

CZE Rapid Charge Variant Analysis Kit

For the PA 800 Plus Pharmaceutical Analysis System
Application Guide

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CZE Rapid Charge Variant Analysis Kit

The SCIEX CZE Rapid Charge Variant Analysis Kit provides reagents necessary to analyze the charge variants of a protein based on their mobility using the PA 800 Plus Pharmaceutical Analysis System. This method is a fast and simple separation that can be used to quantify charge variants. It utilizes a buffer that serves as both a separation matrix and a dynamic coating for the bare fused silica capillary.

This document provides instructions for sample preparation using the CZE Rapid Charge Variant Analysis Kit. It also provides instructions for data acquisition and data analysis using the PA 800 Plus Software.

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 CZE Rapid Charge Variant Analysis 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.

CZE Rapid Charge Variant Analysis Kit

Table 1 CZE Rapid Charge Variant Analysis Kit (PN C44790)

Component	Quantity	Reorder Part Number
Acid Wash/Regenerating solution (0.1 M HCl)	125 mL	C44790
CZE Rapid Charge Variant Separation buffer	140 mL	N/A
CE-Grade Water	140 mL	C48034
Protein Test Mix	3 mg	477436

Table 2 Additional Supplies from SCIEX

Component	Quantity	Part Number
PCR micro vials (200 µL)	100	144709
Universal vial caps, blue	100	A62250
Universal vials	100	A62251

Table 3 Additional Required Reagents or Supplies

Description	Vendor	Part Number
National Institute of Standards and Technology (NIST) Humanized IgG1κ Monoclonal Antibody	NIST	8671
(Optional) Amicon Ultra-4 Centrifugal Filter Unit with Ultracel-10 membrane	Millipore	UFC801024

Storage Conditions

- Upon receipt, store the CZE Rapid Charge Variant Analysis kit at 2 °C to 8 °C.
- Upon receipt, store the NIST IgG sample at –35 °C to –15 °C. After thawing the NIST IgG sample for the first time, remove the amount needed and then store the rest in aliquots at –35 °C to –15 °C.

Customer-Supplied Equipment and Supplies

- Powder-free gloves, neoprene or nitrile recommended
- Safety glasses
- Laboratory coat
- Microcentrifuge, or equivalent, and microcentrifuge tubes
- Vortex mixer
- Pipettes and appropriate tips.
- Analytical balance

Required Detector

A UV detector with a 214 nm filter is required.

Required Cartridge or Capillary

One of the following:

- Pre-assembled cartridge (PN A55625)
- Capillary cartridge (PN 144738) and capillaries, bare-fused silica, 50 µm i.d. (PN 338451)

Methods and Sequences

Download the methods and the sequence from sciex.com. The methods and sequence can also be created manually using the 32 Karat™ Software. Refer to [Methods](#).

Save the methods to the PA 800 Plus controller: C:\32Karat\projects\CZE\Method.

Save the sequence to: C:\32Karat\projects\CZE\Sequence.

As of publication, the sequence and the following methods are available on the SCIEX website:

- **CZE Analysis.seq:** The sequence.
- **CZE Rapid Charge Variant Analysis.met:** Performs a separation of the sample.
- **CZE Capillary Conditioning.met:** Conditions the capillary at the start of each day.
- **CZE Shutdown.met:** Cleans the capillary at the end of a sequence and turns off the UV lamp.

Prepare the Samples

Prepare the IgG Test Sample

1. Prepare the IgG test sample.
 - For the initial run, allow the vial containing the NIST IgG sample to thaw at room temperature and then aliquot the solution in 10 μL portions. Reserve one aliquot and then store the remaining aliquots at $-35\text{ }^{\circ}\text{C}$ to $-15\text{ }^{\circ}\text{C}$ for up to five years.
 - For subsequent runs, thaw one of the 10 μL aliquots at room temperature.
2. Add 90 μL of CE-grade water to the vial containing the stock solution.
The final concentration is 1 mg/mL.
3. Vortex the vial for 10 seconds at the maximum speed to mix.
4. In a microcentrifuge, centrifuge the vial for 5 seconds at the maximum speed to bring the sample to the bottom of the vial.
5. Carefully transfer 95 μL to a micro vial.
Make sure that there is not an air bubble at the bottom of the vial. If a bubble is present, repeat step 4.

Prepare the Protein Test Mix Sample

The Protein Test Mix can be used for troubleshooting. Perform a few runs with the test mix or include it as a control in the same run with the regular samples. If the results for the Protein Test Mix are correct, then the separation is working and there are issues with the other samples.

1. Add 2 mL of CE-grade water to the vial containing the Protein Test Mix and then vortex the vial for 1 minute to mix.
The solution will contain lysozyme, cytochrome C, and ribonuclease A at 0.5 mg/mL for each analyte.
2. To limit repeated freezing and thawing, aliquot portions of the solution into 0.5 mL microfuge vials. Choose a volume appropriate for the experimental design.
When not in use, store at $-35\text{ }^{\circ}\text{C}$ to $-15\text{ }^{\circ}\text{C}$ for up to two years.

Note: The protein test mix is used to confirm the performance of the CZE separation buffer. It is not indicative of a molecules performance.

Prepare the Protein Sample

Similar to the IgG test sample, a final protein concentration of 1 mg/mL is recommended.

1. Add the sample to a vial and then dilute it with the CE-Grade Water.
The final concentration is 1 mg/mL.
2. Vortex the vial for 10 second at the maximum speed to sufficiently mix.
3. In a microcentrifuge, centrifuge the vial for 5 seconds at the maximum speed to bring the sample to the bottom of the vial.

Note: The CZE Separation Buffer is designed for a molecule with a pI of 7 or greater.

If no peaks appear in the electropherogram, dilute the sample to a final concentration of 0.5 mg/mL and then run the sample again.

