
PA 800 Plus Pharmaceutical Analysis System

Maintenance Guide



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Installation Procedures

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Note: For instructions about how to use the system safely, refer to the document: *Overview Guide*.



WARNING! Electrical Shock Hazard. Turn off the power to the system before any system disassembly. Failure to do so can cause electrical shock or other injury.



WARNING! Electrical Shock Hazard. To prevent the risk of electrical shock or injury, do not do maintenance or repair procedures that are not specifically described in this manual. Contact a SCIEX Field Service Employee (FSE) for maintenance service and support.



WARNING! Electrical Shock Hazard. Do not try to disable any of the system interlocks or safety mechanisms.

Install a UV or PDA Detector

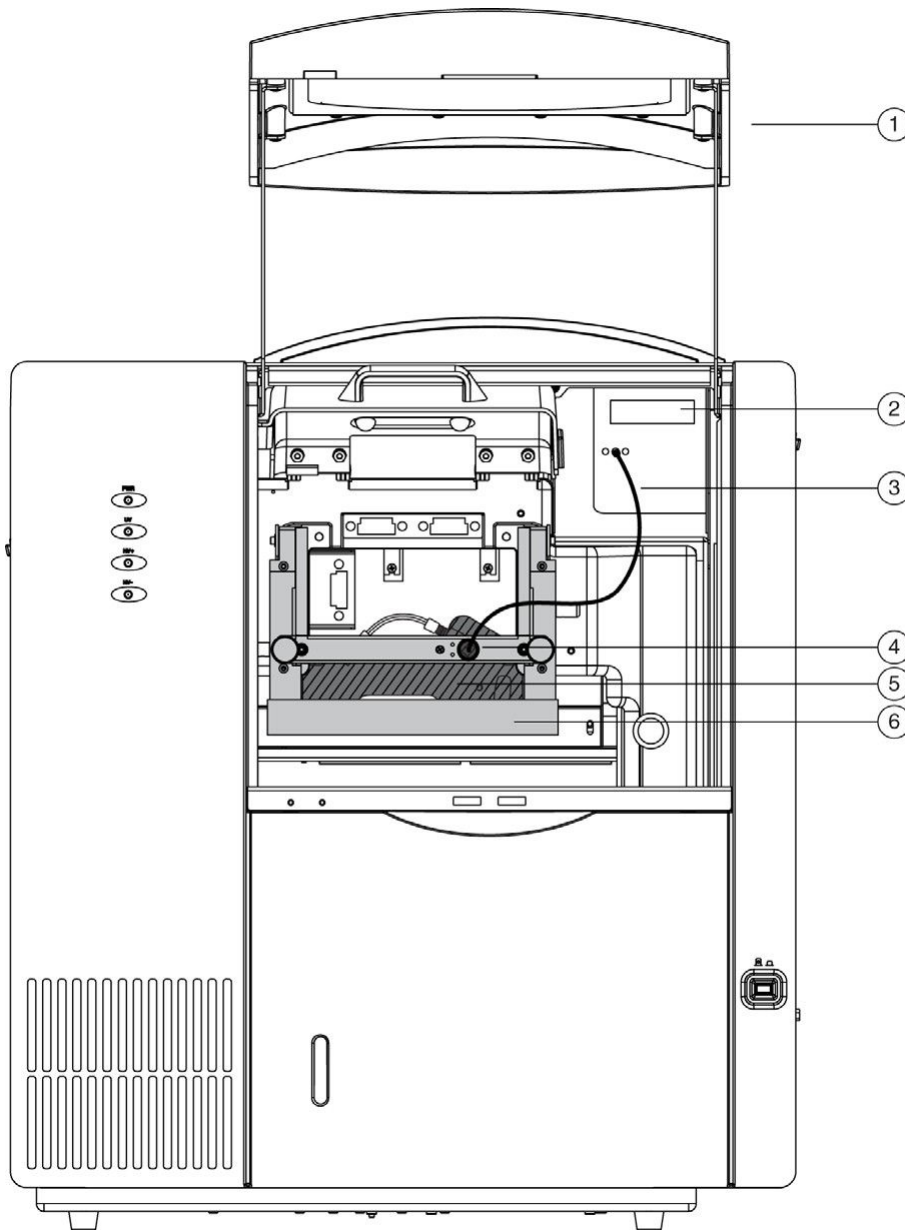
Prerequisites
<ul style="list-style-type: none">• UV or PDA detector• Fiber-optic cable for a UV or PDA detector• UV source optic module is installed

- UV or PDA detector
- Fiber-optic cable for a UV or PDA detector
- UV source optic module is installed

1. Make sure that the power to the system is off and a capillary cartridge is not installed.
2. Open the sample cover.

Installation Procedures

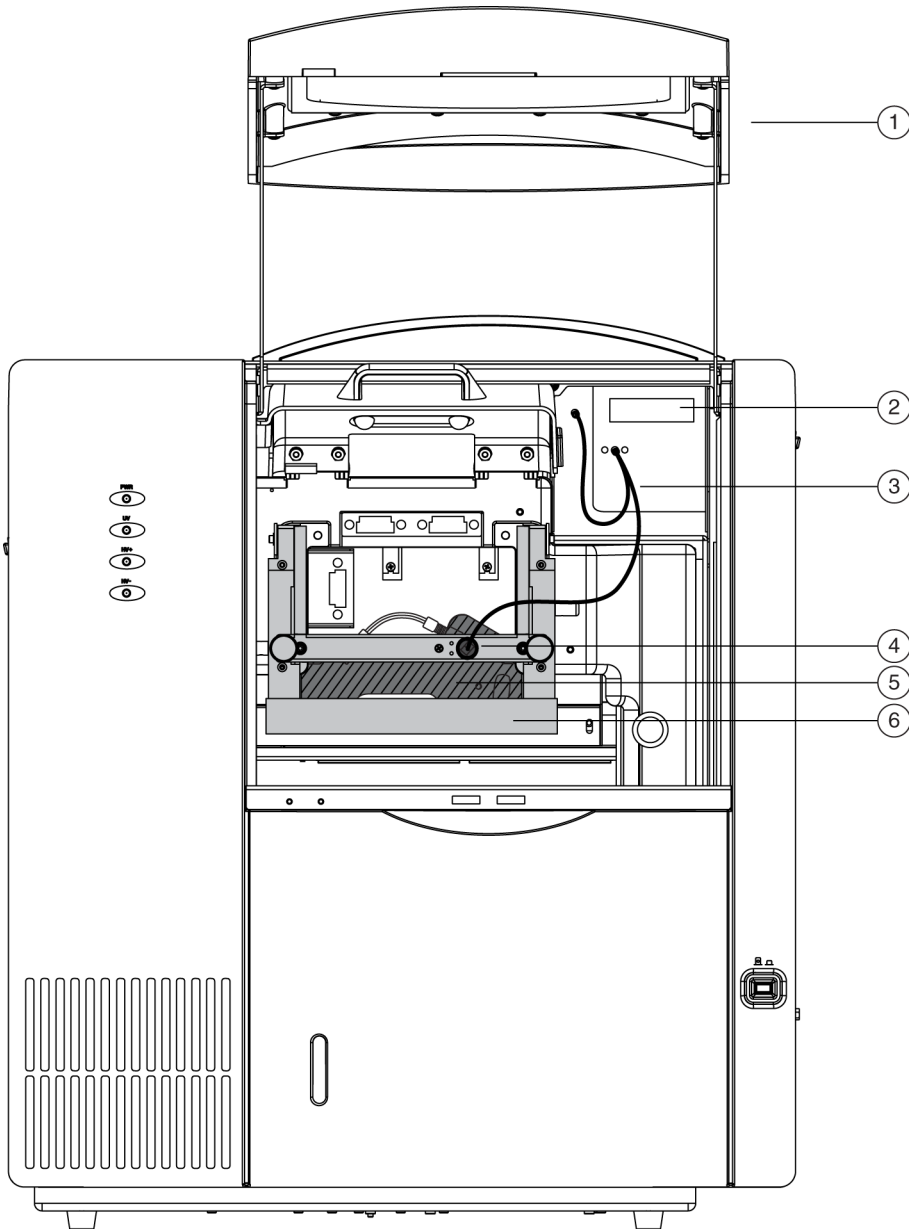
Figure 1-1 PA 800 Plus System with a UV Detector



Item	Description
1	Sample cover or outer door
2	UV detector
3	Two-ended fiber-optic cable
4	Insertion bar

Item	Description
5	Capillary cartridge
6	Interface block

Figure 1-2 PA 800 Plus System with a PDA Detector



Installation Procedures

Item	Description
1	Sample cover or outer door
2	PDA detector
3	Three-ended fiber-optic cable
4	Insertion bar
5	Capillary cartridge
6	Interface block

3. Remove the detector from the packaging, and then remove the protective connector caps. The connector caps can be discarded.
4. Put the detector in the mounting location, and then carefully install the detector in the system until the detector touches the backplane receptacle.
5. Tighten the thumbscrew.
6. Remove the fiber-optic cable from the packaging, and then remove the protective covers from the ends of the cable.

The UV cable has two protective covers. The PDA cable has three protective covers.

Keep the protective covers to use when the system is in storage.

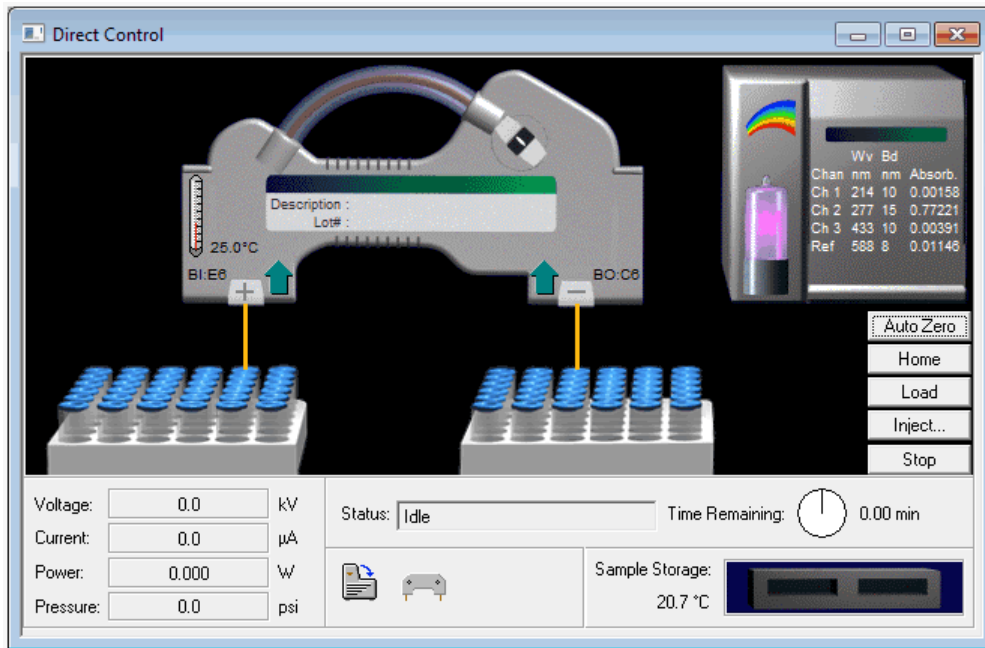
7. Connect the fiber-optic cable to the insertion bar and then to the detector.

Calibrate the PDA Detector

Do this procedure to calibrate the PDA detector after installation, after the lamp or another optical component is replaced, and when required as part of maintenance.

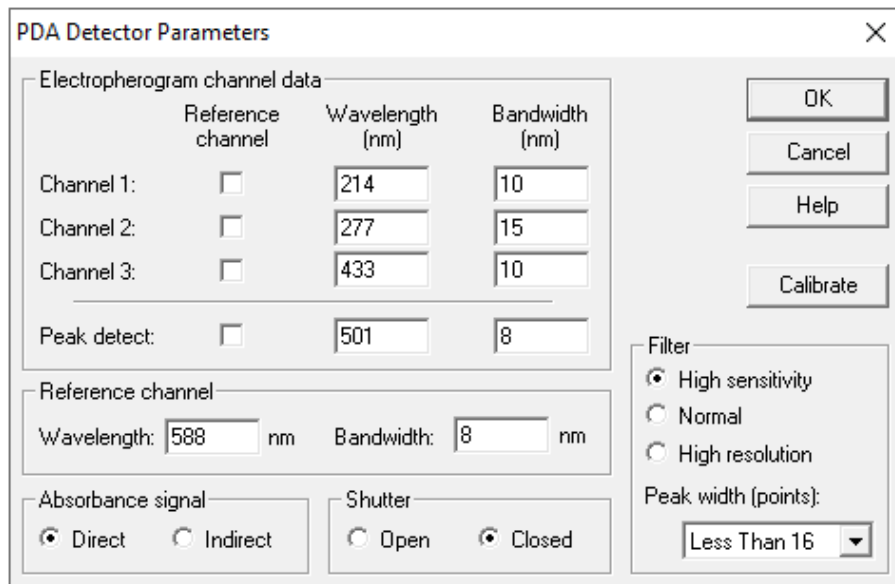
1. Make sure that a cartridge is installed. Refer to the section: [Install the Cartridge](#).
2. From the main menu, open a system that is configured with PDA detection.
3. Click **Control > Direct Control**.

Figure 1-3 Direct Control Window



- Click the rainbow that shows above the lamp.

Figure 1-4 PDA Detector Parameters Dialog



- To start the calibration sequence, click **Calibrate**.

Installation Procedures

The calibration requires approximately 2 minutes to complete. Do not try to get access to the system during the calibration process.

Install the UV Detector Filters

Required Materials
<ul style="list-style-type: none">• Filter• Powder-free gloves (nitrile or neoprene recommended)• Tweezers

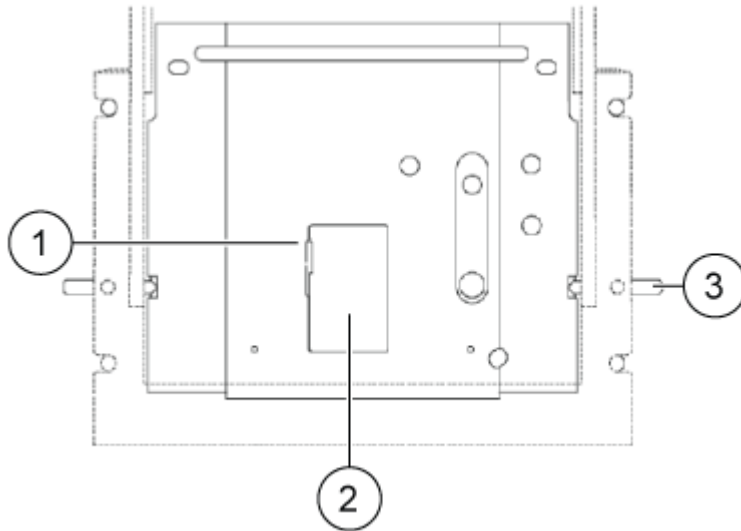
Note: For systems that use both UV and PDA detection, the last filter position (position 8) must be left open, with no filter installed.

CAUTION: Potential System Damage. Do not touch the optical surfaces of the filters without gloves. Oils from skin can cause incorrect filter performance.

The PA 800 Plus system includes 200 nm, 214 nm, 230 nm, 254 nm, and 280 nm filters. Heat and time have an effect on UV filters. At regular intervals, do a check of filter performance with a spectrophotometer or replace the filters. Refer to the section: [Evaluate the UV Filters](#).

1. Go to the Direct Control window, and then click **Load**.
2. Open the sample cover and cartridge cover.
3. Loosen the two thumbscrews on the insertion bar, and then lift the insertion bar.
4. Remove the capillary cartridge from the interface block.
5. Turn off the power to the system.
6. Remove the optics source assembly:
The optics source assembly is on the right behind the interface block. The assembly has a horizontal bar handle.
 - a. Loosen the two thumbscrews.
 - b. Use the handle to pull the assembly forward and remove it from the system.
 - c. Put the assembly on a clean work surface.
7. Remove the access cover for the filter wheel, and then turn the filter wheel until the correct filter can be seen.

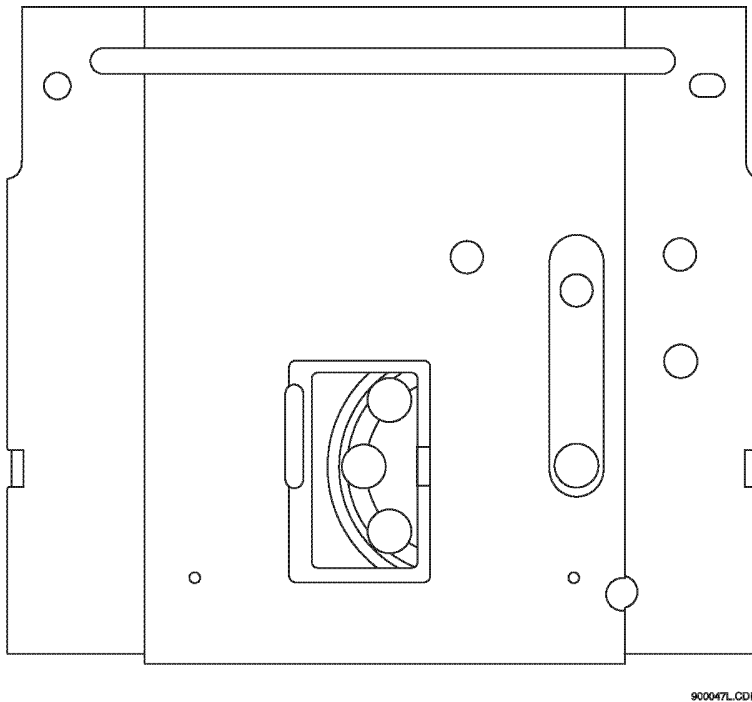
Figure 1-5 Remove the Access Cover for the Filter Wheel



Item	Description
1	Access cover latch
2	Access cover for the filter wheel
3	Thumbscrews, one on each side

8. Put on powder-free gloves.
9. Use tweezers to remove the applicable filters from the filter wheel.
10. Examine each filter for a burned spot. Replace any filter that shows damage.
11. Install the new filter so that the reflective side points inward.

