

# ssDNA 100-R Kit

For the PA 800 Plus Pharmaceutical Analysis System
Application Guide

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### ssDNA 100-R Kit

This document provides instructions for sample preparation using the ssDNA 100-R Kit. It also provides instructions for data acquisition and data analysis using the 32 Karat<sup>TM</sup> Software.

The ssDNA 100-R Kit contains the supplies necessary to perform rapid separation and analysis of oligonucleotides between 10 and 100 bases long. This kit features a replaceable gel buffer and a coated capillary for maximum reproducibility.

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 ssDNA 100-R 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 ssDNA 100-R Kit (PN 477480)

Component	Quantity	Reorder Part Number
DNA Capillary, 65 cm, 100 µm i.d.	2	477477
ssDNA 100-R Gel, lyophilized	1.0 g	477621

Table 1 ssDNA 100-R Kit (PN 477480) (continued)

Component	Quantity	Reorder Part Number
Tris-Borate buffer	1	(see following note)
7M Urea	1	(see following note)
pd(A) 40-60 Test Mix	0.2 O.D.	477626

Note: The Tris-Borate buffer and urea are available for reorder as a kit, use PN 338481.

**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

### **Storage Conditions**

- Store the DNA capillary and ssDNA 100-R Gel at 2 °C to 8 °C.
- Store the pd(A) 40-60 Test Mix at -35 °C to -15 °C.
- Store the unreconstituted Tris-borate buffer and urea bottles 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  $\mu$ m filter and with resistance above 18 M $\Omega$ )
- Magnetic stir plate and stir bar
- 10 mL disposable syringes
- Membrane syringe filters, 0.2 μm and 0.45 μm pores
- (Optional) LABQUAKE rotator (Barnstead International PN 400110)
- 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.

#### **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.

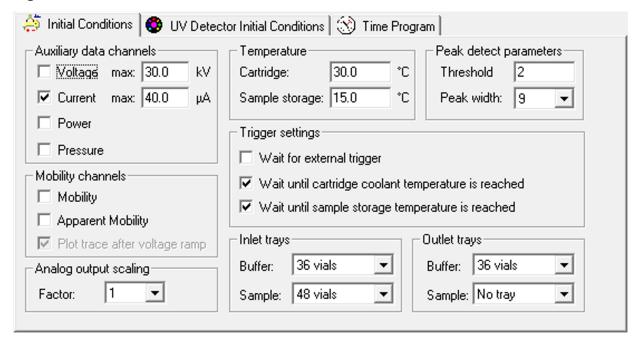
The ssDNA 100-R kit requires the following methods: a conditioning method, a gel filling method, a separation method, and a shutdown method. Refer to Initial Conditions, UV Detector Initial Conditions, and Time Programs to create the methods.

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 scientist 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.

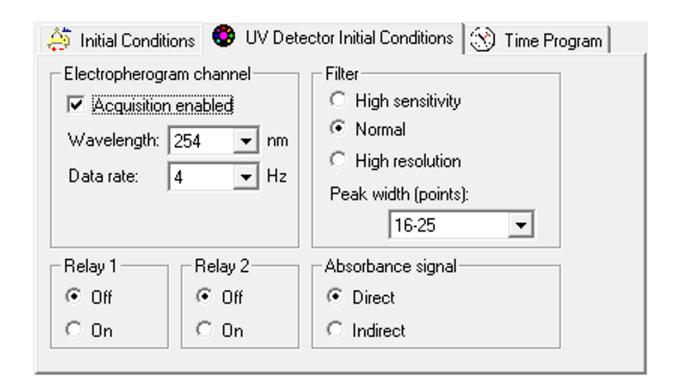
Figure 1 Initial Conditions Tab for ssDNA 100-R Kit Methods



#### **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.

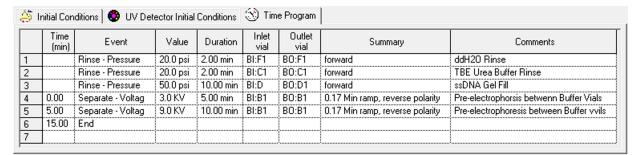
Figure 2 UV Detector Initial Conditions Tab for ssDNA 100-RKit Methods