Perform a Buffer Exchange for the Protein Sample

The signal intensity and resolution of this assay is sensitive to the salt concentration in the sample. If the salt concentration is too high, low signal and peak tailing occurs. Perform a buffer exchange with a Microcon-10 kDa centrifugal filter unit by doing the following:

1. Add 1 mL of sample to a Microcon-10 kDa centrifugal filter unit.
2. Centrifuge for 15 minutes at 4,000 *g*.
3. Add 2 mL of 20 mM Tris, pH 7.8 to a filter unit and then centrifuge for 25 minutes at 4,000 *g*.
4. Repeat step 3 twice.
5. Install the Microcon-10 kDa centrifugal filter unit to a vial and then centrifuge for 3 minutes at 1,000 *g*.

The sample solution is collected in the vial.

6. Transfer the collected protein solution to an appropriate sterile tube.
7. Add SDS-MW Sample Buffer to the final 1 mL volume.

The final concentration of the protein sample must be 1 mg/mL.

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 buffer to crystallize on the electrodes, opening levers, capillary ends, and interface block. Salt crystals 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 separation buffer can evaporate resulting in salt deposits in the system unless regular and thorough cleaning is performed.

Install the Cartridge

1. Remove the cartridge from the box and, if necessary, install the capillary.
2. Install the cartridge in the PA 800 Plus System.

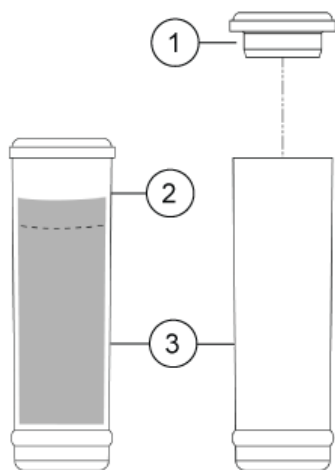
Load the Buffer Trays

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 1](#). For each set of eight samples, prepare:
 - 5 universal vials, each containing 1.5 mL CZE Rapid Charge Variant Separation buffer
 - 2 universal vials, containing 1.5 mL CE-grade water
 - 1 universal vial, containing 1.5 mL Acidic Wash solution

- 2 universal vials, containing 1 mL CE-grade water, for the Waste positions in the outlet buffer tray

Figure 1 Universal Vial and Cap Setup

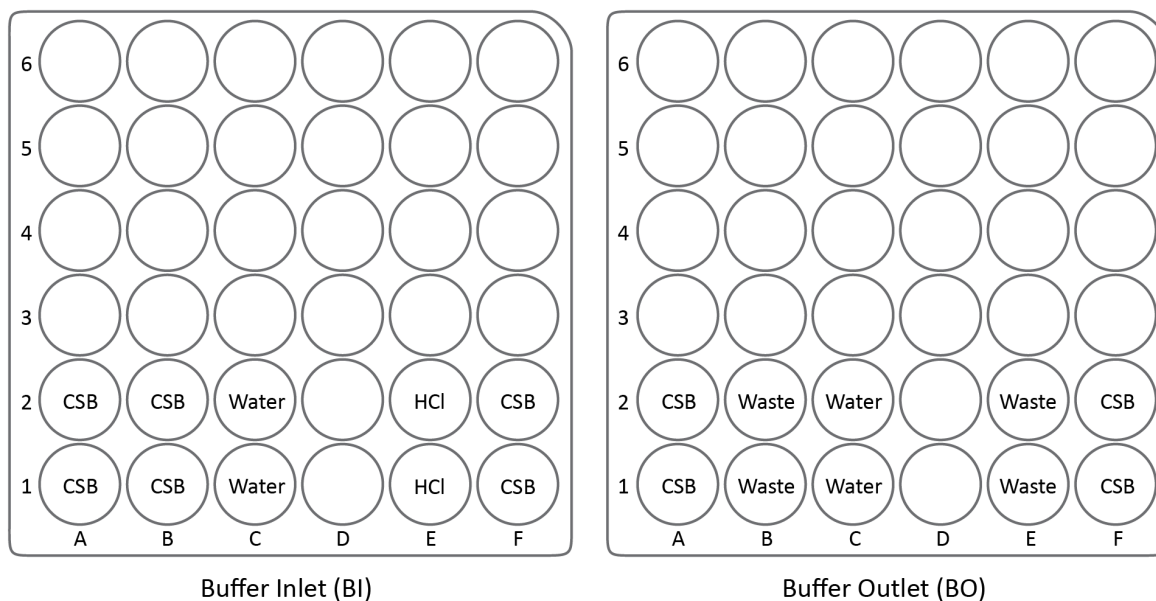


Item	Description
1	Universal vial cap
2	Maximum fill line
3	Universal vial

- Put the vials in the buffer trays as shown in the following figure. Refer to [Figure 2](#). Each row is sufficient for at least eight runs.

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.

Figure 2 Buffer Tray Layout for 16 Runs



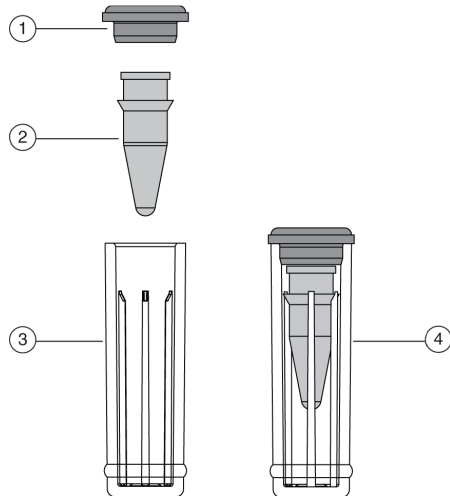
- CSB: CZE Separation buffer
- Water: CE-grade water
- HCL: 1.5 mL Acid Wash solution
- Waste: 1 mL CE-grade water

Note: During electrophoresis the ionic strength of the buffer will change. The separation method is programed to increment the buffer vials after eight runs to avoid ionic depletion.

Load the Sample Tray

1. Prepare the samples. For each sample:
 - a. If necessary, thaw the sample at room temperature for 5 minutes to 10 minutes.
 - b. Transfer at least 50 μ L of the sample to a micro vial.
 - c. Put the micro vial containing the sample in a universal vial.
 - d. Put a blue cap on the universal vial.

