SCIEX

The Power of Precision

dsDNA 1000 Kit

For the PA 800 Plus Pharmaceutical Analysis System

Application Guide

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EC REP

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Contents

Contents

dsDNA 1000 Kit

The dsDNA 1000 Kit contains the supplies necessary to perform rapid separation and analysis of double-stranded DNA (dsDNA) fragments. A linear relationship between migration time and number of base pairs can be obtained from dsDNA fragments in the range of 100 to 1,000 base pairs. In addition, this kit can be used for analysis of dsDNA fragments with sizes up to 15,000 base pairs.

This document provides instructions for sample preparation using the dsDNA 1000 Kit. It also provides instructions for data acquisition and data analysis using the 32 Karat software.

Use the information in this application guide as a starting point. As needed, modify injection time, voltage, injection type, or other parameters to find the best conditions for your needs.

Note: For instructions for safe use of the system, refer to the document: *System Overview Guide*.

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. For hazardous substance information, refer to the section: Hazardous Substance Information.

Intended Use

The dsDNA 1000 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 dsDNA 1000 Kit (PN 477410)

Component	Quantity	Reorder Part Number
DNA Capillary, 65 cm, 100 µm i.d.	2	477477
dsDNA 1000 Gel Buffer, dehydrated	3 vials	477628
dsDNA 1000 Test Mix (10 µg in 10 µL with 11 DNA fragments ranging from 72 to 1,353 base pairs)	2 vials	477414

Table 1 dsDNA 1000 Kit (PN 477410) (continued)

Component	Quantity	Reorder Part Number
Orange G Reference Marker, 0.1% solution in water	1 mL	241524

Table 2 Additional Supplies from SCIEX

Component	Quantity	Part Number
(Optional) LIFluor EnhanCE dsDNA 1000 Dye	1	477409
Capillary cartridge, blank	1	144738
Cartridge rebuild kit	1	144645
CE Grade Water	140 mL	C48034
NanoVials	100	5043467
PCR microvials (200 µL)	100	144709
Universal vial caps, blue	100	A62250
Universal vials	100	A62251

Note: For instructions on how to use the LIFluor EnhanCE dsDNA 1000 Dye, refer to the document: *LIFluor EnhanCE dsDNA 1000 Dye Application Guide*.

Storage Conditions

- Store the dsDNA 1000 Gel Buffer, Orange G Reference Marker, and DNA capillary at 2 °C to 8 °C.
- Store the dsDNA 1000 Test Mix at –35 °C to –15 °C.
- Never heat the gel buffer because it might lead to poor separation.

Customer-Supplied Equipment and Supplies

- Powder-free gloves, neoprene or nitrile recommended
- Safety glasses
- Laboratory coat
- Vortex mixer
- Pipettes and appropriate tips.
- Disposable syringes, 10 mL

- Membrane syringe filters, 0.2 µm and 0.45 µm pores
- (Optional) LABQUAKE rotator (Barnstead International PN 400110)
- 10× concentrated stock solution of Tris-Borate-EDTA buffer (10× TBE) (Sigma PN T4323)
- Tris-EDTA buffer, 10 mM Tris-HCl, 1 mM disodium EDTA, pH 8.0 (Sigma)
- Analytical balance

Required Detector

A UV detector with a 254 nm filter is required.

Note: This kit is not recommended for use with a photodiode array (PDA) detector.

A laser-induced fluorescence (LIF) detector is needed if LIFluor EnhanCE dsDNA 1000 Dye is added to the gel buffer for analysis of samples with low DNA concentrations. Refer to the *LIFluor EnhanCE dsDNA 1000 Dye Application Guide*.

Required Cartridge or Capillary

CAUTION: Potential Wrong Result. If a capillary is being used with the dsDNA 1000 kit, then do not use the same capillary for another application. Different buffers and types of samples can lead to sample carryover, non-specific binding, or poor separation.

