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The Power of Precision

LIFluor™ EnhanCE dsDNA 1000 Dye

For the PA 800 Plus Pharmaceutical Analysis System

Application Guide

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LIFluor™ EnhanCE dsDNA 1000 Dye Kit

This document provides instructions for sample preparation using the LIFluor™ EnhanCE dsDNA 1000 Dye Kit. It also provides instructions for data acquisition and data analysis using the 32 Karat™ Software.

The LIFluor™ EnhanCE dsDNA 1000 Dye 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.

Note: Refer to the *System Overview Guide* for instructions for safe use of the system.

Safety

Refer to the Safety Data Sheets (SDS), available at sciex.com/tech-regulatory, for information about the proper handling of materials and reagents. Always follow standard laboratory safety guidelines. Refer to Hazardous Substance Information for hazardous substance information.

Intended Use

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

Equipment and Materials Required

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

Table 1 LIFluor™ EnhanCE dsDNA 1000 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
PCR micro vials, 200 µL	100	144709
NanoVials	100	5043467
Universal vial caps, blue	100	A62250
Universal vials	100	A62251
Capillary cartridge, blank	1	144738
Cartridge Rebuild Kit	1	144645
dsDNA 1000 kit	1	477410
LIF Performance Test Mix	1	726022
488 mn Notch filter	1	144941
LIF Cartridge Aperture Plug Assembly	1	721125
LIF Cartridge Probe Guide Assembly	1	721126
520 nm Emission filter	1	144940

Storage Conditions

- Store the vial of LIFluor™ EnhanCE dsDNA 1000 Dye at –35 °C to –15 °C.

Note: The LIFluor™ EnhanCE Dye is light sensitive. Do not leave it exposed to light when not in use. Aluminum foil can be used to cover the vial to reduce exposure to light.

Note: Always keep the LIFluor™ EnhanCE Dye vial capped tightly when not in use. If the vial is left opened, the methanol will evaporate, thus increasing the dye concentration.

Note: The LIFluor™ EnhanCE Dye can show deterioration after 10 hours if left at room temperature.

Customer-Supplied Equipment and Supplies

- Powder-free gloves, neoprene or nitrile recommended

- Safety glasses
- Laboratory coat
- Vortex mixer
- Pipettes and appropriate tips.
- Double-deionized (DDI) water (MS-grade water filtered through a 0.2 µm filter and with resistance above 18 MΩ)
- Parafilm
- Magnetic stir plate and stir bar
- 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)

Methods

This guide provides methods for separating the test mix that is provided with the kit.

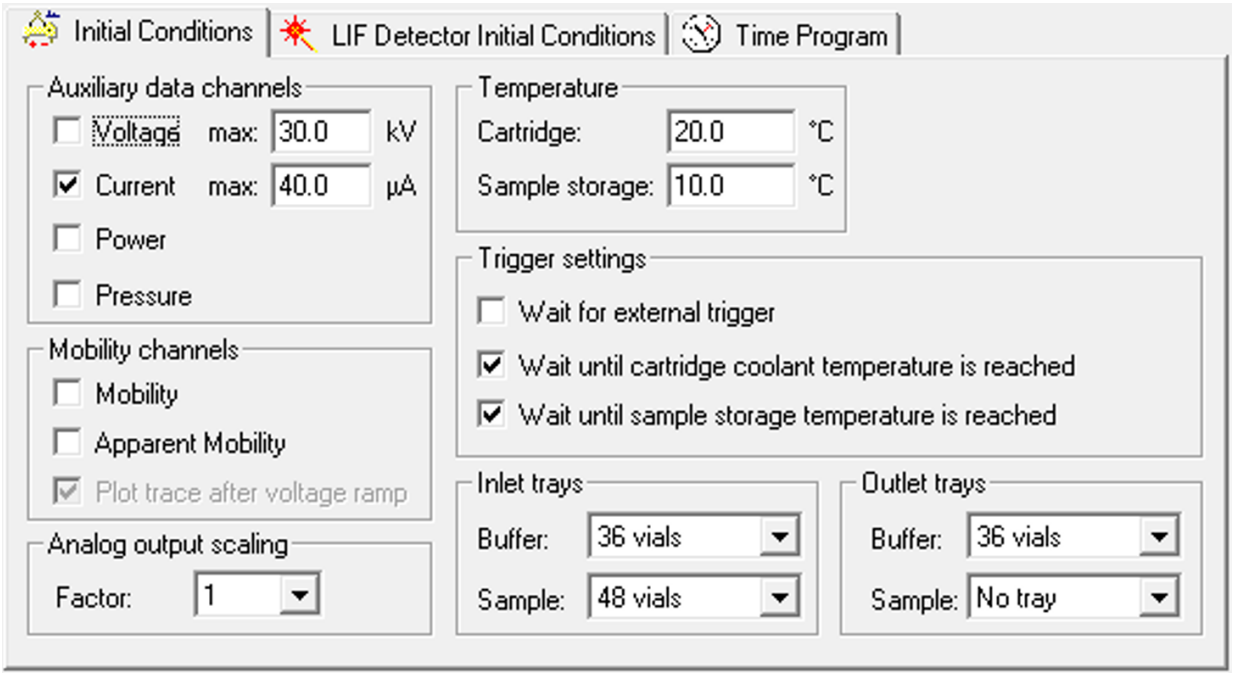
The LIFluor™ EnhanCE dsDNA 1000 Dye kit requires the following methods: a conditioning method, a separation method, and a shutdown method. Refer to Initial Conditions, LIF Detector Initial Conditions, and Time Programs to create the methods.