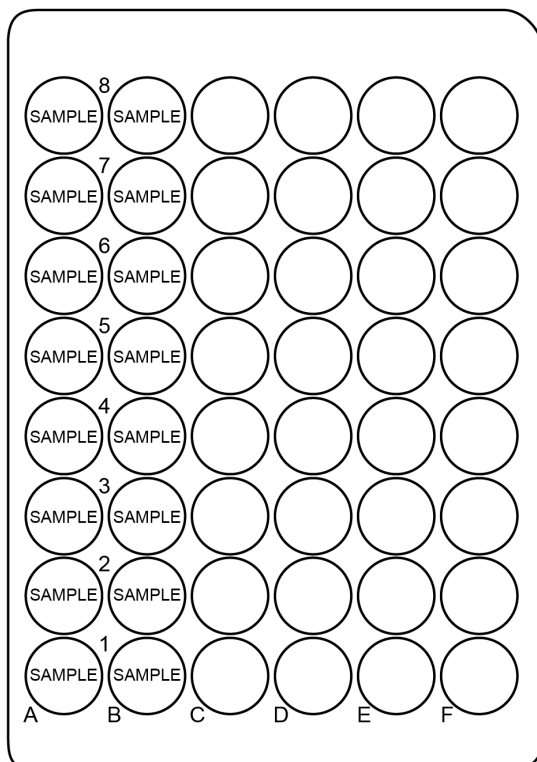
Figure 3 Sample Vial Setup



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

- Put the universal vial in the sample tray as shown in [Figure 4](#).

Figure 4 Sample Tray Layout



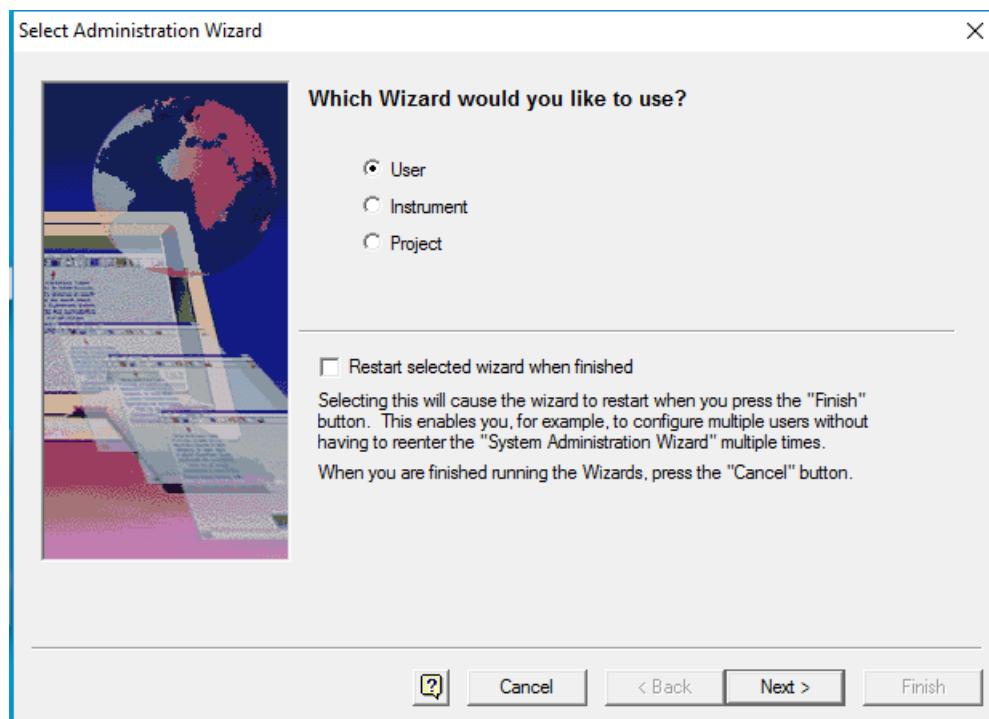
Run the Samples

Create the Example Project

This procedure requires the user name and password for a user with administrative privileges for the 32 Karat™ Software.


1. Double-click the 32 Karat™ Software icon on the desktop.
2. Click **Tools > Enterprise Login**, type the user name and password, and then click **Login**.
3. Click **Tools > System Administration Wizard**.

Figure 5 Select Administration Wizard Window



4. Click **Project** and then click **Next**.
5. Follow the instructions in the wizard to create the CZE project.
Refer to the 32 Karat™ Software help or the *System Administration Guide* for detailed instructions.
6. If necessary, download the method and sequence files from the SCIEX website. Refer to [Methods and Sequences](#).
7. Copy the methods to the methods folder for the project. By default, this is C:\32Karat\projects\CZE\Method.
8. Copy the sequence to the sequence folder for the project. By default, this is C:\32Karat\projects\CZE\Sequence.

Create the Sequence and Start the Run

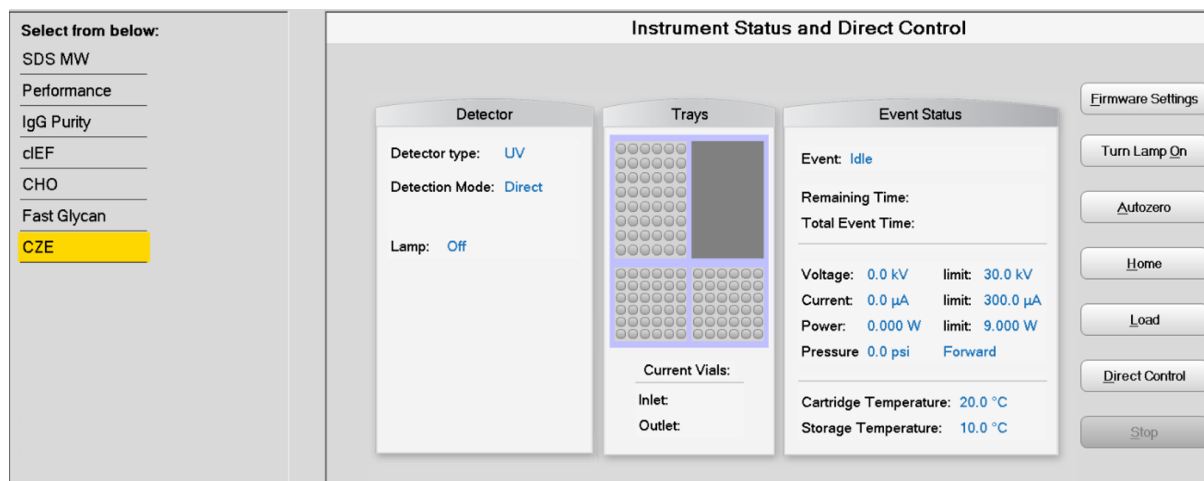
1. Double-click the PA 800 Plus Software icon on the desktop to open the PA 800 Plus Software.
2. In the **PA 800 plus** window, click  (**Run**) in the upper right corner of the window.


