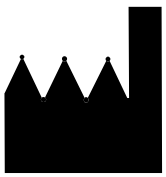
Neutral OptiMS Cartridge

Instruction Guide

RUO-IDV-05-2782-B
August 2017



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SCIEX

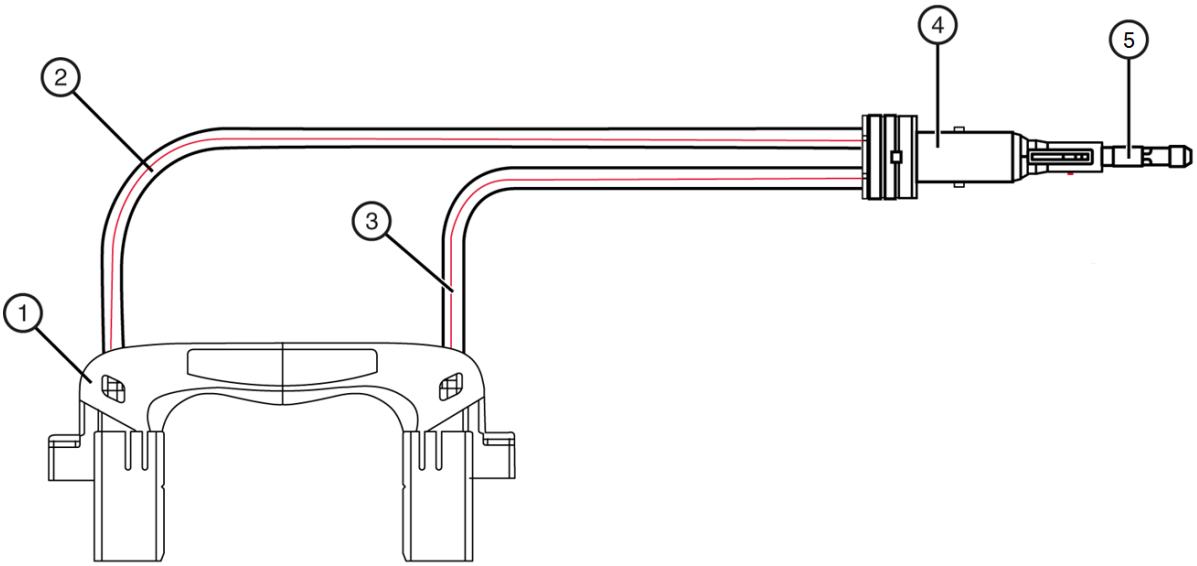
Introduction

The Neutral OptiMS cartridge is for use with the SCIEX CESI 8000 and CESI 8000 Plus High Performance Separation-ESI Module for the capillary electrophoresis-electrospray ionization-mass spectrometry (CESI-MS) analysis of:

- intact proteins and peptide mixtures (such as those commonly used in proteomics experiments)
- analytes, which require no electroosmotic flow, including non-peptides/non-proteins, such as glycans and small molecules.

The internal surface of the separation capillary is covalently coated with a neutral polymer, which prevents the interaction between protein samples and the capillary surface.programs.

Figure 1 Neutral OptiMS Cartridge



Item	Description
1	Neutral OptiMS Cartridge Body
2	Neutral-coated Separation Capillary
3	Conductive Liquid Capillary (CLC)
4	Neutral OptiMS Sprayer Housing
5	Protective Sleeve

Intended Use

For Research Use Only. Not for use in diagnostic procedures.

Safety

Refer to the Safety Data Sheets (SDS), available at sciex.com/tech-regulatory, regarding the proper handling of SCIEX materials and reagents. Always follow standard laboratory safety guidelines.

Material and Reagents

Table 1 Required Reagents

Reagent	Vendor	Part Number
0.1 N (0.1 M) Hydrochloric acid (HCl)	Labchem	LC152204
7.5 M Ammonium acetate (AmAc)	Sigma	A2706
Ammonium hydroxide (30%)	JT Baker	9733-01
Double-deionized (DDI) water (MS grade water filtered through 0.2 µm filter and with resistance above 18 megaohms)	Various	Various
Glacial acetic acid (HAc)	Sigma	27225-1L-M
(Optional) cIEF Peptide pI Marker Kit	SCIEX	A58481
Protein Test Mix	SCIEX	477436

Table 2 Additional Required Materials

Description	Part Number
From SCIEX	
CESI-MS vials (100-pack)	B11648
CESI-MS green caps (100-pack)	B24699
Micro vials (100-pack)	144709
nanoVials (100-pack)	5043467
Neutral OptiMS cartridge	B07368
From Other Laboratory Suppliers	
15 mL and 50 mL Falcon tubes	Various
30 mL, 50 mL, and 500 mL volumetric flasks	Various
Adequate pipettes and pipette tips	Various
Glass bottles	Various
Nalgene bottle (500 mL)	Various

Required Laboratory Equipment

- pH meter
- Magnetic stirrer and stir bar

Using the Cartridge

General Precautions

Upon receipt, store the Neutral OptiMS cartridge at 2 °C to 8 °C.

Caution: Potential System Damage. Do not expose the Neutral OptiMS cartridge to temperatures below 2 °C.

Before using the Neutral OptiMS cartridge for the first time, run the Neutral CESI Washing method to rehydrate the neutral coating followed by the Neutral CESI Electrical Conditioning method to establish the electrical connection within the OptiMS sprayer tip. Failure to use both methods may lead to earlier migration times, poor separation performance, loss of electrospray and irreproducible CE separation electrical current. Refer to Conditioning a New Cartridge.

For temperatures below 2 °C, the coating may become frozen. In this case, run the Neutral CESI Washing method to rehydrate the neutral coating, as described above. When finished, store the capillary in the instrument for 24 hours. it is not necessary to continue flushing. After 24 hours, proceed with the Neutral CESI Electrical Conditioning method as described in Conditioning a New Cartridge.

Caution: Potential System Damage. Do not rinse the separation capillary with solutions with pH higher than 9. The capillary is stable in the pH range from 2 to 9. Exposure to solutions with a pH less than 2 and greater than 9 might degrade the neutral coating leading to poor separation and limited run life.

Caution: Potential System Damage. Use of buffers that will lead to a CE separation current above 5 µA might cause permanent damage to the separation capillary coating. Always ramp down *voltage* to 1 kV with a duration of 5 minutes using the Direct Control window. A CE separation current above 5 µA might cause permanent damage to the separation capillary coating.

Caution: Potential System Damage. The ion spray voltage should not exceed 2 kV. A high ion source temperature can result in damage and clogging to the emitter.

Caution: Potential System Damage. Avoid capillary blockages by using non-volatile buffers such as phosphate and borate buffers.

Cleaning the Cartridge Interface

Carefully clean the CESI 8000 and CESI 8000 Plus system electrodes, ejectors, and interface block as described in Appendix E of the *CESI 8000 Plus High Performance Separation-ESI Module User Manual* (PN B11949). Perform this procedure after every week of operation or as needed.

Conditioning a New Cartridge

Before using a new Neutral OptiMS cartridge, remove the protective sleeve and check for flow of liquid through the separation capillary by performing a forward rinse at 100 psi for 10 min with water. Stop applying pressure when the liquid droplet appears at the end of the spray tip, and then perform a reverse rinse at 100 psi for 10 min with water, to check for flow of liquid through the conductive liquid capillary (CLC). Stop applying pressure when the liquid droplet appears at

the end of the needle. Replace the protective sleeve on the sprayer tip. Proceed with the Neutral CESI Washing method with the sprayer tip submerged in a 50 mL Falcon tube containing 5 mL of DDI water. Refer to Figure 2 and Figure 3 for the method.

After completion of the washing method, remove the sprayer from the Falcon tube and dry the exterior of the sprayer with a dry laboratory tissue. Make sure the electrospray voltage is off on the mass spectrometer system, remove the protective sleeve from the sprayer, and then install the sprayer on the MS adapter. Perform Neutral CESI Electrical Conditioning method (Figure 5). Once this method is completed, the Neutral OptiMS Cartridge is ready for use.

Storing the Cartridge After Use

1. Perform the Neutral CESI Rest method shown in Figure 2 and Figure 3.
2. Disconnect the sprayer from the ion source.
3. Remove the cartridge from CE system.
4. Replace the protective sleeve and store at 2 °C to 8 °C.
5. Put the sprayer tip into a 2 mL Eppendorf tube containing water and seal loosely with Parafilm.