Because this kit can be used in a wide variety of other applications, use the methods described here as a starting point to develop a method that is appropriate for relevant applications. Refer to technical notes on the SCIEX website or contact a SCIEX field applications scientist for specific recommendations or support.

Initial Conditions

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

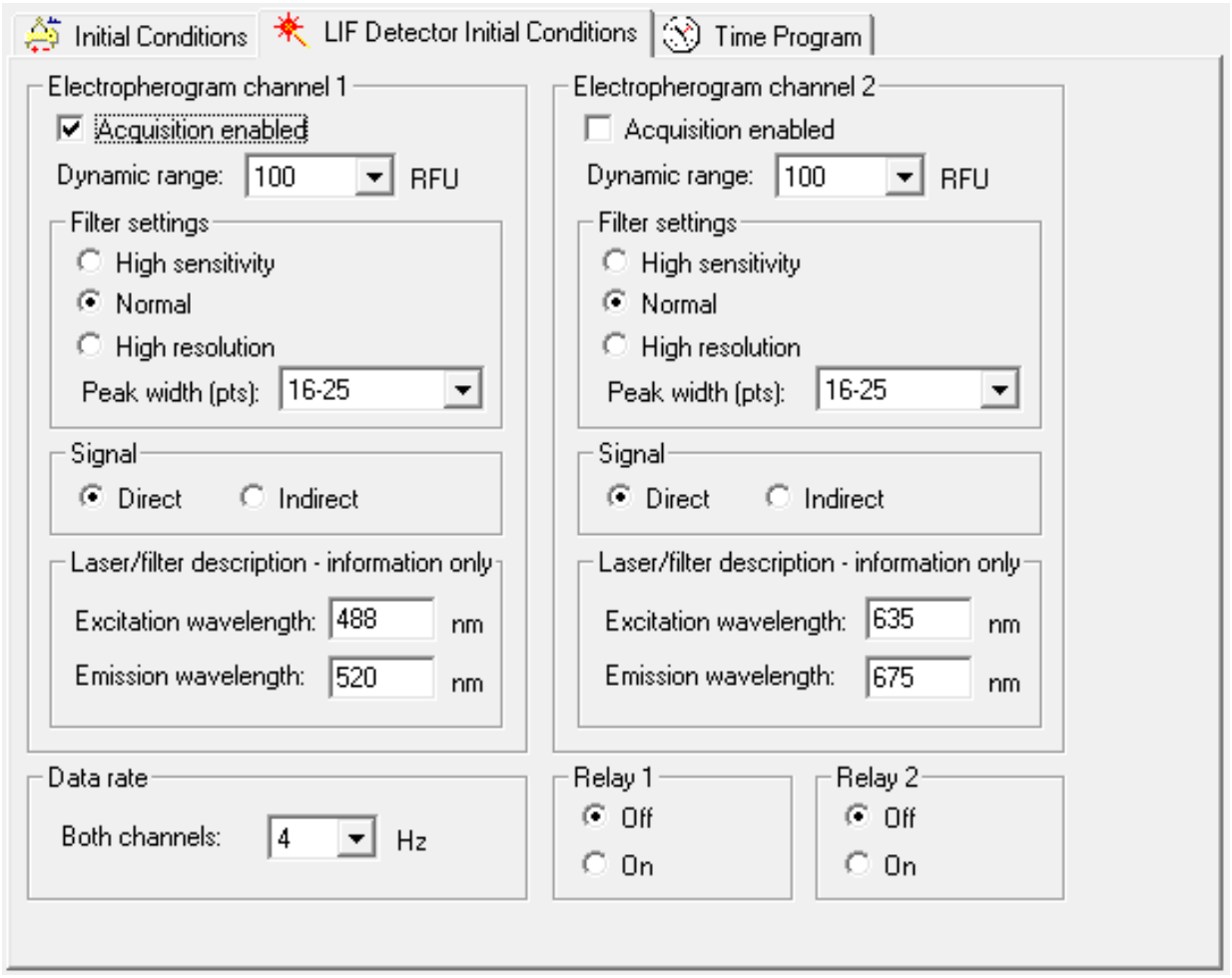
Figure 1 Initial Conditions Tab for LIFluor™ EnhanCE dsDNA 1000 Dye Kit Methods



