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The Power of Precision

LIFluor EnhanCE dsDNA 1000 Dye Kit

For the PA 800 Plus Pharmaceutical Analysis System

Application Guide

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EC Authorized Person

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LIFluor EnhanCE dsDNA 1000 Dye Kit

The LIFluor EnhanCE dsDNA 1000 Dye kit contains one vial of fluorescent DNA intercalating dye for use with the dsDNA 1000 kit. The vial contains 500 µg of dye in 500 µL of methanol. When used with laser-induced fluorescent (LIF) detection, the detection limit for the CE analysis of DNA fragments is substantially increased.

This document gives instructions for sample preparation with the LIFluor EnhanCE dsDNA 1000 Dye kit. It also gives instructions for data acquisition and data analysis with the 32 Karat software.

Use the information in this application guide as a place to start. As required, change the injection time, voltage, injection type, or other parameters to find the best conditions for the requirements.

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

Safety

Refer to the safety data sheets (SDSs), which are available at sciex.com/tech-regulatory, for information about the correct handling of materials and reagents. Always follow standard laboratory safety guidelines. For information about hazardous substances, refer to the section: Hazardous Substance Information.

Intended Use

The LIFluor EnhanCE dsDNA 1000 Dye kit is for laboratory use only.

Required Equipment and Materials

Note: For items with a reorder part number, sometimes the reorder quantity is different than the quantity in the kit.

Table 1 LIFluor EnhanCE Dye (PN 477409)

Component	Quantity	Reorder Part Number
Fluorescent DNA intercalating dye, 500 µg in 500 µL of methanol	1	477409

Table 2 Additional Supplies from SCIEX

Component	Quantity	Part Number
Capillary cartridge, blank	1	144738
Cartridge rebuild kit	1	144645
CE Grade Water	140 mL	C48034
dsDNA 1000 kit	1	477410
Filter, 488 mn notch filter	1	144941
Filter, 520 nm emission filter	1	144940
LIF Cartridge Aperture Plug Assembly	1	721125
LIF Cartridge Probe Guide Assembly	1	721126
LIF Performance Test Mix (20 mL)	1	726022
nanoVials	100	5043467
PCR microvials (200 µL)	100	144709
Universal vial caps, blue	100	A62250
Universal vials	100	A62251

Storage Conditions

- Keep the vial of LIFluor EnhanCE Dye at –35 °C to –15 °C.

Note: The LIFluor EnhanCE Dye is sensitive to light. Prevent exposure to light. Make sure that a cover or cap is attached to the vial when it is not in use. Aluminum foil can be used as a cover to decrease exposure to light.

Note: When the LIFluor EnhanCE Dye vial is not in use, make sure that the cap is attached tightly. If a cover or cap is not attached to the vial, then the methanol will evaporate and increase the dye concentration.

Note: If the LIFluor EnhanCE Dye is kept at room temperature for more than 10 hours, then deterioration will occur.

Customer-Supplied Equipment and Supplies

- Powder-free gloves, neoprene or nitrile recommended
- Safety glasses
- Laboratory coat

- Vortex mixer
- Pipettes and appropriate tips
- Parafilm
- Volumetric pipet, 20 mL
- Micropipets to deliver 1 µL to 100 µL volume
- Membrane syringe filters, 0.45 µm pores
- Tris-EDTA buffer, 10 mM Tris-HCl, 1 mM disodium EDTA, pH 8.0 (Sigma)
- Analytical balance

Required Detector

A laser-induced fluorescence (LIF) detector with an excitation wavelength of 488 nm and a 520 nm emission filter is required.

Required Cartridge or Capillary

- Capillary cartridge (PN 144738)
- DNA capillary, 65 cm, 100 µm i.d. (PN 477477)

Note: The capillary is included with the kit.

Methods

This guide supplies methods to do separations of the test mix that is supplied with the kit.

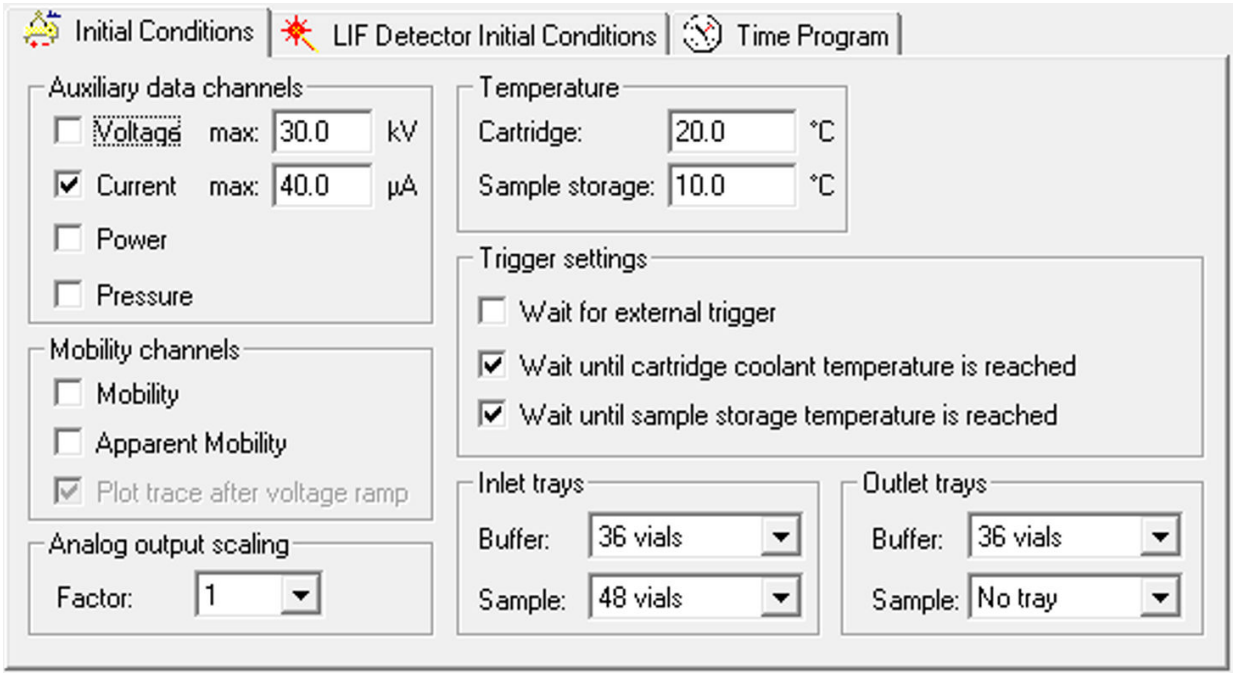
The LIFluor EnhanCE dsDNA 1000 Dye kit requires: a conditioning method, separation method, and shutdown method. To create the methods, refer to the sections: Initial Conditions, LIF Detector Initial Conditions, and Time Programs.