Before using the cartridge again, run the Neutral CESI Washing method. Make sure that the sprayer tip is submerged in 5 mL of DDI water and there is liquid flowing through the separation capillary and the CLC. Run the Neutral CESI Electrical Conditioning method with sprayer installed in MS adapter and make sure the electrospray voltage is off.

Solutions and Buffers

Refer to Table 3 for the required buffers.

Table 3 Recommended Reagents by Sample Type

Sample	Reagent	Purpose
Single Peptide	10% HAc or other BGE such as 50 mM AmAc, pH 3.0 buffer	We recommend optimizing the sprayer tip position at the mass spectrometer entrance by peptide infusion.
Protein Test Mix or intact proteins at pH 3	100 mM AmAc, pH 3.0 buffer	To make 50 mM AmAc, pH 3.0 buffer.
	50 mM AmAc, pH 3.0 buffer	CESI-MS separation buffer.
	10 mM AmAc, pH 3.0 buffer	To make the Protein Test Mix sample.
	Reconstituted Protein Test Mix	
	Protein Test Mix Sample	To check the performance of the Neutral OptiMS cartridge.
Peptide mixtures	10% HAc	Background electrolyte separation solution (BGE), used in the separation capillary and the CLC.
	20% HAc	To adjust pH when making 200 mM Leading Electrolyte (LE) buffer.
	200 mM LE pH 4.0	Added to sample to cause it to concentrate during separation.

100 mM Ammonium Acetate (AmAc), pH 3.0 Buffer

1. In a 500 mL volumetric flask, dilute 2.873 mL of glacial acetic acid with double-deionized (DDI) water to make 500 mL of 100 mM acetic acid.
2. Transfer the entire solution into a new 500 mL Nalgene bottle and add a clean, dry stir bar.
3. In a 50 mL volumetric flask, dilute 0.666 mL of 7.5 M AmAc with DDI water to make 50 mL of 100 mM AmAc.
4. Using a pH meter, measure the initial pH value of the 100 mM acetic acid solution.
5. Adjust the pH of the 100 mM acetic acid solution to pH 3.0 ± 0.1 by adding aliquots of the 100 mM AmAc solution, while stirring the solution.
6. Label the bottle with “100 mM Ammonium Acetate, pH 3.0 buffer”, the final pH, and the preparation date.
7. Store the buffer at 2 °C to 8 °C. The buffer can be used for up to one year after preparation.

50 mM Ammonium Acetate (AmAc), pH 3.0 Buffer

1. In a new 50 mL Falcon tube, mix 10.0 mL of DDI water with 10.0 mL of 100 mM AmAc, pH 3.0 buffer.
2. Mix the contents by inverting the tube 3 times.
3. Label the tube with “50 mM Ammonium Acetate” and the preparation date.
4. Store the buffer at 2 °C to 8 °C. The buffer can be used for up to 3 months after preparation.

10 mM Ammonium Acetate (AmAc), pH 3.0 Buffer

1. In a new 15 mL Falcon tube, mix 9.0 mL of DDI water with 1.0 mL of 100 mM AmAc, pH 3.0 buffer.
2. Mix the contents by inverting the tube 3 times.
3. Label the tube with “10 mM Ammonium Acetate” and the preparation date.
4. Store the buffer at 2 °C to 8 °C when not in use. The buffer can be used for up to 3 months after preparation.

20 mM Ammonium Acetate (AmAc) pH 8.0 Buffer

1. In a clean 50 mL beaker, mix 25 mL of DDI water with 34.4 µL of glacial acetic acid (HAc).
2. Using a calibrated pH meter and a magnetic stirrer, titrate the HAc solution to pH 8.0 (± 0.1) by drop-wise addition of ammonium hydroxide.
3. Transfer the pH 8.0 buffer to a 30 mL volumetric flask and add enough DDI water to make 30 mL.
4. Store the buffer at 2 °C to 8 °C when not in use. The buffer can be used for up to 1 week after preparation.

10% Acetic Acid (HAc) Solution (performed in fume hood)

1. In a 15 mL Falcon tube, mix 1.00 mL of HAc with 9.00 mL of DDI water.
2. Mix the solution by inverting the tube 3 times.
3. Store the solution at room temperature and discard at the end of the day.

20% Acetic Acid (HAc) Solution

Note: Prepare this fresh only when preparing Leading Electrolyte (LE) Buffer. Do not stock this solution.

1. Add 15 mL of DDI water to a clean 50 mL Falcon tube.
2. Inside a fume hood, add 5.0 mL of acetic acid to the DDI water.
3. Mix the solution by inverting the bottle 3 times.
4. Label the bottle with “20% Acetic Acid” and the preparation date.
5. Store the solution at room temperature and discard at the end of the day.

200 mM Leading Electrolyte (LE) Buffer pH 4.0

1. Make 50 mL of 400 mM ammonium acetate (AmAc) solution:
   a. Add 47.3 mL of DDI water to a 50 mL volumetric flask.
   b. Inside a fume hood, add 2.7 mL of 7.5 M ammonium acetate to the same flask.
   c. Mix the solution by inverting the flask 3 times.
2. Pour the 50 mL of 400 mM AmAc solution to a clean 100 mL beaker.
3. Using a calibrated pH meter, measure and record the initial pH value of the solution.
4. Adjust the pH of the solution to 4.0 by adding aliquots of freshly-prepared 20% HAc.
5. Transfer the solution to a clean 100 mL volumetric flask.
6. Add enough DDI water to make 100 mL.
7. Mix the solution by inverting the flask 3 times.
8. Label the flask with “200 mM LE Buffer” and the preparation date.
9. Store at 2 °C to 8 °C when not in use. The solution can be used for up to 2 years after preparation.

Preparing the Protein Test Mix

Reconstituting the Protein Test Mix

1. Add 2.0 mL of DDI water to a vial of Protein Test Mix.
2. Gently vortex the vial until all solid material has dissolved.
3. Aliquot in 20 µL volumes and store them frozen (–35 °C to –15 °C). The reconstituted protein test mix can be used for up to one year after preparation.

Note: When preparing protein digest samples, refer to Chapter 6 of the *CESI 8000 Plus User Guide* for detailed instructions.

Preparing the Protein Test Mix Sample

1. If necessary, allow a vial of reconstituted Protein Test Mix to thaw at room temperature.
2. In a 0.5 mL centrifuge tube, add 20 µL of reconstituted Protein Test Mix.
3. Add 80 µL of 10 mM AmAc, pH 3.0 buffer.
4. Close the tube and vortex for 3 seconds.
5. Store at 2 °C to 8 °C when not in use.

Preparing the Single Peptide Sample for Infusion

1. Transfer 5 µL of the peptide pI 9.5 marker to a centrifuge tube.
2. Add 95 µL of 50 mM AmAc, pH 3.0 buffer (or 10% HAc, pH 3.0; depending on whether BGE was used for the run).
3. Close the tube and vortex for 3 seconds.
4. Store at 2 °C to 8 °C before use. Discard after use.

Methods

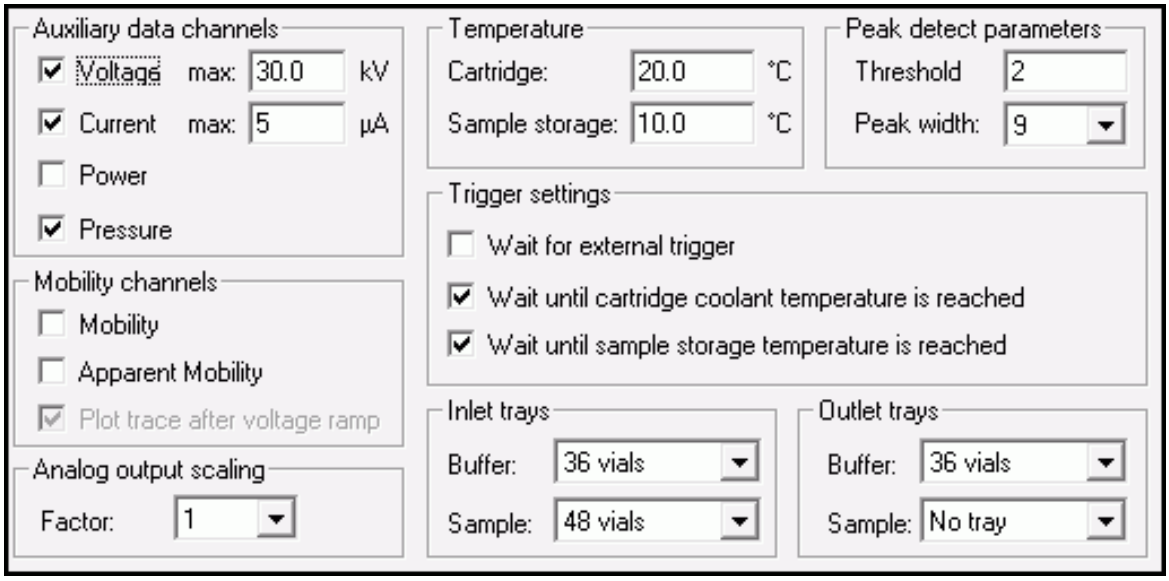
Download the methods from the SCIEX website at sciex.com/CESI8000Plus_methods.