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.

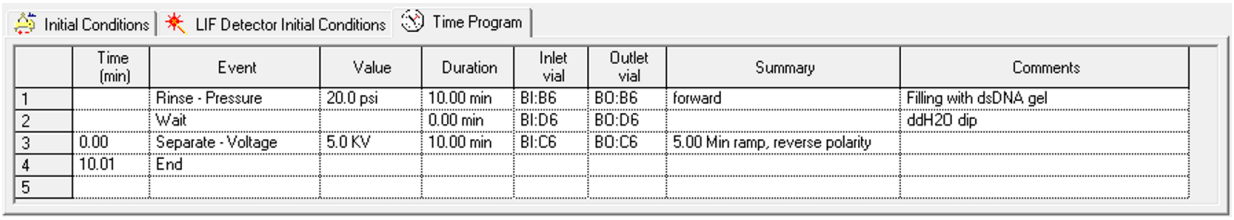
Figure 2 LIF Detector Initial Conditions Tab for LIFluor™ EnhanCE dsDNA 1000 DyeKit Methods



Time Programs

Note: The time programs are different for each method.

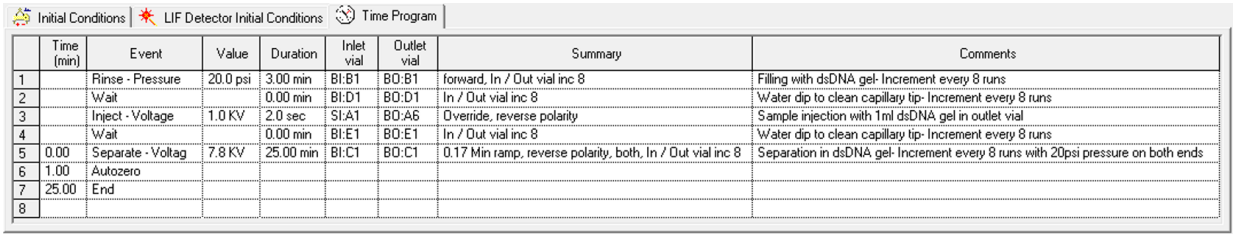
Figure 3 Time Program Tab for the Conditioning Method



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	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								

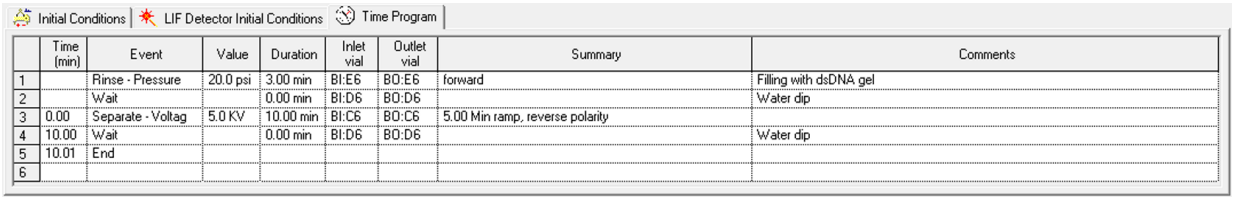
Figure 4 Time Program Tab for the dsDNA 1000 Test Mix Method



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

1. Add 20.0 mL DDI water to the dsDNA 1000 Gel Buffer vial.
2. Using a magnetic stir bar and stir plate, stir the solution until the dsDNA 1000 Gel Buffer is completely dissolved.

