

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. Place the PCR vial in a PCR holder equipped with a vial spring. Seal the PCR vial with a clean gray cap and place it in the inlet sample tray.

**Figure 2.** PCR vial setup



## Running Methods

Three methods are required for performing anion analysis:

- Anion Capillary Conditioning
- Anion Separation
- Anion Shutdown

All three methods utilize the same **Initial Conditions** (Figure 3) and **UV Detector Settings** (Figure 4). However, each method has a different **Time Program** (Figures 5, 6 and 7). Save all three methods, with their corresponding names, in the 32 Karat folder.



### NOTE

When dealing with inorganic anions or organic acids that absorb in the low UV (such as nitrate and azide), the detection wavelength of the method should be set at 254 nm for optimal detection. Save this method with another name, such as Anion Separation Method at 254 nm.



### NOTE

These three methods can be downloaded from [www.CELeader.com/downloads](http://www.CELeader.com/downloads).

**Figure 3.** Initial conditions for **Anion Capillary Conditioning**, **Anion Separation**, and **Anion Shutdown** methods

Figure 4. UV Detector initial conditions for Anion Capillary Conditioning, Anion Separation, and Anion Shutdown methods

Figure 5. Time program for Anion Capillary Conditioning method

	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								

Figure 6. Time program for the Anion Separation method

	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								

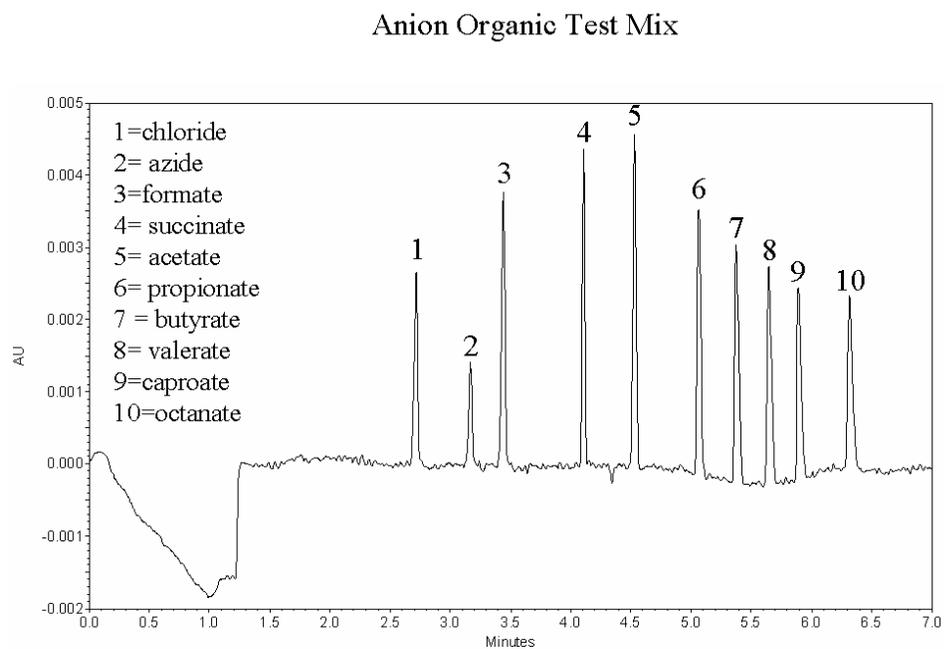
**Figure 7.** Time program for the **Anion Shutdown** method

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	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 Anion Organic Test Mix

To check the P/ACE MDQ system performance, run the Anion Organic Test Mix after performing the Capillary Conditioning method. Compare the electropherogram obtained with the one shown in Figure 8. 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.