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

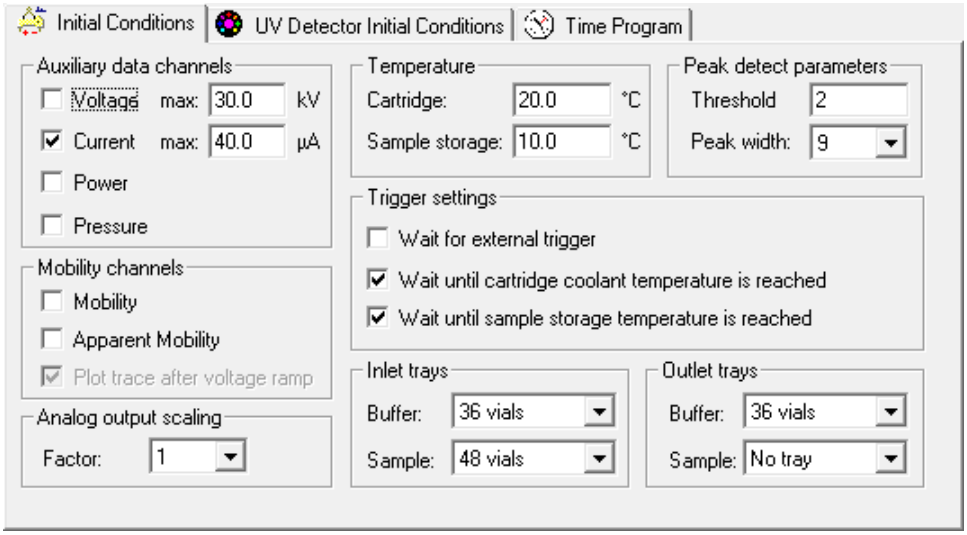
The dsDNA 1000 kit requires the following methods: a conditioning method, a separation method, and a shutdown method. Refer to Initial Conditions, UV Detector Initial Conditions, and Time Programs to create the methods.

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 Specialist for specific recommendations or support.

Initial Conditions

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

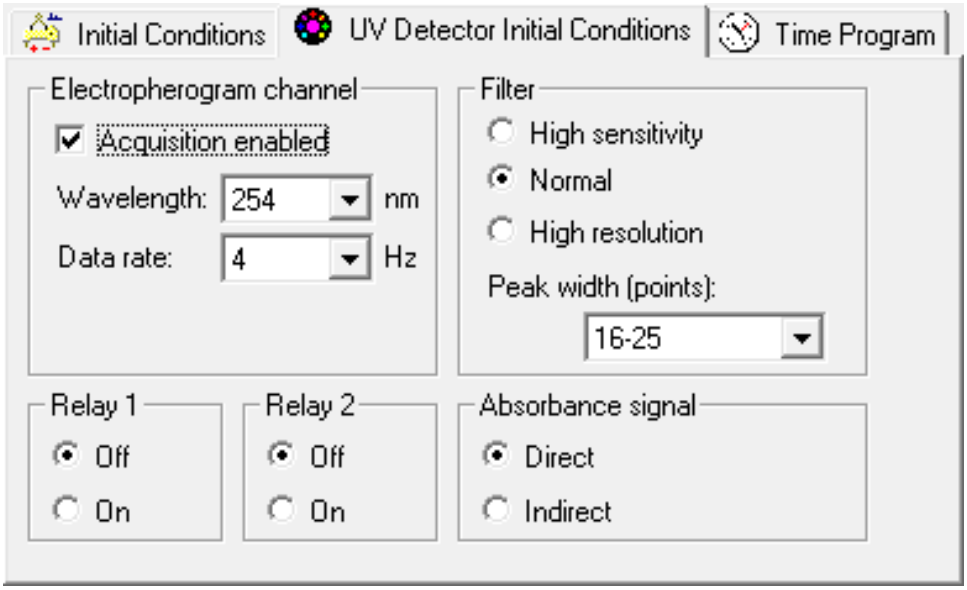
Figure 1 Initial Conditions Tab for dsDNA 1000 Kit Methods



UV Detector Initial Conditions

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

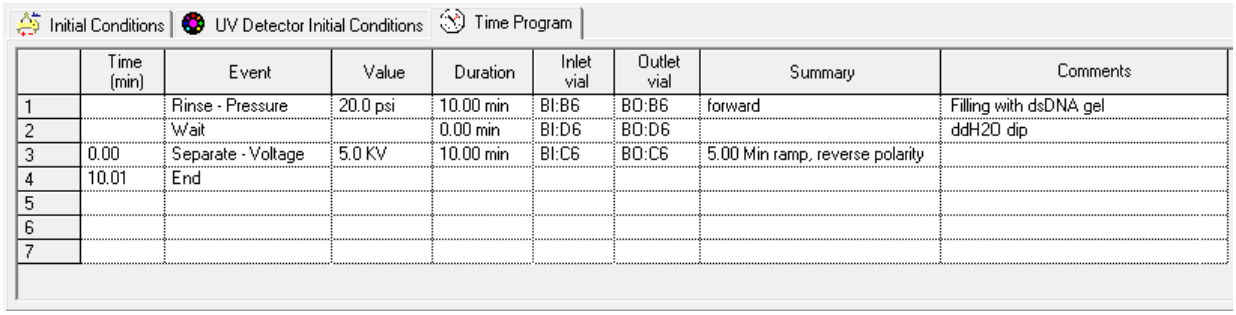
Figure 2 UV Detector Initial Conditions Tab for dsDNA 1000 Kit Methods