   Use a stir bar that is slightly shorter than the diameter of the dsDNA 1000 Gel Buffer bottle in order to stir more effectively.

   It might take up to 24 hours to completely dissolve the lyophilized gel.

3. Immediately before using the gel buffer, filter it with a 0.45 µm filter and then sonicate it for 1 minute to remove any small bubbles.

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

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

   12 mL of gel buffer is enough for the conditioning, eight sample injections, and the shutdown.

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

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 light sensitive. Do not leave it exposed to light when not in use. Aluminum foil can be used to cover the vial to reduce exposure to light.

Note: Always keep the LIFluor™ EnhanCE Dye vial capped tightly when not in use. If the vial is left opened, the methanol will evaporate, thus increasing the dye concentration.

Note: The LIFluor™ EnhanCE Dye can show deterioration after 10 hours if left at room temperature.

CAUTION: Potential Data Loss. Do not heat the dsDNA 1000 Gel Buffer. Heating the gel buffer can lead to poor separation.

Prepare the Samples

Prepare the Test Mix

1. Add 1 mL of DDI water to the dsDNA 1000 Test Mix vial and then use the vortex mixer to mix well.
2. Transfer 100 µL of the reconstituted dsDNA 1000 Test Mix to a PCR micro vial. Add 1 µL LIF Performance Test Mix in 100 µL of dsDNA 1000 Test Mix to the micro vial as a marker, and then mix well.

3. Put the PCR micro vial in a universal vial. Seal it with a blue cap.
4. Transfer 100 µL aliquots of the remaining reconstituted dsDNA 1000 Test Mix into additional vials. Prior to use, add 1 µL of LIF Performance Test Mix per 100 µl of dsDNA 1000 Test Mix.
5. Store the reconstituted test mix at –35 °C to –15 °C when not in use. The reconstituted test mix degrades when stored at room temperature.

Prepare the Sample

1. Dilute the DNA sample with DDI water or Tris-EDTA buffer to a concentration of about 10 ng/µL DNA.
2. Add 1 µL LIF Performance Test Mix to 100 µL of diluted DNA sample, and then mix thoroughly. The LIF Performance Test Mix serves as a marker.
3. Transfer 100 µL of the sample to a PCR micro vial for analysis.

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

Prepare the PA 800 Plus System

This section describes the steps to prepare the PA 800 Plus System to acquire data.

The procedures described in this section assume that the system has already been properly installed and initialized.

Install the LIF Detector

1. Turn off the PA 800 Plus System and then install the LIF detector. Refer to the *System Maintenance Guide*.
2. Turn on the system.
3. Turn on the laser and allow the lamp to warm up for at least 30 minutes.

Clean the Interface Block

CAUTION: Potential System Damage. Do not allow the gel to accumulate 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 changing chemistries. Refer to the *System Maintenance Guide* for detailed instructions.

The gel buffer is very viscous and can accumulate in the system unless regular and thorough cleaning is performed.

Install the Capillary

CAUTION: Potential System Damage. Do not allow the capillary to become dehydrated. Within 5 to 10 minutes of trimming the end of a capillary, the coating inside the capillary begins to dehydrate.

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

- Install the DNA capillary into a capillary cartridge using the *Capillary Cartridge Rebuild Instructions*.

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

  For details about separating longer DNA samples, refer to technical notes on the SCIEX website or contact a SCIEX field applications scientist.

  Follow these modifications to the *Capillary Cartridge Rebuild Instructions* to minimize damage to the capillary coating.

  a. Fill two universal vials with 1.5 mL DDI water, and then cover them with blue caps.

  b. Cut off the end-cap on the inlet side of the capillary and then install the capillary in the cartridge. After inserting the capillary in the cartridge, cut the end-cap from the outlet side and finish assembling the cartridge.

  c. Trim the capillary ends to the recommended length and then submerge both ends of the capillary in the DDI water-filled vials. Do not expose the capillary ends to air for more than 5 to 10 minutes during cartridge assembly.