- Neutral CESI Washing.met – Use this method to rehydrate the neutral coating of a new Neutral OptiMS cartridge or when using a Neutral OptiMS cartridge after long-term storage.
- Neutral CESI Electrical Conditioning.met – Use this method to establish the electrical path within the OptiMS sprayer of a new Neutral OptiMS Cartridge or when using a Neutral OptiMS cartridge after long-term storage.
- Neutral CESI Protein Separation.met – Use this method to inject and separate a protein sample.
- Neutral CESI Peptide Separation.met – Use this method to inject and separate a peptide mixture.
- Neutral CESI Rest.met – Use this to clean the separation capillary and the conductive line capillary (CLC) at the end of a sequence before removing the cartridge for long-term storage.

Initial Conditions

All methods use the initial conditions in Figure 2 but have different time programs.

Figure 2 Initial Conditions for CESI Methods



Time Programs

Figure 3 Time Program Tab–Neutral CESI Washing Method

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	100.0 psi	5.00 min	BI:C1	BO:B1	forward	0.1 N HCl- Separation capillary rinse
2		Rinse - Pressure	100.0 psi	10.00 min	BI:A1	BO:B1	forward	BGE-Separation capillary fill
3		Rinse - Pressure	100.0 psi	5.00 min	BI:D1	BO:B1	reverse	DDI water-Conductive line fill
4		Rinse - Pressure	100.0 psi	30.00 min	BI:D1	BO:B1	forward	DDI water- Separation capillary rinse

Figure 4 Time Program Tab–Neutral CESI Electrical Conditioning Method

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	100.0 psi	3.00 min	BI:A1	BO:A1	reverse	Fill CLC with BGE.
2		Rinse - Pressure	100.0 psi	5.00 min	BI:A1	BO:A1	forward	Fill separation capillary with BGE.
3	0.00	Separate - Voltage	30.0 KV	60.00 min	BI:B1	BO:A1	1.00 Min ramp, normal polarity, both	30 kV voltage application with 5 psi pressure at both ends.
4	60.00	Separate - Voltage	1.0 KV	5.00 min	BI:B1	BO:A1	5.00 Min ramp, normal polarity, both	Ramp down with 5 psi pressure at both ends.
5	65.00	End						

For the electrical conditioning method, make sure steps 3 and 4 use a pressure of 5 psi at both ends. Use 50 mM AmAc, pH 3 as BGE in Lines 1 and 2 of Figure 4.

Figure 5 Time Program Tab–Neutral CESI Protein Separation Method

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	100.0 psi	3.00 min	BI:C1	BO:A1	forward, In / Out vial inc 12	0.1 N HCl rinse
2		Rinse - Pressure	75.0 psi	3.00 min	BI:A1	BO:A1	reverse, In / Out vial inc 12	BGE conductive line fill
3		Rinse - Pressure	100.0 psi	10.00 min	BI:A1	BO:A1	reverse	BGE separation capillary fill
4		Inject - Pressure	2.5 psi	15.0 sec	SI:A1	BO:A1	Override, forward	Hydrodynamic injection of sample
5		Wait		0.00 min	BI:D1	BO:A1	In / Out vial inc 12	Water dip
6		Inject - Pressure	2.5 psi	10.0 sec	BI:B1	BO:A1	No override, forward	Hydrodynamic injection of BGE plug
7	0.00	Separate - Voltage	30.0 KV	10.00 min	BI:B1	BO:A1	1.00 Min ramp, normal polarity, both, In / Out vial inc 12	Separation at low
8	1.00	Relay On					1: 0.10 2: 0.10	Trigger MS
9	10.00	Separate - Voltage	30.0 KV	8.00 min	BI:B1	BO:A1	1.00 Min ramp, normal polarity, both, In / Out vial inc 12	Separation at higher flow
10	18.00	Separate - Voltage	1.0 KV	5.00 min	BI:B1	BO:A1	5.00 Min ramp, normal polarity, forward, In / Out vial inc 12	Voltage ramp down to 1 kV with 50 psi FWD pressure
11	23.00	End						

For the protein separation method, make sure to use the following parameters:

- Step 7 – Use forward pressure of **0.5** psi and reverse pressure of **0.5** psi to prevent drying the conductive line needle.
- Step 9 – Use forward pressure of **1.5** psi and reverse pressure of **1.5** psi to prevent drying the conductive line needle.
- Step 10 – Use forward pressure of **50** psi and **5** min ramp to lower the voltage.

The method increments the buffer vials after 15 consecutive repetitions.

Figure 6 Time Program Tab–Neutral CESI Peptide Separation Method

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	100.0 psi	5.00 min	BI:C1	BO:A1	forward, In / Out vial inc 6	0.1 N HCl rinse
2		Rinse - Pressure	75.0 psi	3.00 min	BI:A1	BO:A1	reverse, In / Out vial inc 6	BGE conductive line fill
3		Rinse - Pressure	100.0 psi	10.00 min	BI:A1	BO:A1	forward	BGE separation capillary fill
4		Inject - Pressure	5.0 psi	60.0 sec	SI:A1	BO:A1	Override, forward	Hydrodynamic injection of sample with LE
5		Wait		0.00 min	BI:D1	BO:A1	In / Out vial inc 6	Water dip
6		Inject - Pressure	2.5 psi	15.0 sec	BI:B1	BO:A1	No override, forward	Hydrodynamic injection of BGE
7	0.00	Separate - Voltage	30.0 KV	25.00 min	BI:B1	BO:A1	1.00 Min ramp, normal polarity, both, In / Out vial inc 6	Separation at low flow
8	1.00	Relay On					1: 0.10 2: 0.10	Trigger MS
9	25.00	Separate - Voltage	30.0 KV	55.00 min	BI:B1	BO:A1	1.00 Min ramp, normal polarity, both, In / Out vial inc 6	Separation at highe flow
10	80.00	Separate - Voltage	1.0 KV	5.00 min	BI:B1	BO:A1	5.00 Min ramp, normal polarity, forward, In / Out vial inc 6	Voltage ramp down to 1 kV with 50 psi FWD pressure
11	85.00	End						

For the peptide separation method, make sure to use the following parameters:

- Step 7 – Use forward pressure of **0.5** psi and reverse pressure of **0.5** psi to prevent drying.
- Step 9 – Use forward pressure of **2.0** psi and reverse pressure of **2.0** psi to prevent drying.
- Step 10 – Use the values in Figure 7 to lower the voltage. This step is mandatory for the method.