Time Programs

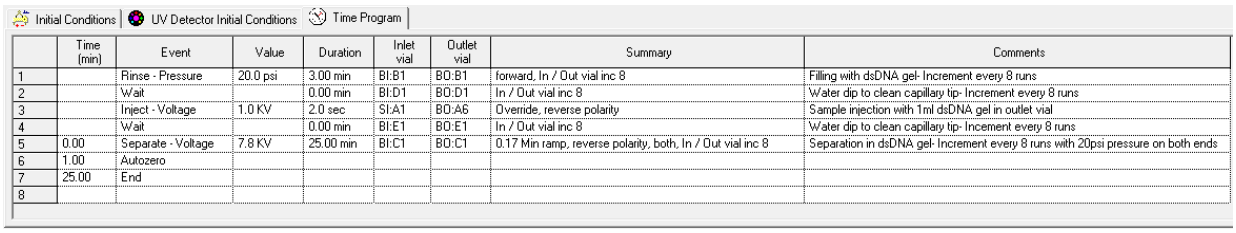
Note: The time programs are different for each method.

Figure 3 Time Program Tab for the dsDNA 1000 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								
6								
7								

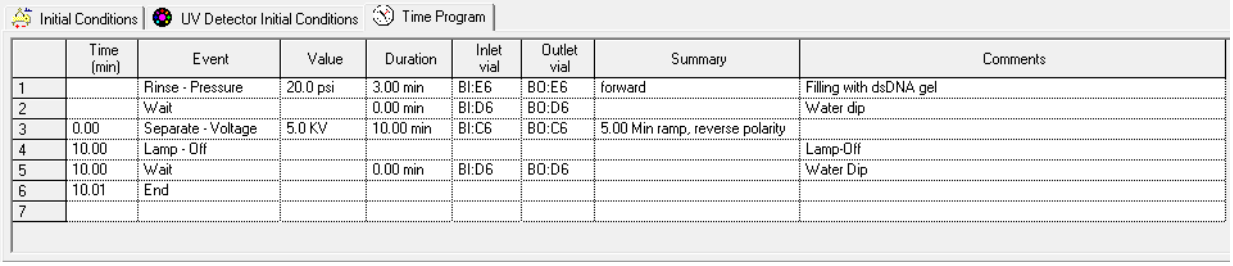
Figure 4 Time Program Tab for the dsDNA 1000 Separation Method



	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- Incement every 8 runs
5	0.00	Separate - Voltage	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								

Note: For analysis of samples with a concentration lower than 10 µg/mL, optimize the injection parameters. Refer to the section: Recommended Sample Injection for the Separation Method.

Figure 5 Time Program Tab for the dsDNA 1000 Shutdown Method



	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 - Voltage	5.0 KV	10.00 min	BI:C6	BO:C6	5.00 Min ramp, reverse polarity	
4	10.00	Lamp - Off						Lamp-Off
5	10.00	Wait		0.00 min	BI:D6	BO:D6		Water Dip
6	10.01	End						
7								

Prepare the Reagents and Stock Solutions

Prepare the dsDNA 1000 Gel Buffer

1. Add 20.0 mL 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.

   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.

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

Note: LIFluor EnhanCE dsDNA 1000 Dye can be added to the dsDNA 1000 Gel Buffer if LIF detection is required. Refer to the instructions supplied with the LIFluor EnhanCE dsDNA 1000 Dye for use with this kit.

Prepare the Samples

Prepare the Test Mix

1. Add 40 µL of CE Grade Water and 0.5 µL of the Orange G Reference Marker to the dsDNA 1000 Test Mix vial and then mix well.

   This makes a 200 µg/mL solution of total DNA.

2. Transfer all of the diluted dsDNA 1000 Test Mix to a PCR microvial. Alternatively, add 5 to 10 µL of the sample to a nanoVial.
3. Put the microvial in a universal vial. Seal it with a blue cap.
4. 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.