Install the Cartridge and Calibrate the Detector

Note: To make sure that analysis results are consistent over time, we strongly recommend calibrating the detector each time it is installed in the PA 800 Plus System. Also calibrate the detector after replacing the capillary in the cartridge or installing a different cartridge.

1. Remove the cartridge from the vials.

2. Install the cartridge in the PA 800 Plus System.
3. Calibrate the detector.

   Use the Calibration wizard, available from the Instrument Configuration dialog in the 32 Karat™ Software. Refer to the *System Maintenance Guide* for detailed instructions. Use the values in the following table in step two of the Calibration wizard.

Table 3 Calibration Parameters

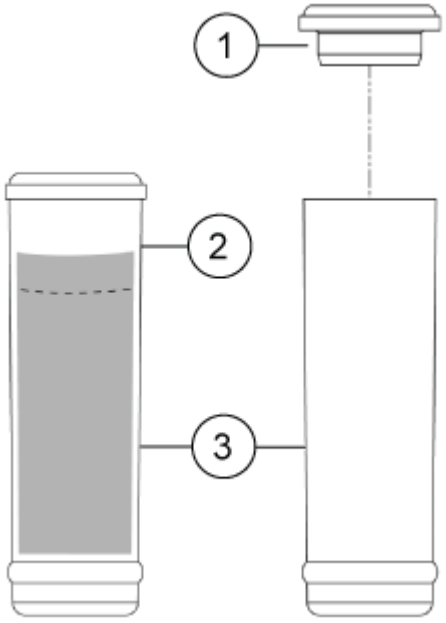
Detector Channel	1
Target RFU	62
Capillary Dimensions:	
Internal Diameter	100 µm
Total Length	40 cm or 50 cm, depending on the length of the capillary

Load the Buffer Trays

CAUTION: Potential System Damage. Do not fill any vial with more than 1.8 mL of liquid. Also, do not allow more than 1.8 mL to accumulate in the waste vials. If a vial contains more than 1.8 mL, then the pressure system might be damaged.

1. Depending on the number of samples to be run, fill the appropriate number of vials and then cap them. Refer to Figure 7.

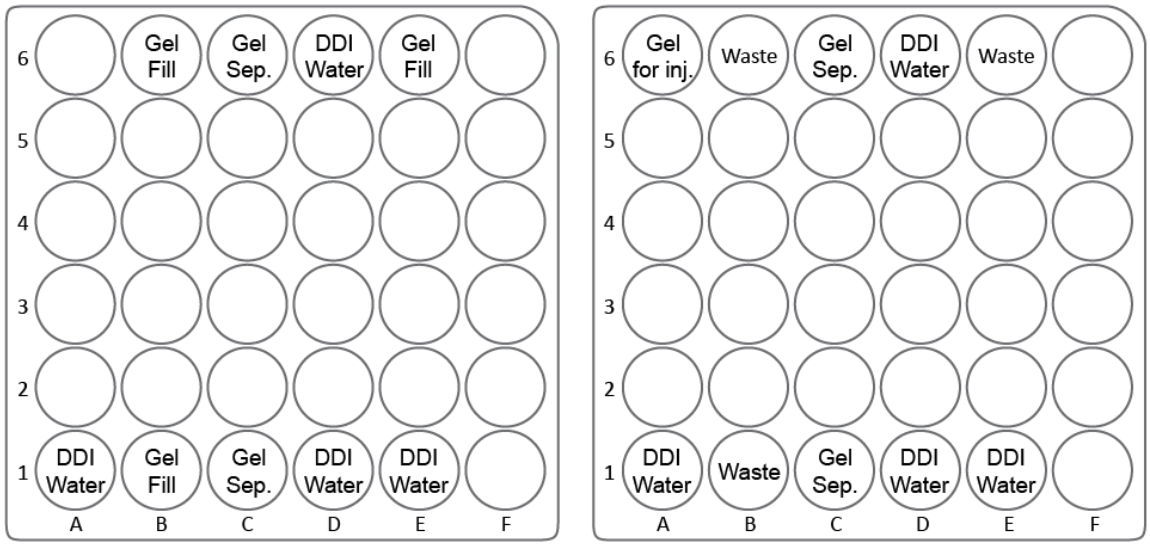
Figure 6 Universal Vial and Cap Setup



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

2. 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 7 Buffer Tray Inlet (BI), Left and Buffer Tray Outlet (BO), Right