Because this kit can be used in a wide variety of other applications, use the methods given here as a place to start to create a method that is applicable for relevant applications. For additional application and workflow support, refer to technical notes found on the SCIEX website or contact SCIEX Technical Support at sciex.com/request-support.

Initial Conditions

Note: The values on the Initial Conditions and LIF Detector Initial Conditions tabs are the same for all of the methods.

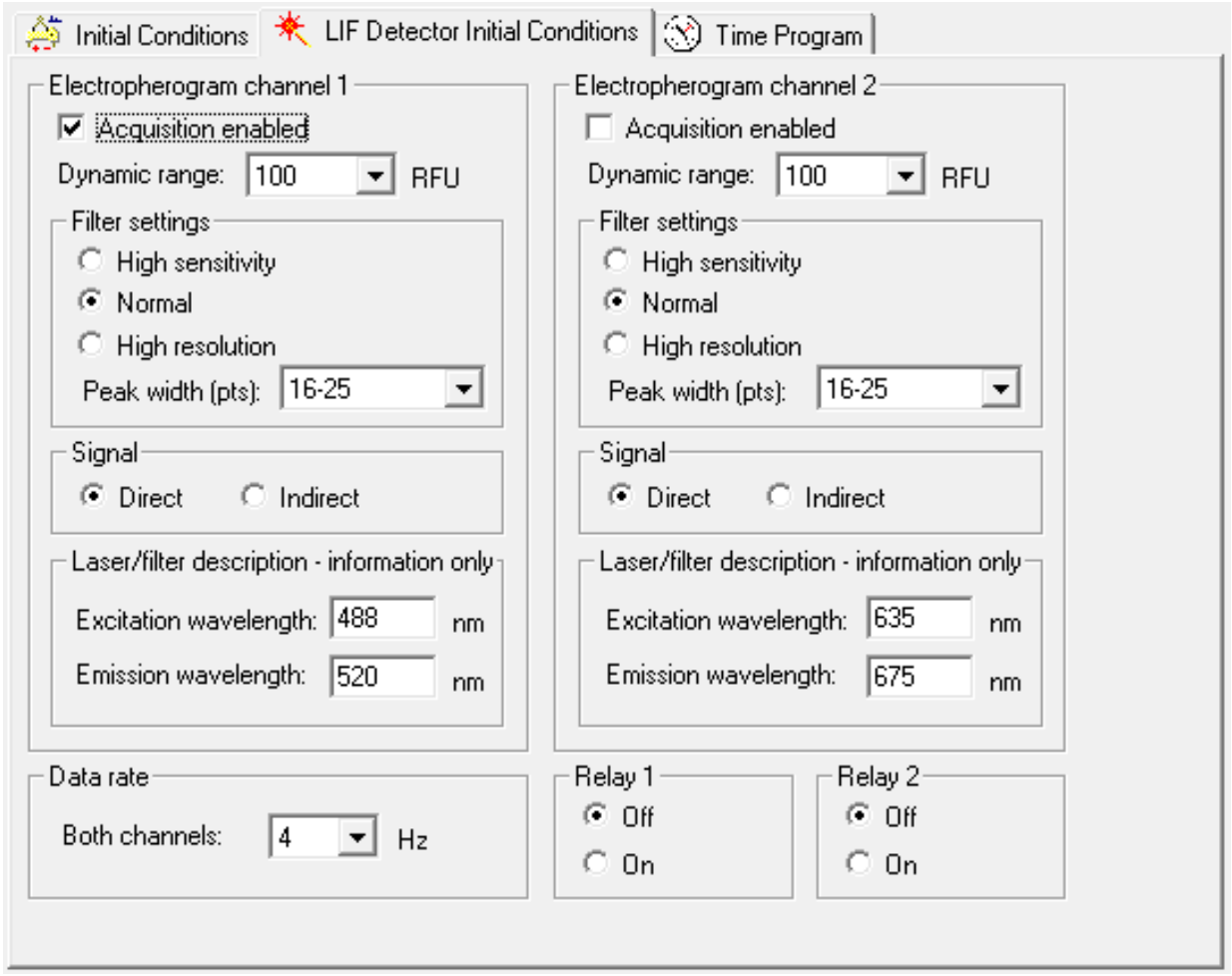
Figure 1 Initial Conditions Tab for LIFluor EnhanCE dsDNA 1000 Dye Kit Methods



LIF Detector Initial Conditions

Note: The values on the Initial Conditions and LIF Detector Initial Conditions tabs are the same for all of the methods.

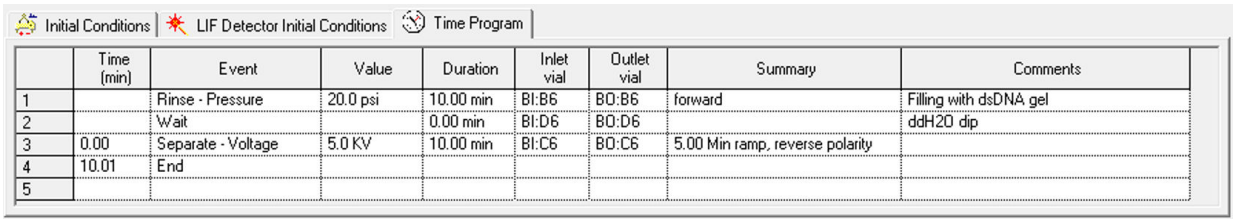
Figure 2 LIF Detector Initial Conditions Tab for LIFluor EnhanCE dsDNA 1000 Dye Kit Methods



Time Programs

Note: The time programs are different for each method.