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3. Click the **CZE** instrument, and then click **CZE Analysis**. If system administration is enabled, type a user name and password when prompted.


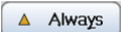
The Instrument Status and Direct Control page opens.

Figure 6 Instrument Status and Direct Control Page



4. Click  (**Describe**).
5. In the **Application** list, click **CZE**. In the **Sequence** list, click **Browse** and then browse to the **CZE Analysis** sequence. If prompted, type a user name and password.

The page updates to show the selected sequence and all rows in the sequence are designated as samples.

6. Set the type for the first and last rows in the sequence. The first row is for capillary conditioning and the last row is for system shutdown.
 - a. Click the first row (with the CZE Conditioning method) to select it and then click  (**Always**) in the **Rows** area.
 - b. Click the last row (with the CZE Shutdown method) to select it and then click  (**Always**) in the **Rows** area.

The icon in the **Type** column first and last rows in the sequence is now a triangle.

Figure 7 Describe sequence rows and columns Page with Conditioning Method Set to "Always"

Describe sequence rows and columns

Application:

Sequence:

Rows: Sample Control Always

Columns: Optional Required Fixed

Verification: Samples

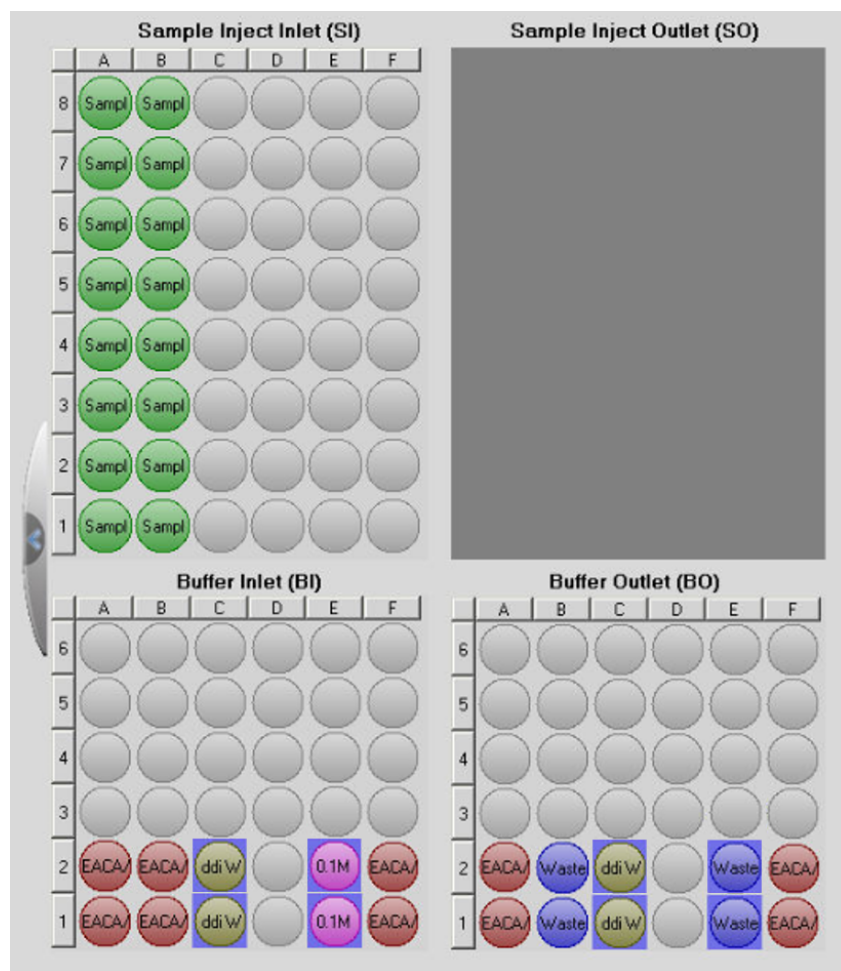
Run#	Type	Run Type	Reps	Inject Inlet	Sample ID	Method	Data File
1	▲	Unknown	1	None		C:\32Karat\project...	Capillary con...
2	●	Unknown	1	SI:A1	NIST IqG	C:\32Karat\project...	NIST_1.dat
3	●	Unknown	1	SI:A2	NIST IqG	C:\32Karat\project...	NIST_2.dat
4	●	Unknown	1	SI:A3	NIST IqG	C:\32Karat\project...	NIST_3.dat
5	●	Unknown	1	SI:A4	NIST IqG	C:\32Karat\project...	NIST_4.dat
6	●	Unknown	1	SI:A5	NIST IqG	C:\32Karat\project...	NIST_5.dat
7	●	Unknown	1	SI:A6	NIST IqG	C:\32Karat\project...	NIST_6.dat
8	●	Unknown	1	SI:A7	NIST IqG	C:\32Karat\project...	NIST_7.dat
9	●	Unknown	1	SI:A8	NIST IqG	C:\32Karat\project...	NIST_8.dat
10	●	Unknown	1	SI:B1	PTM	C:\32Karat\project...	PTM_1.dat
11	●	Unknown	1	SI:B2	PTM	C:\32Karat\project...	PTM_2.dat
12	●	Unknown	1	SI:B3	PTM	C:\32Karat\project...	PTM_3.dat
13	●	Unknown	1	SI:B4	PTM	C:\32Karat\project...	PTM_4.dat
14	●	Unknown	1	SI:B5	PTM	C:\32Karat\project...	PTM_5.dat
15	●	Unknown	1	SI:B6	PTM	C:\32Karat\project...	PTM_6.dat
16	●	Unknown	1	SI:B7	PTM	C:\32Karat\project...	PTM_7.dat
17	●	Unknown	1	SI:B8	PTM	C:\32Karat\project...	PTM_8.dat
18	▲	Unknown	1	None		C:\32Karat\project...	Shutdown.dat