- DDI H_2O: 1.5 mL DDI water
- Gel Fill: 1.5 mL dsDNA 1000 Gel Buffer containing LIFluor™ EnhanCE Dye
- Gel Sep.: 1.5 mL dsDNA 1000 Gel Buffer containing LIFluor™ EnhanCE Dye
- Gel Inj.: 1 mL dsDNA 1000 Gel Buffer containing LIFluor™ EnhanCE Dye
- Waste: 1 mL DDI water

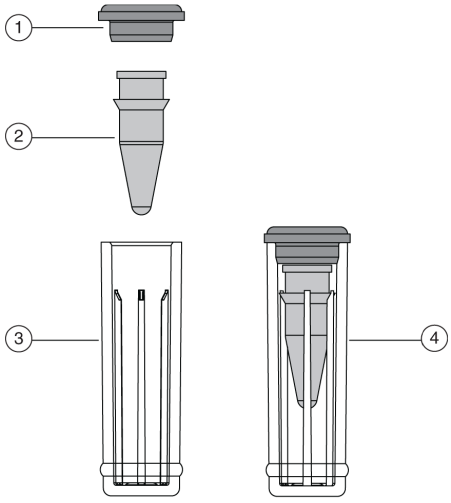
Note: For this application, all vials and caps are designed for a maximum of eight runs. Do not reuse the caps, because they might be contaminated with dried gel and other chemicals.

Load the Sample Tray

1. Prepare the samples.
   - For samples in PCR micro vials, put the PCR micro vial in a universal vial and then cover the vial with a cap. Refer to Figure 8.

- For samples in nanoVials, cover the vial with a cap.

Figure 8 PCR Micro Vial in a Universal Vial

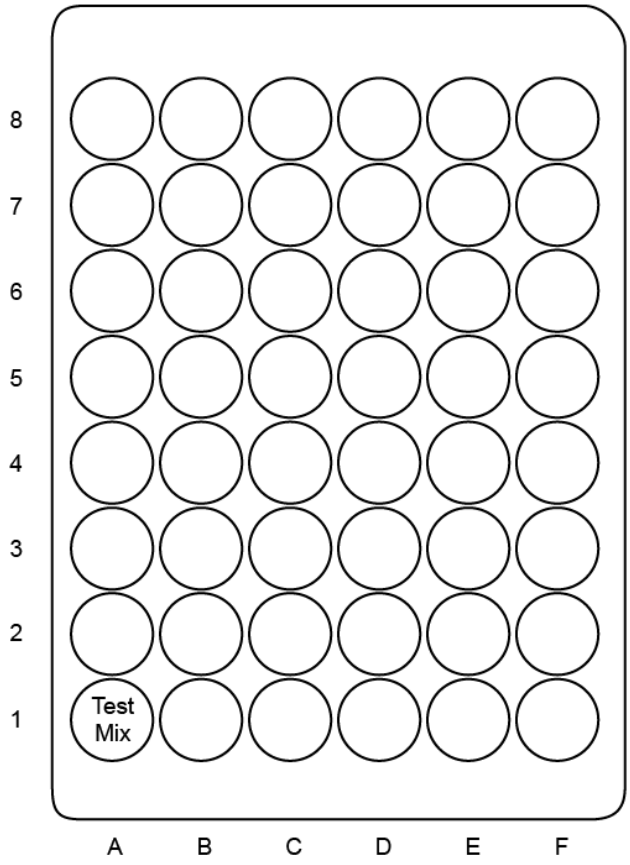


Item	Description
1	Universal vial cap
2	PCR micro vial
3	Universal vial
4	Micro vial inside universal vial

2. Load the sample vials into the sample inlet tray.

   To run the samples, put them in the sample tray starting at position A1 and then filling all the A wells before filling any other wells.

Figure 9 Sample Tray Inlet (SI)



Run the Samples

Create the Sequence and Start the Run

Note: The following instructions assume the user is familiar with how to create and run a sequence using 32 Karat™ Software. For detailed instructions, refer to the *PA 800 Plus Pharmaceutical Analysis System Methods Development Guide*.