Note: The reconstituted test mix should last up to 2 weeks when stored frozen. As it deteriorates, the resolution of the last three peaks decreases.

Prepare the Sample

1. Dilute the dsDNA sample with CE Grade Water or 0.2 µm filtered Tris-EDTA buffer to a concentration of about 200 ng/µL DNA.
2. Add 1 µL Orange G Reference Marker to 100 µL diluted DNA sample and then mix thoroughly. The Orange G Reference Marker serves 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.

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

Install the UV Detector

1. Turn off the PA 800 Plus System and then install the UV detector. Refer to the document: *System Maintenance Guide*.
2. Turn on the system and let the lamp warm up for at least 30 minutes.

Clean the Interface Block

CAUTION: Potential System Damage. Do not let the gel 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. For detailed instructions, refer to the document: *System Maintenance Guide*.

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.

1. Install the DNA 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. Use a 100 µm × 200 µm 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.

2. Follow these modifications to minimize damage to the capillary coating.

   a. Fill two universal vials with 1.5 mL CE Grade 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 then 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 minutes to 10 minutes during cartridge assembly.

For details about separating longer DNA samples, refer to technical notes on the SCIEX website or contact a SCIEX Field Applications Specialist.

Install the Cartridge

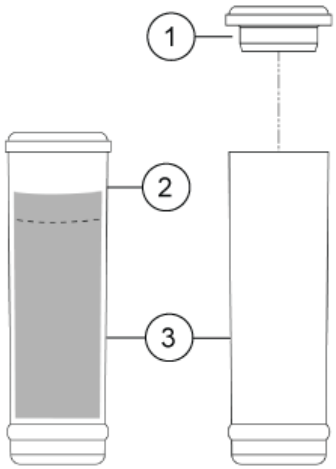
1. Remove the cartridge from the vials.
2. Install the cartridge in the PA 800 Plus system. Refer to the document: *System Maintenance Guide*.

Load the Buffer Trays

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

1. Fill the appropriate number of vials for the number of samples to be run and then cap them. Refer to the figure: Figure 7.

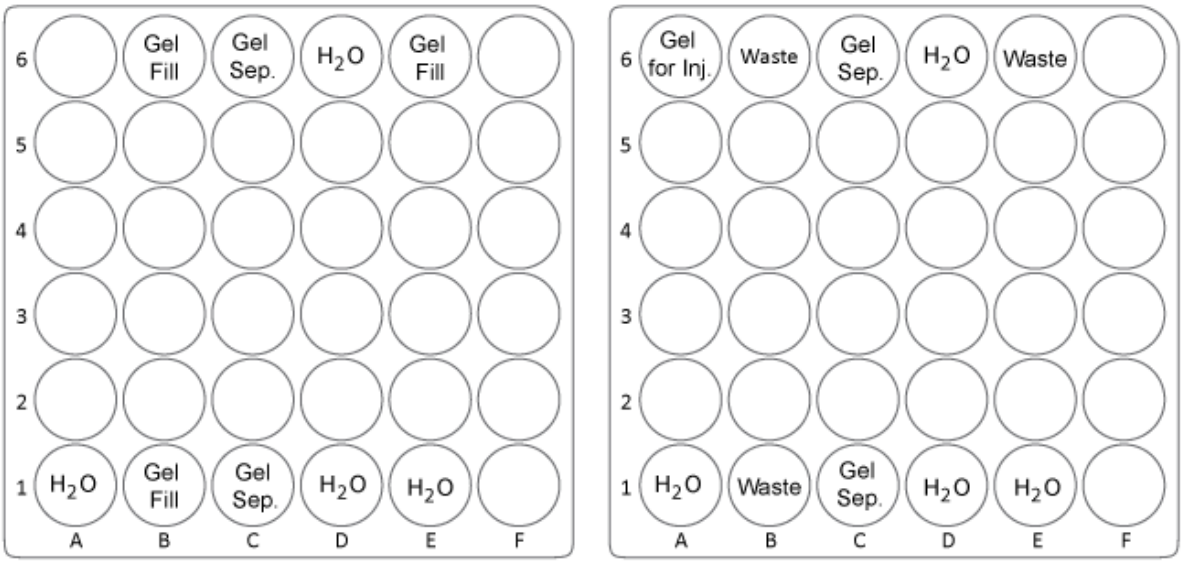
Figure 6 Universal Vial and Cap Setup



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



Item	Description
H_2O	1.5 mL CE Grade Water
Gel Fill	1.5 mL dsDNA 1000 Gel Buffer
Gel Sep.	1.5 mL dsDNA 1000 Gel Buffer
Gel Inj.	1 mL dsDNA 1000 Gel Buffer
Waste	1 mL CE Grade Water

Note: If absolute migration time is being used as a system suitability criteria for assay repeatability and peak shifting is occurring, then use a higher ionic strength buffer such as 10× TBE in vial location C1. To achieve similar migration times to those in data obtained using the dsDNA 1000 Gel Buffer in the C1 vial, the separation voltage might need to be decreased.