Figure 3 Time Program Tab for the Conditioning Method



	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	20.0 psi	10.00 min	BI:B6	BO:B6	forward	Filling with dsDNA gel
2		Wait		0.00 min	BI:D6	BO:D6		ddH2O dip
3	0.00	Separate - Voltage	5.0 KV	10.00 min	BI:C6	BO:C6	5.00 Min ramp, reverse polarity	
4	10.01	End						
5								

Figure 4 Time Program Tab for the dsDNA 1000 Test Mix Method

Initial Conditions | LIF Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	20.0 psi	3.00 min	BI:B1	BO:B1	forward, In / Out vial inc 8	Filling with dsDNA gel- Increment every 8 runs
2		Wait		0.00 min	BI:D1	BO:D1	In / Out vial inc 8	Water dip to clean capillary tip- Increment every 8 runs
3		Inject - Voltage	1.0 KV	2.0 sec	SI:A1	BO:A6	Override, reverse polarity	Sample injection with 1ml dsDNA gel in outlet vial
4		Wait		0.00 min	BI:E1	BO:E1	In / Out vial inc 8	Water dip to clean capillary tip- Increment every 8 runs
5	0.00	Separate - Voltag	7.8 KV	25.00 min	BI:C1	BO:C1	0.17 Min ramp, reverse polarity, both, In / Out vial inc 8	Separation in dsDNA gel- Increment every 8 runs with 20psi pressure on both ends
6	1.00	Autozero						
7	25.00	End						
8								

Figure 5 Time Program Tab for the Shutdown Method

Initial Conditions | LIF Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	20.0 psi	3.00 min	BI:E6	BO:E6	forward	Filling with dsDNA gel
2		Wait		0.00 min	BI:D6	BO:D6		Water dip
3	0.00	Separate - Voltag	5.0 KV	10.00 min	BI:C6	BO:C6	5.00 Min ramp, reverse polarity	
4	10.00	Wait		0.00 min	BI:D6	BO:D6		Water dip
5	10.01	End						
6								

Prepare the Reagents and Stock Solutions

Prepare the dsDNA 1000 Gel Buffer

CAUTION: Potential Data Loss. Do not put the gel buffer through a filter after the LIFluor EnhanCE Dye is added.

CAUTION: Potential Data Loss. Do not apply heat to the dsDNA 1000 Gel Buffer. If heat is applied to the gel buffer, then poor separation can occur.

Note: The concentration of LIFluor EnhanCE Dye in the gel buffer has been optimized to saturate a 10 µg/mL DNA sample. However, for samples with a low concentration of DNA (< 1 µg/mL), add less LIFluor EnhanCE Dye (< 10 µL) to the vial of rehydrated gel buffer.

Note: The LIFluor EnhanCE Dye is sensitive to light. Prevent exposure to light. Make sure that a cover or cap is attached to the vial when it is not in use. Aluminum foil can be used as a cover to decrease exposure to light.

Note: When the LIFluor EnhanCE Dye vial is not in use, make sure that the cap is attached tightly. If a cover or cap is not attached to the vial, then the methanol will evaporate and increase the dye concentration.

Note: If the LIFluor EnhanCE Dye is kept at room temperature for more than 10 hours, then deterioration will occur.

1. Add 20.0 mL of CE Grade Water to the dsDNA 1000 Gel Buffer vial.

2. Use a magnetic stir bar and stir plate to stir the solution until the dsDNA 1000 Gel Buffer is completely dissolved.

   To stir more effectively, use a stir bar that is slightly shorter than the diameter of the dsDNA 1000 Gel Buffer bottle.

   The lyophilized gel might require up to 24 hours to dissolve completely.

3. Immediately before the gel buffer is used, apply a 0.45 µm filter, and then sonicate the gel buffer for 1 minute to remove any bubbles.

   Rehydrated dsDNA 1000 Gel Buffer can be kept for 30 days when stored at 2 °C to 8 °C.

4. Add 15.0 µL of LIFluor EnhanCE Dye to 20 mL of rehydrated, filtered gel buffer, and then mix the solution fully.

   Twelve mL of gel buffer is sufficient for the conditioning method, eight sample injections, and the shutdown method.

Prepare the Samples

Prepare the Test Mix

1. Add 1 mL of CE Grade Water to the dsDNA 1000 Test Mix vial.
2. Use the vortex mixer to mix the solution fully.
3. Transfer 100 µL of the reconstituted dsDNA 1000 Test Mix to a PCR microvial.
4. Add 1 µL LIF Performance Test Mix in 100 µL of dsDNA 1000 Test Mix to the microvial as a marker.
5. Mix the solution fully.
6. Put the microvial in a universal vial. Attach a blue cap.
7. Transfer 100 µL aliquots of the remaining reconstituted dsDNA 1000 Test Mix into more vials.
8. Before use, add 1 µL of LIF Performance Test Mix per 100 µl of dsDNA 1000 Test Mix.
9. Keep the reconstituted test mix at –35 °C to –15 °C when it is not in use. If the reconstituted test mix is kept at room temperature, then it will degrade.

Prepare the Sample

1. Dilute the DNA sample with CE Grade Water or Tris-EDTA buffer to make a concentration of approximately 10 ng/µL DNA.
2. Add 1 µL of LIF Performance Test Mix to 100 µL of diluted DNA sample, and then mix thoroughly. The LIF Performance Test Mix is used as a marker.
3. Transfer 100 µL of the sample to a microvial for analysis.

   If the available sample volume is low, then transfer 5 µL to 10 µL of the sample to a nanoVial for analysis.

Prepare the PA 800 Plus System

Use the procedures in this section to prepare the PA 800 Plus system to acquire data.

Tip! To save time, turn on the light source 30 minutes before the start of the run to let it warm up.