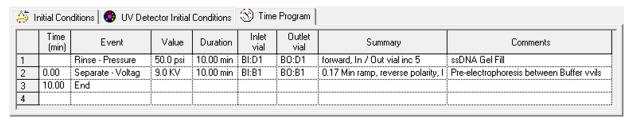
### **Time Programs**

**Note:** The time programs are different for each method.

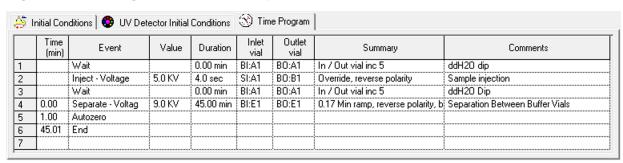
Figure 3 Time Program Tab for the Conditioning Method



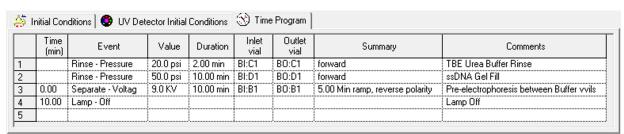
#### Figure 4 Time Program Tab for the Gel-Filling Method



#### Figure 5 Time Program Tab for the Separation Method



#### Figure 6 Time Program Tab for the Shutdown Method



## **Prepare the Reagents and Stock Solutions**

### **Prepare the Tris-Borate-Urea Buffer**

- Add 135 mL of DDI water to the bottle containing the dry Tris-Borate buffer.
- 2. Clean a large magnetic stir bar with methanol and then rinse it with DDI water.
- 3. Use the magnetic stir bar to stir the solution for 20 minutes to 30 minutes until the boric acid is completely dissolved.

Be sure that the boric acid is completely dissolved before proceeding to the next step.

4. Slowly add the dry urea from the 7 M urea bottle to the Tris-Borate buffer while continuing to stir the solution.

**Note:** Do not heat the buffer solution to speed up the process. This decreases the usable life of the buffer.

The dissolution of urea is endothermic, so the bottle can become very cold.

5. Stir the solution for approximately 2 hours at room temperature until the urea is completely dissolved and the buffer is clear.

**Note:** Some magnetic stirrers produce enough heat to degrade the urea. A small piece of corrugated cardboard can be used as an insulator between the buffer and the stirrer to minimize the heating.

6. Store the Tris-Borate-Urea buffer at 2 °C to 8 °C. The reconstituted Tris-Borate-Urea buffer can be used for up to 30 days after preparation.

#### **Prepare the Tris-Borate-Urea Buffer after Storage**

- 1. If the buffer solution was previously reconstituted and refrigerated, bring the entire container of buffer to ambient temperature before use while stirring slowly and continuously with a clean stirring bar.
- 2. Remove the required volume to be used for the day and filter through a 0.2 µm disposable syringe filter into a clean container.

#### Prepare the ssDNA 100-R Gel

- 1. Filter the Tris-Borate-Urea buffer.
  - a. If the buffer solution was previously reconstituted and refrigerated, let the entire container of buffer come to ambient temperature before use while stirring slowly and continuously with a clean stirring bar.
  - b. Remove the volume of Tris-Borate-Urea buffer necessary for the day, and then filter it through a 0.2 μm disposable syringe filter into a clean container large enough to hold the volume.
- 2. Add 5.0 mL of filtered Tris-Borate-Urea buffer to the ssDNA 100-R Gel vial.
- 3. Use a clean, dry magnetic bar that is slightly shorter than the diameter of ssDNA 100-R Gel vial to stir the solution for 4 hours to 6 hours until the gel is completely dissolved. Alternatively, put the gel solution on a rotator in a cold room (2 °C to 8 °C) for 72 hours.
- 4. After the gel is completely dissolved, filter the ssDNA 100-R Gel solution through a 0.45 μm disposable syringe filter.

## **Prepare the Samples**

#### **Prepare the Test Mix**

- 1. Add 500 µL of DDI water to the pd(A) 40-60 Test Mix vial and then mix well.
- 2. Pipette 100  $\mu$ L of the reconstituted pd(A) 40-60 Test Mix into a PCR micro vial. Alternatively, add 5 to 10  $\mu$ L of the sample to a nanoVial.
- 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 test mix to sealable vials.
- 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 oligonucleotide sample with DDI water or 0.2 um filtered Tris-EDTA buffer to a concentration between 5 OD/mL and 10 OD/mL.
- 2. Transfer 100  $\mu$ L of the sample to a PCR micro vial for analysis. If the available sample volume is low, transfer 5 to 10  $\mu$ L 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 UV Detector

- 1. Turn off the PA 800 Plus System and then install the UV detector. Refer to the *System Maintenance Guide*.
- 2. Turn on the system 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 20 cm to the window and 30.2 cm total length. Use a 100  $\mu$ m × 200  $\mu$ m aperture.