Figure 7 Separate Dialog–Parameters for Ramping Down the Separation Voltage

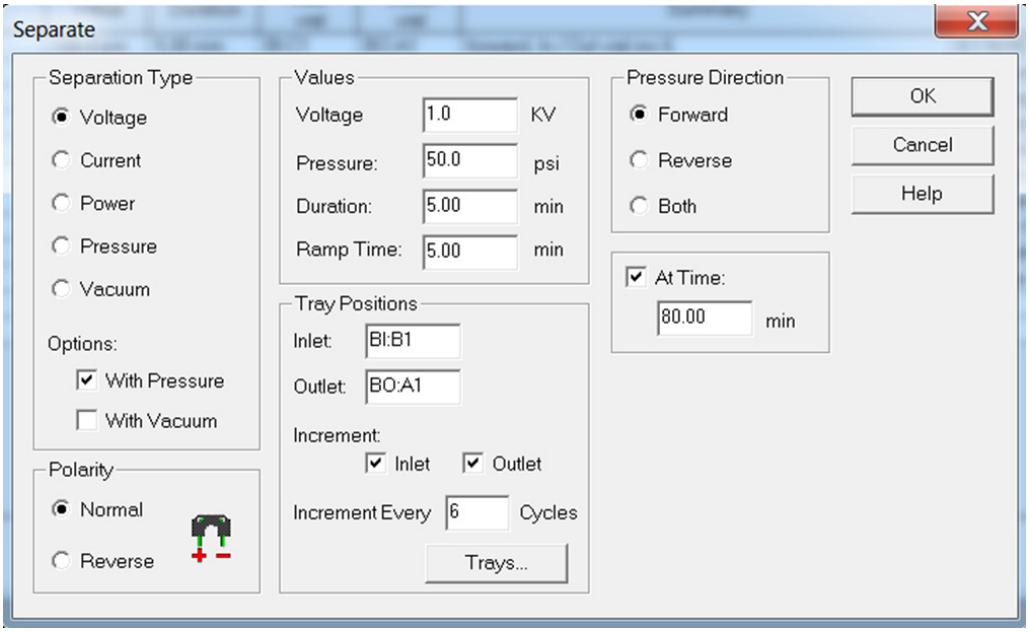


Figure 8 Time Program Tab–Neutral CESI Rest Method

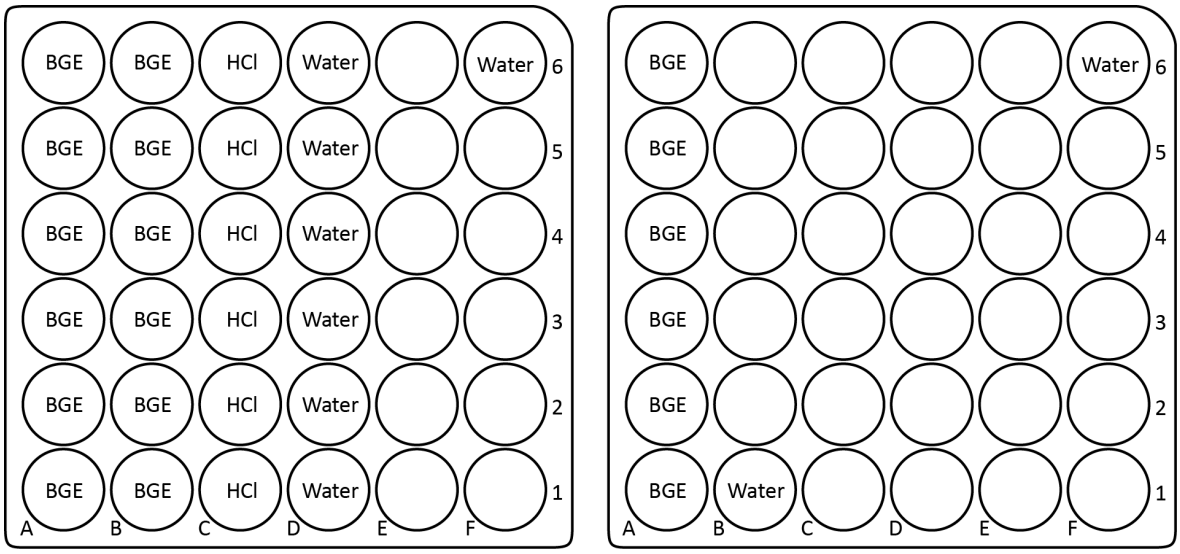
	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	100.0 psi	5.00 min	BI:C1	BO:B1	forward	0.1 N HCl
2		Rinse - Pressure	75.0 psi	5.00 min	BI:F6	BO:F6	reverse	DDI water rinse-conductive capillary line
3		Rinse - Pressure	100.0 psi	10.00 min	BI:F6	BO:F6	forward	DDI water rinse-separation capillary
4								

Note: If the cartridge will be used within 24 hours, extend the rinse pressure and time in step 3 to 5 psi for 999 seconds to avoid plugging the sprayer tip.

Preparing the Buffer Trays

Use only CESI vials (PN B11648) and CESI green caps (PN B24699) in the buffer trays. Fill CESI vials with 1.4 mL of each reagent. Seal each vial with a green cap and then load the inlet and outlet buffer trays as shown in Figure 9.

Figure 9 Buffer Tray Configuration



BI (Inlet Buffer Tray) BO (Outlet Buffer Tray)

Refer to Appendix C of the *CESI 8000 Plus User Manual* for information on the Auto Vial Increment feature.

The chemical composition in the vials labeled “Buffer” varies by sample type and pH, as shown in Table 4.

Table 4 Buffer Vial Contents by Sample Type

Sample Type	Position BI:A1	Position BI:B1	Position BO:A1
Protein Test Mix or intact proteins at pH 3.0	50 mM AmAc, pH 3.0	50 mM AmAc, pH 3.0	50 mM AmAc, pH 3.0
Peptide mixtures	10% HAc	10% HAc	10% HAc

Note: To prevent plugging the separation capillary, use low concentration buffers when performing CESI 8000 Plus separations at pH values greater than 7.

Preparing the Sample

Use the values in Table 5 as starting conditions and optimize as needed for the sensitivity of the mass spectrometer system and injection parameters. Desalting the sample is recommended.

If the sample is a peptide mixture, the final LE concentration in the sample should be 100 mM.

Table 5 Recommended Analyte Protein Concentration by Sample Type

Sample Type	Concentration
Protein Test Mix at pH 3	0.1 mg/mL in 10 mM AmAc, pH 3.0 buffer
Intact proteins at pH 3.0 buffer*	0.1 mg/mL in 10 mM AmAc, pH 3.0 buffer
Peptide mixtures	3 µg/µL or 1.5 µM in 100 mM LE, pH 4.0

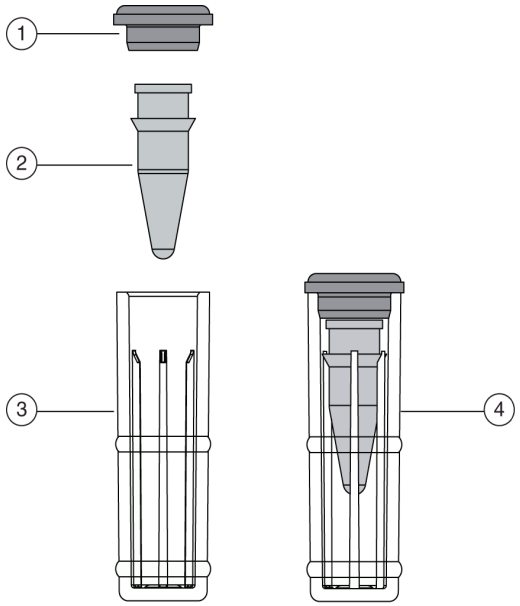
*Use this as starting condition and optimize as necessary.

Micro Vial Setup

For sample volumes between 50 µL and 100 µL, use a micro vial.

1. Transfer 50 µL to 100 µL of sample to a micro vial.
2. Centrifuge for 5 seconds in a bench-top centrifuge to remove any air bubbles at the bottom.
3. Put the micro vial in a CESI vial and seal the vial with a green cap (Figure 10).
4. Put the sample vial in the inlet sample tray at position SI:A1.

Figure 10 Micro Vial Setup



Item	Description
1	CESI-MS cap (PN B24699)
2	Micro vial (PN 144709)
3	CESI-MS vial (PN B11648)
4	Micro vial inside a CESI-MS vial

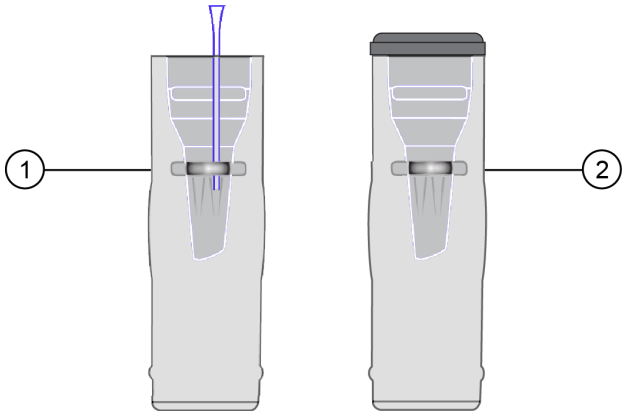
nanoVial Setup

For sample volumes between 5 µL and 50 µL, use a nanoVial.