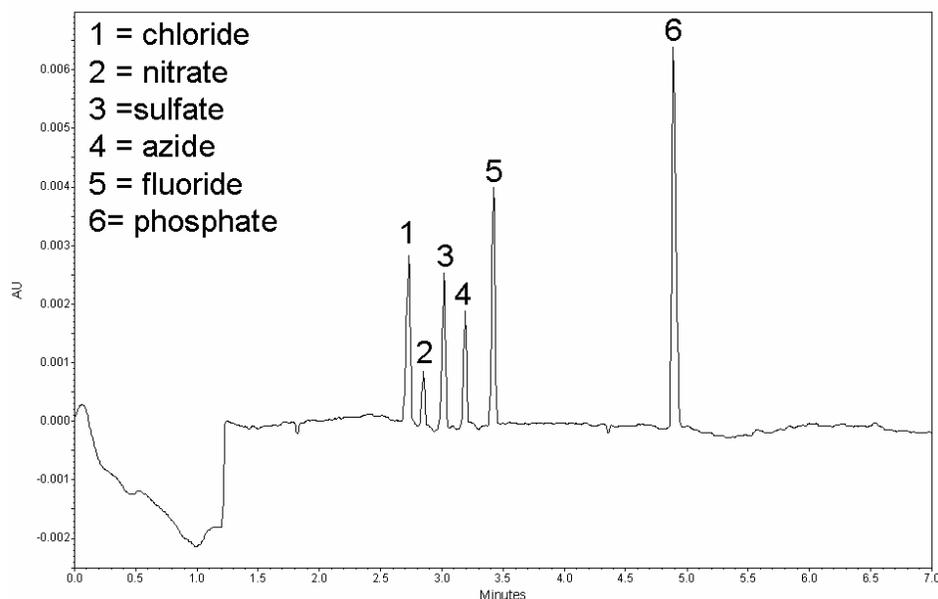
**Figure 8.** Typical electropherogram of the Anion Organic Test Mix. The concentration of each ion in the test mix is approximately 20 ppm, with the exception of azide, which is about 10 ppm.



## Checking System Performance with the Anion Inorganic Test Mix

To check the P/ACE MDQ system performance, run the Anion Inorganic Test Mix after performing the Capillary Conditioning method. Compare the electropherogram obtained with the one shown in Figure 9. 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 9.** Typical electropherogram of the Anion Inorganic Test Mix. The approximate ion concentrations in the test mix is approximately 20 ppm, with the exception of azide (10 ppm), fluoride (10 ppm), and phosphate (50 ppm).



## Integration Parameters

The integration parameters in the analysis method should be optimized for each sample. As a starting point, use the integration values shown in Figure 10. These values will successfully integrate both Anion Test Mixes.

**Figure 10.** Recommended integration parameters and corresponding initial values

#		Event	Start Time	Stop Time	Value
1	<input checked="" type="checkbox"/>	Integration Off <span style="float: right;">▼</span>	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"/>				

- **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.

The integration parameter, known as **Minimum Cluster Distance**, can be used to split peaks when shoulder sensitivity does not provide proper integration. Its value specifies the distance between non-baseline separated peaks so that they are not identified as one peak.

Additional help is provided in the 32 Karat Help files.

## Troubleshooting

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<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



# Filter Installation

## Installing the 230 nm Filter

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Before installing the 230 nm filter, consult the **Installation UV detector wavelength filters** section in the *P/ACE MDQ Installation and Maintenance Guide* (A36419). In summary:

1. Set the buffer trays to the **load** position using Direct Control.
2. Lift the cartridge cover door and allow the coolant to drain from the capillary cartridge.
3. Turn off the P/ACE MDQ system.
4. Loosen the two thumb screws and lift the insertion bar.
5. Remove the capillary cartridge.
6. Loosen the thumb screws and remove the optics source assembly.
7. Wearing clean gloves, remove the filter wheel access cover and rotate the filter wheel to position 6.
8. Place the 230 nm filter at position 6 with the reflective side facing inward (toward the back of the instrument). Do not touch the filter with your hands.
9. Reinstall the filter wheel cover on the optics source assembly.
10. Replace the optics source assembly and tighten the two thumb screws.
11. Place the cartridge inside the instrument, lower the insertion bar, and tighten the two thumb screws.
12. Close the cartridge cover door.
13. Turn on the instrument.

Next, the 32 Karat software must be configured for performing anion analysis.

