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Agilent

ExD AQ-25x Option

AQ-251 • AQ-252

for Agilent LC/Q-TOF

User Guide

R011 • November 2023

Notices

Patents

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e-MSion welcomes your feedback, questions, and suggestions for improvement on this guide.

You can reach us at emsion-support@agilent.com. We deeply appreciate your assistance in our efforts to continuously improve the quality of our documentation.

Contact Us

For technical questions regarding the ExD Cell, contact e-MSion via the following:

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About this Guide

The purpose of this document is to provide:

- A functional description of the ExD AQ-25x Option for Agilent LC/Q-TOF.
- Instructions for operation and maintenance.
- An introduction to the fundamental concepts of electron-based fragmentation.

Related Documentation

- *e-MSion ExD AQ-25x Option Site Preparation Checklist*
- *e-MSion ExDControl Software User Guide*
- *e-MSion ExD Controller User Guide*
- *Agilent 6200/6400/6500 LC/MS Maintenance Guide* (found on the Agilent TOF and Q-TOF LC/MS Resource App)
- *e-MSion ExD AQ-25x Option Quick Start Guide*

Terms Used

In this document:

- *ExD* refers to a family of electron-based gas-phase molecular ion dissociation techniques.
- *MassHunter* refers to Agilent MassHunter Data Acquisition software.
- *MS1* corresponds to **Total Ion Mode** and *MS2* corresponds to **Isolation Mode** in MassHunter.
- *Tuning mix* refers to Agilent ESI-L Low Concentration Tuning Mix.

Safety and Compliance

Symbols

WARNING **A Warning indicates a hazard. If the contents of the message are not observed, the health and/or safety of personnel may suffer.**

CAUTION **A Caution indicates a hazard. If the contents of the message are not observed, equipment may be damaged and/or data may be lost.**

NOTE **A Note contains helpful information and tips.**

Safety Precautions

- Always wear gloves when handling ExD hardware to avoid contamination.
- Handle all ExD hardware with care to avoid physical damage.
- Do not place liquids near the ExD Controller or other electronics.
- Run cables cleanly to minimize trip hazards and reduce the risk of dislodging the ExD Controller. Refer to the *Site Preparation Checklist* for guidance on ExD Controller placement.
- Exceeding the voltage range specification for the ExD Controller BNC float input can create a shock hazard. For ExD Controller Model ExD19: [-120, +120 V].
- Even when the instrument is disconnected from power, if the ExD Controller is ON, hazardous voltages can exist in the wiring between the ExD Controller D-sub cable and the ExD cell.
- When the ExD Controller is connected to a power source, even if the power switch is OFF, hazardous voltages can exist in wiring between the ExD Controller, the power supply, and the power switch.
- Before performing maintenance, ensure that both the instrument and ExD Controller are disconnected from all power sources. Wait for a sufficient time before removing any protective covers, as internal capacitors may still be charged.
- Always set the ExD cell filament current to 0 A and turn OFF the ExD Controller before shutting down the instrument.
- The ExD Controller is not user-serviceable. Do not remove covers. Hazardous voltages may be present.
- Apart from replacing the filament, the ExD cell is not user-serviceable. Powerful magnets inside the cell can disrupt medical pacemakers and create a crush hazard by attracting small objects.
- The ExD cell filament operates at temperatures high enough to cause serious burns. Allow parts to cool and use the insulated grip when you remove the filament cassette from the instrument.
- While the filament access door is open, keep surrounding area clear. Loose parts that enter the instrument can obstruct the turbopumps and create ejected material and smoke inhalation hazards.

Compliance

For products with ExD Controller model ExD19 ONLY:

The e-MSion Electron-based Fragmentation Option for Agilent Q-TOF LC/MS was tested to the following regulations on Electromagnetic Compatibility (EMC):

- AS/NZS CISPR 11: 2011 Class A
- EN 61326-1:2013
- ICES-003: Class A
- FCC 15:107 Class A
- FCC 15: 109 Class A

WEE Compliance

This product is required to comply with the European Union's Waste Electrical & Electronic Equipment (WEE) Directive 2002/96/EC.

Contents

1. Parts and Function 6

Overview 6

ExD cell 6

Magnets and lenses 7

Filament insert and cassette 8

ExD Controller and ExDControl software 8

2. Operation 9

Basics of Operation 9

ExD cell operating modes 9

Filament states 9

Lens profiles 10

Before Operation 11

Shutting Down 12

Daily Use 13

To set default profiles 14

To autotune the ExD cell 15

To add/edit autotune samples 16

To optimize the filament current 17

To run MassHunter instrument tunes with the ExD cell installed 18

Advanced Use 19

To manually tune the ExD cell 19

To add collision energy to an ECD profile 20

To alter the collision cell gas pressure 21

Tuning Standards 22

To prepare substance P 22

To set up direct infusion 23

To set up loop infusion 24

3. Data Acquisition and Analysis 25

Acquisition 25

Analysis 25

ExDViewer 26

Third-party tools for top- and middle-down analysis 26

4. Maintenance **27**
To check for filament burnout 27
To check for filament current leakage 27
To replace the filament 28
Step 1. Inspect the replacement filament insert 29
Step 2. Prepare the replacement filament cassette 29
Step 2. Shut down the system 30
Step 3. Replace the filament cassette assembly 31
Step 4. Restart the system 32
Step 5. Turn the filament ON 32
To evaluate the cause of filament failure 33
To resolve ExDControl connection issues 34
To reboot the instrument SmartCard 35
Troubleshooting Table 36
5. Concepts: Gas-Phase Fragmentation **38**
Collision Induced Dissociation 38
Electron-Based Dissociation 38
ExD Efficiency 41