- (Optional) For rows containing system suitability samples, click the row to select it and then click Control (**Control**).
- In the lower right corner of the window, click (**Save**) and then click (**Finish**).
- In the **Number of samples** field, click the arrow buttons to set the number of samples for the run.

Number of samples:

As the number of samples changes, the images of the buffer and sample trays on the right are updated to show the correct number of vials and their locations for the run. For example, in [Figure 2](#), one row of reagents is required for eight samples. Two rows of reagents are required for 16 samples.

Figure 8 Tray Map for CZE Separation





10. If the buffer and sample trays have not been loaded, click  (**Load**), load the buffer and sample trays in the PA 800 Plus System, and then close the door.
11. Click  (**Next**) and then click **Yes - run now**.

Figure 9 Samples Loaded Prompt

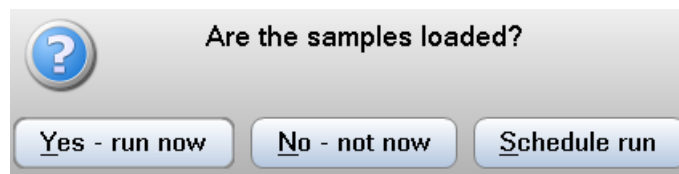
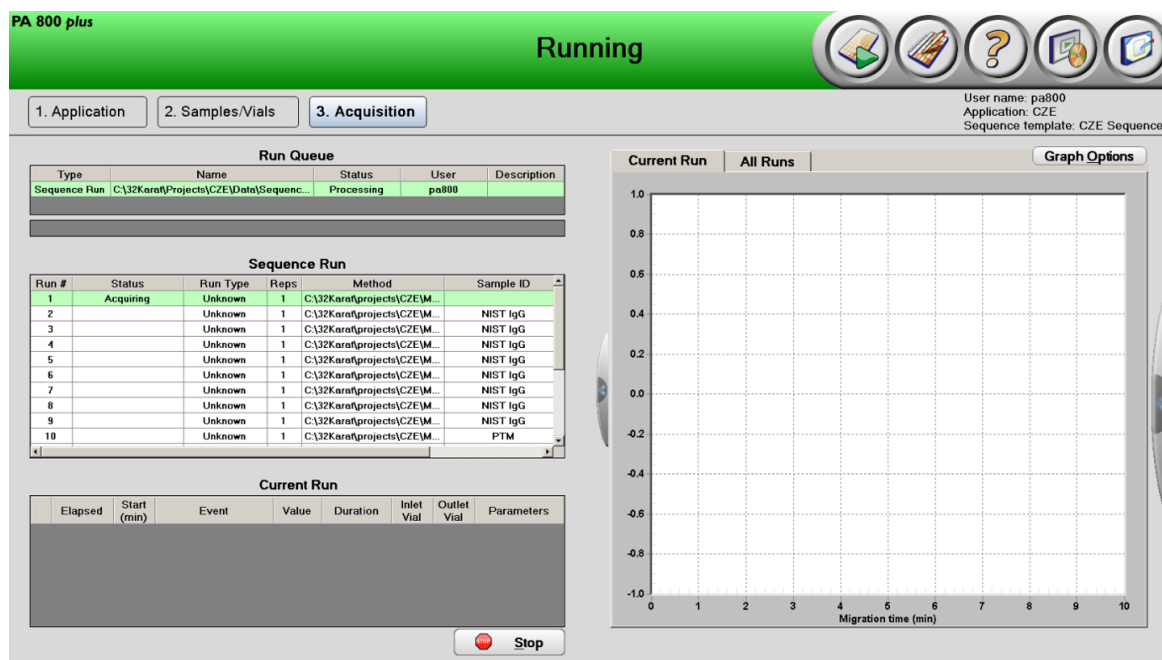


Figure 10 PA 800 Software During Data Acquisition



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

1. Perform the shutdown method to clean the capillary.
2. Rinse the capillary with 0.1M HCl for 5 min at 50 psi.
The capillary will be filled with 0.1M HCl.
3. Remove the cartridge from the system.

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4. Store the cartridge upright in the refrigerator between 2 °C and 8 °C, with the capillary ends immersed in vials of CE-Grade Water.