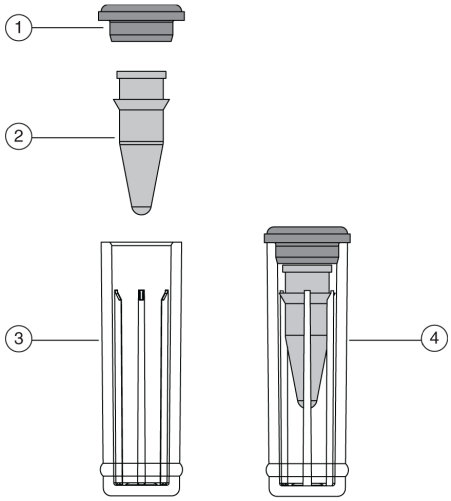
Note: For this application, all vials and caps are designed for a maximum of eight runs. Do not reuse the vials or caps, because they might be contaminated with dried gel and other chemicals.

Load the Sample Tray

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

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

Figure 8 Microvial in a Universal Vial

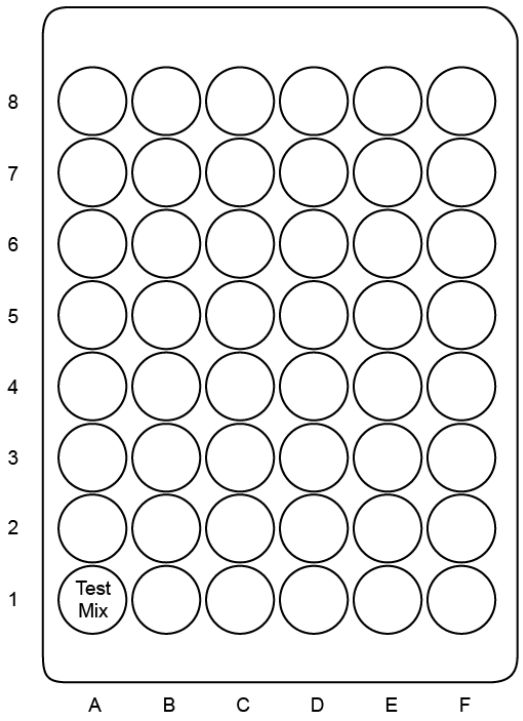


Item	Description
1	Universal vial cap
2	Microvial
3	Universal vial
4	Microvial inside universal vial

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

   To run the samples, put them in the sample tray starting at position A1 and fill all of 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 knows how to create and run a sequence using 32 Karat software. For detailed instructions, refer to the document: *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 a UV 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 UV lamp is turned on, that the sample and buffer trays are loaded, and then click  .
   The Run Sequence dialog opens.
6. Make any required changes, 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. Use 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 CE Grade Water.

Store the Cartridge for More Than 48 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. 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 if it has been stored for an extended time, then condition the capillary using the conditioning method.