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**

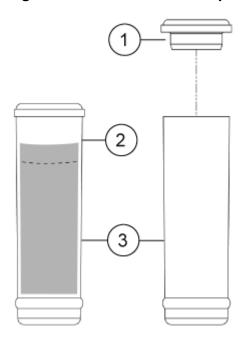
- 1. Remove the cartridge from the vials.
- 2. Install the cartridge in the PA 800 Plus System.

### **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 8.

Figure 7 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 five runs.

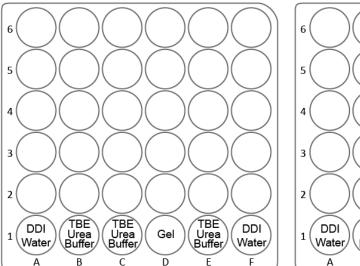
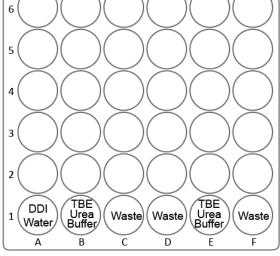


Figure 8 Buffer Tray Inlet (BI), Left and Buffer Tray Outlet (BO), Right



- DDI water: 1.5 mL DDI water.
- TBE Urea Buffer: 1.5 mL Tris-Borate-Urea buffer
- Gel:
  - For five runs or fewer: 200 μL ssDNA 100-R Gel.
  - For six or more consecutive runs: 1.5 mL ssDNA 100-R Gel.

**Note:** The ssDNA 100-R Gel in the capillary should be replaced every five runs using the gel filling method.

Waste: 1 mL DDI water

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

**Note:** The ssDNA 100-R Gel is stable for 24 hours at room temperature. If a sequence lasts longer than 24 hours, replace the ssDNA 100-R Gel.

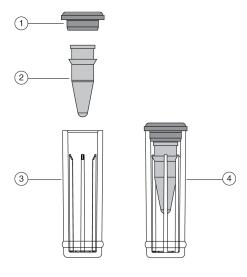
**Note:** On-board stability for a vial filled with gel buffer is 24 hours.

**Note:** If using 200  $\mu$ L of gel buffer, do not leave the gel buffer in the buffer tray for more than 5 hours. This can result in an increased migration time due to an increase in the viscosity of the gel buffer.

### **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 9.
  - For samples in nanoVials, cover the vial with a cap.

#### Figure 9 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.

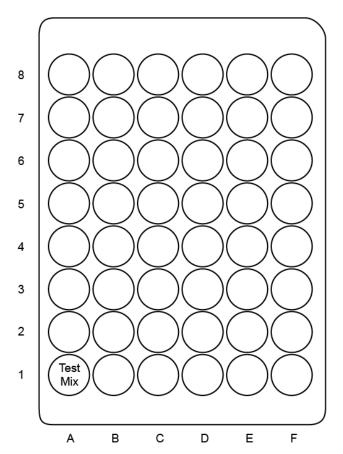


Figure 10 Sample Tray Inlet (SI)

## **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<sup>™</sup> Software. For detailed instructions, refer to the *PA 800 Plus Pharmaceutical Analysis System Methods Development Guide*.

- Open the 32 Karat<sup>™</sup> Software.
- 2. In the 32 Karat<sup>™</sup> 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 sample, create a sequence with four rows:
  - Row 1 Conditioning method
  - Row 2 Gel Filling method to be repeated after every five separations
  - Row 3 Separation method
  - Row 4 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



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 ssDNA 100-R Gel.
- 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 pd(A) 40-60 Test Mix should give a baseline separation of all 21 oligonucleotides in 45 minutes when using a 30.2 cm capillary at a field strength of 300 V/cm. Refer to Figure 11. Peak intensity can vary as a result of batch-to-batch variation. Refer to the electropherogram provided with the test mix to make sure that the results are correct. The electrical current should remain fairly stable between 6  $\mu$ A and 8  $\mu$ A.

**Note:** The peak that occurs in Figure 11 at the 12.5-minute interval is a system peak.

The high resolving power of this kit can not only achieve single-base resolution of each oligonucleotide, but it can also show the appearance of shoulders on the major peaks which might be the de-phosphorylated form of each oligonucleotide or other contamination. However, the intensity of these shoulders can vary due to the lot-to-lot variation in test mix preparation.