**Filter Installation**  
*Installing the 230 nm Filter*

# System Configuration

## Configuring the P/ACE MDQ System

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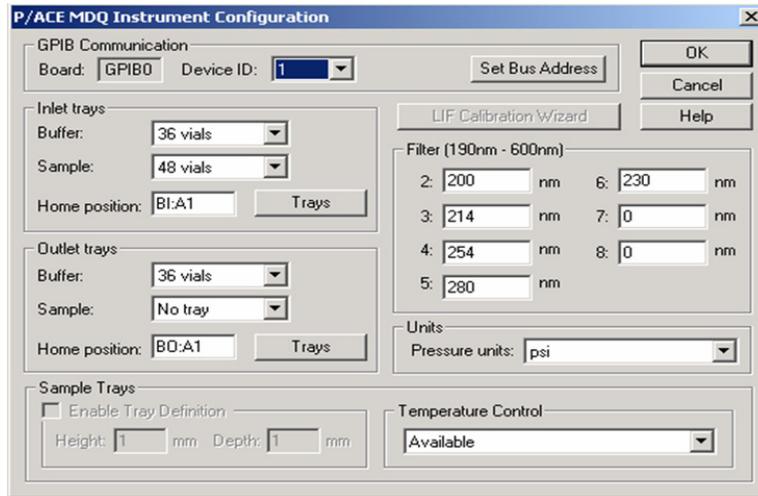
This procedure describes how to configure the P/ACE MDQ system to perform anion analysis.

 **IMPORTANT**

Make sure that the P/ACE MDQ 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**.
4. A new icon that looks like a question mark appears. Right click on this icon, and select **Rename**.
5. Rename this icon **Anion**.
6. Right click on the **Anion** icon and select **Configure**.
7. Select **P/ACE MDQ CE** as the instrument type and click **Configure**.
8. A new window opens. Click on the **UV detector** icon on the left.
9. Click on the **Green arrow**. The UV detector icon should now be on the right side under **Configured Modules**.
10. Double click the **UV Detector** icon to display the configuration settings.
11. The Configuration settings should match the ones shown in Figure B.1.
12. Click **OK** to accept the detector configuration.
13. Go to the next section to activate Caesar integration.

Figure B.1 P/ACE MDQ instrument configuration required for running anion analysis

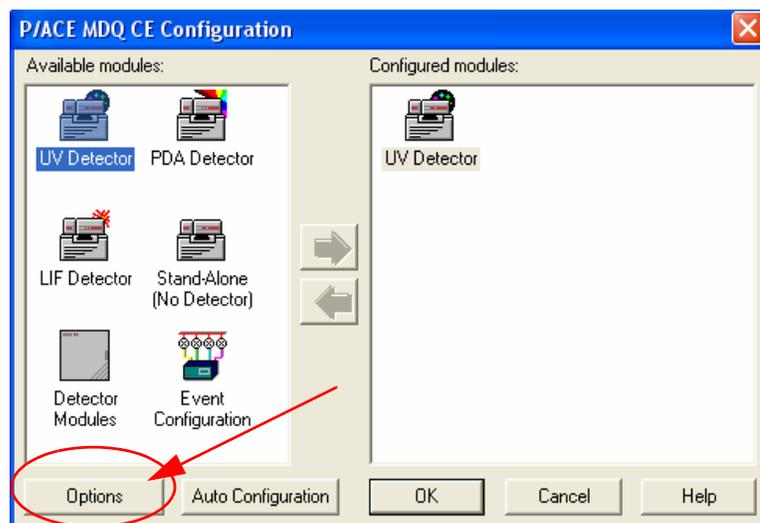


## Activating Caesar Integration in 32 Karat

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

1. In the P/ACE MDQ System Configuration window, click **Options**, as shown in Figure B.2.
2. Under **General**, make sure that only **Qualitative Analysis** and **Caesar Integration** have been selected.
3. Click **OK** in the next three windows to approve the changes.

Figure B.2 P/ACE MDQ CE configuration for anion analysis





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