Prepare the Cartridge After Storage

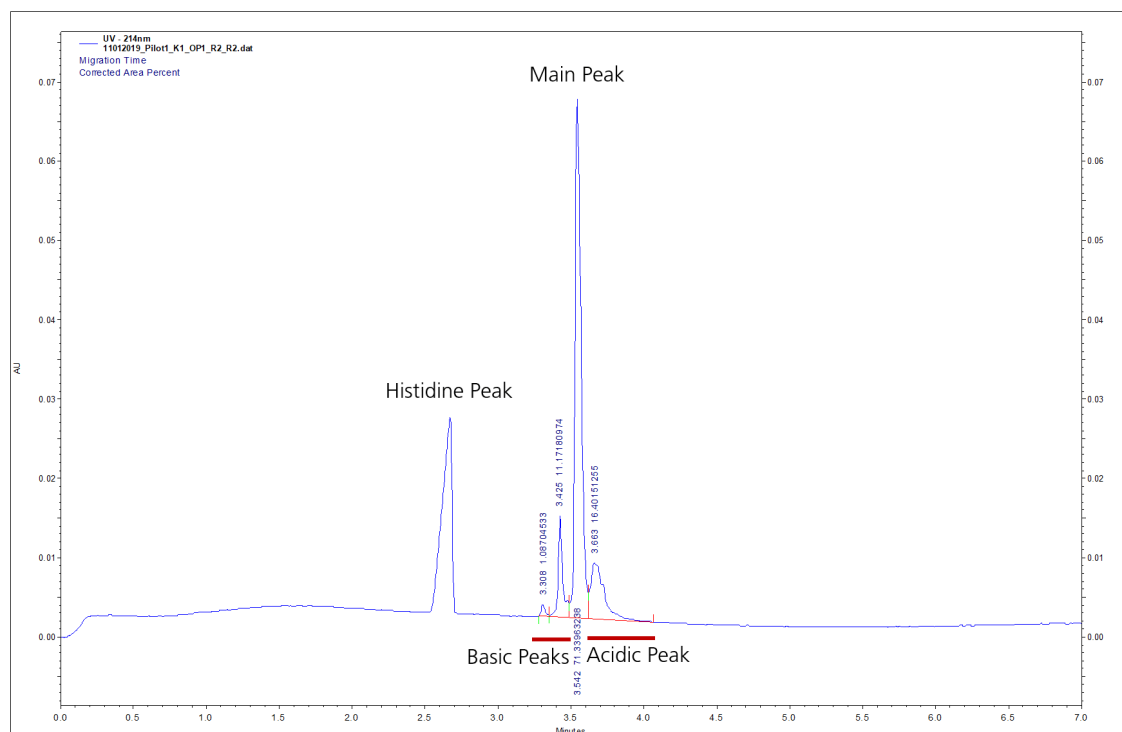
- If the cartridge has not been used for more than a day or it has been stored for an extended time, then condition the capillary using the CZE conditioning method.

Analyze the Data

Analyze the Data for the NIST IgG Samples

1. In the 32 Karat™ Software, open the sequence from the run.
2. Open the data file for the first run.
3. Click **File > Open > Method**, select **CZE Rapid Charge Variant Data Analysis** and then click **OK**.
4. Adjust the integration events until all the peaks in the test sample are integrated correctly. Refer to the "Integration" chapter in the *Methods Development Guide*.

Figure 11 Example Electropherogram for the NIST IgG Sample

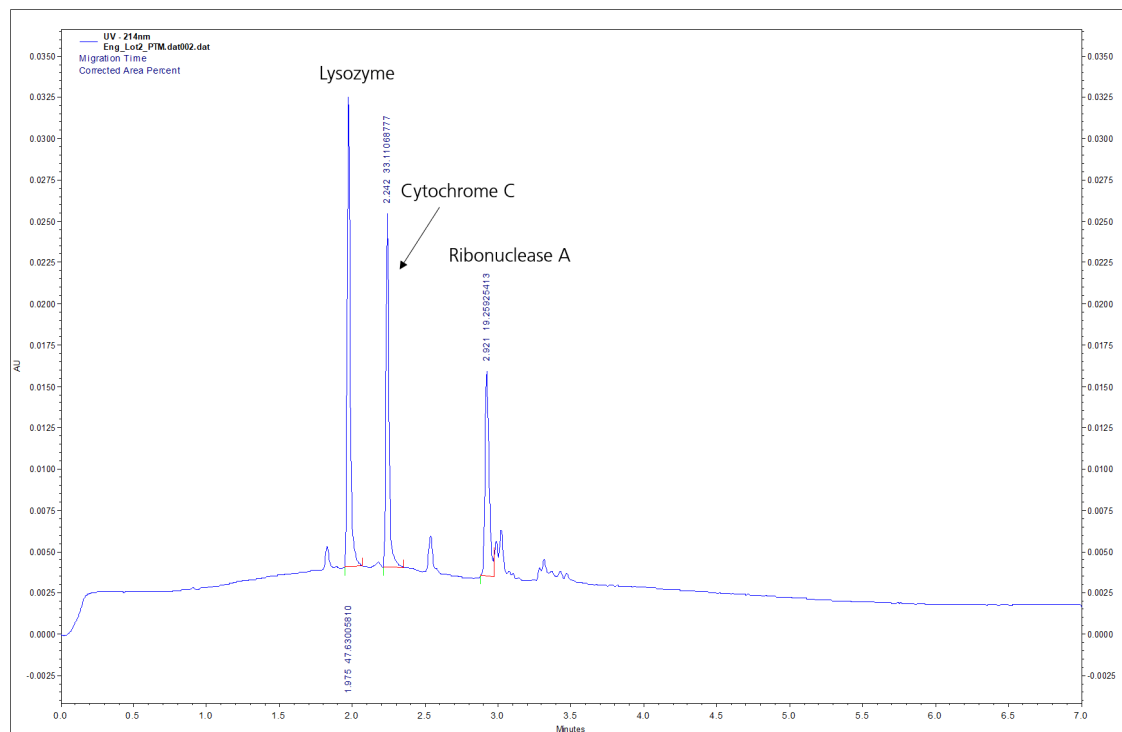


5. Click **File > Save** to save the method and then apply it to the rows in the sequence that contain the samples.

Analyze the Data for the Protein Test Mix Sample

1. In the 32 Karat™ Software, open the sequence from the run.
2. Open the data file for the first run.
3. Click **File > Open > Method**, select **CZE Rapid Charge Variant Data Analysis** and then click **OK**.
4. Adjust the integration events until all the peaks in the test sample are integrated correctly. Refer to the "Integration" chapter in the *Methods Development Guide*.

Figure 12 Example Electropherogram for the Protein Test Mix Sample



5. Click **File > Save** to save the method and then apply it to the rows in the sequence that contain the Protein Test Mix samples.