Figure 1-6 Install a New Filter



12. When all of the filters have been examined or replaced, install the access cover for the filter wheel.
13. Install the optics source assembly:
 - a. Install the assembly in the mounting location.
 - b. Align the two top guide pins.
 - c. Tighten the two thumbscrews.
14. Install the capillary cartridge in the interface block.
15. Lower the insertion bar, and then tighten the two thumbscrews.
16. Close the cartridge cover and sample cover.
17. Turn on the power to the system.
18. If required by one of the following conditions, then in the 32 Karat software, change the instrument configuration for the new filters:
 - At least one of the filters has a different wavelength.
 - The filters have the same wavelength but are in different positions.

For detailed instructions, refer to the document: *Methods Development Guide*.

Install the Solid-State Laser Module



WARNING! Electrical Shock Hazard. Before component installation is started, turn off the power to the system.



WARNING! Potential System Damage. Do not operate the 488 nm solid-state laser module on any CE system other than the PA 800 Plus Pharmaceutical Analysis system or upgraded PA 800 Enhanced Protein Characterization system. The 488 nm solid-state laser uses more current than the interconnect module with the 488 nm argon ion laser, and causes damage to systems other than the PA 800 Plus Pharmaceutical Analysis system or upgraded PA 800 Enhanced Protein Characterization system. The PA 800 Plus Pharmaceutical Analysis system and upgraded PA 800 Enhanced Protein Characterization system include a wiring harness with a higher rating to operate safely with the increased current.

The 488 nm solid-state laser is part of the interconnect module in the PA 800 Enhanced Protein Characterization system upgrade and PA 800 Plus system. The 488 nm solid-state laser is compatible with these two instruments only. However, the 488 nm module does permit the connection of an external laser.

A SCIEX field service employee (FSE) must complete the initial installation of the solid-state laser module. This installation procedure sets the correct laser configuration, installs an updated wiring harness, installs and configures the LIF detector (if required), and makes sure that system operation, including system safety features, is correct.

Before the FSE arrives to complete the installation of the solid-state laser, do the following:

1. Close the 32 Karat software.
2. If a UV or PDA detector is installed, then remove the detector module.
3. Install the solid-state laser module in the system, and then tighten the thumbscrews at the front of the module.

Install the LIF Detector Filters

Install the filters and filter housing before the LIF detector is installed in the system.

The PA 800 Plus system is supplied with a single-color LIF detector. The filters that are installed for channel 1 have a 488 nm rejection filter and a 520 nm emission filter. The use of channel 2 requires a change to the detector. To set up and use channel 2, contact SCIEX Technical Support at sciex.com/request-support.

The filter housing is on the right side of the LIF detector. The housing has two filter holders, one for each detector channel. One or more filters can be used in each filter holder. The application and the width of the filter control the number of filters that can be used in the filter holder. Typical operation uses two filters: one rejection filter and one emission filter. The rejection filter prevents

Installation Procedures

scattered laser light from going into the detector. The emission filter is a band-pass filter that permits only the fluorescent signal at a selected wavelength to go into the detector.

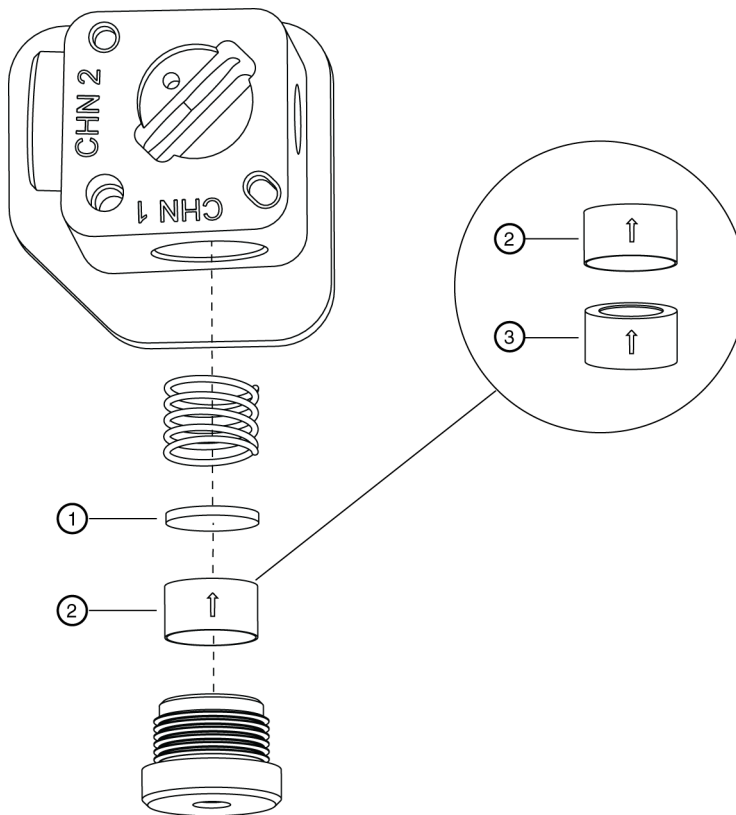
Any standard filter with a 0.5-inch diameter in the range of 350 nm to 750 nm that is required by the application and laser can be used. The total thickness of the filters cannot be more than 0.350 inches.

Install a Filter in the Filter Housing

CAUTION: Potential System Damage. Do not touch the optical surfaces of the filters without gloves. Oils from skin can cause incorrect filter performance.

1. Hold the filter housing in the orientation shown in the following figure.

Figure 1-7 Install Filters in the Housing



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Item	Description
1	Rejection filter
2	Emission (band-pass) filter, bottom view

Item	Description
3	Emission (band-pass) filter, top view

2. Hold the filter so that the arrow on the outside edge of the filter points up.

Note: Make sure to touch only the edges of the filters.

Note: If an arrow does not show on the edge of the filter, then look at both sides of the filter and find the side that has the more reflective surface. The more reflective side has a wide-edge rim that extends beyond the filter surface. Install the filter with the more reflective side toward the light source (the laser). In LIF detection, laser light comes from the center of the filter housing. While holding the housing in the correct orientation, install a filter so that the arrow (or the reflective side with the wider rim) points up, toward the filter housing.

3. Carefully install the filters in the filter holder.

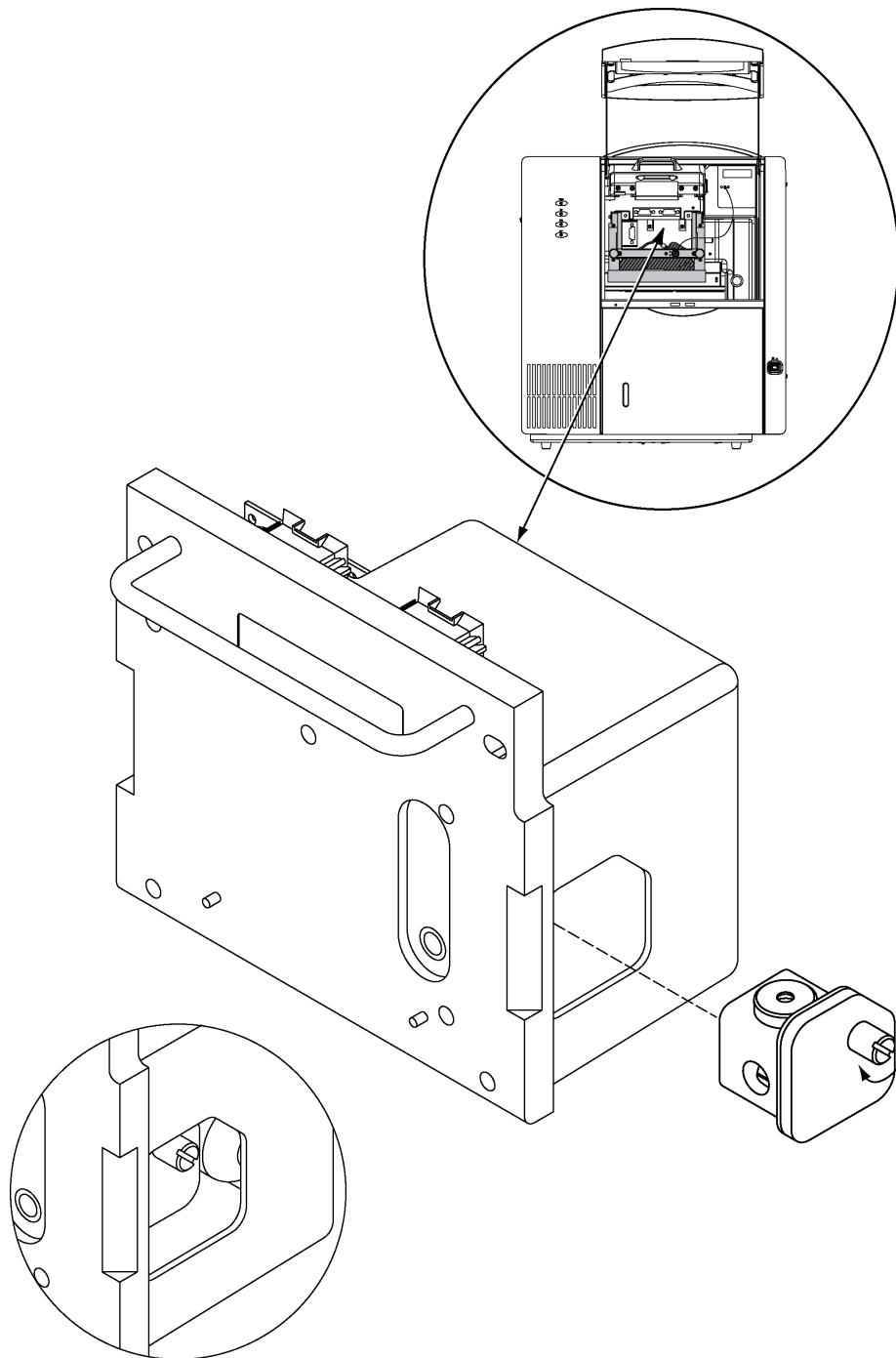
Note: If a filter is installed incorrectly, then performance decreases even though the filter might seem to be serviceable.

4. When all of the filters have been installed, do this:
 - a. Install the spring.
 - b. Install the filter holder in the applicable location (channel 1 or channel 2) in the filter housing.

Install the Filter Housing in the Detector

1. Install the filter housing in the opening on the right side of the detector.
 Make sure that the keyed corner of the filter housing aligns with the keyed corner of the detector module. The housing can only be installed in the opening in the orientation shown in the following figure.

Figure 1-8 Install the Filter Housing



2. After the filters have been changed, let the system stabilize for 15 minutes before use.

Install the LIF Detector in the System



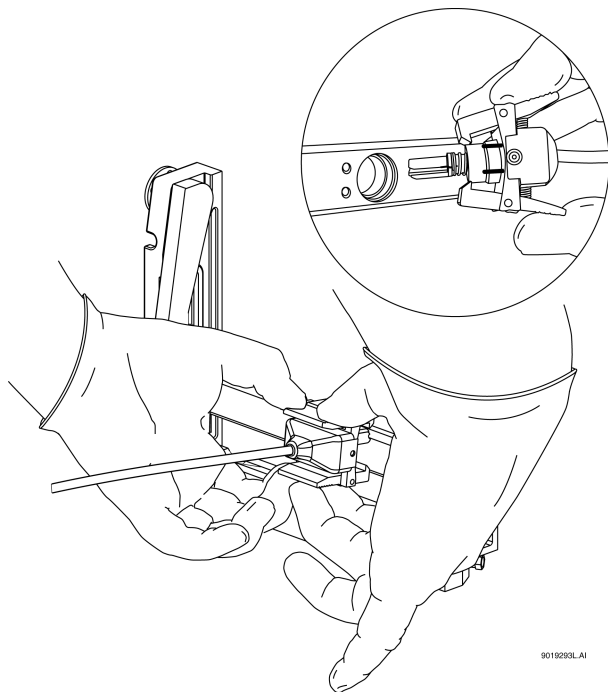
WARNING! Electrical Shock Hazard. Before component installation is started, turn off the power to the system.

CAUTION: Potential System Damage. When the LIF detector is not in use, keep the probe in the holder on the 488 nm laser module to protect it from mechanical damage, dust, and other particles.

CAUTION: Potential System Damage. Install the probe carefully to prevent the capillary window from breaking.

1. Remove the UV source optics module:
 - a. Loosen the knobs on the sides of the interface block.
 - b. Use the handle to remove the module.
2. Install the LIF detector.
3. Connect the LIF detector to the system:
 - a. Keep the two female connectors on the detector level with the two male connectors on the system.
 - b. Connect the female and male connectors.
4. To lock the detector module into position, tighten the captive screws until they are finger tight.
5. Make sure that a LIF aperture is installed in the cartridge.
6. Install the cartridge, but do not close the cartridge cover or sample cover. Refer to the section: [Install the Cartridge](#).
7. To connect the probe to the cartridge, align the two pins on the left side of the probe and probe body with the two pin holes and probe hole in the insertion bar.

Figure 1-9 Connect the Probe to the Cartridge



8. Squeeze the probe to open its jaws and then carefully attach the probe to the insertion bar.
9. Close the cartridge cover and sample cover.

Calibrate the LIF Detector (Optional)

This procedure is optional. If there is a requirement for consistency from system to system or cartridge to cartridge, then do this procedure. For information about the calibration, refer to the section: [About Automatic Calibration](#).

Calibrate the LIF detector after the LIF detector is installed, when the cartridge is installed again, and after a new capillary is installed in the cartridge.

Note: The following procedure technically does normalization, not calibration. Normalization uses a measured quality, such as the fluorescence of the LIF Performance Test Mix. Calibration uses an external standard. Because the software user interface uses the term *calibration*, that term is used in this guide.

For the best results, we recommend the use of the following:

- The same capillary that will be used for the separations
- For each system, a dedicated LIF aperture and probe guide

Required Materials
<ul style="list-style-type: none">• LIF Performance Test Mix• CE Grade Water

1. Turn on the PA 800 Plus system.
2. Open the 32 Karat software.
The 32 Karat Software Enterprise window opens.
3. Open the LIF instrument, open the Direct Control window, and then turn on the laser.
4. In the 32 Karat Software Enterprise window, click **Tools > Enterprise Login**, and then log on as a user with Administrative privileges.
5. Right-click the applicable instrument icon, and then click **Configure > Instrument**.
The Instrument Configuration dialog opens.
6. Click **Configure**.
The PA 800 plus Configuration dialog opens.
7. In the right pane, click the **LIF Detector** icon, and then right-click and click **Open**.

Figure 1-10 PA 800 plus System Instrument Configuration Dialog for LIF Detectors

PA 800 plus System Instrument Configuration

Firmware Version: 10.2.5-R Serial Number: A746035298

GPIB Communication
Board: GPIB0 Device ID: 1 Set Bus Address

Inlet trays
Buffer: 36 vials
Sample: 48 vials
Home position: BI:A1 Trays

Outlet trays
Buffer: 36 vials
Sample: No tray
Home position: BO:A1 Trays

Sample Trays
 Enable Tray Definition
Height: 1 mm Depth: 1 mm

LIF Calibration Wizard

Filter (190nm - 600nm)
2: 200 nm 6: 220 nm
3: 214 nm 7: 0 nm
4: 254 nm 8: 0 nm
5: 280 nm

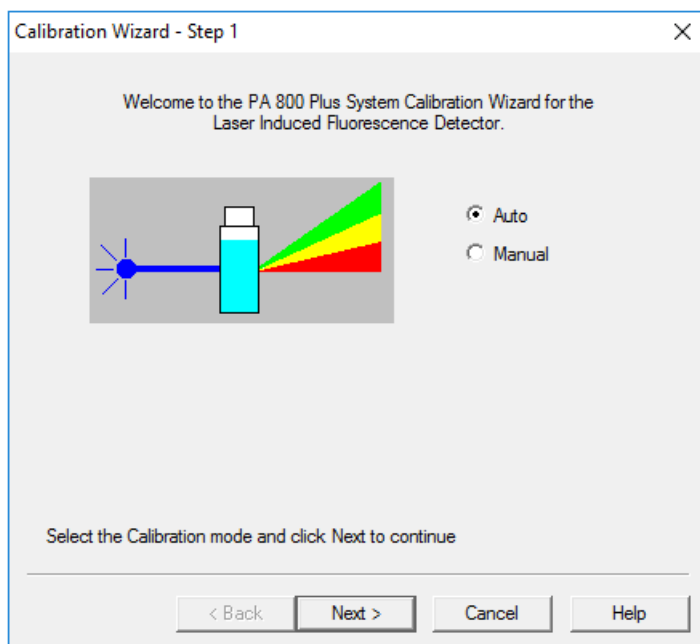
Units
Pressure units: psi

Temperature Control
Available

OK Cancel Help

8. Click **LIF Calibration Wizard**.
9. Do the calibration:
 - a. Click **Auto**, and then click **Next**.

Figure 1-11 Calibration Wizard - Step 1



- b. In the **Target RFU value** field, type the recommended RFU value. Refer to the following table.

Table 1-1 Recommended Target RFU by Capillary Type

Type of Capillary	Internal Diameter (μm)	Total Length (cm)	Recommended Target RFU
Bare fused-silica	50	User specified	15
Bare fused-silica	75	User specified	35
DNA capillary	100	User specified	62
N-CHO coated capillary	50	User specified	7

- c. In the **Internal diameter** and **Total length** fields, type the correct values for the capillary in use, and then click **Next**.

Figure 1-12 Calibration Wizard - Step 2

Calibration Wizard - Step 2

Please enter the following calibration parameters

Detector channel: 1 2

Target RFU value: RFU

Capillary dimensions

Internal diameter: um

Total length: cm

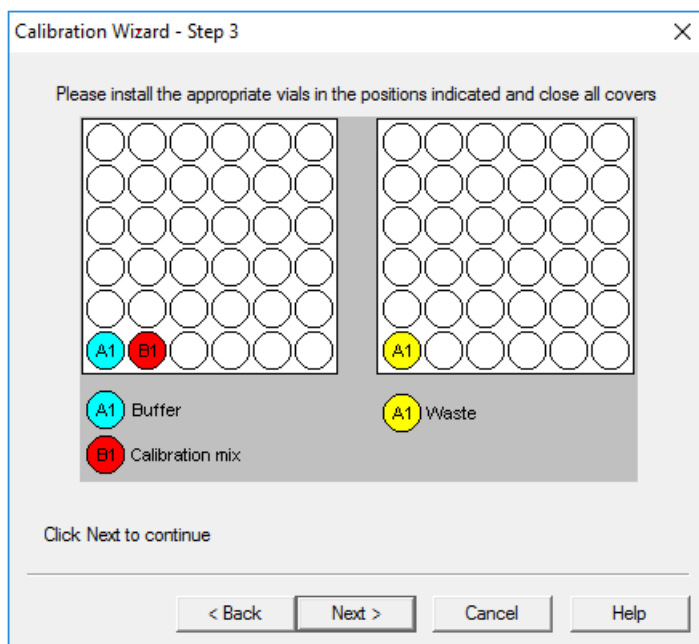
Click Next to continue

< Back Next > Cancel Help

10. Put a universal vial in position A1 in the buffer outlet tray.
11. Put universal vials in positions A1 and B1 in the buffer inlet tray.