Analyze the Data

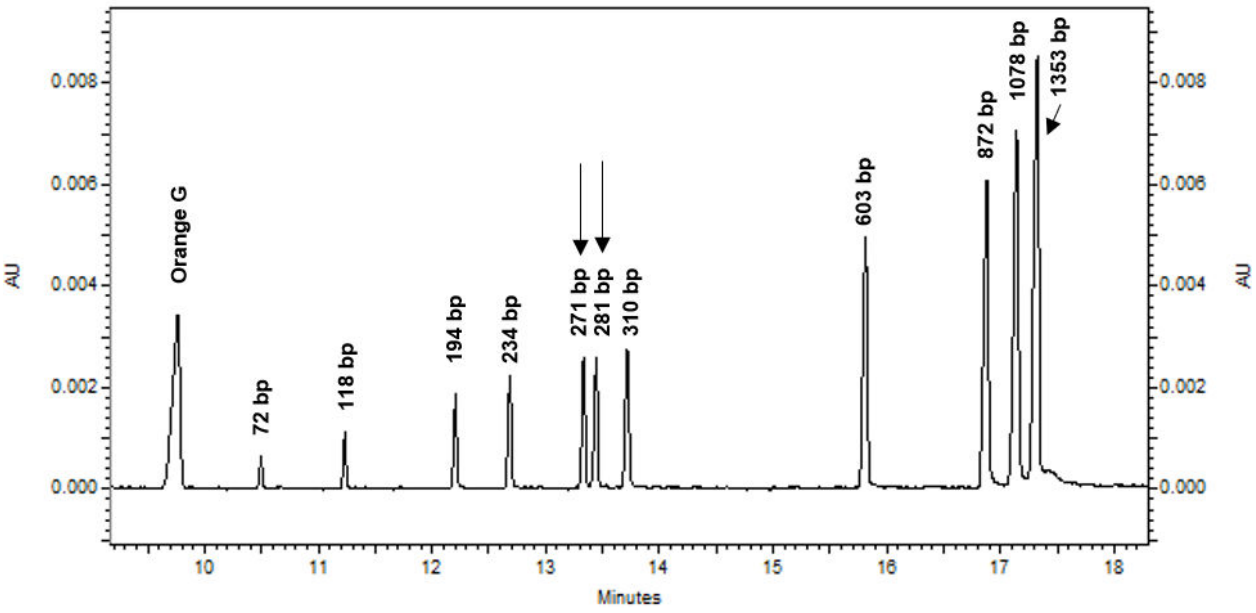
Analyze the Data for the Test Mix

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 the figure: 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 that air bubbles are present in the gel buffer. These variations in the current 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 Specialist.

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 duration can be increased to a period of 5 seconds if required.

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

Tips for Best Results

- If resolution decreases over time, then replace the dsDNA 1000 Gel Buffer. Do a run with the text mix to make sure that the resolution has improved.
- If baseline separation is not achieved, then use the same field strength (V/cm) and increase the capillary length as required.

- 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.

Troubleshooting

Table 3 dsDNA Troubleshooting

Symptom	Possible Cause	Corrective Action
Low or unsteady current	1. The capillary is blocked. 2. There is a problem with the PA 800 Plus system.	1. Either: • Rinse the capillary with CE Grade Water for 10 min at 20 psi to remove the gel buffer and then condition the capillary using the conditioning method. • Replace the capillary. Refer to the section: Install the Capillary. 2. Verify the PA 800 Plus system performance by using the Performance Test Kit.
Broad peaks or migration time changes from run to run	1. The gel has dried on the electrodes. 2. The gel buffer or test mix is deteriorating. 3. The ionic strength of the buffer is too low.	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. 3. Use a higher ionic strength buffer such as 10x TBE in vial C1 instead of dsDNA 1000 Gel Buffer. To achieve similar migration times to those in data obtained using the dsDNA 1000 Gel Buffer in the C1 vial, the separation voltage might need to be lower.

Table 3 dsDNA Troubleshooting (continued)

Symptom	Possible Cause	Corrective Action
No peaks or low signal	1. The capillary is blocked. 2. The capillary window is not aligned with the detector.	1. Either: • Rinse the capillary with CE Grade Water for 10 min at 20 psi to remove the gel buffer and then condition the capillary using the conditioning method. • Replace the capillary. Refer to the section: Install the Capillary. 2. Make sure that the capillary window is positioned over the detector aperture.
Spikes in the electropherogram	The gel buffer has air bubbles.	Make sure that the reconstituted buffer is at room temperature. 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, and then sonicate for 5 seconds to remove the air bubbles.

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. The safety data sheets are available upon request or can be downloaded from our website, at sciex.com/tech-regulatory.

Hazard classification according to HCS 2012.

dsDNA 1000 Gel Buffer, dehydrated

DANGER! May damage fertility of the unborn child. May form combustible dust concentrations in air.

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

These components are not classified as hazardous:

- dsDNA 1000 Test Mix
- Orange G Reference Marker

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 Now Learning Hub

Purchase Supplies and Reagents

Reorder SCIEX supplies and reagents online at store.sciex.com. To set up an order, use the account number, found on the quote, order confirmation, or shipping documents. Currently, customers in the United States, United Kingdom, and Germany have access to the online store, but access will be extended to other countries in the future. For customers in other countries, contact a 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 documentation DVD for 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.