Troubleshooting

Symptom	Possible Cause	Corrective Action
Broad peaks, poor resolution	<ol style="list-style-type: none"> The protein has a pI lower than 7. The sample concentration is too high. The salt concentration in the sample is too high. The end cuts on the capillary are not even. The end of the capillary tip is dirty or plugged. The protein is adsorbed to the capillary wall. The capillary was cleaned with a basic solution such as 0.1 N NaOH. 	<ol style="list-style-type: none"> The protein is not compatible with this separation technique. Further dilute the sample to a concentration of 1.0 mg/mL to decrease sample loading. Perform a buffer exchange to remove salt from the sample. Refer to Perform a Buffer Exchange for the Protein Sample. Inspect the capillary ends under magnification. If a cut is jagged, then cut the end again or replace the capillary. Clean the capillary. Refer to Clean the Capillary. Do the following: <ol style="list-style-type: none"> In the separation method, decrease the interval between vial increments below eight runs. This changes the separation buffer vials more frequently. In the shutdown method, replace the final capillary water rinse step with rinse with acidic solution, such as 0.1 M HCl or 10 mM phosphoric acid. The capillary can be filled with the acidic solution before next use. Store the capillary with the ends in CE-Grade Water. Replace the capillary. <p>Note: Do not use a basic solution to clean the capillary as it negatively ionizes the capillary wall causing interactions with the protein.</p>

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Symptom	Possible Cause	Corrective Action
Carryover	<ol style="list-style-type: none"> 1. The sample concentration is too high. 2. The vials or caps are contaminated. 	<ol style="list-style-type: none"> 1. Make sure that the sample concentration is between 0.5 mg/mL and 1.0 mg/mL. If necessary, dilute the sample with water and then run the sample again. 2. Replace the vials and caps or modify the method: <ul style="list-style-type: none"> • Fill clean vials with freshly prepared reagents, cover the vials with clean caps, and then replace the vials in the tray. • Make sure that the waste vials contain 1.0 mL of water and are present in the outlet buffer tray. • Add one or more water dip steps to the time program after the sample injection step.
High current	The buffer trays are not set up correctly.	Make sure that the vials in the buffer tray contain the correct reagents and are in the correct location. Refer to Load the Buffer Trays .
Low signal	<ol style="list-style-type: none"> 1. The end of the capillary tip is dirty or plugged. 2. The sample volume is too low. 3. The salt concentration in the sample is too high. 	<ol style="list-style-type: none"> 1. Clean the capillary. Refer to Clean the Capillary. 2. Make sure that there is a minimum of 50 μL of sample in the sample vial. 3. Perform a buffer exchange to remove salt from the sample. Refer to Perform a Buffer Exchange for the Protein Sample.
Low intensity peaks	The sample concentration is too low.	<p>Make sure that the final sample concentration is at least 0.5 mg/mL. If the sample concentration is significantly lower, then concentrate the sample using a spin filter with a 10 kDa molecular weight cutoff (MWCO).</p> <p>Alternatively, increase the duration of the sample injection in the separation method.</p>

Symptom	Possible Cause	Corrective Action
Low current	<ol style="list-style-type: none"> 1. The capillary temperature is not correct. 2. The end of the capillary tip is dirty or plugged. 3. The capillary window or end is broken. 4. The reagents are contaminated. 	<ol style="list-style-type: none"> 1. Open the separation method in the software, and then make sure that the capillary temperature is correct. 2. Clean the capillary. Refer to Clean the Capillary. 3. Inspect the capillary window and tip. If either is broken, then replace the cartridge. 4. Fill clean vials with freshly prepared reagents, cover the vials with clean caps, and then replace the vials in the tray.
No peaks	<ol style="list-style-type: none"> 1. The separation method is incorrect. 2. The sample is not in the correct position in the sample tray. 3. There are air bubbles at the bottom of the sample vial. 4. The capillary window or end is broken. 5. The sample interacted with the capillary wall. 	<ol style="list-style-type: none"> 1. Open the separation method in the 32 Karat™ Software and then make sure that: <ul style="list-style-type: none"> • The voltage is correct. • The Normal polarity check box is selected. During the run, observe the amber LED on the PA 800 Plus System. It should be lit when the normal polarity voltage is applied. 2. Make sure that the samples are in the correct locations in the sample tray. Refer to Load the Sample Tray. 3. Centrifuge the sample tube to make sure that there are no bubbles at the bottom. 4. Inspect the capillary window and tip. If either is broken, then replace the cartridge. 5. Inspect the separation current. If it is stable, then the sample has adsorbed to the capillary wall. Replace the capillary.
Slower migration time with or without concurrent low current	The end of the capillary tip is dirty or plugged.	Clean the capillary. Refer to Clean the Capillary .
Unsteady current resulting in slow migration	There are air bubbles in the separation buffer.	Sonicate the buffer from 10 s to 20 s to remove air bubbles.

Clean the Capillary

Use the following procedure to clean a capillary that is plugged or dirty.

1. Rinse for 2 minutes at 75 psi with CE-Grade Water.
2. Rinse for 2 minutes at 75 psi with Acid Wash solution (0.1 M HCl).
3. Rinse for 2 minutes at 75 psi with CE-Grade Water.
4. If there are still problems with the capillary, trim 1 mm from the outlet end of the capillary and then rinse with CE-Grade Water for 5 minutes at 75 psi.

If the issues are still not resolved, then replace the capillary.

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.

Acid Wash/Regenerating Solution (0.1 M HCl)



DANGER! Causes severe skin burns and eye damage.

CZE Rapid Charge Variant Separation Buffer



WARNING! Causes mild skin irritation. May cause an allergic skin irritation. Harmful to aquatic life.

Protein Test Mix



DANGER! May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Other Reagents

These components are not classified as hazardous:

- CE-Grade Water

For reagents from other vendors, read the *Safety Data Sheet* from the vendor before use.

The CZE Rapid Charge Variant Analysis application requires three methods.