1. Open the 32 Karat™ Software.
2. In the 32 Karat™ Software window, either select an instrument with an 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 an additional row after the conditioning method for each sample. Fill sufficient vials in the buffer trays according to the number of samples to be run. Each buffer vial row runs five samples.
5. Make sure that the LIF detector laser is turned on, that the sample and buffer trays are loaded, and then click

   to open the Run Sequence dialog.
6. Make any required changes in the dialog and then click **Start**.

Waste Disposal

WARNING! Biohazard or Toxic Chemical Hazard. Follow local directives when disposing of chemicals, vials and caps, and the remains of the prepared samples, if applicable. They might contain regulated compounds and biohazardous agents.

Store the Cartridge

Store the Cartridge for Less Than 48 Hours

1. Perform the shutdown method to clean the capillary.

   The shutdown method fills the capillaries with dsDNA 1000 Gel Buffer.
2. Store the cartridge for up to 48 hours in the system with the capillary ends immersed in vials of DDI water.

Store the Cartridge for More Than 48 Hours

1. Perform the shutdown method to clean the capillary.
2. Remove the cartridge from the system.

3. Place the cartridge in the cartridge storage box with the capillary ends immersed in vials of DDI water.
4. Store 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 or it has been stored for an extended time, then condition the capillary using the conditioning method.

Analyze the Results

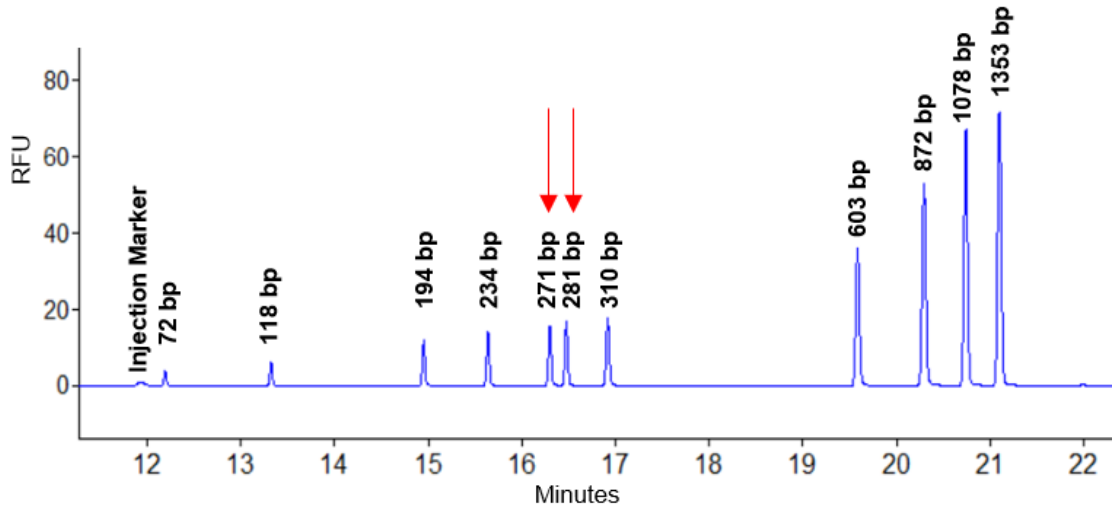
Analyze the Data for the Test Mix

The dsDNA 1000 Test Mix contains a Phi-X 174 DNA Hae III digest consisting of 11 fragments. The test mix should separate in less than 25 minutes using the recommended separation method with baseline separation of the 271 bp and 281 bp fragments. Refer to Figure 10.

The electrical current should remain fairly stable between 14 µA and 24 µA. Small variations in the current can indicate that the capillary is experiencing temperature fluctuations or the presence of air bubbles in the gel buffer. This could result in a noisy baseline, miscellaneous spikes or broad peaks. Remove any air bubbles by sonicating the gel buffer vials for 5 seconds before use.

If the DNA sample is larger than 2 kb, a capillary with a longer total length, such as 50.2 cm, can be used. Modify the method to reflect the longer capillary. For details about separating longer DNA samples, refer to technical notes on the SCIEX website or contact a SCIEX field applications scientist.

Figure 10 Example Electropherogram for Test Mix



Recommended Sample Injection for the Separation Method

Begin with an electrokinetic injection, performed at 1 kV for 2 seconds as shown in the separation method. The injection can be lengthened to a period of 5 seconds if necessary.