1. Using a thin pipette tip, transfer 5 µL to 50 µL of the sample to the deeper well of a nanoVial.

   The deeper well is on the left side of the nanoVial, when the tab is facing you (Figure 11).

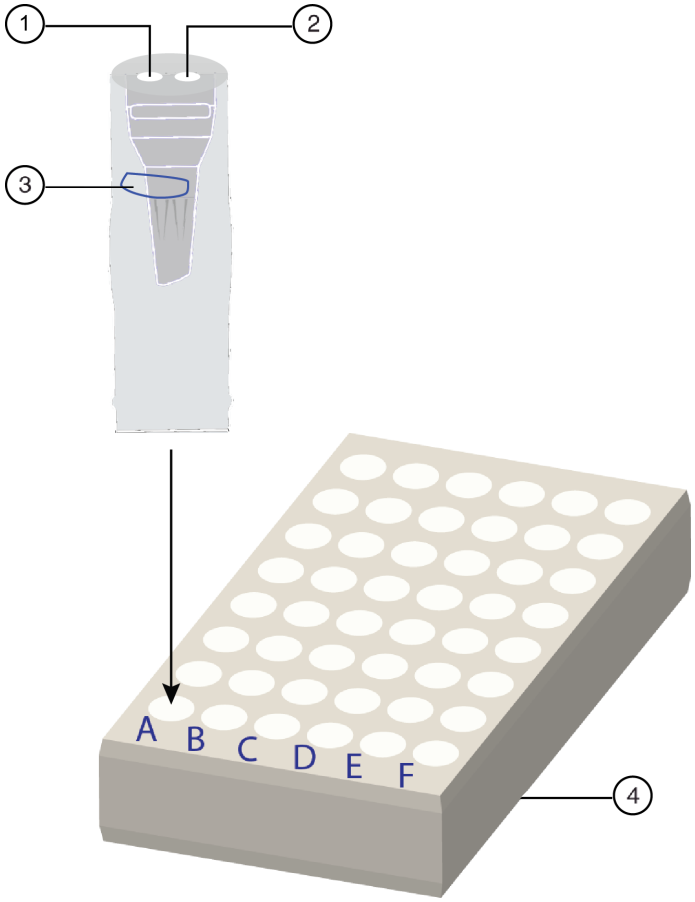
Figure 11 nanoVial Setup



Item	Description
1	nanoVial inside a CESI-MS vial, thin pipette tip is shown transferring sample (right side)
2	nanoVial inside a CESI-MS vial, covered with cap

2. Cap the vial with a green cap.
3. Put the sample vial in the inlet sample tray at position S1:A1. Make sure that the tab on the outside of the vial is facing the front of the sample tray. Refer to Figure 12.

Figure 12 Orientation of nanoVial Tab in Sample Trays



Item	Description
1	Electrode at left side of nanoVial
2	Capillary at right side of nanoVial; for use in loading samples
3	Tab outside of nanoVial
4	Sample tray

Tip! If there are not enough samples to fill the tray, keep every second column in the sample tray empty. This will make it easier to remove nanoVials from tray.

Setting up the CESI 8000 Plus System

Use the following procedures to set up the CESI 8000 Plus system. Refer to the *CESI 8000 Plus User Manual* for detailed instructions.

Prepare the System and Condition the Cartridge

This procedure is only required for a new cartridge or if the cartridge has been stored for more than two weeks. Overnight rehydration is optional.

1. Make sure the mass spectrometer has been calibrated and is connected to the CESI 8000 Plus system.
2. On the mass spectrometer acquisition computer, set the ESI voltage to **0**.
3. Install the Neutral OptiMS cartridge in the CESI 8000 Plus system.
4. Condition the cartridge by performing the Neutral CESI Washing method followed by the Neutral CESI Electrical Conditioning method. Refer to Conditioning a New Cartridge on page 7 for detailed instructions.

Note: The mass spectrometer electrospray voltage must be off before starting the Neutral CESI Electrical Conditioning method.

Caution: Potential System Damage. Perform the Neutral CESI Washing method with the sprayer tip submerged in DDI water. Remove the sprayer from the DDI water and then install on the MS adapter before starting the Neutral CESI Electrical Conditioning method. Failure to do so may result in sprayer tip breakage.

Optimize the Sprayer Position

Note: Optimize the sprayer position with BGE provided that it has a background ion can be detected by mass spectrometer. If the mass spectrometer is unable to detect the BGE, a single peptide solution in BGE should be used in the optimization. Prepare the single peptide solution and transfer it to a micro vial. Place the micro vial into a CESI vial and cover with a green cap. Place the vial containing the single peptide solution in the inlet buffer tray at position BI:F1. Make sure to fill the separation capillary with peptide solution and fill the CLC with BGE. Follow the instructions below.

1. If needed, adjust the height of the CESI 8000 cart so that the CESI buffer vials are at the same height as the sprayer.

   Refer to Chapter 2 of the *CESI 8000 Plus User Manual* for additional information on adjusting the cart height.

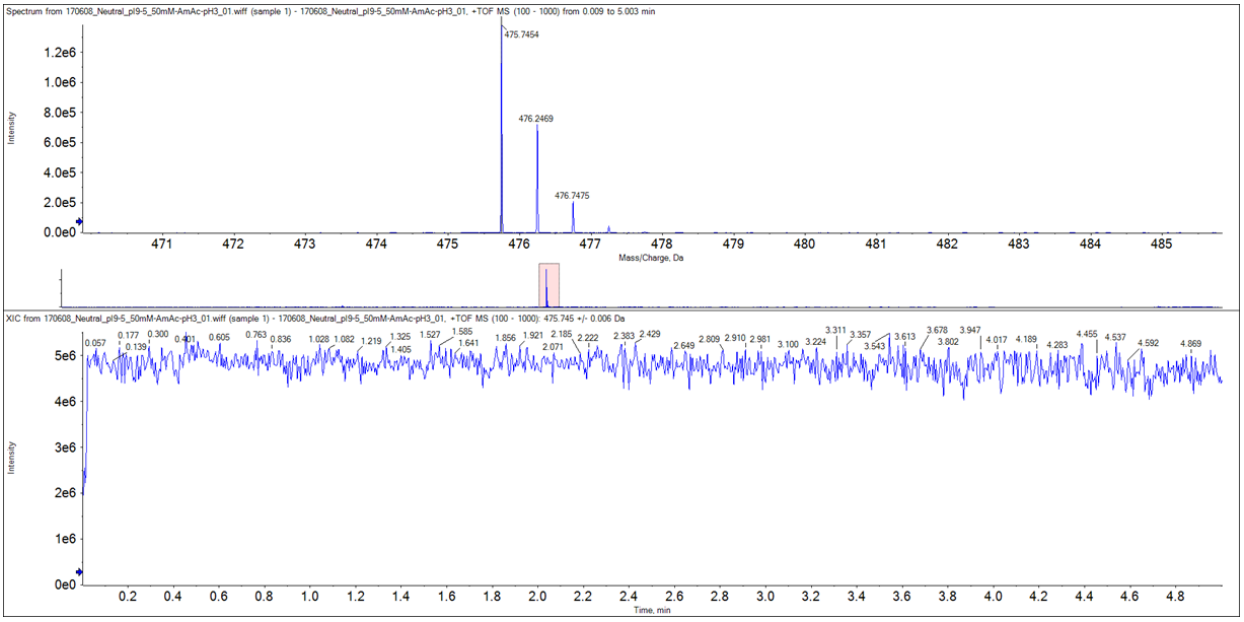
2. In the Direct Control window, rinse the conductive liquid capillary (CLC) for 5 minutes at 100 psi with BGE in the reverse direction.

   Confirm there is flow across the CLC by observing drop formation at the base of the sprayer needle.

3. Rinse the separation capillary with the sample to be infused for 5 minutes at 100 psi in the forward direction. Refer to Preparing the Single Peptide Sample for Infusion on page 11.

   Confirm there is flow across the separation capillary by detecting drop formation at the tip of the sprayer. Move the sprayer tip towards the center of the mass spectrometer inlet. The distance between the sprayer tip and the mass spectrometer inlet should be not more than 2 mm to 3 mm. Make sure the rinse drops forming at the sprayer tip fall outside the mass spectrometer inlet.

Figure 13 Total Ion Electropherogram (TIE) of pI 9.5 Marker in 50 mM Ammonium Acetate, pH 3.0



Experimental conditions were CE: 30 kV, 1.5 psi; mass spectrometer: TripleTOF® 6600 system, accumulation time 250 ms, CE: 10, DP: 80, 1300 V ISVF, curtain gas: 5 psi.