Install the LIF Detector

1. Turn off the PA 800 Plus system.
2. Install the LIF detector. For detailed instructions, refer to the document: *Maintenance Guide*.
3. Turn on the system.
4. Turn on the laser, and then let it warm up for at least 30 minutes.

Clean the Interface Block

CAUTION: Potential System Damage. Do not let the gel collect on the electrodes, opening levers, capillary ends, and interface block. Gel accumulation might cause broken capillaries, bent electrodes, jammed vials, or missed injections.

Clean the electrodes, opening levers, capillary ends, and interface block after every use or when chemistries are changed. For detailed instructions, refer to the section: "Clean the Electrodes, Insertion Levers, and Interface Block" in the document *Maintenance Guide*.

The gel buffer is very viscous. To make sure that gel buffer does not collect in the system, do regular and thorough cleaning.

Install the Capillary

CAUTION: Potential System Damage. Do not let the capillary become dehydrated. The coating inside the capillary starts to dehydrate within 5 minutes to 10 minutes after the end of the capillary is trimmed.

CAUTION: Potential System Damage. Do not cut the capillary to its final length before it is installed in the cartridge.

1. Install the capillary into a capillary cartridge. Refer to the document: *Capillary Cartridge Rebuild Instructions*.

   The recommended capillary length is 30 cm to the window and 40.2 cm total length. If the DNA sample is larger than 2 kb, then a capillary with a longer total length, such as 50.2 cm with a 40 cm length to the window, can be used.

   Use the LIF aperture and probe guide.
2. To minimize damage to the capillary coating, do this:

a. Fill two universal vials with 1.5 mL of CE Grade Water, and then attach blue caps.

b. Cut off the end-cap on the inlet side of the capillary, and then install the capillary in the cartridge. After the capillary is in the cartridge, cut the end-cap from the outlet side, and then complete the cartridge assembly.

c. Trim the capillary ends to the recommended length, and then submerge both ends of the capillary in vials filled with CE Grade Water.

For details about how to separate longer DNA samples, refer to technical notes on the SCIEX website or contact SCIEX Technical Support at sciex.com/request-support.

Install the Cartridge

1. Remove the cartridge from the box.
2. Install the cartridge in the PA 800 Plus system. For detailed instructions, refer to the document: *Maintenance Guide*.
3. (Optional) Calibrate the LIF detector.

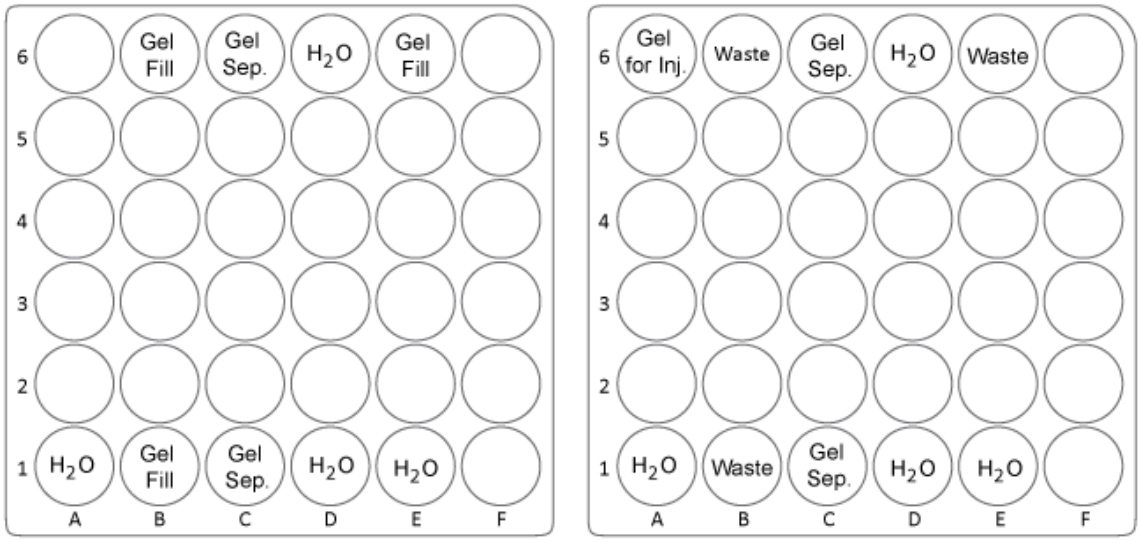
   Use the Calibration wizard, which is available from the Instrument Configuration dialog in the 32 Karat software. For detailed instructions, refer to the section: Calibrate the LIF Detector (Optional).

Load the Buffer Trays

CAUTION: Potential System Damage. Do not fill any vial with more than 1.8 mL of liquid, or let more than 1.8 mL of liquid collect in waste vials. If a vial is filled with more than 1.8 mL of liquid, then the pressure system can be damaged.

1. Put the vials in the buffer trays as shown in the following figure. Each row is sufficient for eight runs. Row 6 does not increment.

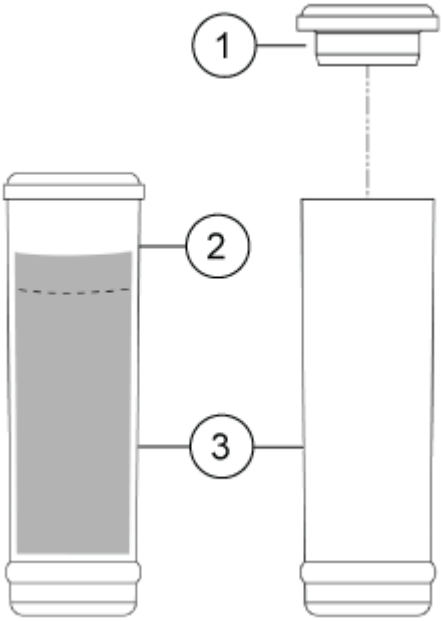
Figure 6 Buffer Tray Inlet (BI), Left and Buffer Tray Outlet (BO), Right



Item	Description
H_2O	1.5 mL of CE Grade Water
Gel Fill	1.5 mL of dsDNA 1000 Gel Buffer containing LIFluor EnhanCE Dye
Gel Sep.	1.5 mL of dsDNA 1000 Gel Buffer containing LIFluor EnhanCE Dye
Gel for Inj.	1 mL of dsDNA 1000 Gel Buffer containing LIFluor EnhanCE Dye
Waste	1 mL of CE Grade Water

2. Fill the vials with the solutions listed in the previous table, and then attach the caps. Refer to the following figure.