Alternatively, for samples with higher salt concentrations, begin with a pressure injection, performed at 0.5 psi for 10 seconds. The injection can be lengthened to a period of 20 seconds if necessary.

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, the migration time of the dsDNA fragments increases.
  - If less than 15 µL of LIFluor™ EnhanCE Dye is used, the dsDNA fragments migrate rapidly. For example, the 72 bp fragment migrates before the LIF Performance Test Mix, if 2 µL of LIFluor™ EnhanCE Dye is used.
  - If any of the dsDNA peaks are followed by a negative baseline upset, then there is not enough LIFluor™ EnhanCE Dye in the gel buffer to stain all of the dsDNA. Either inject less sample or increase the amount of LIFluor™ EnhanCE Dye in the gel buffer to eliminate this artifact.
- 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 indicate 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 for DNA Application Guides

Table 4 LIFluor Troubleshooting

Symptom	Possible Cause	Corrective Action
Low current	The capillary is blocked.	Either: • Rinse the capillary with DDI water for 10 minutes at 20 psi to remove the gel buffer and then condition the capillary using the conditioning method. • Replace the capillary. Refer to 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 Clean the Interface Block. 2. Replace the gel buffer or test mix as needed.
No peaks	1. The capillary is blocked. 2. The capillary window or tip is broken.	1. Either: • Rinse the capillary with DDI water for 10 minutes at 20 psi to remove the gel buffer and then condition the capillary using the conditioning method. • Replace the capillary. Refer to Install the Capillary. 2. Replace the capillary. Refer to Install the Capillary.

Table 4 LIFluor Troubleshooting (continued)

Symptom	Possible Cause	Corrective Action
Spikes in the electropherogram	Air bubbles are in the gel buffer.	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	Microparticles are in the gel buffer.	Filter the gel buffer using a 0.45 µm pore filter to remove microparticles. Sonicate for 5 seconds to remove the air bubbles.
Decrease in peak height	The dye deteriorated.	Prepare fresh LIFluor™ EnhanCE Dye-gel solution. Protect the LIFluor™ EnhanCE Dye-gel solution from light.
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. Protect the LIFluor™ EnhanCE Dye-gel solution from light. 2. Make sure that the LIFluor™ EnhanCE Dye-gel solution has been mixed well. 3. Prepare fresh LIFluor™ EnhanCE Dye-gel solution. Protect the LIFluor™ EnhanCE Dye-gel solution from light.
Current change	1. The dye deteriorated. 2. The electrodes are dirty.	1. Prepare fresh LIFluor™ EnhanCE Dye-gel solution. Protect the LIFluor™ EnhanCE Dye-gel solution from light. 2. Clean the electrodes and lever arms. Refer to Clean the Interface Block.

Table 4 LIFluor Troubleshooting (continued)

Symptom	Possible Cause	Corrective Action
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. Reduce dye volume added to gel for diluted 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.	Dilute the sample or inject less sample. Reduced sample loads improve both resolution and peak efficiency.

Hazardous Substance Information A

The following information must be noted and the relevant safety measures taken. Refer to the respective safety data sheets for more information. These are available upon request or can be downloaded from our website 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.

Contact Us

Customer Training

- In North America: NA.CustomerTraining@sciex.com
- In Europe: Europe.CustomerTraining@sciex.com
- Outside the EU and North America, visit sciex.com/education for contact information.

Online Learning Center

- SCIEX University™

Purchase Consumables

Reorder SCIEX consumables online at store.sciex.com. To set up an order, use the account number, found on the quote, order confirmation, or shipping documents. The SCIEX online store is currently limited to the US, UK, and Germany but will be expanding to other countries in the future. For customers in other countries, contact the local SCIEX representative.

SCIEX Support

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:

- sciex.com/contact-us
- sciex.com/request-support

CyberSecurity

For the latest guidance on cybersecurity for SCIEX products, visit sciex.com/productsecurity.

Documentation

This version of the document supercedes all previous versions of this document.

To view this document electronically, Adobe Acrobat Reader is required. To download the latest version, go to https://get.adobe.com/reader.

To find software product documentation, refer to the release notes or software installation guide that comes with the software.

To find hardware product documentation, refer to the *Customer Reference* DVD that comes with the system or component.

The latest versions of the documentation are available on the SCIEX website, at sciex.com/customer-documents.

Note: To request a free, printed version of this document, contact sciex.com/contact-us.