Determine the Minimum Ion Spray Voltage

1. In the Direct Control window, apply voltage for 60 minutes, using the settings in Figure 14 while the cartridge inlet and outlet are submerged in BGE.

Figure 14 Voltage Settings Dialog–Initial Parameters

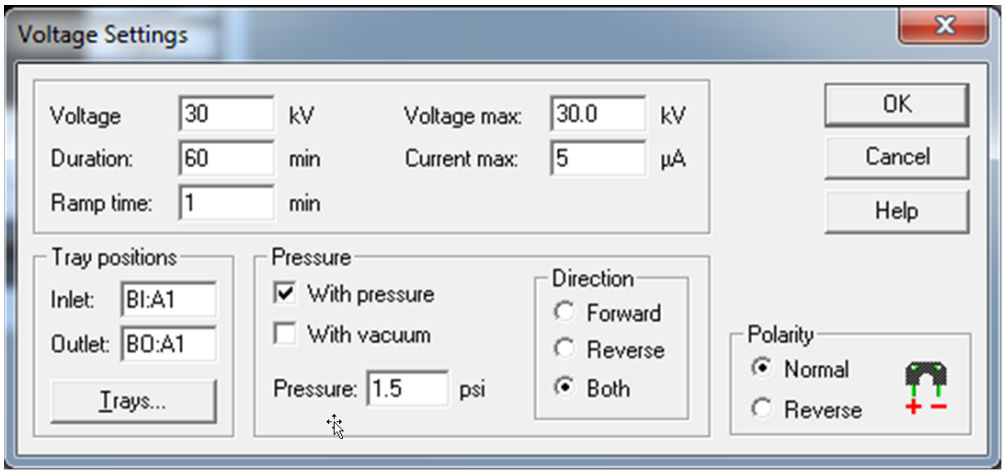
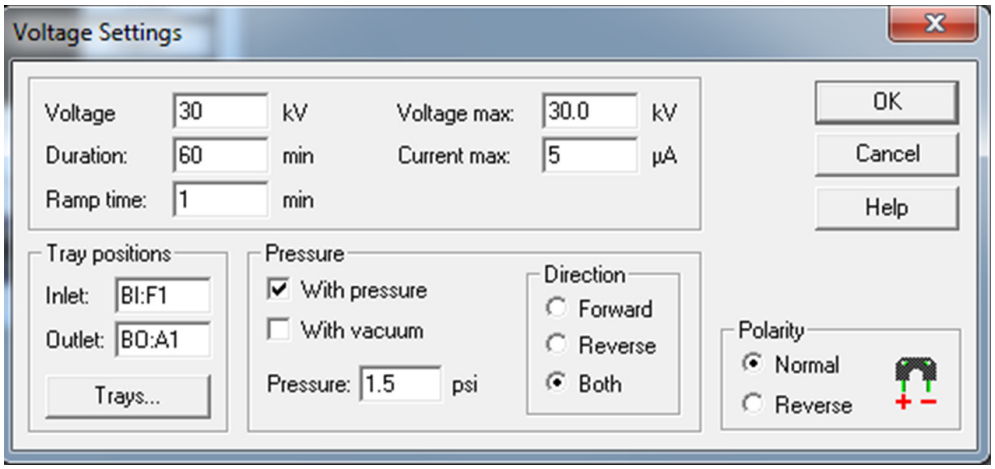


Figure 15 Analyte Voltage Settings Dialog–Initial Parameters; pI Marker Solution is at Position BI:F1



2. On the mass spectrometer acquisition computer, set the ESI voltage to **0** and begin acquiring data (use a scan range of 200 m/z to 1000 m/z for peptides and 300 m/z to 2000 m/z for the Protein Test Mix).

   There should be no spray and therefore no data in the mass spectrum. If there is spray, refer to Troubleshooting on page 26.

3. On the mass spectrometer acquisition computer, set the ion spray voltage to **1.0** kV and continue acquiring data.

4. Increase the ion spray voltage in **0.1** kV increments until a continuous signal is achieved at the mass spectrum window.

5. Optimize the position of the sprayer tip in relation to the mass spectrometer inlet by maximizing the XIC (eXtracted Ion Electropherogram) signal for the pI 9.5 marker in use (refer to Figure 13) while minimizing its fluctuations.

6. After optimizing the sprayer position, decrease the ESI voltage in 0.1 kV increments until spraying stops.

7. Increase the ESI voltage in 0.1 kV increments until continuous spray is detected, and then write down the voltage value.

   This is the minimum ESI voltage.

8. Add **0.2** kV to the minimum ESI voltage,

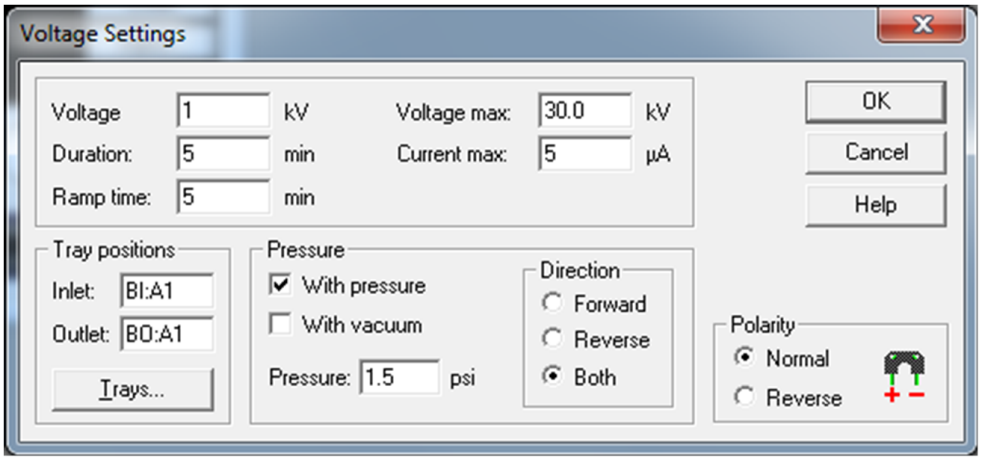
9. Monitor the spray stability for 20 minutes to 30 minutes. The baseline fluctuation must be less than or equal to 40%.

   If the baseline fluctuation is above 40%, repeat the electrical conditioning method. Refer to Conditioning a New Cartridge on page 7.

10. Turn off the mass spectrometer electrospray voltage first and then turn off the CE voltage using the settings in Figure 16.

Tip! To prolong the life of the Neutral OptiMS cartridge, always use a 5 minute ramp down before turning off the CE voltage.

Figure 16 Voltage Settings Dialog–Parameters for Ramping Down the Separation Voltage



Note: If the infusion was done with the analyte at the BI:F1 position, ramping down should be done from the same vials.

Edit the Method, the Batch, and the Sequence

1. Open the mass spectrometer acquisition method.
2. Enter the value determined in step 8 above as the ESI voltage.
3. Set the duration in the acquisition method based on the type of mass spectrometer.
   - For Bruker, Waters, and ThermoFisher mass spectrometers – Set the duration 1.0 minute less than the total CE separation time.
   - For SCIEX mass spectrometers – Set the duration 2.5 minutes less than the total CE separation time.
4. Save the acquisition method.

5. Create the mass spectrometer batch and the CE sequence.

   The number of separations in the CE sequence should be the same as the number of samples in the mass spectrometer batch.

6. At the end of the CE sequence, add the Neutral CESI Rest method (refer to Figure 8). A corresponding mass spectrometer method is not required.

Run the Protein Test Mix and Evaluate the Results

After setting up the mass spectrometer and the CESI 8000 Plus system, verify the performance of the Neutral OptiMS cartridge using 50 mM AmAc, pH 3.0 buffer and the Protein Test Mix sample.

1. Put the sample vial in the inlet sample tray at position S1:A1.

   Note: Do not store samples in the CESI 8000 Plus sample cooler at 10 °C for more than 5 days.

2. If necessary, update the sample location in the CE sequence and then save the CE sequence.
3. Check the coolant level in the CESI 8000 Plus system and add coolant if necessary.
4. Start the mass spectrometer batch and make sure the mass spectrometer is ready to acquire.
5. Start the CE sequence protein method.