Note: To prevent splashing, put the vials in the tray before liquid is added and the cap is attached.

Figure 1-13 Calibration Wizard - Step 3



12. Fill the vials, and then put caps on the vials:

- Inlet buffer tray position A1 (labeled `Buffer`): 1.5 mL CE Grade Water
- Inlet buffer tray position B1 (labeled `Calibration mix`): 1.5 mL LIF Performance Test Mix

Note: For the N-CHO capillary, dilute the LIF Performance Test Mix 1:1 with CE Grade Water.

- Outlet buffer tray position A1 (labeled `waste`): 1.0 mL CE Grade Water

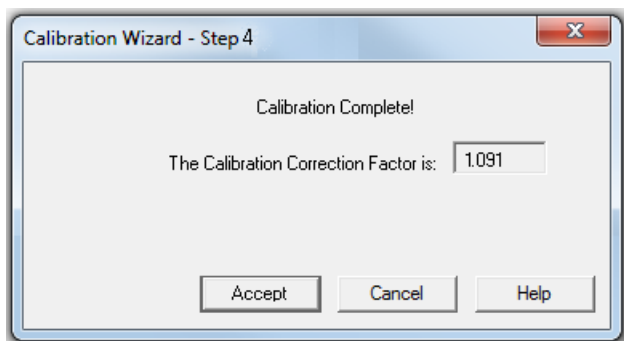
13. Click **Next**.

The 32 Karat software does the calibration. When the calibration is complete, the Calibration Wizard - Step 4 window opens.

14. Use the value in the **Calibration Correction Factor** field to examine the results:

- If the number is less than 0.1, then click **Cancel**, and refer to the section: [CCF Values for LIF Detector Calibration](#).
- If the number is between 0.1 and 10, then the calibration is successful. Click **Accept** to save the results and close the Calibration Wizard.
- If the number is more than 10, then click **Cancel**, and refer to the section: [CCF Values for LIF Detector Calibration](#).

Figure 1-14 Calibration Wizard - Step 4



15. If the system will be used immediately for separation, in the Direct Control window, set the sample storage temperature to a value that is correct for the samples to be run.
By default, after the calibration completes, the sample storage temperature is set to 25 °C.
16. Close all of the dialogs and windows.

Troubleshoot the LIF Detector Calibration

CCF Values for LIF Detector Calibration

Reported CCF Value	Action
Less than 0.1 or System performance is not satisfactory	<ul style="list-style-type: none"> • Make sure that the correct capillary was used, and that it is not broken. • Make sure that the laser output for the laser in use on the system is correct. • Make sure that the correct filters are installed in the LIF detector: <ul style="list-style-type: none"> • Excitation: 488 nm • Emission: 520 nm • Replace the test mix, buffer, and capillary, and then do the calibration again. If the issue continues, then contact SCIEX Technical Support at sciex.com/request-support.
Between 0.1 and 10.0	There is no issue with the system. Run a standard and make sure that the system performance is satisfactory.

Reported CCF Value	Action
<p>More than 10 or System performance is not satisfactory</p>	<ul style="list-style-type: none"> • Make sure that the laser output for the laser in use on the system is correct. • Make sure that the correct filters are installed in the LIF detector: <ul style="list-style-type: none"> • Excitation: 488 nm • Emission: 520 nm • Replace the test mix, buffer, and capillary, and then do the calibration again. If the issue continues, then contact SCIEX Technical Support at sciex.com/request-support.

No Step Change Detected

The LIF calibration does a comparison of detector signals with a nonfluorescent solution and a known fluorescent solution. When a rinse with nonfluorescent solution is done and then followed by a rinse with fluorescent solution, the first part of the detector signal should be near zero and the second part should be near the target fluorescent value. This detector output is in the shape of a step and is referred to as a *step change*. If a step change is not seen, then the applicable solutions are not passing the detector or the detector cannot detect the solutions.

1. Make sure that the switch on the right side of the laser is in the ON position.
2. Make sure that the laser that was supplied with the system is connected and the LASER ON light is illuminated.
3. To make sure that the solution goes through the capillary, from buffer inlet position A1 to an empty buffer vial in outlet position B1, use Direct Control to do a pressure rinse with CE Grade Water at 20 psi for 5 minutes.
4. When the rinse starts, open the sample cover. Look at the outlet end of the capillary in position B1.
 - If there are droplets on the outlet end of the capillary, then continue with step 6.
 - If there are no droplets on the outlet end of the capillary, then the capillary is blocked or the system has a pressure failure. Continue with the next step.
5. Replace the capillary, and then do the pressure rinse again.
 - If there are still no droplets on the outlet end of the capillary, then contact SCIEX Technical Support at sciex.com/request-support.
 - If there are droplets on the outlet end of the capillary, then the detection system is the only possible cause when no step change is detected. Continue with the next step.
6. Make sure that the correct filters are installed in the LIF detector.

Installation Procedures

7. Manually set the calibration correction factor (CCF) to 1.0.
8. Calibrate the LIF detector. Refer to the section: [Calibrate the LIF Detector \(Optional\)](#).
If there is no step change detected, then contact SCIEX Technical Support at sciex.com/request-support.

Capillary Cartridge

For the application to operate correctly, the correct capillary must be installed in the cartridge. To identify the correct capillary, refer to the application documentation for the system in use.

If a cartridge that has been used successfully starts to leak coolant, then immediately remove the cartridge from the system and refer to the section: [Rebuild a Capillary Cartridge](#).

To install the capillary, refer to the section: [Install a New Capillary](#).

To install the cartridge, refer to the section: [Install the Cartridge](#).

Rebuild a Capillary Cartridge

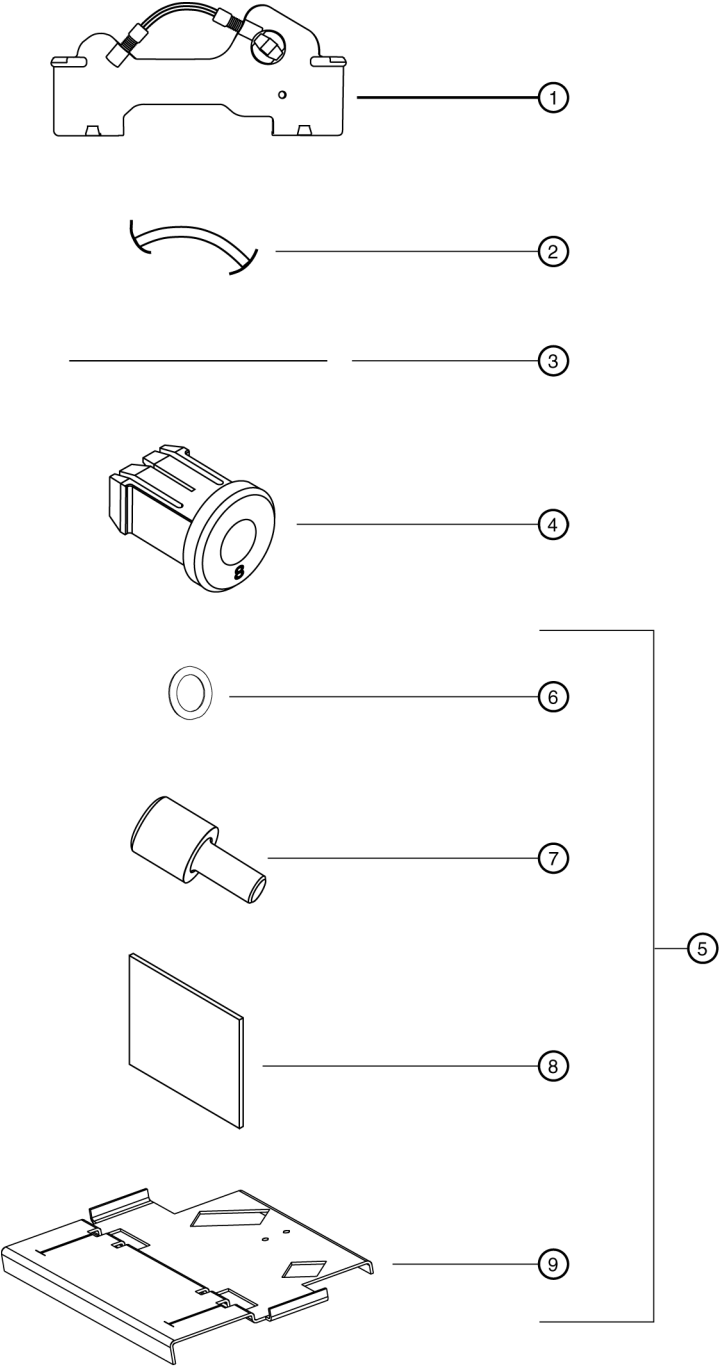
The procedure to rebuild a capillary cartridge is also available on video from the PA 800 Plus release CD, SCIEX website, and PA 800 Plus software.

Required Materials

- Capillary
- Capillary Rebuild Kit
 - Aperture O-ring
 - Capillary length template
 - Cleaving stone
 - O-ring installation tool
 - Tweezers
- Cartridge
- LIF detection: Probe guide and probe retainer
- PDA detection: Aperture, 100 μm x 800 μm
- UV detection: Aperture, 100 μm x 200 μm
- Coolant tubing kit
- CE Grade Water
- Methanol
- Felt-tip pen
- Lint-free wipes
- Magnifying lens
- Ruler
- Protective eyewear

Installation Procedures

Figure 1-15 Capillary Cartridge Rebuild Components



Item	Description
1	Cartridge

Item	Description
2	Coolant tubing
3	Capillary
4	Capillary Rebuild Kit
5	Aperture
6	Aperture O-ring
7	O-ring installation tool
8	Cleaving stone
9	Capillary length template

Do these tasks in the order given:

1. [Remove the Capillary.](#)
2. [Replace the Coolant Tubing and O-rings.](#)
3. [Install a New Capillary.](#)
4. For UV/PDA detection: [Replace the Aperture \(UV or PDA Detection\).](#)
5. For LIF detection: [Install the Probe Guide and Probe Retainer \(LIF Detection\).](#)
6. [Install the Seal-Retainer Clips.](#)
7. [Trim the Capillary Ends.](#)

Remove the Capillary

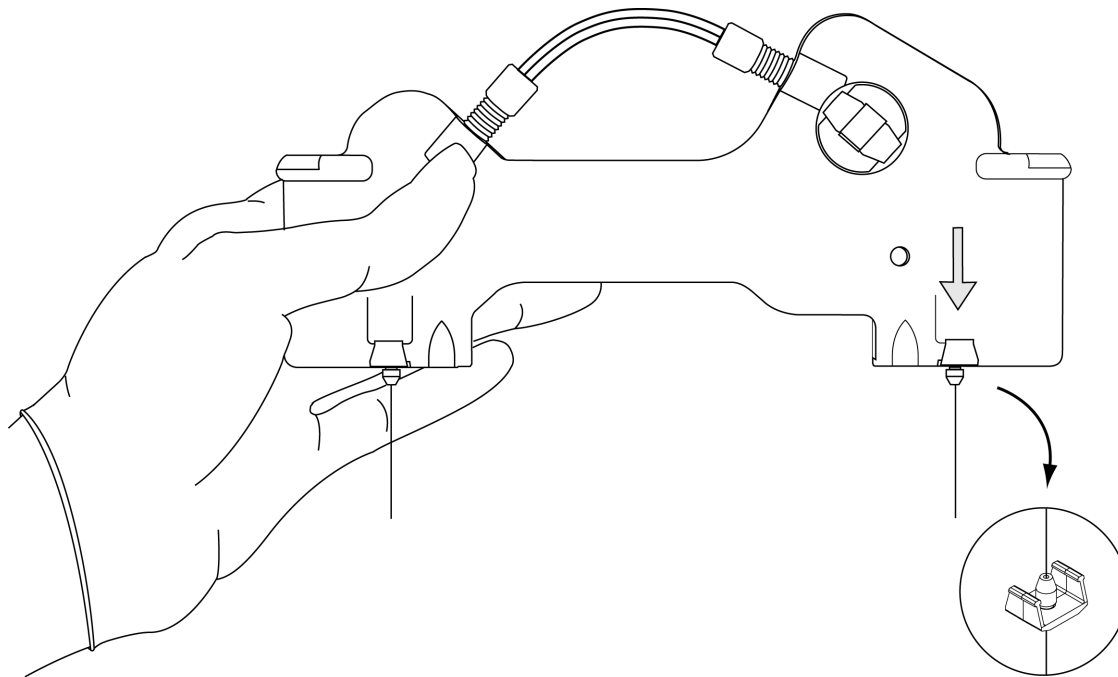


WARNING! Puncture Hazard. Be careful when handling the cartridge. The tips of the capillaries are extremely sharp.

CAUTION: Potential System Damage. Do not remove the capillary from the inlet side of the cartridge. To prevent the capillary from breaking, remove the capillary from the outlet side of the cartridge only.

1. Carefully remove the seal-retainer clips from the capillary ends.

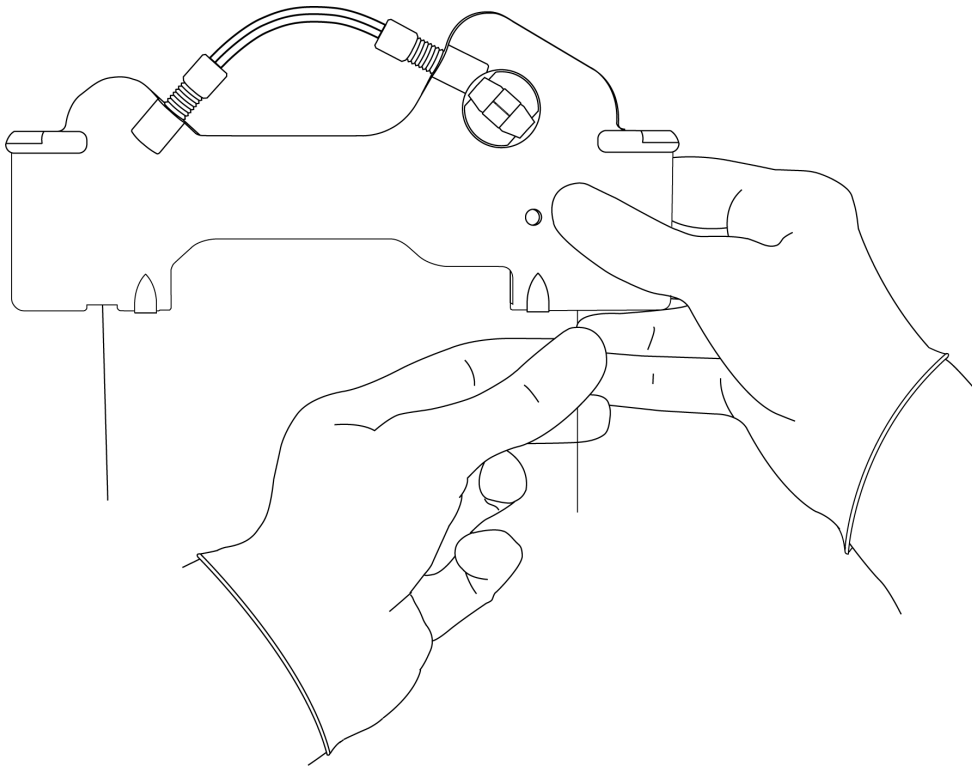
Figure 1-16 Remove the Seal-Retainer Clips



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2. Examine the seals. If the seals show signs of wear, damage, or leaking, then replace the seals.
3. At the cartridge outlet, hold the capillary tightly and pull to remove it. When the capillary window has been removed from the cartridge, hold the capillary above the window and continue to pull until the capillary is fully removed from the cartridge.

Figure 1-17 Remove the Capillary (Outlet Side)



4. If the capillary will be used again, then prepare the capillary for storage.
Refer to the applicable application guide for storage instructions.

Replace the Coolant Tubing and O-rings

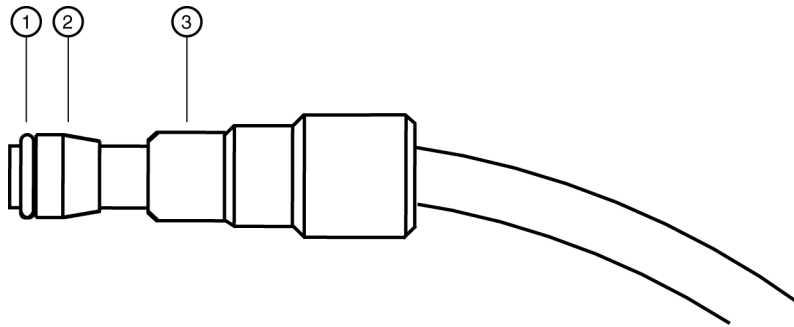
This procedure is only required to replace damaged coolant tubing or O-rings, or to use a different length of coolant tubing.

1. From the outlet side of the cartridge, do this:
 - a. Loosen and remove the tubing nut.
 - b. Remove the coolant tubing.

Note: The tubing nut and ferrule stay attached to the coolant tubing.

Installation Procedures

Figure 1-18 Coolant Tubing, Tubing Nut, Ferrule, and O-ring



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Item	Description
1	O-ring
2	Ferrule
3	Tubing nut

2. From the inlet side of the cartridge, do this:
 - a. Loosen and remove the tubing nut.
 - b. Remove the coolant tubing.

Note: The tubing nut, ferrule, and O-ring stay attached to the coolant tubing.