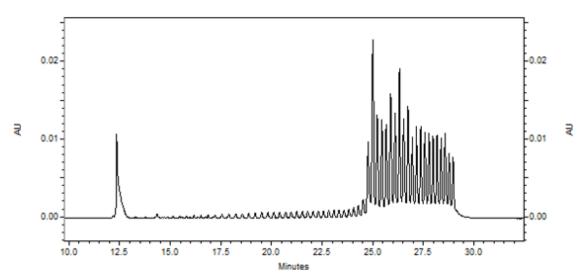


Figure 11 Example Electropherogram for Test Mix

### **Tips for Best Results**

- If resolution decreases over time, then replace the ssDNA 100-R Gel using the gel filling method. Do a test run to make sure that the resolution has improved.
- If baseline separation is not achieved, use the same field strength (V/cm) and increase the capillary length as needed.
- 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 for DNA Application Guides**

#### **Table 3 ssDNA Troubleshooting**

Symptom	Possible Cause	Corrective Action
Reduced resolution	The buffer or test mix is deteriorating.	Change to a new buffer or test mix.
	<ul><li>2. The gel is deteriorating inside the capillary.</li><li>3. The capillary coating is bad.</li></ul>	2. Rinse the capillary with DDI water for 10 minutes at 20 psi to remove the gel and then condition the capillary using the conditioning method.
		3. Replace the capillary. Refer to Install the Capillary.
Low or unsteady current	1. The buffer is deteriorating.	Replace the buffer.
	2. The capillary is blocked.	2. Either:
	<ul><li>3. Air bubbles are in the gel.</li><li>4. The capillary window or tip is broken.</li></ul>	<ul> <li>Rinse the capillary with DDI water for 10 minutes at 20 psi to remove the gel and then condition the capillary using the conditioning method.</li> </ul>
		<ul> <li>Replace the capillary.</li> <li>Refer to Install the Capillary.</li> </ul>
		<ol> <li>Rinse the capillary with DDI water for 10 minutes at 20 psi to remove the gel and then condition the capillary using the conditioning method.</li> </ol>
		Replace the capillary. Refer to Install the Capillary.

**Table 3 ssDNA Troubleshooting (continued)** 

Symptom	Possible Cause	Corrective Action
Inconsistent migration time from run to run	<ol> <li>The capillary is not equilibrated.</li> <li>The gel in the universal vials is deteriorating.</li> <li>The electrodes are contaminated.</li> </ol>	<ol> <li>Rinse the capillary with DDI water for 10 minutes at 20 psi to remove the gel and then condition the capillary using the conditioning method.</li> <li>Replace the gel vials.</li> <li>Clean the electrodes and then the capillary ends.</li> </ol>
No peaks or low UV absorption	<ol> <li>The capillary is blocked.</li> <li>The capillary window or tip is broken.</li> <li>The capillary is not equilibrated.</li> </ol>	<ol> <li>Either:         <ul> <li>Rinse the capillary with DDI water for 10 minutes at 20 psi to remove the gel and then condition the capillary using the conditioning method.</li> <li>Replace the capillary. Refer to Install the Capillary.</li> </ul> </li> <li>Replace the capillary. Refer to Install the Capillary.</li> <li>Rinse the capillary with DDI water for 10 minutes at 20 psi to remove the gel and then condition the capillary using the conditioning method.</li> </ol>

#### **Table 3 ssDNA Troubleshooting (continued)**

Symptom	Possible Cause Corrective Action	
Spikes in the electropherogram	<ol> <li>Air bubbles are in the gel.</li> <li>The gel or buffer have been contaminated or contain microparticles.</li> </ol>	Make sure that the gel is at room temperature and that the air bubbles are removed.
	·	2. Replace the used vials with clean vials. Replace the gel and buffer with freshly filtered solutions.
Unstable or shifting baseline	The capillary is not equilibrated.	Rinse the capillary with DDI water for 10 minutes at
	The gel in the universal vials is deteriorating.	20 psi to remove the gel and then condition the capillary using the
	3. The Tris-Borate-Urea buffer	conditioning method.
	has degraded.	2. Replace the gel vials.
		3. Replace the buffer.

## **Hazardous Substance Information**



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.

#### **Tris-Borate Buffer**



DANGER! May be harmful if swallowed. May be harmful in contact with skin. Causes skin irritation. Causes serious eye irritation. May cause respiratory irritation. May damage fertility or the unborn child.

#### **Other Reagents**

These components are not classified as hazardous:

- 7M Urea
- ssDNA 100-R Gel
- pd(A) 40-60 Test Mix

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<sup>™</sup>

#### **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.

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