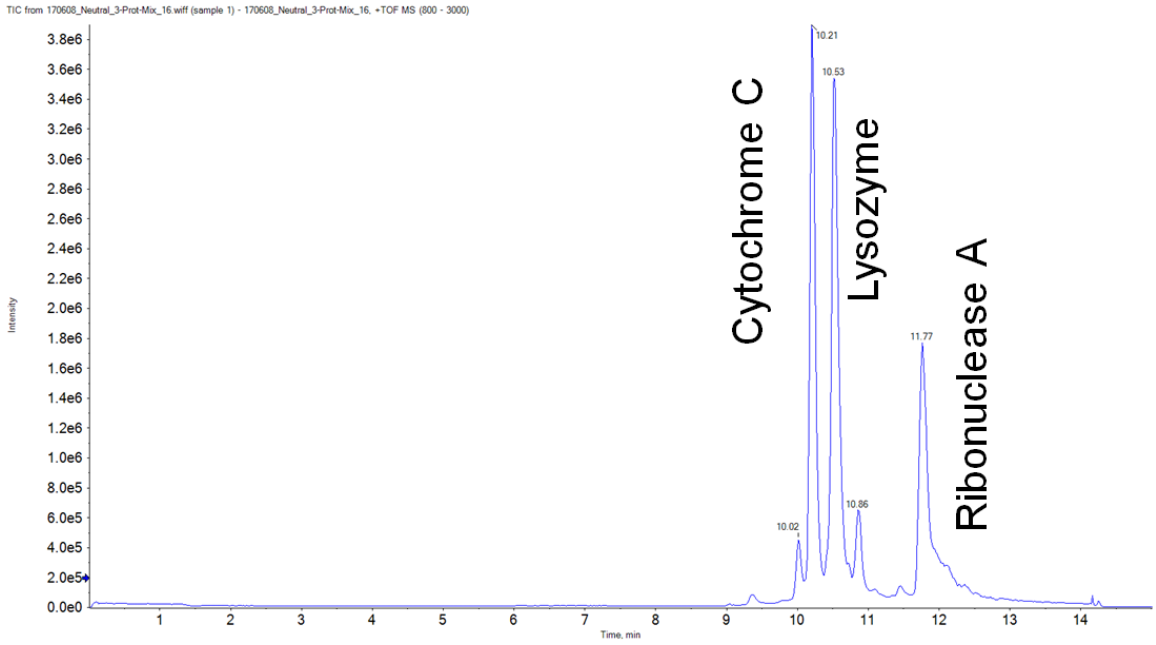
Evaluate the Protein Test Mix Results

When the separation of the Protein Test Mix is complete, inspect the detection times for the three proteins. All three proteins should be present in the 8 minute to 15 minute range.

If the proteins are not separated, refer to Troubleshooting on page 26.

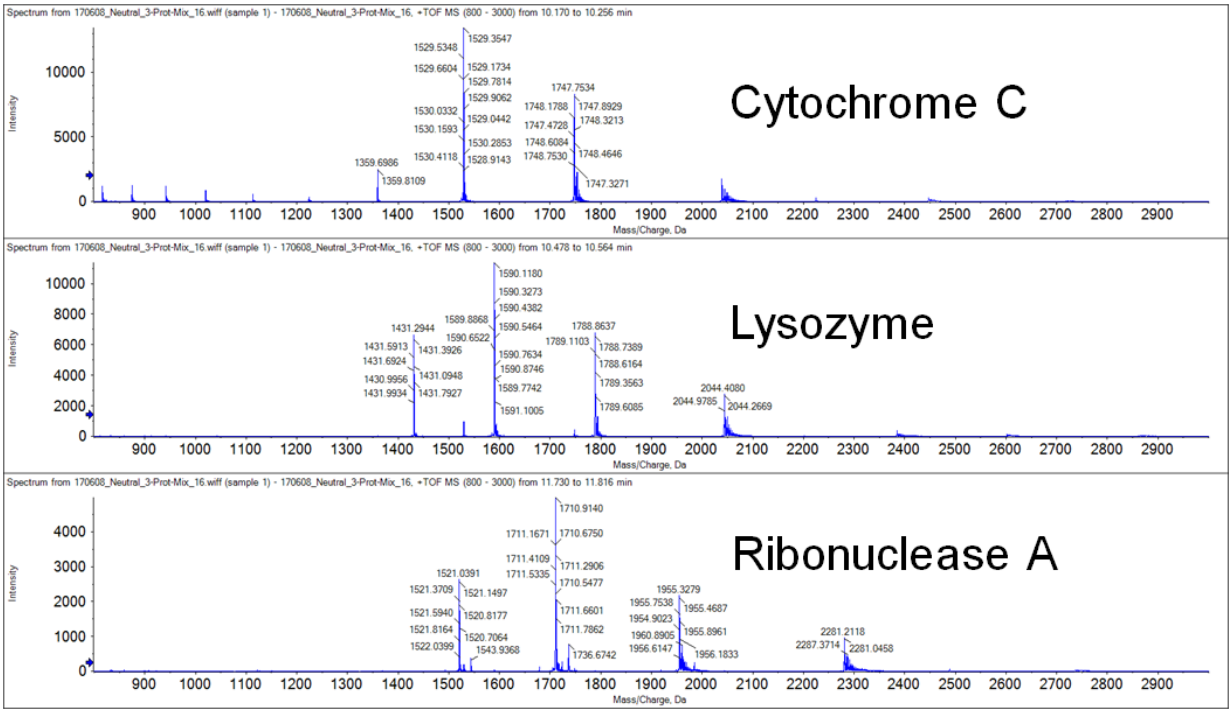
Figure 17 shows a total ion electropherogram (TIE) for a typical CESI 8000 Plus separation of the Protein Test Mix using a SCIEX TripleTOF® 6600 system and 50 mM AmAc, pH 3.0 buffer. Figure 18 shows the mass spectrum for each protein in the Protein Test Mix.

Figure 17 TIE of Protein Test Mix in 50 mM Ammonium Acetate, pH 3.0



Experimental conditions were CE: 30 kV, 1.5 psi; mass spectrometer: TripleTOF® 6600 system, accumulation time 1000 ms, CE: 20, DP: 80, 1500 V ISVF, curtain gas: 5 psi.

Figure 18 Mass Spectra of Cytochrome C (top panel), Lysozyme (middle panel), and Ribnuclease A (bottom panel) Protein Test Mix



CESI-MS Separation of Peptide Mixtures

As a starting point, use the Neutral CESI Peptide Separation method (Figure 2 and Figure 6) with 10% HAc as the background electrolyte. Make sure the sample contains 100 mM LE.

Additional optimization of the separation method might be required.

Figure 19 is an example of a TIE for a CESI 8000 Plus system separation of an *E. coli* cell lysate digest. The protein concentration was 3 μg/μL The sample was injected at 5 psi for 60 seconds and separated using the method shown in Figure 6.