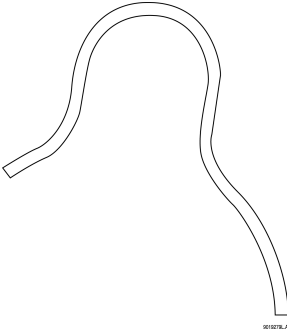
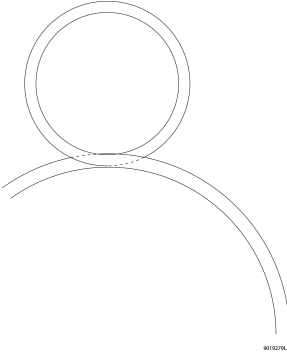
3. From the outlet side of the cartridge, remove the O-ring. If required, then use tweezers.
4. Use the following table to find the correct length of pre-formed coolant tubing and loop shape to use with the capillary length. Use only the lengths of pre-formed coolant tubing given in the table.

Note: To prevent kinks and blockages, use the pre-formed coolant tubing that is supplied in the coolant tubing kit.

For a length of coolant tubing that is not pre-formed, use the following guidelines to make loops in the coolant tubing:

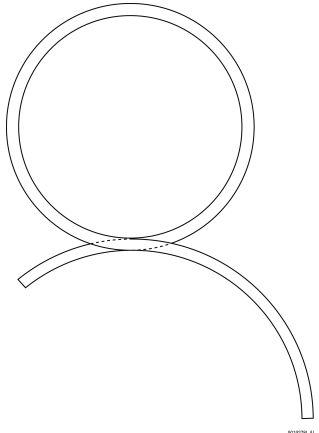
- For total capillary lengths that are longer than 60.2 cm and less than 90 cm, make one loop in the coolant tubing.
- For total capillary lengths that are 90 cm or longer, make two loops in the coolant tubing.

Table 1-2 Preformed Coolant Tubing and Related Capillary Dimensions

Capillary Length to Detector	Total Capillary Length	Description
20 cm	30.2 cm	Pre-formed coolant tubing for a total capillary length of 30.2 cm 
30 cm	40.2 cm	Pre-formed coolant tubing for a total capillary length of 40.2 cm 
40 cm	50.2 cm	Pre-formed coolant tubing for a total capillary length of 50.2 cm 

Installation Procedures

Table 1-2 Preformed Coolant Tubing and Related Capillary Dimensions (continued)

Capillary Length to Detector	Total Capillary Length	Description
50 cm	60.2 cm	Pre-formed coolant tubing for a total capillary length of 60.2 cm 

5. Install one tubing nut, ferrule (with the tapered edge toward the middle of the tube length), and O-ring over each end of the new coolant tubing.

Note: Always install a new ferrule. Used ferrules can cause leaks.

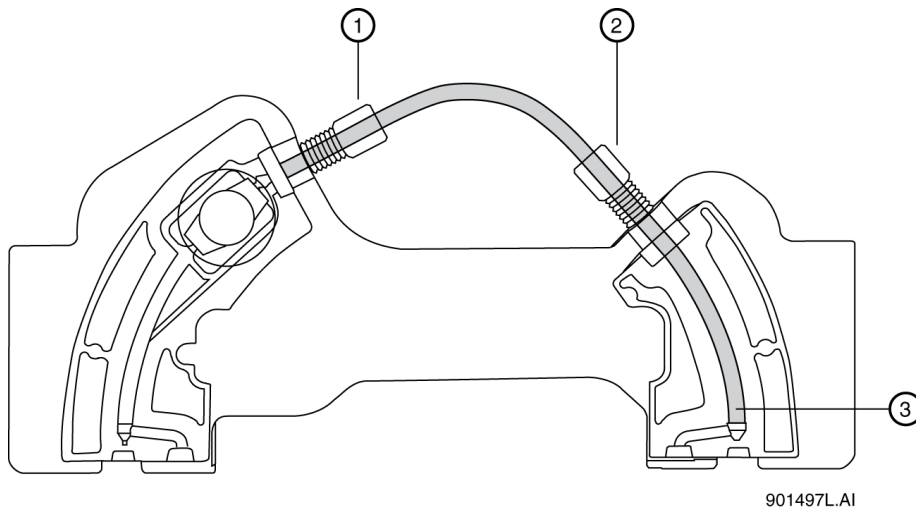
6. On the inlet side of the cartridge, do this:
 - a. Install the coolant tubing in the cartridge, and then press the coolant tubing tightly and fully into the cartridge base.
 - b. Install the tubing nut, ferrule, and O-ring.
 - c. Tighten the tubing nut until it is finger tight.

CAUTION: Potential System Damage. Do not overtighten the tubing nut. If the tubing nut is overtightened, then the cartridge might break.

Note: If leakage occurs, then examine the cartridge housing for cracks. If no cracks or other damage can be seen, then make sure that the O-ring is centered on the coolant tubing. If the coolant tubing or ferrule is damaged, then replace it.

Note: To prevent kinks and blockage of the coolant flow, do not sharply bend the coolant tubing.

Figure 1-19 Coolant Tubing in the Cartridge (Back View)



Item	Description
1	Cartridge outlet side
2	Cartridge inlet side
3	Coolant tubing installed fully in the cartridge base

7. On the outlet side, do this:
 - a. Install the other end of the coolant tubing in the cartridge, and then press the coolant tubing tightly and fully into the cartridge base.
 - b. Continue to press the coolant tubing into the cartridge until the tubing nut, ferrule, and O-ring are installed in the cartridge.
 - c. Tighten the tubing nut until it is finger tight.

Install a New Capillary

CAUTION: Potential System Damage. Do not install a pre-cut capillary. A pre-cut capillary can cause the capillary window to break.

CAUTION: Potential System Damage. To prevent breakage, do not bend the capillary at the capillary window.

CAUTION: Potential System Damage. Do not touch the capillary window, which is fragile. Oils from skin can decrease the sensitivity of detection.

1. Remove one new capillary from the storage container.

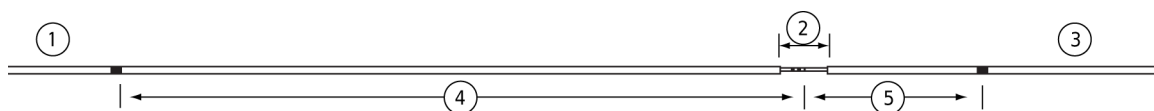
Installation Procedures

2. Carefully uncoil and straighten the capillary.
The capillary is protected by clear plastic tubing. The capillary window is protected by an additional rectangular shield.
3. Tape down the protective tubing near the end of the capillary that is farthest from the capillary window.
4. From the end of the capillary that is closest to the capillary window, pull the capillary out of the protective tubing. When the capillary window is out of the protective tubing, hold the capillary between the capillary window and the edge of the protective tubing and continue to pull the capillary. Do not pull on the capillary window.
5. Measure 10.2 cm from the middle of the capillary window to the outlet end. Use a felt-tip pen to make a mark on the capillary just outside the measured length.

This is the short side of the capillary, which is closest to the capillary window.

Note: Make sure that the position markings are just outside the measured end points so that the markings are removed when the capillary is trimmed.

Figure 1-20 Capillary Position Markers



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Item	Description
1	Inlet
2	Window
3	Outlet
4	Applicable length to the detector
5	10.2 cm

Tip! To make sure that measurements are consistent, put tape on the edge of a workbench and make measurements marks on the tape. When a new capillary is prepared, use the marks on the tape to make marks on the capillary.

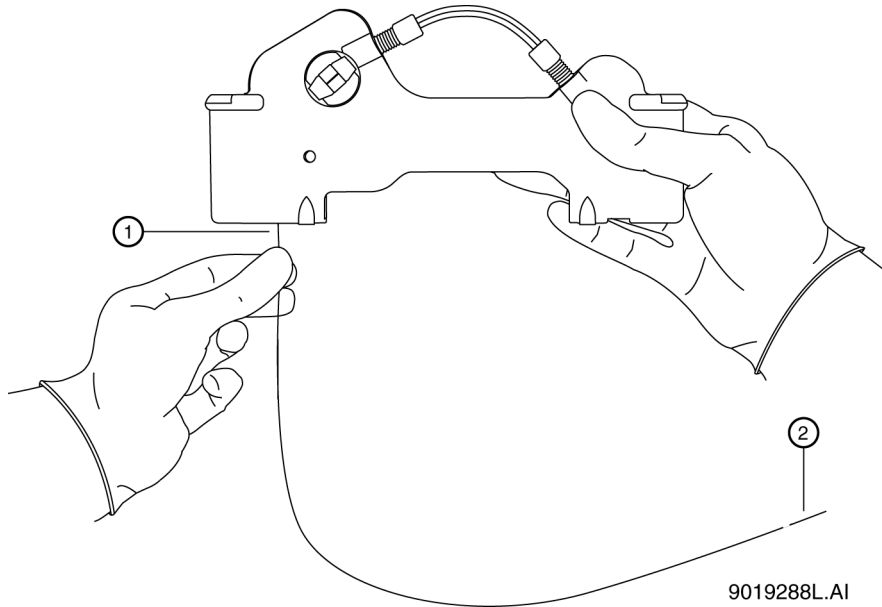
6. Measure the required length from the middle of the capillary window to the inlet end. Use a felt-tip pen to make a mark on the capillary just outside the measured length.
This is the long side of the capillary, which is farthest from the window.

Note: Do not cut the capillary at this time.

7. Install the long side of the capillary (the end farthest from the capillary window) in the outlet side of the cartridge.

Note: If the cartridge will be used for the first time, then it might be required to spin the capillary while installing it to make a hole in the aperture.

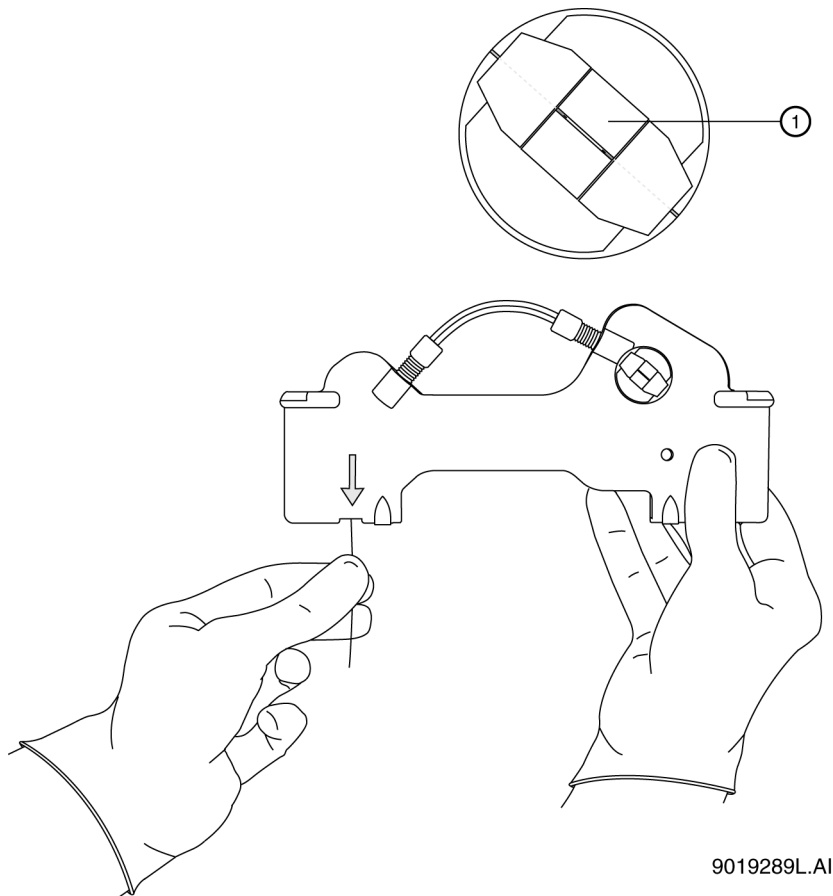
Figure 1-21 Install the Long End of the Capillary in the Outlet Side of the Cartridge (Back View)



Item	Description
1	Capillary outlet
2	Capillary window

8. Carefully push the capillary in through the cartridge outlet until the capillary shows at the cartridge inlet.
9. From the inlet side of the cartridge, pull the capillary through the cartridge until the capillary window shows in the center of the cartridge window.

Figure 1-22 Center the Capillary Window in the Cartridge Window



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Item	Description
1	Capillary window

10. For UV/PDA detection, continue with the section: [Replace the Aperture \(UV or PDA Detection\)](#). For LIF detection, continue with the section: [Install the Probe Guide and Probe Retainer \(LIF Detection\)](#).

Replace the Aperture (UV or PDA Detection)

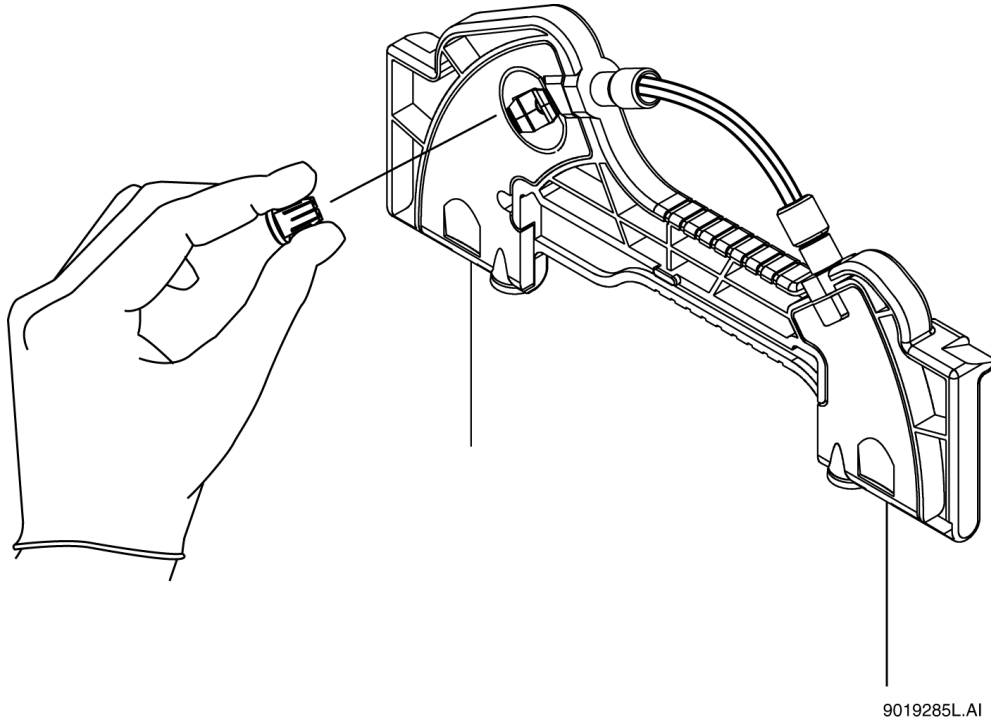
Note: For UV or PDA detection, use a standard cartridge and an aperture. Refer to the applicable application guide to find the correct aperture for the application.

Note: Before the aperture is installed, remove the O-ring from the previous installation.

1. To remove the aperture, do this:

- a. To remove the aperture, push the aperture from the front of the cartridge and pull the aperture from the back of the cartridge.

Figure 1-23 Remove the Aperture (Back View)

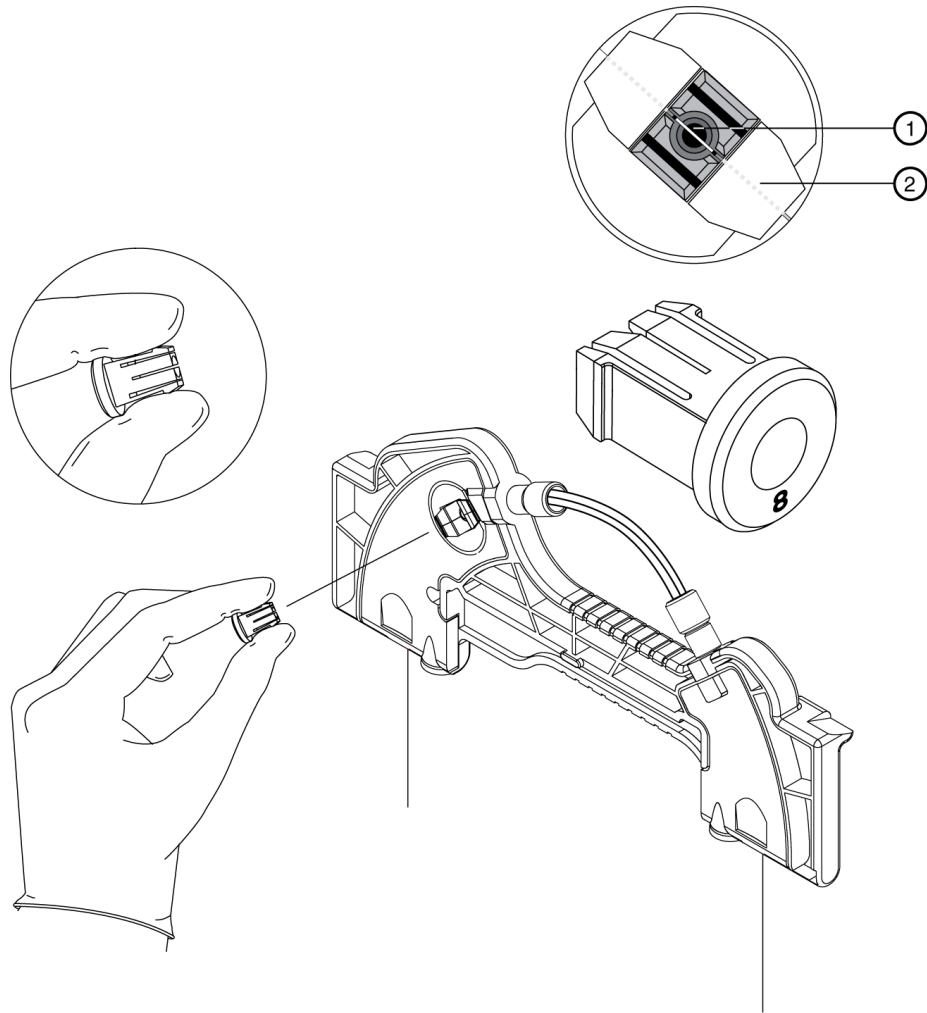


- b. To remove the O-ring from the aperture, lightly tap the aperture on the work surface or use tweezers.

Note: Be careful not to cause damage to the aperture.

2. To install the aperture, do this:
 - a. From the back of the cartridge, carefully align the center groove of the aperture with the capillary window.