Figure 7 Universal Vial and Cap Setup

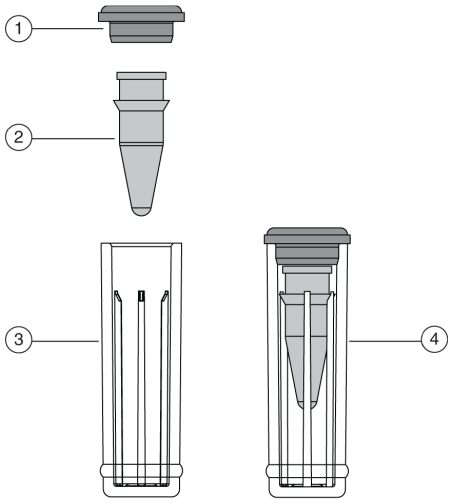


Item	Description
1	Universal vial cap
2	Maximum fill line
3	Universal vial

Load the Sample Tray

1. Prepare the samples.
   - For samples in microvials, put the microvial in a universal vial, and then attach a cap. Refer to the figure: Figure 8.
   - For samples in nanoVials, attach a cap.

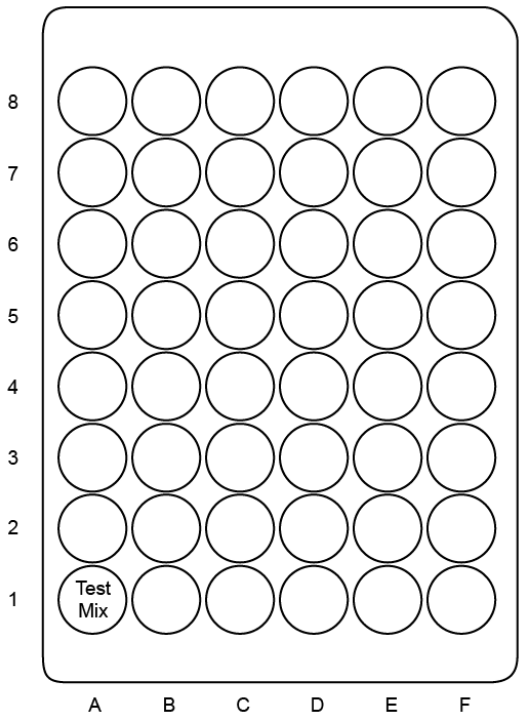
Figure 8 Microvial in a Universal Vial



Item	Description
1	Universal vial cap
2	Microvial
3	Universal vial
4	Microvial inside universal vial

2. Put the sample vials in the sample inlet tray.
   Start at position A1. Fill the A wells first, and then fill the other wells.

Figure 9 Sample Tray Inlet (SI)



Run the Samples

Create the Sequence and Start the Run

Note: To do the following procedure, the user must know how to use the 32 Karat software to create and run a sequence. For detailed instructions, refer to the document: *Methods Development Guide*.

1. Open the 32 Karat software.
2. In the 32 Karat Software window, either select an instrument with a LIF detector or create a new one, and then open the instrument.
3. To run only the test mix, create a sequence with three rows:
   - Row 1: Conditioning method
   - Row 2: Separation method
   - Row 3: Shutdown method
4. To run additional samples, add another row after the conditioning method for each sample. Fill the vials in each of the buffer trays.

Note: Each buffer vial row is sufficient for five samples.

5. Make sure that the LIF detector laser is turned on.
6. Make sure that the sample and buffer trays are loaded.
7. Click  .
   The Run Sequence dialog opens.
8. Make any required changes, and then click **Start**.

Waste Disposal

WARNING! Biohazard or Toxic Chemical Hazard. Follow local directives to dispose of chemicals, cartridges, buffer trays, sample trays, vials and caps, and the remains of the prepared samples. They might contain regulated compounds and biohazardous agents.

Store the Cartridge

Store the Cartridge for Less Than 24 Hours

1. Use the shutdown method to clean the capillary.
   The shutdown method fills the capillaries with dsDNA 1000 Gel Buffer.
2. Keep the cartridge up to 24 hours in the system with the capillary ends immersed in vials of CE Grade Water.

Store the Cartridge for More Than 24 Hours

1. Use the shutdown method to clean the capillary.
2. Remove the cartridge from the system.
3. Put the cartridge in the cartridge storage box with the capillary ends immersed in vials of CE Grade Water.
4. Keep the cartridge storage box upright in the refrigerator between 2 °C and 8 °C.

Prepare the Cartridge After Storage

- If the cartridge has not been used for more than a day, then use the conditioning method to condition the capillary.