Figure 19 TIE of *E. coli* Cell Lysate Digest

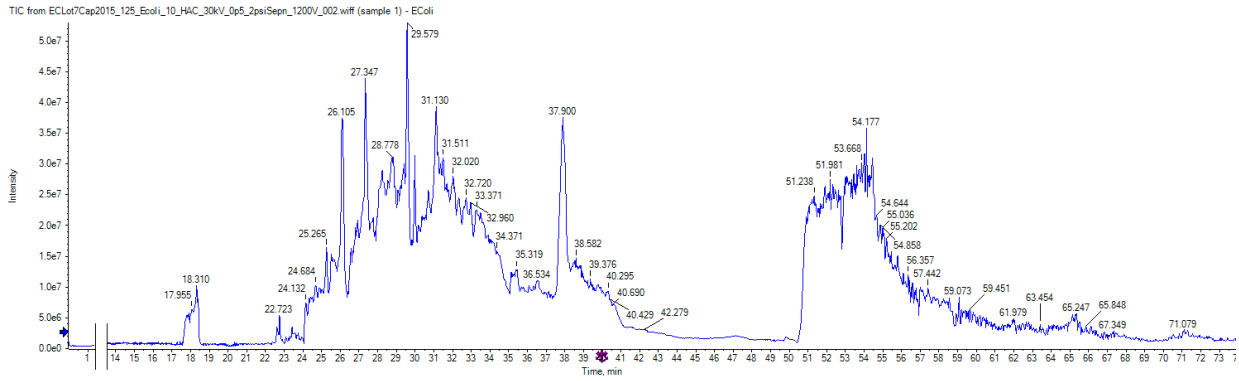
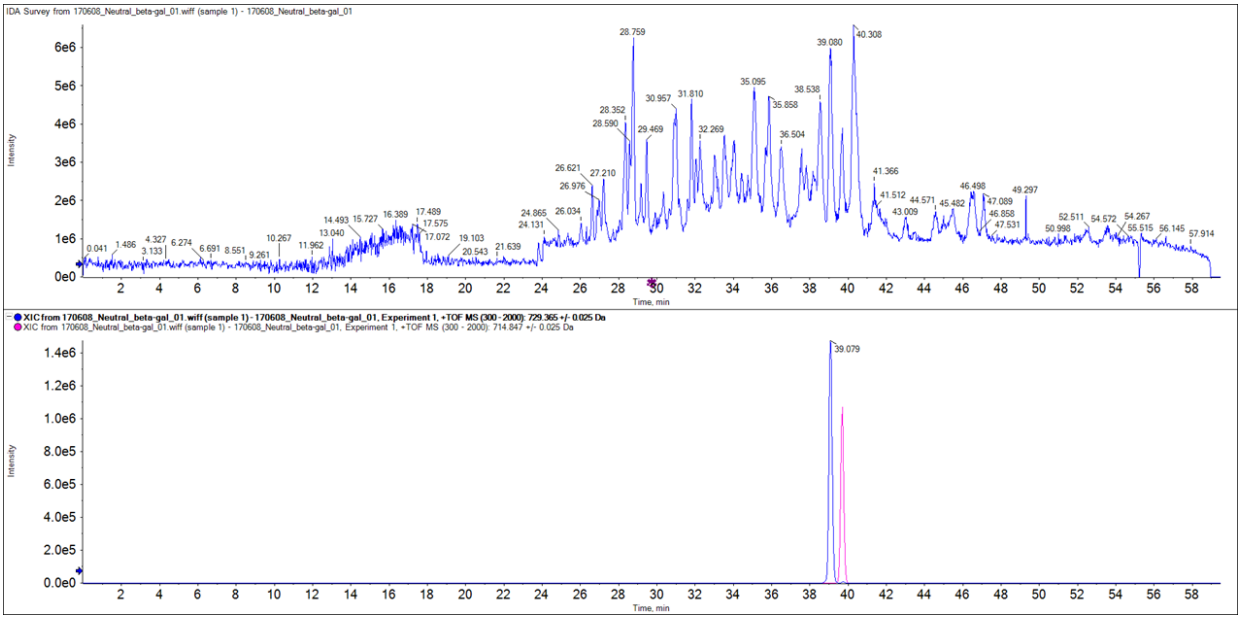


Figure 20 is an example of a TIE for a CESI 8000 Plus system separation of a beta-galactosidase trypic digest. The protein concentration was 1 μM. The sample was injected at 5 psi for 60 seconds and separated using the method shown in Figure 6.

Figure 20 TIE of 1 μM Beta-galactosidase Tryptic Digest Diluted 50:50 in Leading Electrolyte (200 mM Ammonium Acetate, pH 4) with BGE, 10% Acetic Acid



Neutral OptiMS Cartridge Log Book

Use the table to keep track of runs using the Neutral OptiMS Cartridge.

Table 6 Cartridge Log Book

Cartridge Type:			PN:	SN:	
Date	**Operator**	**Purpose**	**Application**	**# of Runs**	**Comments**

Troubleshooting

Additional troubleshooting information can be found in Appendix F of the *CESI 8000 Plus User Manual*.

Symptom	Possible Cause	Corrective Action
Electrospray is detected when the ion spray voltage (IS) is zero while applying CE voltage with forward pressure.	Missing cable or cable is only partially connected.	Make sure that all cables are present and connected. Refer to Chapter 2 in the *CESI 8000 Plus User Manual*.
	MS adapter is not making contact with the cartridge.	Remove the OptiMS sprayer from the MS adapter and pull up its metal contact. After replacing the sprayer, make sure that the OptiMS sprayer is in the locked position in the MS adapter.
	Cartridge capillaries are not filled with separation buffer.	Make sure that the separation capillary and CLC are filled with separation buffer by rinsing each one for 3 minutes at 100 psi.
	Other	Contact the local SCIEX Field Application Specialist (FAS).
No flow through the separation capillary.	Capillary is plugged.	Attempt to remove the plug by running the washing method while the sprayer tip is submerged in a 50 mL Falcon tube filled with 10 mL of DDI water. If the point of clogging is at the inlet side, apply a vacuum.
	Source temperature is too high resulting in precipitation of protein at the emitter.	Addition of an organic, for example, 10% IPA, will help to increase the solubility. Attempt to remove the plug by running the washing method with 10% IPA while the sprayer tip is submerged in a 50 mL Falcon tube filled with 10 mL of 10% IPA.
	Capillary is broken.	Replace the cartridge.

Symptom	Possible Cause	Corrective Action
Electrospray is not stable.	ESI voltage is not optimized.	Determine the minimum ESI voltage and increase its working value by 0.2 kV.
	Sprayer position is not optimized in front of mass spectrometer inlet.	Optimize the sprayer position while applying CE voltage and forward pressure of 1.5 psi.
	Forward pressure in separation capillary is below 1.5 psi.	Increase pressure value to 1.5 psi or above.
	The OptiMS sprayer needle is not completely filled.	Increase the filling time of conductive liquid capillary (CLC) in the Neutral CESI Separation method. If humidity is below 20%, apply pressure at both capillary ends during the separation.
	Buffer concentration and pH are not optimized.	Optimize buffer concentration and pH.
	Curtain gas value is too high.	Decrease the curtain gas to value recommended in the *CESI 8000 Plus User Manual*.
	Cartridge was exposed to temperatures below 2 °C.	Condition the cartridge again. Refer to Conditioning a New Cartridge on page 7. If the electrospray is still not stable, replace the cartridge.
Carryover is observed between runs.	Buffer vials are contaminated with sample.	Replace buffer vials. To prevent contamination of the buffer vials, make sure that a water dip step follows sample injection in the separation method.
	Separation capillary was not sufficiently rinsed between separations.	Make sure to rinse with 0.1 M HCl at the start of each separation.
		Increase rinsing times between separations.

Symptom	Possible Cause	Corrective Action
Sample peaks are tailing in the TIE.	Separation capillary was not rinsed effectively between separations.	Make sure to rinse with 0.1 M HCl at the start of each separation.
		Increase rinsing times between separations.
	Buffer concentration and pH are not optimized, resulting in high electro-migration dispersion.	Optimize buffer concentration and pH for the sample under study.
	Forward pressure value is too high distorting peak shape.	Reduce forward pressure to 1.5 psi.
	Neutral coating is damaged.	Run a Protein Test mix sample to confirm separation performance. If the separation fails, then replace the Neutral OptiMS cartridge.
No sample is detected.	Sample vial is in the wrong position.	Make sure sample vial is in the correct position in sample tray, sequence and/or method.
	Insufficient sample volume.	Increase sample volume above 50 μL for micro vials or 5 μL for nanoVials.
	Injection plug is too short.	Increase injection time and/or injection pressure.
	Sample is too dilute.	Use a more concentrated sample.
	Wrong polarity used in method.	Use normal polarity.
	Separation method is too short.	Increase the separation time in both CE and mass spectrometer methods.
	MS settings are not optimized.	Make sure that mass spectrometer settings are correct and that ionization parameters have been optimized for the sample under CESI 8000 Plus system analysis.

Symptom	Possible Cause	Corrective Action
Low sensitivity due to highly oxidized proteins.	High ESI voltage.	Lower the ESI voltage and optimize sprayer tip position.
	Sprayer tip is too close to mass spectrometer inlet.	Move sprayer tip away from mass spectrometer inlet. Refer to “Fine Tuning Sprayer Tip Position” in Appendix F of the *CESI 8000 Plus User Manual*.
	Mass spectrometer entrance temperature is too high.	Lower mass spectrometer entrance temperature.


SCIEX

Revision History

Revision	Description of Change	Date
RUO-IDV-05-2782-A | B80092AA	First release of document.	October 2015
RUO-IDV-05-2782-B | B80092AB	Rebranded, Added Revision History, instructions for using the pI marker, nanoVial setup, Methods URLs, and a step to monitor spray stability to “Determine the Minimum Ion Spray Voltage”. Replaced all references to normality with molarity. Updated legal page, system name, and company name.	August 2017