Figure 1-24 Align the Groove of the Aperture with the Capillary Window

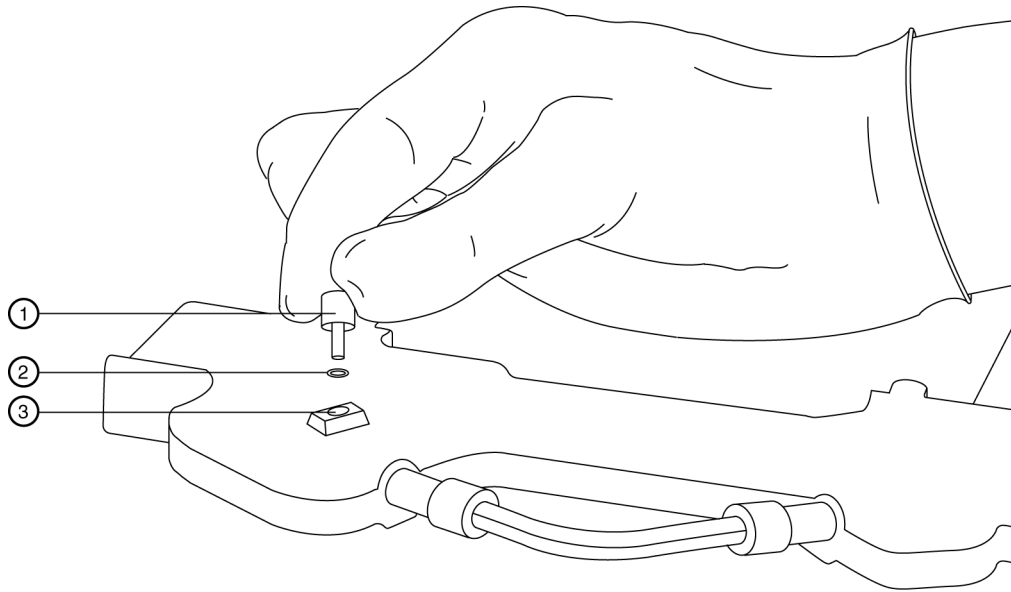


Item	Description
1	Aperture
2	Capillary

- b. From the back of the cartridge, carefully align the center groove of the aperture with the capillary window.
- c. Press the aperture into position.
3. To install an o-ring for the aperture, do this:
 - a. From the front side of the cartridge, install the retainer O-ring in the aperture clip hole.

- b. Use an O-ring insertion tool to press the O-ring carefully into the aperture clip until the O-ring is fully installed.

Figure 1-25 Install the O-ring in the Aperture Clip Hole (Front View)



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Item	Description
1	O-ring insertion tool
2	O-ring
3	Aperture clip hole

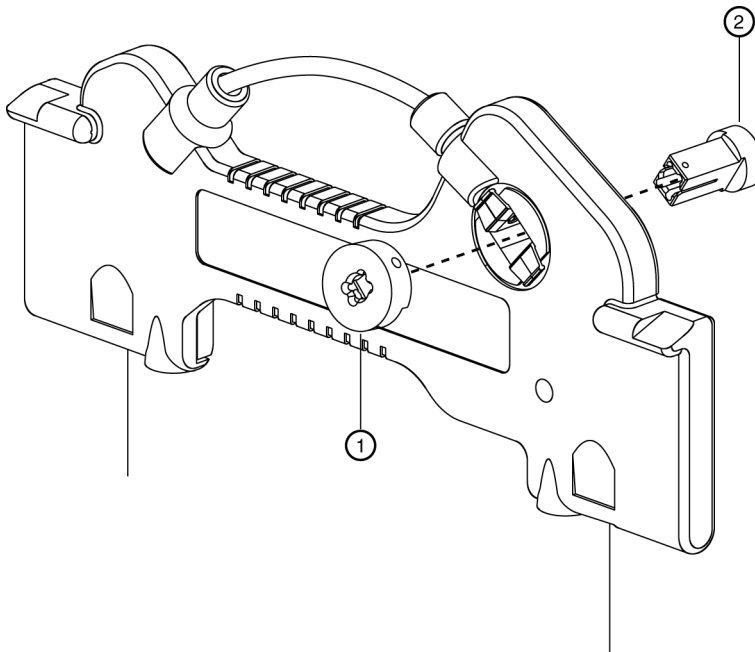
4. Continue with the section: [Install the Seal-Retainer Clips](#).

Install the Probe Guide and Probe Retainer (LIF Detection)

Note: For LIF detection, use a standard cartridge, probe guide, and probe retainer.

1. From the back of the cartridge, carefully install the probe guide.
2. Install the probe retainer in the probe guide.
The lock pins on the probe retainer make a click when they engage with the dimples in the probe guide.

Figure 1-26 Install the Probe Guide and Probe Retainer



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Item	Description
1	Probe guide
2	Probe retainer

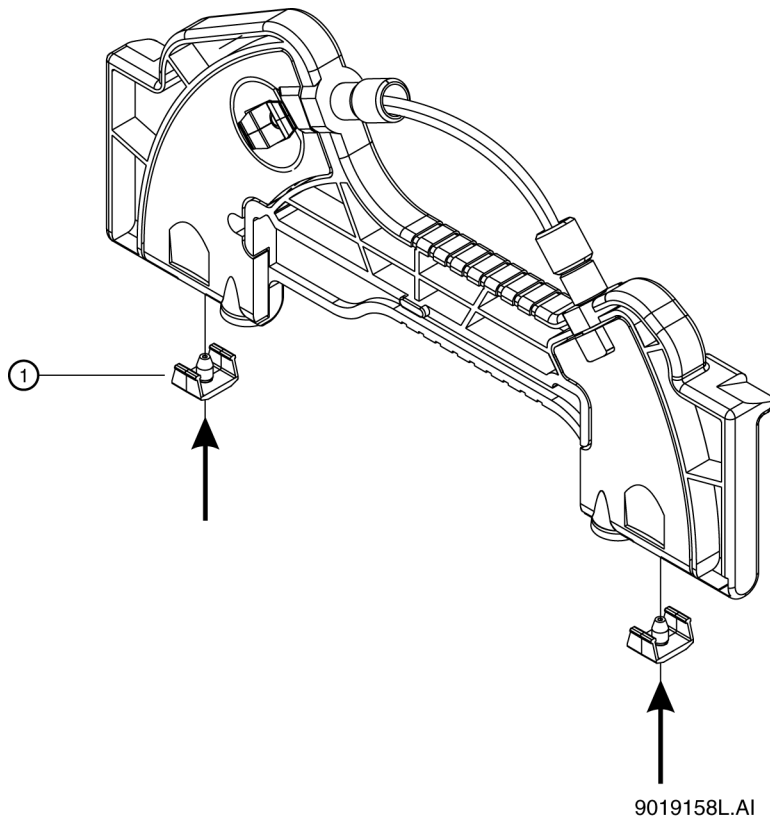
3. Continue with the section: [Install the Seal-Retainer Clips](#).

Install the Seal-Retainer Clips

1. Carefully install a seal-retainer clip on each capillary end, and then close the clip edges.

Note: Make sure that both the front and back edges of each clip fully close into position with a click.

Figure 1-27 Install the Capillary Seal-Retainer Clips



Item	Description
1	Seal-retainer clips

2. Examine the capillary ends. If the ends are not straight, then remove the seal-retainer clips and install them again.

Trim the Capillary Ends



WARNING! Puncture Hazard. Be careful when handling the cartridge. The tips of the capillaries are extremely sharp.



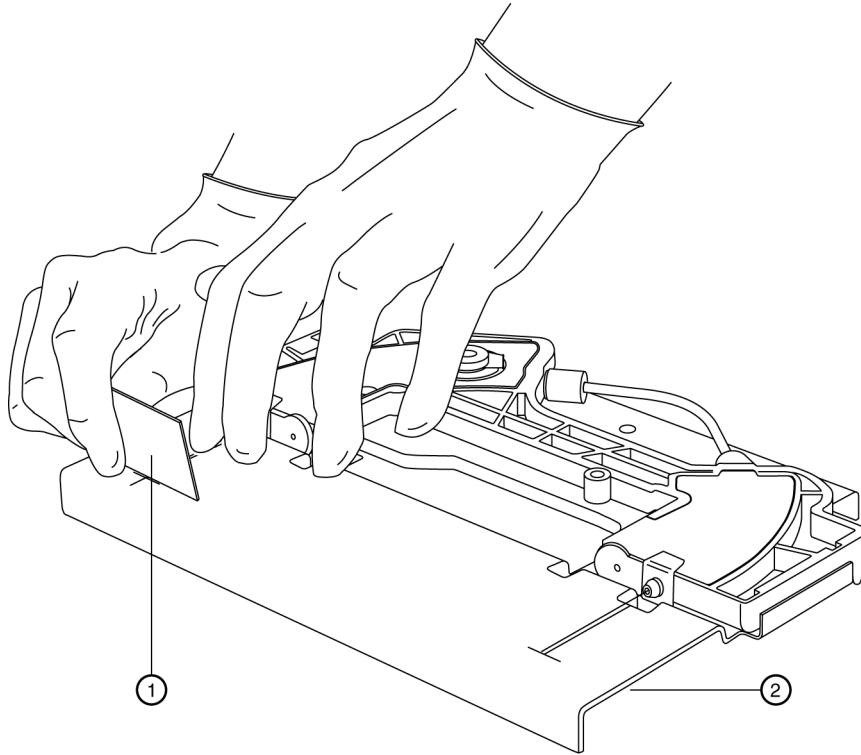
WARNING! Personal Injury Hazard. Wear protective eyewear when cleaving and breaking the capillary.

Note: Only trim the capillary when the wet chemistry sample, buffer trays, and sequence are ready and the system is ready to operate. After the capillary is cut, make sure to keep the protruding capillary ends in a solution that is recommended in the applicable application guide.

Installation Procedures

1. Put the cartridge face down against the capillary length template.

Figure 1-28 Trim the Capillary



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Item	Description
1	Cleaving stone
2	Template

2. Align the measurement mark on the inlet side of the capillary end just below the score line on the capillary length template.

Note: Make sure that the capillary end is straight between the two lines on the template.

Note: Do not make a sawing motion (back and forth) with the cleaving stone.

3. While holding the capillary against the template, make a score on the inlet end of the capillary at the template cross-mark.

Note: Hold the cleaving stone at a 30-degree angle while scoring.

Note: Make sure to cut the measurement mark off of the capillary.

4. Remove the excess inlet capillary. Do not bend the capillary.
5. Align the measurement mark on the outlet side of the capillary end to just below the score line on the capillary length template.

Note: Make sure that the capillary end is straight between the two lines on the template.

6. While holding the capillary against the template, make a score on the outlet end of the capillary at the template cross-mark.

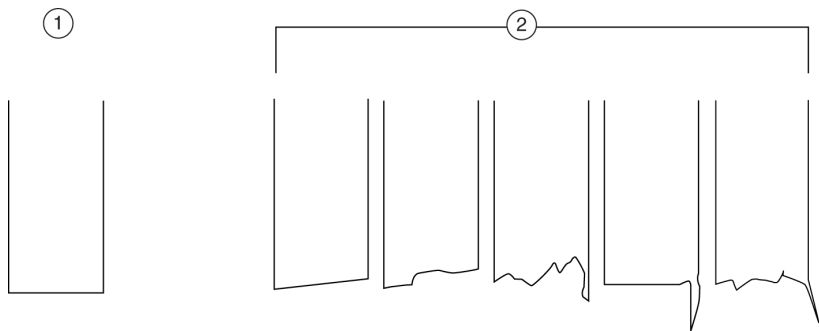
Note: Hold the cleaving stone at a 30-degree angle while scoring.

Note: Make sure to cut the measurement mark off of the capillary.

7. Remove the excess outlet capillary. Do not bend the capillary.
8. Use a magnifying lens to examine the capillary ends. Make sure that the ends are smooth, not jagged.

In the following figure, only cut 1 is satisfactory.

Figure 1-29 Capillary Cuts



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Item	Description
1	Acceptable cuts
2	Unacceptable cuts

9. Use the rough side of the cleaving stone to make the capillary ends smooth.
10. Use lint-free wipes dampened with methanol and then lint-free wipes dampened with CE Grade Water to wipe the ends of the capillary.
The cartridge is ready to use.

Installation Procedures

Note: For information about capillary equilibration and storage conditions, refer to the applicable application guide.

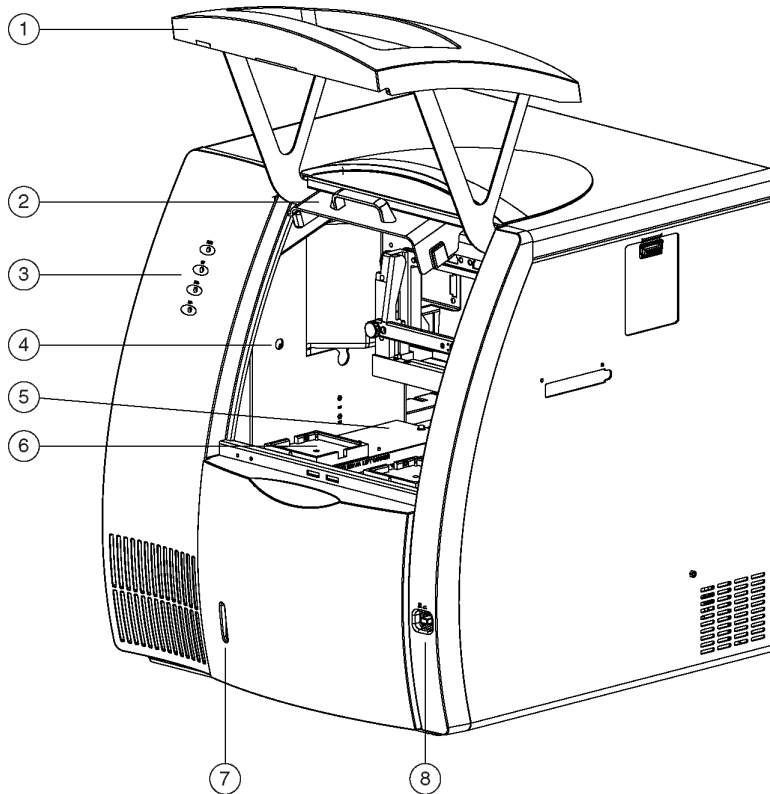
Install the Cartridge

Required Materials

- Fully assembled capillary cartridge, including an installed capillary

1. Open the sample cover and cartridge cover.

Figure 1-30 PA 800 Plus System



Item	Description
1	Sample cover or outer door (open)
2	Cartridge cover or inner door (open)
3	Indicators
4	Coolant fill port

Item	Description
5	Sample trays
6	Buffer trays
7	Coolant level indicator
8	Power switch

2. Loosen the two thumbscrews on the insertion bar, and then lift the insertion bar.
3. Put the capillary cartridge over the interface block, and then carefully lower the cartridge into position.
4. Lower the insertion bar, and then tighten the thumbscrews.
5. Close the cartridge cover and sample cover.

Note: To make sure that performance is correct, replace the aperture after 3 months of use. Refer to the section: [Replace the Aperture \(UV or PDA Detection\)](#).

About Vials

CAUTION: Potential System Damage. Do not fill any vial with more than 1.5 mL of liquid. Fill waste vials with 1.0 mL of liquid. Do not let more than 1.5 mL of liquid collect in waste vials. If a vial is filled with more than 1.5 mL of liquid, then the pressure system can be damaged.

CAUTION: Potential System Damage. Do not underfill the vials or let the liquid level get too low. If the liquid level in the vials is too low, then the separation capillary can fill with air and cause the vials to break if voltage is applied.

Note:

- Always use the applicable vial cap.
 - Do not use any vial or cap more than once.
 - Make sure that the vial caps are clean and fully dry before use.
-

Fill a Universal Vial

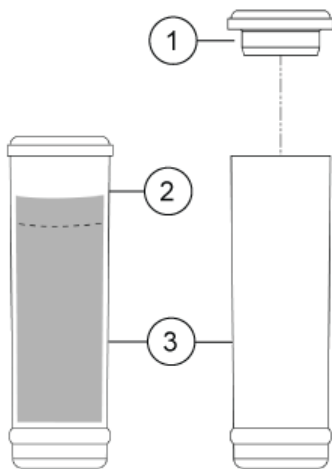
Required Materials

- | |
|---|
| <ul style="list-style-type: none">• Universal vials• Universal vial caps |
|---|

Note: To prevent splashing, put the vials in the tray before liquid is added and the cap is attached.

1. Fill the universal vial as shown in the following figure.
For the fill volume, refer to the applicable application guide.

Figure 1-31 Universal Vial



Item	Description
1	Universal vial cap
2	Maximum fill level
3	Universal vial

2. Put a universal cap on the universal vial, and then push the cap into position.

Fill a Microvial

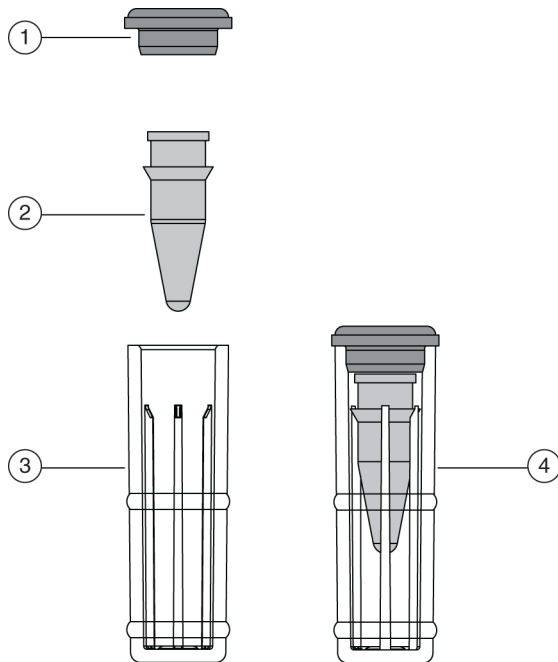
Required Materials
<ul style="list-style-type: none"> • Microvials • Universal vials • Universal vial caps

Note: To prevent splashing, put the vials in the tray before liquid is added and the cap is attached.

1. Fill the microvial with at least 100 μ L of sample.

Note: When filling the vials, do not make air bubbles. If there are air bubbles in the vial, then spin the vial in a centrifuge for a few seconds to remove the bubbles.