Analyze the Data

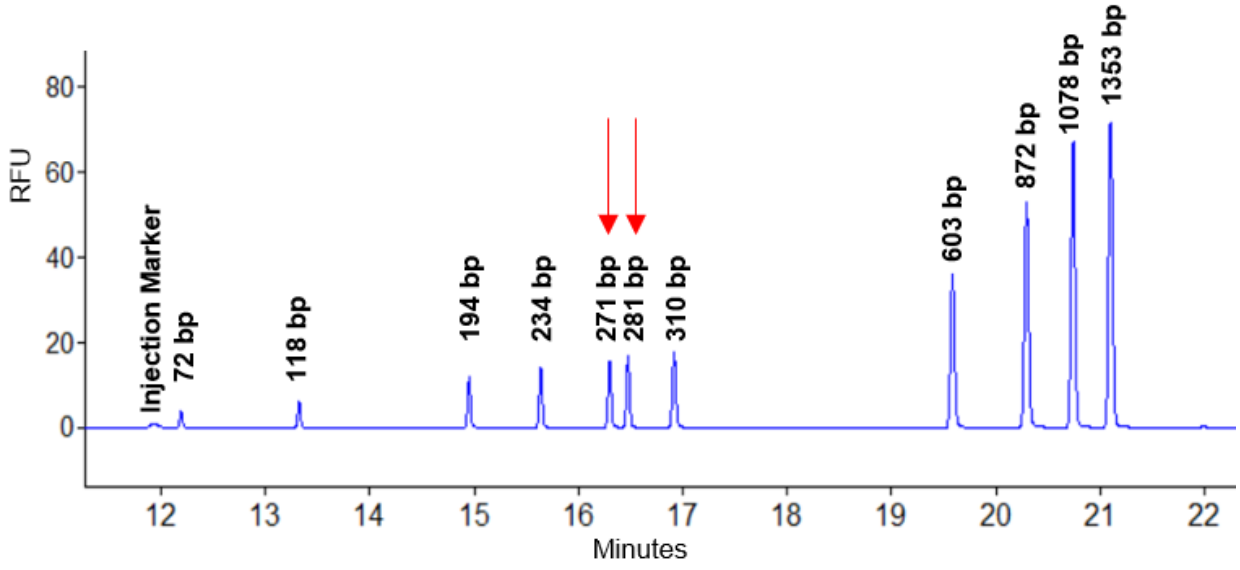
Analyze the Data for the Test Mix

The dsDNA 1000 Test Mix contains a Phi-X 174 DNA Hae III digest that has 11 fragments. When the recommended separation method is used, the test mix should separate in less than 25 minutes and there should be baseline separation of the 271 bp and 281 bp fragments. Refer to the figure: Figure 10.

The electrical current should stay stable between 14 µA and 24 µA. Small variations in the current can be caused by temperature fluctuations in the capillary or air bubbles. The result of these variations in the current might be a noisy baseline, miscellaneous spikes, or broad peaks. To remove any air bubbles, sonicate the gel buffer vials for 5 seconds before use.

If the DNA sample is larger than 2 kb, then a capillary with a longer total length, such as 50.2 cm, can be used. Change the method to use the longer capillary. For details about how to separate longer DNA samples, refer to technical notes on the SCIEX website or contact SCIEX Technical Support at sciex.com/request-support.

Figure 10 Example Electropherogram for Test Mix



Recommended Sample Injection for the Separation Method

Start with an electrokinetic injection, done at 1 kV for 2 seconds, as shown in the separation method. If required, then the injection duration can be increased to a period of 5 seconds.

As an alternative, for samples with higher salt concentrations, start with a pressure injection, done at 0.5 psi for 10 seconds. If required, then the injection duration can be increased to a period of 20 seconds.

Tips for Best Results

- When different dye concentrations are used, variations in migration time can occur.
  - If more than 15 µL of LIFluor EnhanCE Dye is used, then the migration time of the dsDNA fragments increases.
  - If less than 15 µL of LIFluor EnhanCE Dye is used, then the dsDNA fragments migrate quickly. For example, if 2 µL of LIFluor EnhanCE Dye is used, then the 72 bp fragment migrates before the LIF Performance Test Mix.
  - If any of the dsDNA peaks are followed by a negative baseline upset, then there is not sufficient LIFluor EnhanCE Dye in the gel buffer to stain all of the dsDNA. To remove this artifact, either inject less sample or increase the amount of LIFluor EnhanCE Dye in the gel buffer.
- If LIFluor EnhanCE Dye is added directly to the dsDNA sample, then extreme peak tailing might occur. Add LIFluor EnhanCE Dye to the separation gel buffer only.
- Monitor the current at all times. Changes in the average current or fluctuations in the current can identify changes in ionic strength, gel buffer degradation, or the formation of bubbles. This can result in a noisy baseline, miscellaneous spikes, or broad peaks.

Troubleshooting

Table 3 LIFluor Troubleshooting

Symptom	Possible Cause	Corrective Action
Low current	The capillary is blocked.	Either: • Rinse the capillary with CE Grade Water for 10 minutes at 20 psi to remove the gel buffer, and then condition the capillary with the conditioning method. • Replace the capillary. Refer to the section: Install the Capillary.
Broad peaks or changing migration times	1. The gel has dried on the electrodes. 2. The gel buffer or test mix is deteriorating.	1. Clean the electrodes, the ends of the capillary, and the lever arms. Refer to the section: Clean the Interface Block. 2. Replace the gel buffer or test mix as required.

Table 3 LIFluor Troubleshooting (continued)

Symptom	Possible Cause	Corrective Action
No peaks	1. The capillary is blocked. 2. The capillary window or end is broken.	1. Either: • Rinse the capillary with CE Grade Water for 10 minutes at 20 psi to remove the gel buffer, and then condition the capillary with the conditioning method. • Replace the capillary. Refer to the section: Install the Capillary. 2. Replace the capillary. Refer to the section: Install the Capillary.
Spikes in the electropherogram	The gel buffer has air bubbles.	Make sure that the reconstituted buffer is at room temperature. Degas the gel buffer before use. Sonicate the gel buffer vials for 5 seconds to remove the air bubbles.
Noisy baseline	The gel buffer has microparticles.	Put the gel buffer through a 0.45 µm pore filter to remove microparticles, and then sonicate the gel buffer vials for 5 seconds to remove the air bubbles.
Decrease in peak height	The dye deteriorated.	Prepare fresh LIFluor EnhanCE Dye-gel solution. Prevent exposure to light.