Capillary Conditioning Method

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

Figure B-1 Initial Conditions Tab

The screenshot shows the 'Initial Conditions' tab of a software interface. It contains several sections for configuring experimental parameters:

- Auxiliary data channels:** Includes checkboxes for Voltage (unchecked), Current (checked), Power (unchecked), and Pressure (unchecked). Voltage is set to 30.0 kV and Current to 300.0 μ A.
- Mobility channels:** Includes checkboxes for Mobility (unchecked), Apparent Mobility (unchecked), and Plot trace after voltage ramp (checked).
- Analog output scaling:** Factor is set to 1.
- Temperature:** Cartridge is set to 25.0 $^{\circ}$ C and Sample storage is set to 20.0 $^{\circ}$ C.
- Peak detect parameters:** Threshold is set to 2 and Peak width is set to 9.
- Trigger settings:** Includes checkboxes for Wait for external trigger (unchecked), Wait until cartridge coolant temperature is reached (checked), and Wait until sample storage temperature is reached (unchecked).
- Inlet trays:** Buffer is set to 36 vials and Sample is set to 48 vials.
- Outlet trays:** Buffer is set to 36 vials and Sample is set to No tray.

Figure B-2 UV Detector Initial Conditions Tab

Initial Conditions | UV Detector Initial Conditions | Time Program

Electropherogram channel

Acquisition enabled

Wavelength: 214 nm

Data rate: 4 Hz

Filter

High sensitivity

Normal

High resolution

Peak width (points): 16-25

Relay 1

Off

On

Relay 2

Off

On

Absorbance signal

Direct

Indirect

Figure B-3 Capillary Conditioning Method Time Program Tab

Initial Conditions | UV Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	50.0 psi	5.00 min	BI:E1	BO:E1	forward	Rinse with 0.1 M HCl
2		Rinse - Pressure	50.0 psi	5.00 min	BI:B1	BO:B1	forward	Rinse with CZE Buffer
3	0.00	Separate - Voltage	30.0 KV	30.00 min	BI:F1	BO:F1	0.17 Min ramp, normal polarity	Equilibrate with Voltage Separation
4	30.00	End						
5								

Separation Method

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

Methods

Figure B-4 Initial Conditions Tab

The screenshot shows the 'Initial Conditions' tab with three sub-tabs: 'Initial Conditions', 'UV Detector Initial Conditions', and 'Time Program'. The 'Initial Conditions' sub-tab is active. It contains several sections:

- Auxiliary data channels:** Voltage max: 30.0 kV; Current max: 300.0 μ A; Power; Pressure.
- Mobility channels:** Mobility; Apparent Mobility; Plot trace after voltage ramp.
- Analog output scaling:** Factor: 1.
- Temperature:** Cartridge: 25.0 $^{\circ}$ C; Sample storage: 20.0 $^{\circ}$ C.
- Peak detect parameters:** Threshold: 2; Peak width: 9.
- Trigger settings:** Wait for external trigger; Wait until cartridge coolant temperature is reached; Wait until sample storage temperature is reached.
- Inlet trays:** Buffer: 36 vials; Sample: 48 vials.
- Outlet trays:** Buffer: 36 vials; Sample: No tray.

Figure B-5 UV Detector Initial Conditions Tab

The screenshot shows the 'UV Detector Initial Conditions' sub-tab. It contains several sections:

- Electropherogram channel:** Acquisition enabled; Wavelength: 214 nm; Data rate: 4 Hz.
- Filter:** High sensitivity; Normal; High resolution; Peak width (points): 16-25.
- Relay 1:** Off; On.
- Relay 2:** Off; On.
- Absorbance signal:** Direct; Indirect.

Figure B-6 Separation Method Time Program Tab

Initial Conditions			UV Detector Initial Conditions			Time Program		
	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	30.0 psi	1.00 min	BI:E1	BO:E1	forward, In / Out vial inc 8	Rinse with 0.1 M HCl
2		Rinse - Pressure	30.0 psi	2.00 min	BI:B1	BO:B1	forward, In / Out vial inc 8	Rinse with CZE Buffer
3		Inject - Pressure	0.5 psi	10.0 sec	SI:A1	BO:A1	Override, forward	Sample Injection
4	0.00	Separate - Voltage	30.0 KV	7.00 min	BI:A1	BO:A1	0.17 Min ramp, normal polarity, In / Out vial inc 8	Separation
5	7.00	End						
6								

Shutdown Method

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

Figure B-7 Initial Conditions Tab

Initial Conditions			UV Detector Initial Conditions			Time Program		
Auxiliary data channels <input type="checkbox"/> Voltage max: 30.0 kV <input checked="" type="checkbox"/> Current max: 300.0 μ A <input type="checkbox"/> Power <input type="checkbox"/> Pressure			Temperature Cartridge: 25.0 $^{\circ}$ C Sample storage: 20.0 $^{\circ}$ C			Peak detect parameters Threshold: 2 Peak width: 9		
Mobility channels <input type="checkbox"/> Mobility <input type="checkbox"/> Apparent Mobility <input checked="" type="checkbox"/> Plot trace after voltage ramp			Trigger settings <input type="checkbox"/> Wait for external trigger <input checked="" type="checkbox"/> Wait until cartridge coolant temperature is reached <input type="checkbox"/> Wait until sample storage temperature is reached					
Analog output scaling Factor: 1			Inlet trays Buffer: 36 vials Sample: 48 vials			Outlet trays Buffer: 36 vials Sample: No tray		

Methods

Figure B-8 UV Detector Initial Conditions Tab

Initial Conditions | UV Detector Initial Conditions | Time Program

Electropherogram channel

Acquisition enabled

Wavelength: 214 nm

Data rate: 4 Hz

Filter

High sensitivity

Normal

High resolution

Peak width (points): 16-25

Relay 1

Off

On

Relay 2

Off

On

Absorbance signal

Direct

Indirect

Figure B-9 Shutdown Method Time Program Tab

Initial Conditions | UV Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1	0.00	Separate - Pressure	50.0 psi	5.00 min	BI:E1	BO:E1	forward	Rinse with 0.1 M HCl
2	5.00	Wait		0.00 min	BI:C1	BO:C1		Place Capillary Tips in Water Vials
3	5.01	Lamp - Off						
4	5.01	End						
5								

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