Figure 1-32 Microvial



Item	Description
1	Universal cap
2	Microvial

Installation Procedures

Item	Description
3	Universal vial
4	Microvial in a universal vial

- Put the microvial in the universal vial.
- Put the universal cap on the universal vial, and then push the cap into position.

Note: Small amounts of sample can evaporate into the space in the microvial holder. To minimize the evaporation, put 200 μ L to 800 μ L of CE Grade Water in the bottom of the universal vial.

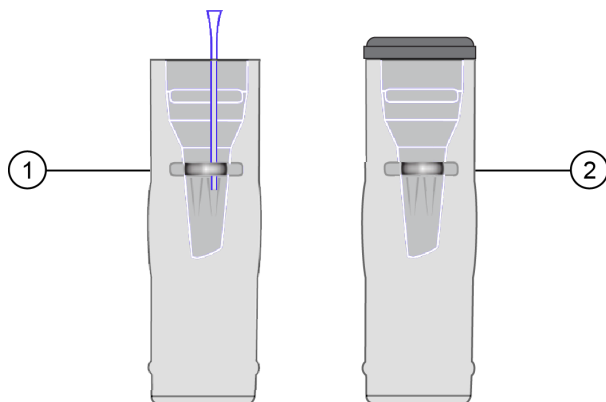
Fill a nanoVial

For sample volumes between 5 μ L and 50 μ L, use a nanoVial.

1. Use a thin pipette tip to transfer the sample to the deeper well of a nanoVial.

When the tab points to the user, the deeper well is on the left side of the nanoVial.

Figure 1-33 nanoVial



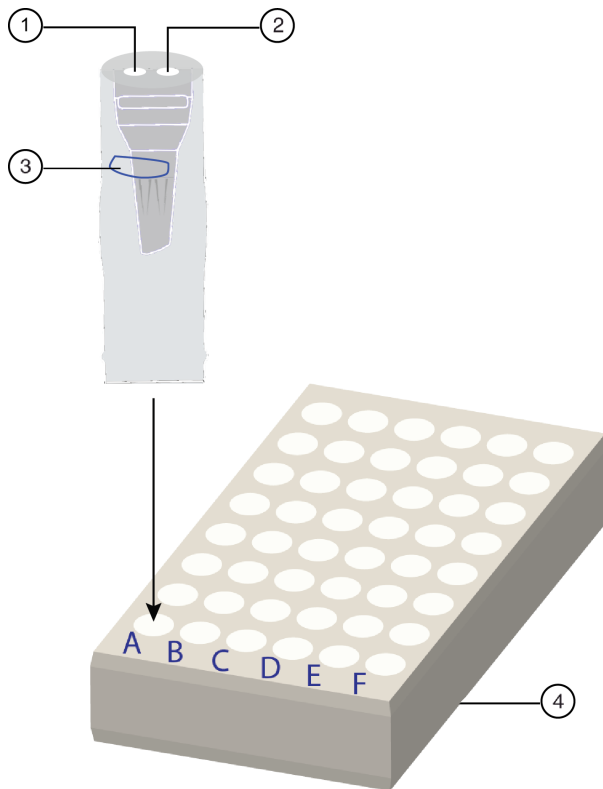
Item	Description
1	nanoVial inside the CESI vial with the thin pipette tip transferring sample
2	nanoVial inside the CESI vial with the cap attached

2. Put the CESI cap on the CESI vial, and push the cap into position.
3. Put the sample vial in the inlet sample tray at position S1:A1.

Note: Make sure that the tab on the outside of the vial points to the front of the sample tray.

Installation Procedures

Figure 1-34 Orientation of the nanoVial Tab in the Sample Tray



Item	Description
1	Electrode at the left side of the nanoVial
2	Capillary at the right side of the nanoVial (for use in loading sample)
3	Tab outside of the nanoVial
4	Sample tray

Note: If there are not enough sample vials to fill the tray, then keep every second column in the sample tray empty. This will make it easier to remove the nanoVials from the tray.

Note: When filling the vials, do not make air bubbles. If there are air bubbles in the vial, then spin the vial in a centrifuge for a few seconds to remove the bubbles.



WARNING! Electrical Shock Hazard. Turn off the power to the system before any system disassembly. Failure to do so can cause electrical shock or other injury.



WARNING! Electrical Shock Hazard. To prevent the risk of electrical shock or injury, do not do maintenance or repair procedures that are not specifically described in this manual. Contact a SCIEX Field Service Employee (FSE) for maintenance service and support.



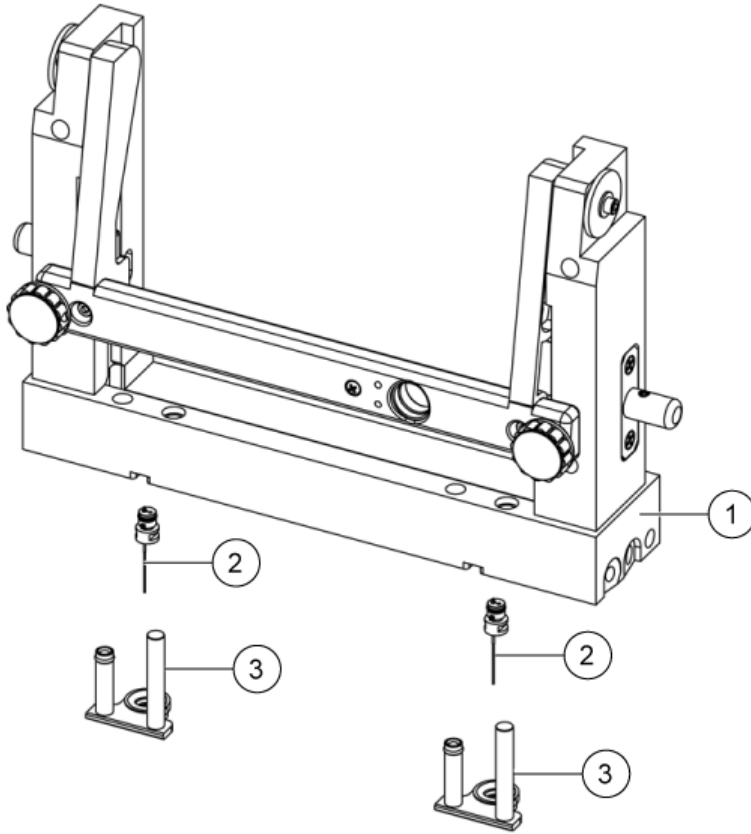
WARNING! Electrical Shock Hazard. Do not try to disable any of the system interlocks or safety mechanisms.



WARNING! Ionizing Radiation Hazard, Biohazard, or Toxic Chemical Hazard. Before cleaning or maintenance, identify whether decontamination is required. If radioactive materials, biological agents, or toxic chemicals have been used with the system, then the customer must decontaminate the system before cleaning or maintenance.

Interface Block, Electrodes, and Insertion Levers

Figure 2-1 Interface Block, Electrodes, and Insertion Levers



Item	Description
1	Interface block
2	Electrodes
3	Insertion levers

Replace the Insertion Levers

Required Materials
<ul style="list-style-type: none">• Insertion Lever Interface Parts Kit



WARNING! Puncture Hazard. Do not put fingers directly below the electrodes during installation of the insertion levers. The electrode ends are sharp. Handle them carefully.

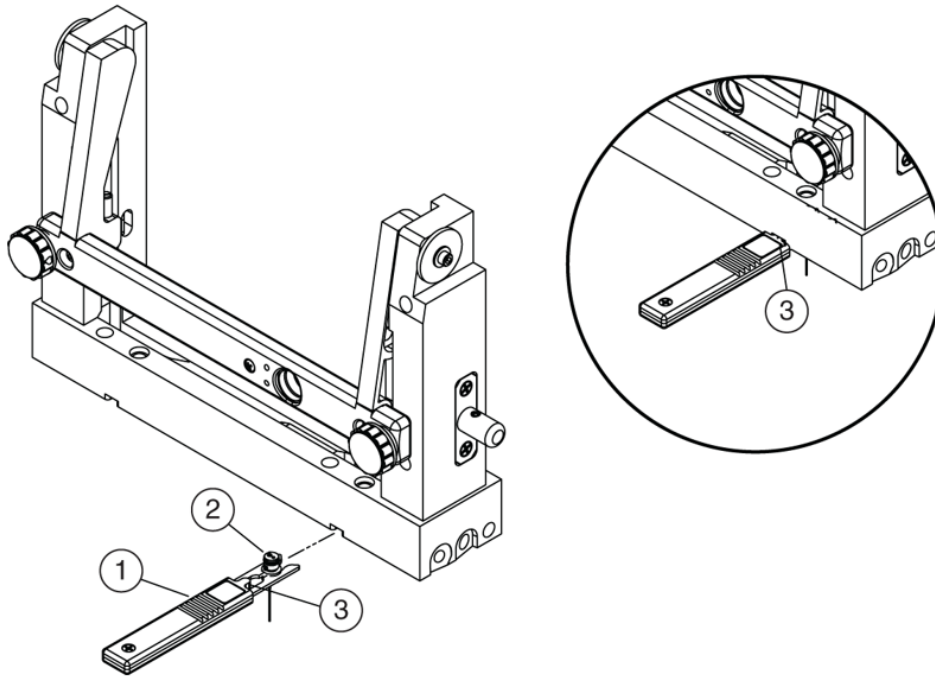
1. To remove an insertion lever, do this:
 - a. Go to the Direct Control window, and then click **Load**.
 - b. Open the cartridge cover, and then wait for the coolant to drain from the cartridge.
 - c. Turn off the power to the system.
 - d. Loosen the two thumbscrews on the insertion bar, and then lift the insertion bar.
 - e. Remove the cartridge from the interface block.
 - f. If the trays prevent access to the insertion levers, then remove the trays.
 - g. Hold the insertion lever with both hands and pull down firmly.
2. To install an insertion lever, do this:
 - a. Align the O-ring and electrode hole in the insertion lever directly under the electrode. The short cylinder side of the insertion lever should be under the spring.
 - b. With fingers on the sides of the insertion lever, push the insertion lever up into the interface block until the insertion lever moves into position with a click.

Replace the Electrodes

Required Materials
<ul style="list-style-type: none">• Electrodes• Electrode tool

1. To remove an electrode from the interface block, do this:
 - a. Remove the insertion levers.
Refer to the section: [Replace the Insertion Levers](#).
 - b. Align the electrode tool so that it is flush with the bottom of the interface block.
 - c. Push straight forward under the interface block to catch the electrode with the tool.
 - d. Make sure that the nub at the end of the electrode tool handle goes into the notch on the interface block.
 - e. Pry carefully with the electrode tool to remove the electrode from the interface block.
 - f. Remove the electrode from the electrode tool.

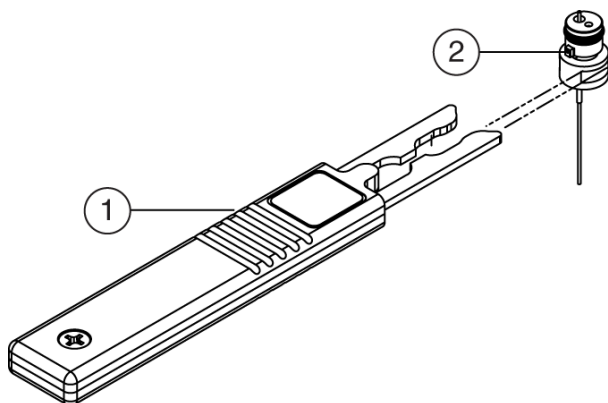
Figure 2-2 Remove an Electrode



Item	Description
1	Electrode tool
2	Electrode
3	Nub at the end of the electrode tool handle

2. To install an electrode in the interface block, do this:
 - a. Put an electrode in the electrode tool so that the electrode key points toward the user.
 - b. Align the electrode tool below the notch and parallel to the bottom of the interface block.
 - c. Push up to install the electrode in the interface block with a click.
 - d. To remove the electrode tool, pull straight back.
 - e. Install the insertion levers.
Refer to the section: [Replace the Insertion Levers](#).

Figure 2-3 Install an Electrode



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Item	Description
1	Electrode tool
2	Electrode key

Clean the Electrodes, Insertion Levers, and Interface Block

Required Materials

- Beaker
- Cotton swabs
- Lint-free wipes
- 150 mL CE Grade Water
- Methanol
- Mirror
- Pen light

Note: To prevent corrosion of the ion source, frequently clean the dry chemical waste that collects below the sprayer.

1. Remove the insertion levers.
Refer to the section: [Replace the Insertion Levers](#).
2. Immerse both insertion levers in a beaker with at least 150 mL of CE Grade Water.
3. Remove the electrodes.

Maintenance

Refer to the section: [Replace the Electrodes](#).

4. Immerse both electrodes in the CE Grade Water with the insertion levers for 2 hours.
5. Remove the parts from the CE Grade Water, and then use lint-free wipes to dry them fully.
6. Use cotton swabs dampened with CE Grade Water and then cotton swabs dampened with methanol to clean the interface block.
7. Use a mirror and pen light to examine the interface block.
8. Do steps 4 through 7 until the interface block is clean.
9. Let the interface block surface dry.
10. Install the electrodes.
11. Install the insertion levers.

Refer to the section: [Replace the Insertion Levers](#).

12. Install the cartridge.
13. Lower the insertion bar, and then tighten the two thumbscrews.
14. Close the cartridge cover.
15. Turn on the power to the system.

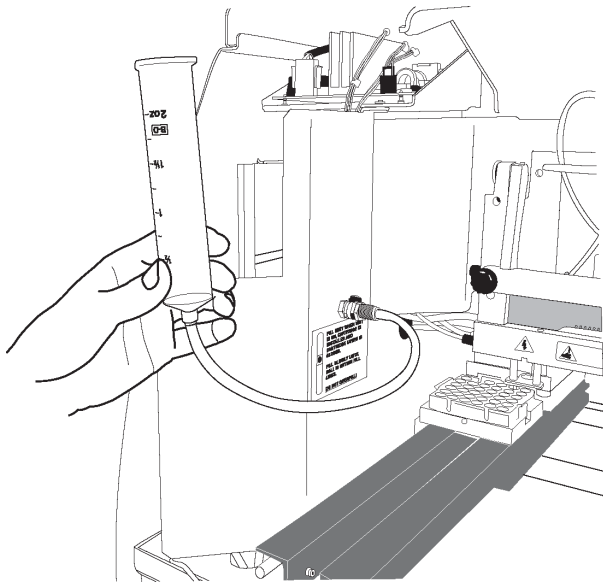
Add Capillary Cartridge Coolant

Required Materials
<ul style="list-style-type: none">• Capillary cartridge coolant (PN 359976)• Coolant fill tool (PN 144647)

CAUTION: Potential System Damage. To prevent damage, do not use the plunger in the coolant fill tool when coolant is added. Gravity supplies sufficient force to pull the coolant into the system.

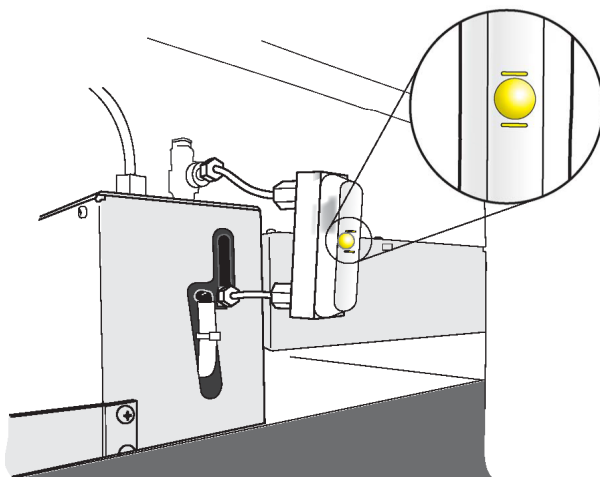
1. Open the sample cover.
2. Connect the coolant fill tool to the coolant fill port.

Figure 2-4 Coolant Fill Tool Connected to the Coolant Fill Port



3. Fill the syringe with 120 mL of coolant.
4. Slowly add coolant until the fill indicator is between the yellow lines in the coolant sight glass.

Figure 2-5 Coolant Sight Glass



5. Remove the coolant fill tool, and then close the sample cover.

Clean the Fiber-Optic Cable Connectors (UV/PDA Detectors)

Required Materials
<ul style="list-style-type: none">• Nonabrasive lens tissue• Analytical methanol, ethanol, or isopropanol

CAUTION: Potential Wrong Result. Do not touch the surface of the fiber-optic cable with fingers. Oils from the skin can decrease performance.

1. Go to the Direct Control window, and then click **Load**.
2. Open the cartridge cover.
3. Turn off the power to the system.
4. Loosen the two thumbscrews on the insertion bar, and then lift the insertion bar.
5. Disconnect the fiber-optic cable at the detector.
6. Disconnect the fiber-optic cable at the insertion bar.
7. Dampen a nonabrasive lens tissue with analytical methanol, ethanol, or isopropanol.
8. Clean the surface of each fiber-optic cable connector. Make sure that each connector is dry before it is connected.
9. Connect the fiber-optic cable at the insertion bar.
10. Connect the fiber-optic cable at the detector.
11. Lower the insertion bar, and then tighten the two thumbscrews.
12. Close the cartridge cover.

Change the Deuterium Lamp

Required Materials
<ul style="list-style-type: none">• Deuterium lamp• 7/64 in. hex key• Powder-free gloves



WARNING! Hot Surface Hazard. Before a lamp is replaced, turn off the power to the system, and then let the lamp cool fully. A hot lamp will cause burns.

CAUTION: Potential Wrong Result. Before the deuterium lamp is installed, make sure that an orange O-ring is installed on the lamp flange. A missing O-ring decreases lamp performance.