Table 3 LIFluor Troubleshooting (continued)

Symptom	Possible Cause	Corrective Action
Migration time change	1. The dye deteriorated. 2. The LIFluor EnhanCE Dye-gel solution is nonhomogenous. 3. The LIFluor EnhanCE Dye has evaporated.	1. Prepare fresh LIFluor EnhanCE Dye-gel solution. Prevent exposure to light. 2. Make sure that the LIFluor EnhanCE Dye-gel solution has been mixed well. 3. Prepare fresh LIFluor EnhanCE Dye-gel solution. Prevent exposure to light.
Current change	1. The dye deteriorated. 2. The electrodes are dirty.	1. Prepare fresh LIFluor EnhanCE Dye-gel solution. Prevent exposure to light. 2. Clean the electrodes and lever arms. Refer to the section: Clean the Interface Block.
Low peak signal	1. The sample concentration is too low. 2. The dye concentration is too high. 3. The capillary window is not aligned with the detector.	1. Increase the injection volume. 2. Add less dye to the gel for the prepared sample. 3. Examine the cartridge to make sure that the capillary window is aligned with the cartridge correctly.
High peak signal with poor peak resolution	The sample concentration is too high.	Decrease the concentration of the sample or inject less sample. Decreased sample loads increase both resolution and peak efficiency.

Hazardous Substance Information A

The following information must be noted and the relevant safety measures must be obeyed. For more information, refer to the respective safety data sheets. The safety data sheets are available on request or can be downloaded from our website, at sciex.com/tech-regulatory.

Hazard classification according to HCS 2012.

LIFluor EnhanCE Dye

DANGER! Highly flammable liquid and vapor. Toxic if swallowed. Toxic in contact with skin. Toxic if inhaled. Causes damage to organs.

Other Reagents

For reagents from other vendors, read the safety data sheet from the vendor before use.

Calibrate the LIF Detector (Optional) B

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" in the document: *Maintenance Guide*.

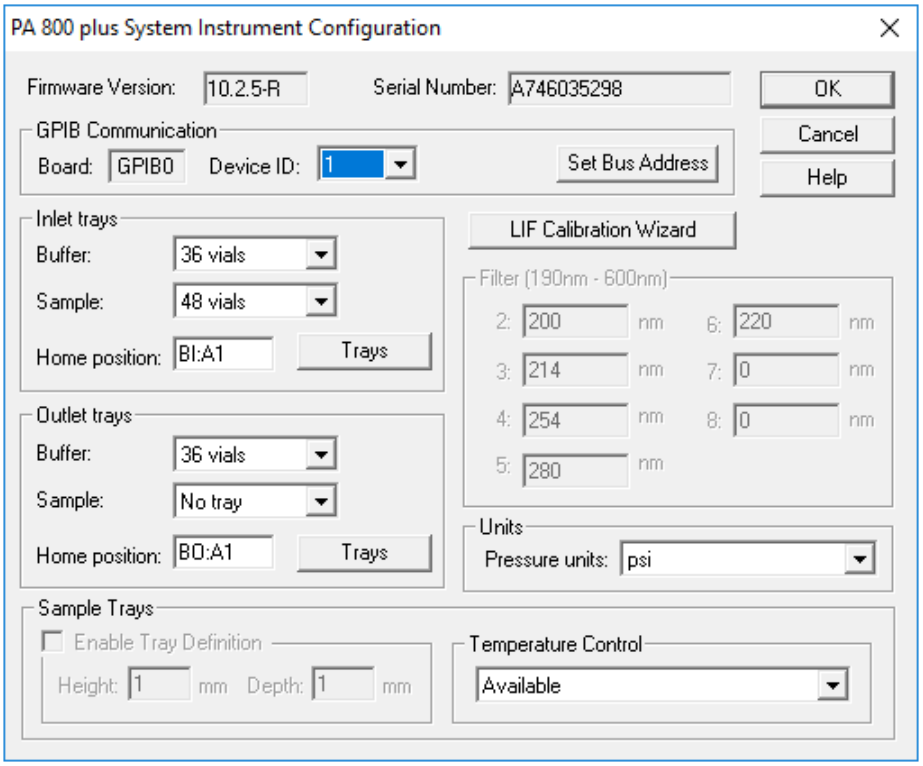
Calibrate the LIF detector after the LIF detector is installed, after a different cartridge is installed, or after a new capillary is installed in the cartridge.

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.

Required Materials
• LIF Performance Test Mix • CE Grade Water

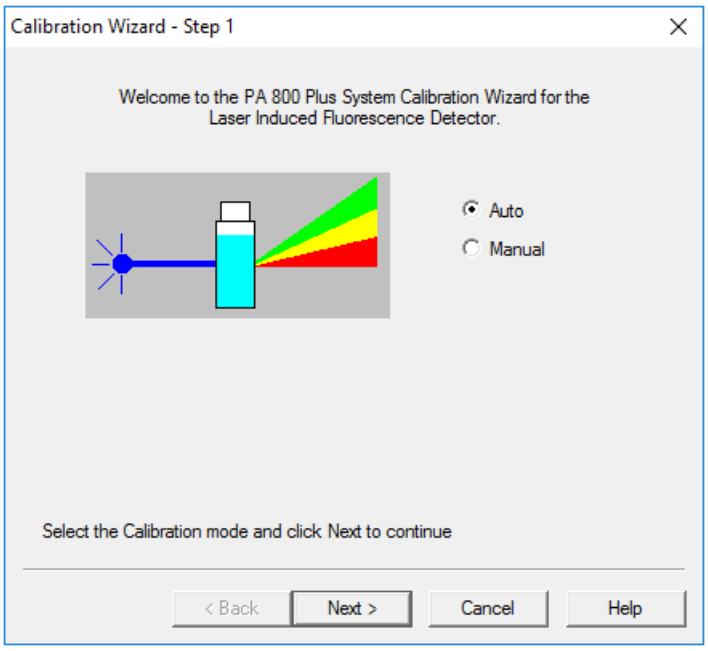
1. Turn on the PA 800 Plus system.
2. Open the 32 Karat software.
   The 32 Karat Software Enterprise window opens.
3. Open the LIF instrument, open the Direct Control window, and then turn on the laser.
4. In the 32 Karat Software Enterprise window, click **Tools** > **Enterprise Login**, and then log 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 B-1 PA 800 plus System Instrument Configuration Dialog for LIF Detectors