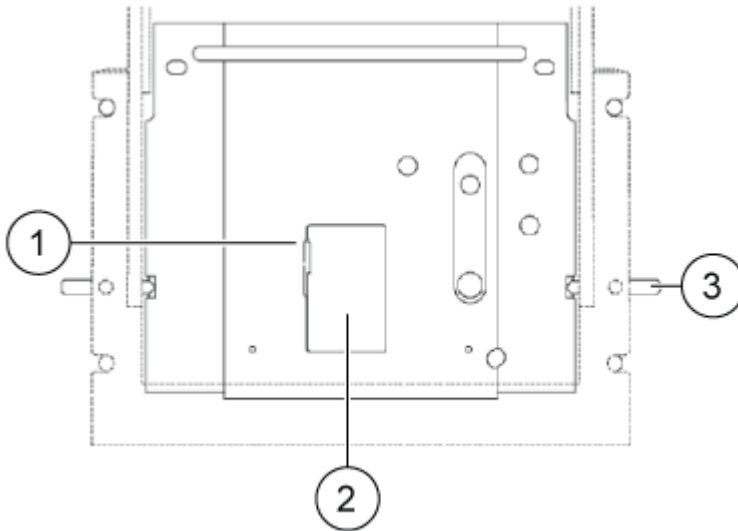
CAUTION: Potential System Damage. Do not touch the envelope of the lamp. Oils from skin can cause damage to the lamp.

CAUTION: Potential System Damage. Use powder-free gloves to handle the UV lamp. Fingerprints, under the high temperatures and strong UV intensity that come from operating the UV lamp, form corrosive compounds that etch the surface of the UV lamp and can cause it to break when it is turned on. When handling the UV lamp, keep the UV optical window dry and protect it from abrasion.

The deuterium lamp is used by the UV and PDA detectors. If the baseline is excessively noisy or the lamp does not illuminate, then the deuterium lamp might need to be replaced.

1. Go to the Direct Control window, and then click **Load**.
2. Open the cartridge cover.
3. Turn off the power to the system, and then let the deuterium lamp cool.
4. Loosen the two thumbscrews on the insertion bar, and then lift the insertion bar.
5. Remove the capillary cartridge from the interface block.
6. Loosen the two thumbscrews on the optics source assembly.

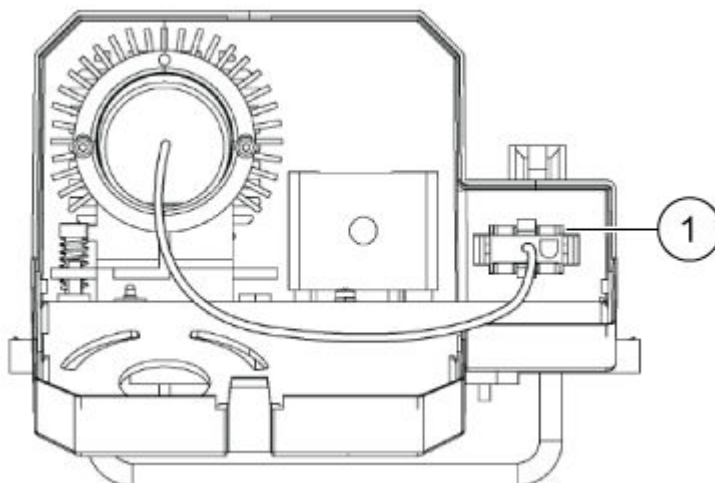
Figure 2-6 Optics Source Assembly



Item	Description
1	Access cover latch
2	Access cover
3	Thumbscrews (one on each side)

7. Pull forward to remove the optics source assembly.
8. Put the assembly on a clean work surface.
9. Open the access cover on the back of the optics source assembly, and then disconnect the power connector.

Figure 2-7 Deuterium Lamp Assembly



Item	Description
1	Power connector

10. Use a 7/64 in. hex key to remove the two screws that hold the deuterium lamp in position, and then remove the lamp from the lamp housing.
11. To install the new deuterium lamp, align the flange guide notch in the lamp with the housing guide pin.
12. Install the two hex screws, and then tighten them until they are snug.
13. Connect the power plug to the optics source assembly, and then close the access cover.
14. Put the optics source assembly in the mounting location, align the two upper guide pins, and then tighten the two thumbscrews.
15. Install the capillary cartridge in the interface block.
16. Lower the insertion bar, and then tighten the two thumbscrews.
17. Close the cartridge cover.
18. Turn on the power to the system.
19. Open the 32 Karat software.
20. Click **Control > Diagnostics > View**.

The applicable Diagnostics dialog opens.

Figure 2-8 PDA Diagnostics Dialog

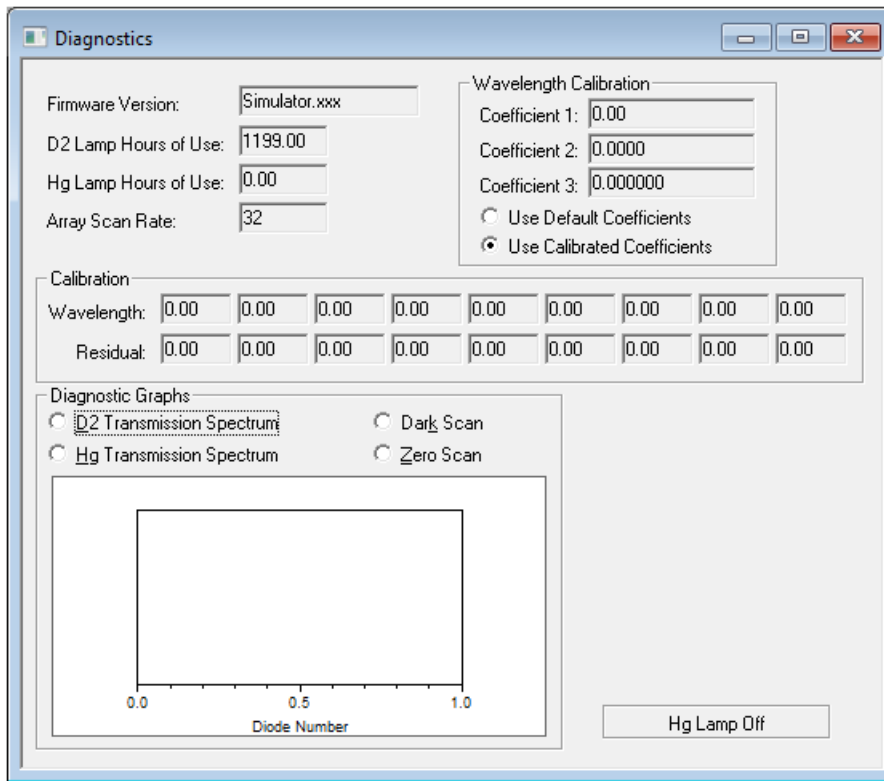
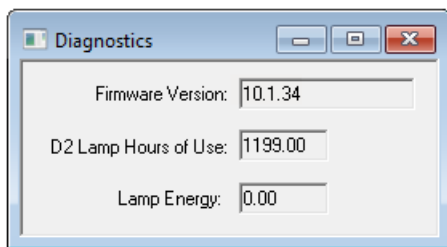
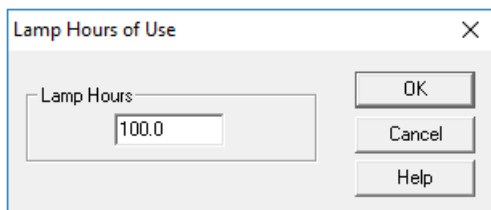


Figure 2-9 UV Diagnostics Dialog



21. Click **Control > Diagnostics > Set D2 Lamp Hours**.

Figure 2-10 Lamp Hours of Use Dialog



22. Type 0, and then click **OK**.

LIF Detector Maintenance



WARNING! Do not do maintenance on the 488 nm laser module. The 488 nm laser module emits laser light that can cause serious injury to the eyes. Laser maintenance must be done only by a SCIEX field service employee (FSE).

Examine the LIF Detector



WARNING! Do not try to defeat the system interlock for the laser module. If the interlock is defeated, then harmful laser power up to 3 mW can be emitted from the interior of the module or the fiber-optic cable.

The LIF detector system is designed to prevent exposure to laser light from the laser box, fiber-optic cable, and detector.

- To make sure that the laser light is contained, do the following steps at regular intervals:
 - a. Examine the full length of the fiber-optic cable to make sure that it is in a satisfactory condition.
 - b. Examine the laser module housing to make sure that panels are not loose. Loose panels can permit dangerous access to laser energy from inside the module.
 - c. Make sure that the interlock operates correctly.

Clean the LIF Detector

Required Materials
<ul style="list-style-type: none">• CE Grade Water or mild detergent• Soft cloths

CAUTION: Potential System Damage. Do not clean a filter unless it is sufficiently dirty to have an effect on system performance. The LIF filters are delicate optical components that can easily be damaged during cleaning.

The LIF filters must be protected from dirt, dust, and fingerprint contamination. When installed in the filter housing, the filters are protected from contamination and usually do not require cleaning.

1. When required, use a clean cloth dampened with CE Grade Water or a mild detergent to wipe the outside surfaces of the LIF detector and laser module.
2. Use a soft, dry cloth to dry the outside surfaces.

Store the LIF Detector

When the LIF detector is not in use, keep it in the probe holder on the 488 nm laser module to protect the probe from mechanical damage, dust, and other particles.

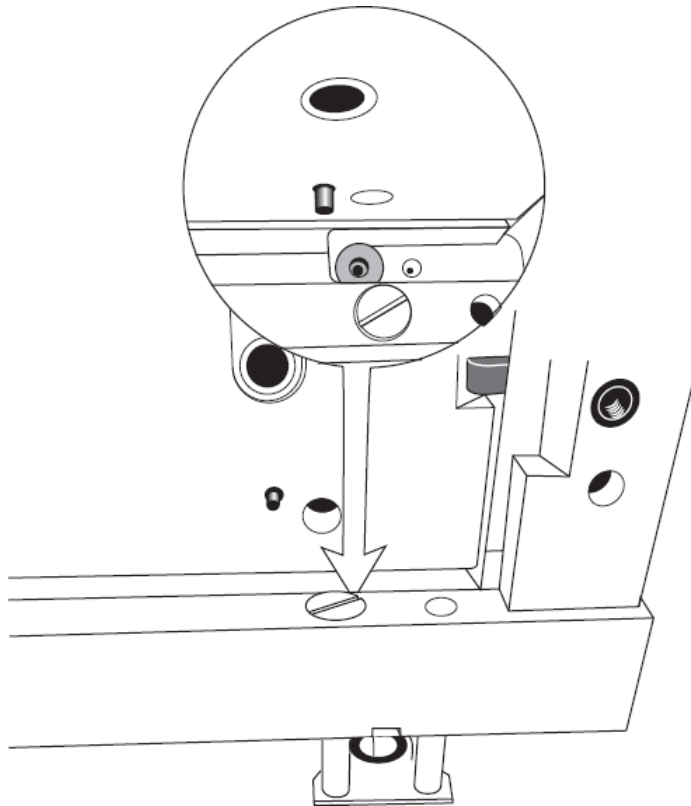
Replace the Quad Rings

Required Materials
<ul style="list-style-type: none">• New quad rings

The quad rings on the interface block make a seal between the interface block and the cartridge. If coolant is leaking between the interface block and the cartridge, then replace the quad rings.

1. Go to the Direct Control window, and then click **Load**.
2. Open the cartridge cover.
3. Loosen the two thumbscrews on the insertion bar, and then lift the insertion bar.
4. Examine the tubing connections for the cartridge coolant for leaks.
5. Remove the capillary cartridge from the interface block.
6. Use tweezers to remove the quad rings.

Figure 2-11 Quad Rings



7. Install the new quad ring in the recess of the interface block.
8. Lower the insertion bar, and then tighten the two thumbscrews.
9. Install the capillary cartridge in the interface block.
10. Close the cartridge cover.

Replace the Fuses

Required Materials

- Flat-bladed screwdriver, #2
- Replacement fuses (Qty: 2)



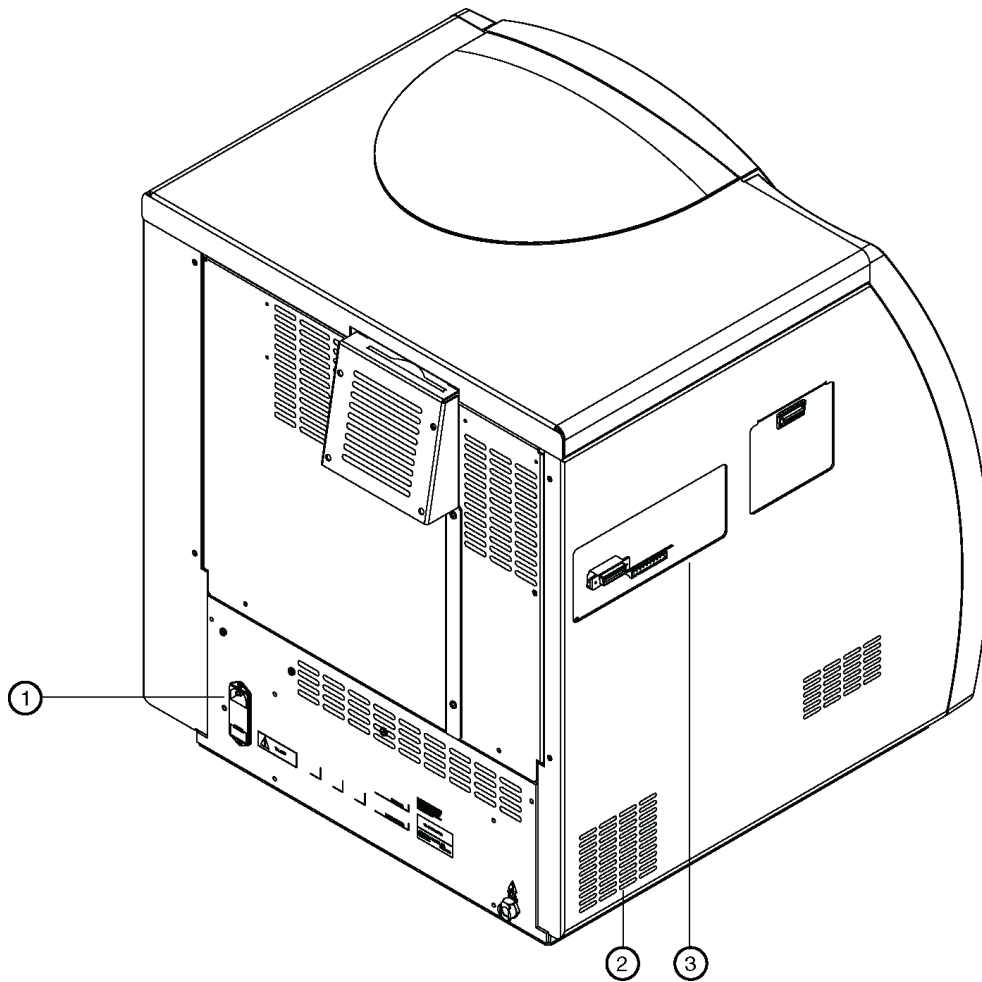
WARNING! Fire Hazard. Make sure to replace the fuses with the correct type and rating for continued protection against the risk of fire and incorrect system operation.

Maintenance

CAUTION: Potential System Damage. Before the fuses are replaced, find the cause of the failure. If the fuses continue to blow after they are replaced, then contact sciex.com/request-support.

1. Turn off the power to the system.
2. Disconnect the power cable from the AC power outlet.
3. Use a flat-bladed screwdriver to remove the fuse block.

Figure 2-12 Fuse Block



Item	Description
1	Fuse block
2	Air conditioning vents

Item	Description
3	External connections panel

4. Replace the fuses.

Table 2-1 Fuse Type and Rating

Line Voltage	100 VAC to 120 VAC	200 VAC to 240 VAC
Fuse Type and Rating	8.0 A Slow Blow, ¼ in.	6.3 A Time Delay, 20 mm

5. Install the fuse block.
6. Connect the power cable for the system to the AC power outlet.

More About LIF Detector Calibration 3

LIF detection and absorbance detection have some important differences. An absorbance detector measures a small intensity difference in a high-intensity light source. With this technique, the percentage of light does not change as the source light intensity changes. This means that the peak response for a sample stays the same as the lamp ages or when a lamp is changed.

With LIF detection, low light intensity is measured from a dark background. These low intensities are more easily influenced by changes in the optical path. A calibration that uses a SCIEX-supplied test mix can be done to correct for these influences. In this section, LIF calibration uses the 488 nm laser with fluorescein.

Relative Fluorescent Units

LIF detection systems can give different responses as changes are made in the optical path. The result can be that a sample of known concentration gives different results after a capillary is changed or if the separation is run on a different system. Because of this uncertainty, the LIF detector response is measured in relative fluorescent units (RFU). If a sample concentration can be related to a detector response, then the use of RFU is generally satisfactory for capillary electrophoresis detection.

The LIF Performance Test Mix, which contains 1×10^{-7} M fluorescein sodium salt in water, was developed to supply performance specifications for the LIF detector.

Calibration Correction Factor

Several different LIF systems were used to identify an expected response value for the LIF Performance Test Mix. For the performance test capillary (75 μm x 60 cm total length, bare fused-silica), the expected detector response is 35. Most of the systems that were tested were within $\pm 10\%$ of this value.

To correct for this variability, use the following equation to calculate a calibration correction factor (CCF) for each system:

$$\text{CCF} = 35 \div M$$

where:

- Expected response (target) = 35
- Measured response = M

To apply the CCF, use the LIF Calibration Wizard. Refer to the section: [Calibrate the LIF Detector \(Optional\)](#).

About Automatic Calibration

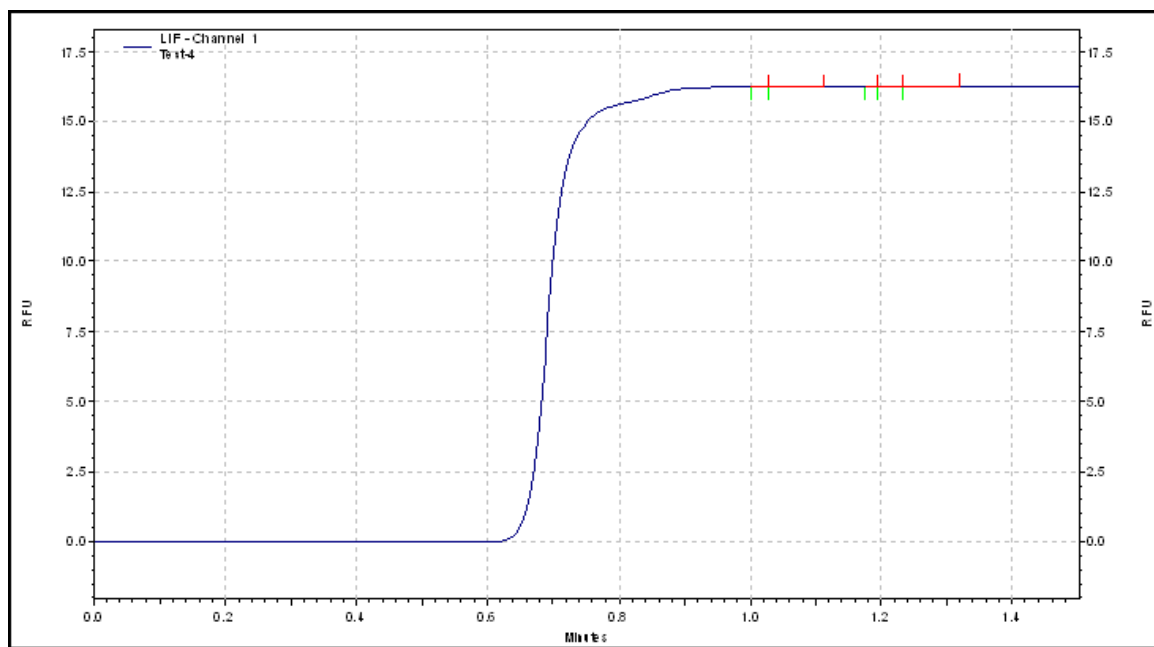
Use the 32 Karat software to do an automatic calibration. For instructions, refer to the section: [Calibrate the LIF Detector \(Optional\)](#).

The following steps are done during automatic calibration:

1. The capillary is rinsed with a nonfluorescent solution.
2. A pressure separation of the LIF Performance Test Mix (or another fluorescent marker) is done.
3. The data is collected.

The result is an electropherogram with the data in the shape of a step.

Figure 3-1 Representative Results from Automatic Calibration



4. To purge the fluorescent marker, the capillary is rinsed with the nonfluorescent solution.
5. The 32 Karat software calculates a calibration correction factor (CCF).

Find the Target RFU for Capillaries with Different Dimensions

When the detector performance tests are done by a SCIEX field service employee (FSE), a 75 μ m x 60 cm total length, bare fused-silica capillary is used. However, many applications

More About LIF Detector Calibration

require capillaries that have different dimensions. The capillary dimensions can have the following effects on the test mix calibration:

- The capillary length has an effect on the detection time of the step change.
- The cross-sectional area of the capillary changes the target RFU value.

The following equations use the target RFU of a 75 µm capillary to find the target RFU for a 50 µm capillary.

$$75 \text{ } \mu\text{m capillary cross-sectional area} = 3.14 \times r^2 = 3.14 \times (75 \div 2)^2 = 4418$$

$$50 \text{ } \mu\text{m capillary cross-sectional area} = 3.14 \times (50 \div 2)^2 = 1963$$

$$\text{Area ratio} = 1963 \div 4418 = 0.44$$

$$50 \text{ } \mu\text{m target RFU} = 0.44 \times 75 \text{ } \mu\text{m target RFU} = 0.44 \times 35 \text{ RFU} = 15.4 \text{ RFU}$$

Because the LIF Calibration Wizard requires an integer for the target RFU, the value calculated for the 50 µm capillary must be rounded to 15. This rounding is not an issue, because RFU are arbitrary units. If the same number is used as the target RFU, then a given concentration should have the same response on any detector, capillary, or laser. Because the target RFU is rounded down, it is likely that the raw detector response will be higher than the target RFU. This causes the CCF to be less than 1. Because the signal and noise are corrected by the same CCF, this does not have an effect on sensitivity.

Calibrate the LIF Detector for a Different Fluorescent Solution

Applications can require the use of a different fluorescent marker. If the marker has an emission wavelength that is significantly different than fluorescein (the fluorophore in the LIF Performance Test Mix), then different filters might be required. With different filters, the standard fluorescein target RFU is not applicable. Even if fluorescein can be detected at the new wavelength, a new target value should be set.

Note: For this procedure, use the fluorescent marker as an alternative to the LIF Performance Test Mix and CE Grade Water that were used for the nonfluorescent solution.

1. Do manual calibration with the **CCF** set to **1.0**. Refer to the section: [Calibrate the LIF Detector \(Optional\)](#).
 - a. In Calibration Wizard - Step 1, click **Manual**, and then click **Next**.
 - b. In Calibration Wizard - Step 2, in the **CCF** field, type 1.0.
2. Run the pressure separation method six times.

Figure 3-2 Time Program for Manual Calibration

The screenshot shows a software window titled "Instrument Setup" with three tabs: "Initial Conditions", "PDA Detector Initial Conditions", and "Time Program". The "Time Program" tab is active and displays a table with the following data:

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	20.0 psi	2.00 min	BI:A1	BO:A1	forward	Water to Waste
2	0.00	Separate - Pressure	10.0 psi	2.00 min	BI:B1	BO:A1	forward	Fluorophore to Waste
3	2.00	Rinse - Pressure	20.0 psi	2.00 min	BI:A1	BO:A1	forward	Water to Waste
4								

An "Apply" button is located at the bottom right of the window.

3. Calculate the average of the responses of the step change in the six runs. This value is the new target RFU.

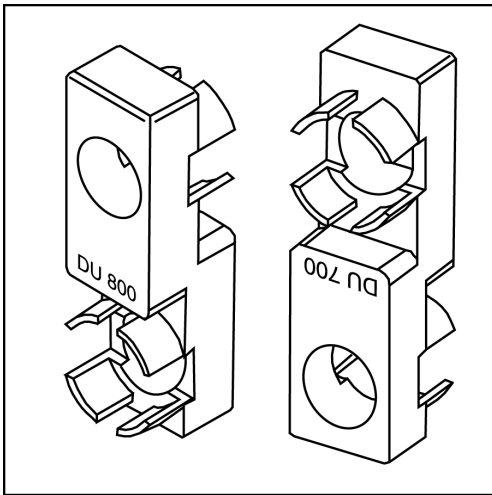
Evaluate the UV Filters

4

Filter characteristics can change with time, heat, humidity, exposure to UV light, and handling. Examine the filters regularly. Replace the filters as required. Use application and sample characteristics to set pass/fail criteria as part of method validation.

This section shows how to use a certified spectrophotometer to test the UV filters. An individual filter must be put into position at a height that lets the optical beam of the spectrophotometer pass through the center of the filter. A special filter holder tool is required. The following figure shows an example of a filter holder tool from Beckman Coulter for DU 800 and DU 700 series spectrophotometers.

Figure 4-1 Filter Holder Tool



A017325L.PNG

All of the UV filters have a center wavelength (λ_c) of ± 2 nm. Use the following equation to find the λ_c :

$$\lambda_c = \frac{2\lambda_1\lambda_2}{\lambda_1 + \lambda_2}$$

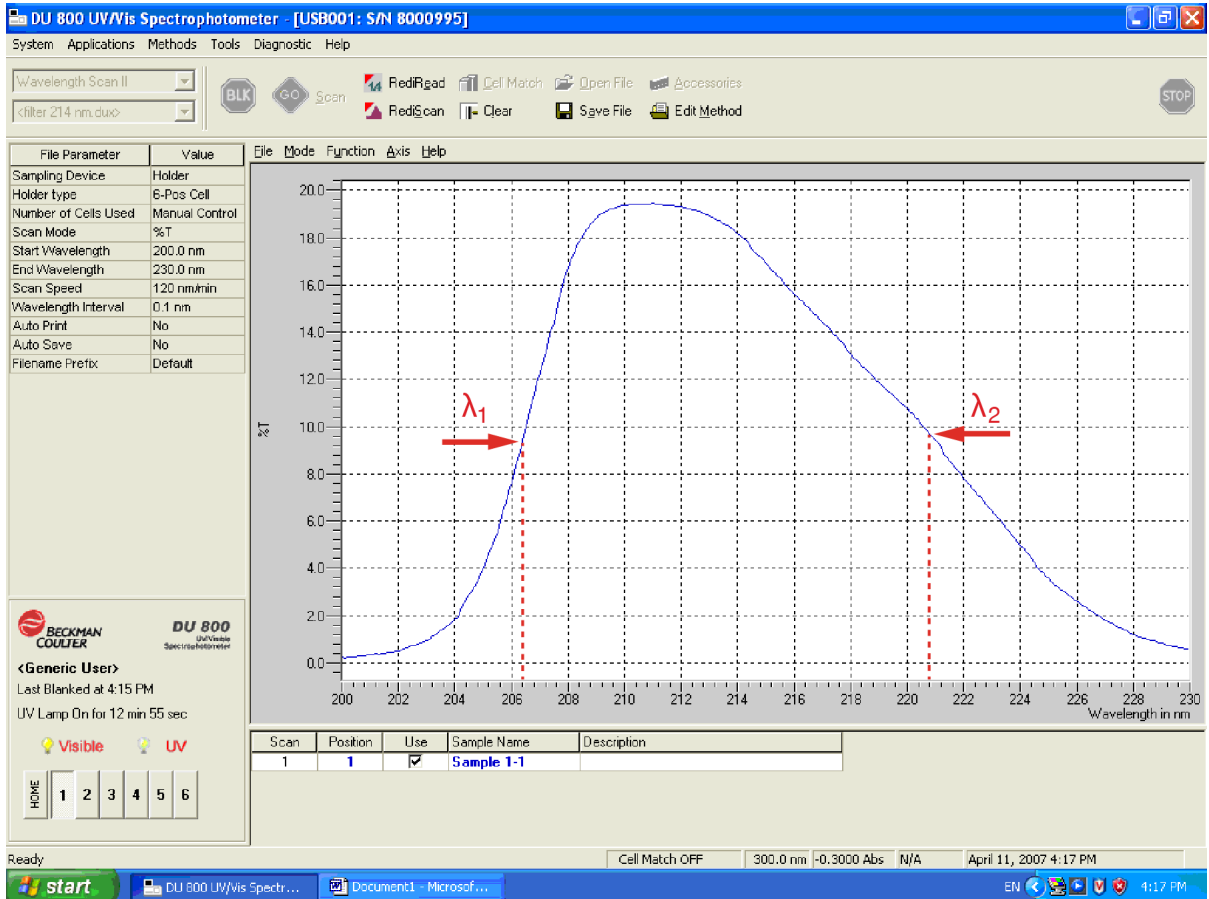
where:

- λ_c = Center wavelength
- λ_1 and λ_2 = Half power points at the starting and ending wavelengths, typically referred to as *full width at half maximum* (FWHM)

- λ_1 = The point of the trace to the left of the apex at half the maximum percent transmission
- λ_2 = The point of the trace to the right of the apex at half the maximum percent transmission

For an example of a scan of a 214 nm filter with a qualified DU 800 spectrophotometer, refer to the following figure.

Figure 4-2 Example of a Center Wavelength Calculation that Uses Full Width at Half Maximum

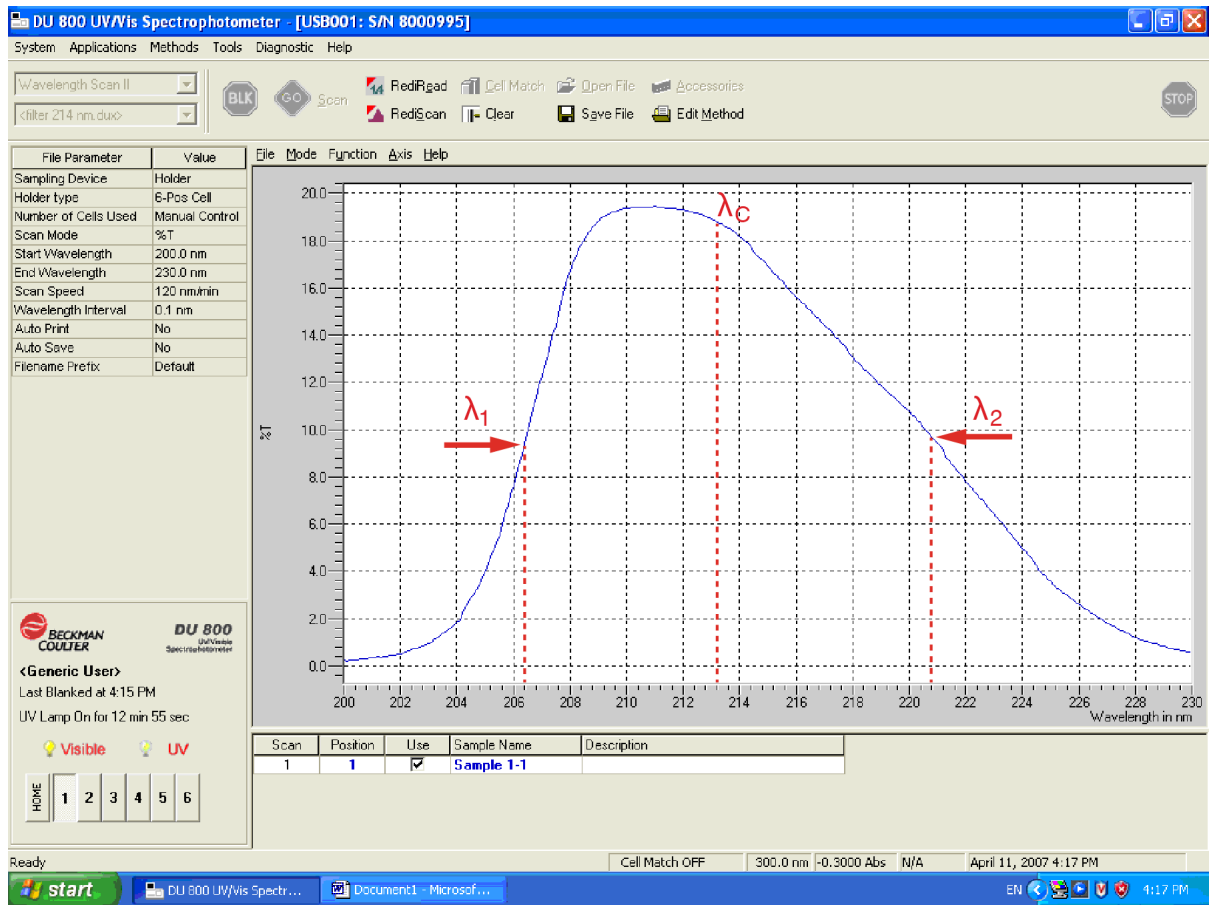


$$\lambda_c = \frac{2 \times (206.4 \text{ nm}) \times (220.8 \text{ nm})}{206.4 \text{ nm} + 220.8 \text{ nm}}$$

$$\lambda_c = 213.2 \text{ nm}$$

Evaluate the UV Filters

Figure 4-3 Calculated Result of Center Wavelength



- Use any of the following options to order parts from SCIEX:
 - **Telephone:** (877) 740-2129, Option 1 (toll-free, United States only), or go to sciex.com/contact-us to find a local office
 - **E-mail:** Sales.Americas@sciex.com
 - **Fax:** (800) 343-1346
 - **Internet:** For customers in the United States, Canada, United Kingdom, Belgium, Netherlands, France, Germany, and Switzerland, go to store.sciex.com

Cartridges and Parts

Note: Parts that have an asterisk (*) after the part number are available only from a SCIEX sales representative.

Table 5-1 Cartridges, Capillaries, and Apertures

Part Number	Description
144712	Aperture, 100 μ m \times 200 μ m (3)
144711	Aperture, 100 μ m \times 800 μ m (3)
338472	Capillary, bare fused-silica, 50 μ m \times 5 m
338473	Capillary, bare fused-silica, 75 μ m \times 5 m
338474	Capillary, bare fused-silica, 75 μ m \times 5 m
338475	Capillary, bare fused-silica, precut, 20 μ m \times 67 cm (3)
338451	Capillary, bare fused-silica, precut, 50 μ m \times 67 cm (3)
338454	Capillary, bare fused-silica, precut, 75 μ m \times 50 cm (3)
477477	Capillary, DNA, 100 μ m \times 65 cm
477601	Capillary, N-CHO, 50 μ m \times 80 cm
477441	Capillary, neutral, 50 μ m \times 67 cm
359976	Capillary cartridge coolant, 450 mL
144647	Capillary cartridge coolant fill tool
A144738	Cartridge, no capillary

Order Parts

Table 5-1 Cartridges, Capillaries, and Apertures (continued)

Part Number	Description
144660	Cartridge, optical calibration (OPCAL)
A55625	Cartridge, pre-assembled with 30 cm bare fused-silica capillary
A11147	Cartridge, semi-assembled, for 30 cm capillaries (capillary not included)
144645	Cartridge rebuild kit
144717	Cartridge tubing kit, connectors, and 100 cm tubing
144689	Cartridge tubing kit, connectors, and tubing, 1 each: 20 cm, 30 cm, 40 cm, and 50 cm
721125	LIF cartridge aperture plug
721126	LIF cartridge probe guide

Table 5-2 Trays, Vials, and Caps

Part Number	Description
A94462	Buffer tray, 6 × 6
5043467	NanoVials, 100 Pack
144709	PCR Microvials, 100 Pack
A94461	Sample tray, 6 × 8
C04895	Sample tray holder, for 48-vial sample tray
A62250	Universal vial caps, 100 Pack
A62251	Universal vials, 100 Pack

Table 5-3 Filters

Part Number	Description
144940	Band-pass filter, 520 nm
149068	Band-pass filter, 560 nm
144942	Band-pass filter, 655 nm
144941	LIF notch filter, 488 nm
144430	UV filter, 200 nm

Table 5-3 Filters (continued)

Part Number	Description
144431	UV filter, 210 nm
144437	UV filter, 214 nm
144432	UV filter, 220 nm
144433	UV filter, 230 nm
144438	UV filter, 254 nm
144434	UV filter, 260 nm
144439	UV filter, 280 nm

Table 5-4 Detector Parts

Part Number	Description
144667	Deuterium lamp
144951*	LIF 2-color upgrade kit
969136*	LIF upgrade module for PS 800 and PA 800 Plus series
A59494*	Packaged laser module upgrade kit, 488 nm, single color
144094	PDA fiber-optic Y-cable
B68372*	Photodiode array detector upgrade
144093	UV/Vis fiber-optic cable

Table 5-5 Other Parts

Part Number	Description
A47775	Electrode
A59525	Electrode tool
A95348	Insertion lever interface parts kit
B78399*	Software reprocessing key

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SCIEX and its representatives maintain a staff of fully-trained service and technical specialists located throughout the world. They can answer questions about the system or any technical issues that might arise. For more information, visit the SCIEX website at sciex.com or contact us in one of the following ways:

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