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

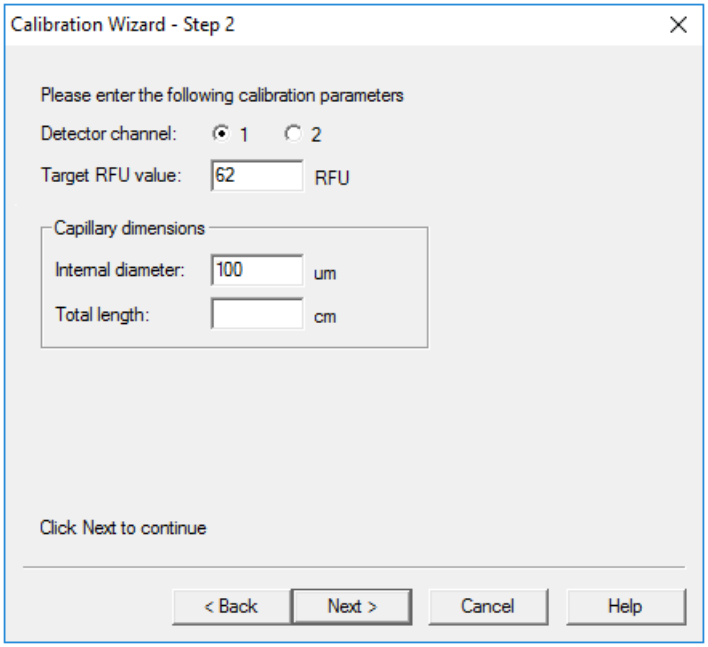
Figure B-2 Calibration Wizard - Step 1



b. In the **Target RFU** field, type `62`.

c. Make sure that the values in the Capillary dimensions section are correct, and then click **Next**.

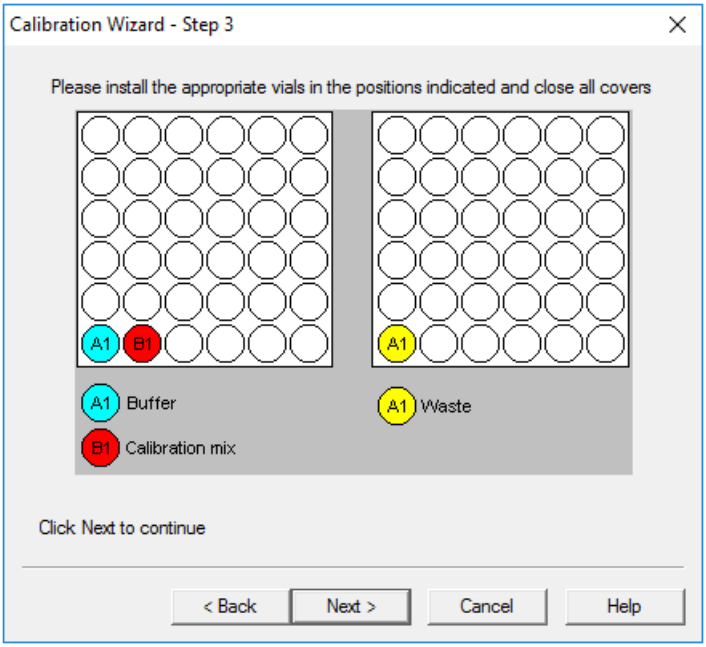
Figure B-3 Calibration Wizard - Step 2



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 empty vials in the tray, and then add liquid and attach the caps.

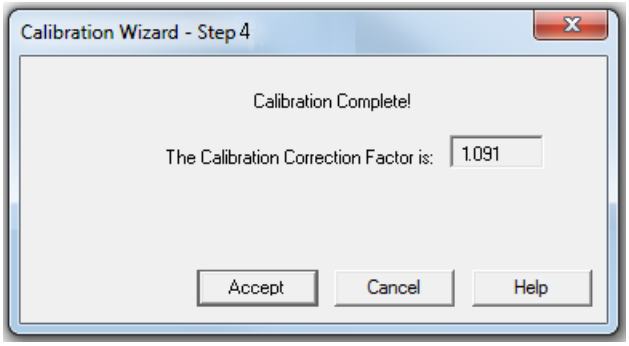
Figure B-4 Calibration Wizard - Step 3



12. Fill the vials, and then put caps on the vials:
    - Inlet buffer tray position A1 (labeled `Buffer`): 1.5 mL of CE Grade Water
    - Inlet buffer tray position B1 (labeled `Calibration mix`): 1.5 mL of LIF Performance Test Mix
    - Outlet buffer tray position A1 (labeled `Waste`): 1.0 mL of 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.

    If the message `No step change detected` is shown, then the detector cannot detect the solution. For troubleshooting procedures, refer to the section: No Step Change Detected.
14. Examine the value in the **Calibration Correction Factor** field:
    - If the CCF value is less than 0.1, then click **Cancel**. Refer to the section: CCF Values for LIF Detector Calibration.
    - If the CCF value is between 0.1 and 10, then the calibration was successful. Click **Accept** to save the results.
    - If the CCF value is more than 10, then click **Cancel**. Refer to the section: CCF Values for LIF Detector Calibration.

Figure B-5 Calibration Wizard - Step 4



15. In the Direct Control window, set the sample storage temperature to 10 °C.
16. Close all of the dialogs and windows.

Troubleshoot the LIF Detector Calibration

CCF Values for LIF Detector Calibration

Issue	Action

Reported CCF value is less than 0.1
 	
or
 	
System performance is not satisfactory
	• 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:
	  • 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.

Reported CCF value is 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.

Issue	Action

Reported CCF value is more than 10
 	
or
 	
System performance is not satisfactory
	• 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:
	  • 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 compares detector signals from 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 do 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. Continue with the next step.
6. Make sure that the correct filters are installed in the LIF detector.

7. If no step change is detected, then do the calibration procedure again. Refer to the section: Calibrate the LIF Detector (Optional).

   If the calibration procedure has been done more than 3 times, then manually set the calibration correction factor (CCF) to 1.0, and then calibrate the LIF detector again.

   If the LIF detector calibration continues to fail, then contact SCIEX Technical Support at sciex.com/request-support.

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