1. Parts and Function

Overview

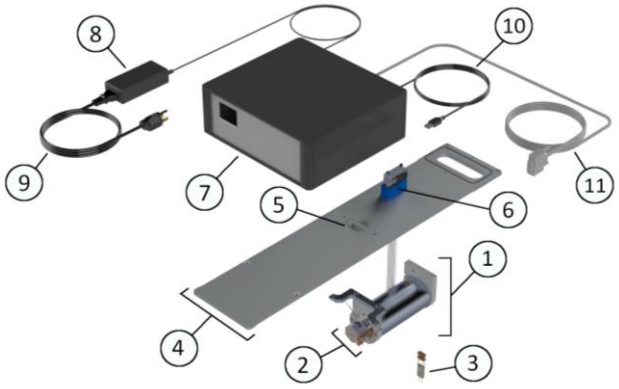


Figure 1. ExD AQ-25x Option. Model -251 is for Agilent 6545XT AdvanceBio LC/Q-TOF. Model -252 is for Agilent 6560 Ion Mobility LC/Q-TOF, and formerly 6545 & 6550 (now discontinued). Model -253 (discontinued) was for the 6546.

Table 1. Components.

Label	Part
1	ExD-collision cell assembly **AQ-251:** 6545XT IBC **AQ-252:** other Q-TOF IBC
2	ExD cell
3	Filament cassette assembly
4	Manifold cover assembly
5	Filament access door
6	D-sub vacuum feedthrough
7	ExD Controller
8	Power supply
9	Power supply cord
10	USB cord & Cat6 Ethernet cable
11	D-sub cable
-	Loop infusion kit
-	Toolkit

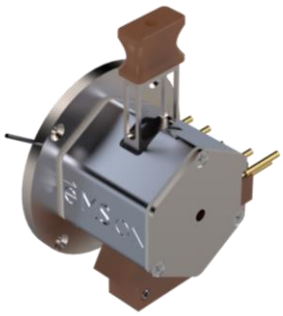
The ExD AQ-25x Option (-251, -252) is a hardware and software package that equips Agilent LC/Q-TOF mass spectrometers with the ability to perform electron-based fragmentation.

Key components include:

- **ExD cell**
- **Filament insert and cassette**
- **ExD Controller and ExDControl software**

"ExD" describes a family of electron-based gas-phase molecular ion dissociation techniques. Electron capture dissociation (ECD) is the principal method of fragmentation enabled by the ExD cell. See **Concepts** for more information.

ExD cell



The *ExD cell* uses a compact arrangement of permanent magnets and electrostatic lenses around an electron-emitting filament to facilitate the ion-electron interactions that produce ExD. The cell works on a microsecond timescale, without reagent, RF potentials, or ion trapping.

The ExD cell may be tuned for ECD or for transmission of ions without electron-based fragmentation. See **Operation**.

Figure 2. ExD cell.

In Agilent LC/Q-TOF instruments modified with the ExD Option, the ExD cell attaches to the entrance of a shortened collision cell, replacing the instrument collision cell.

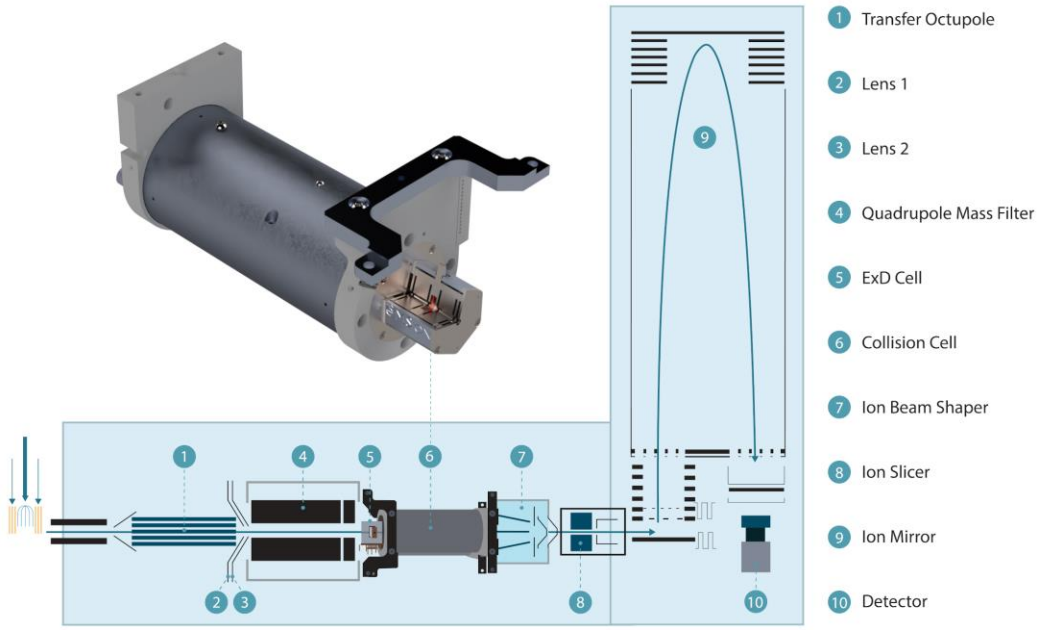


Figure 3. Placement of the ExD cell in an Agilent AdvanceBio 6545XT LC/Q-TOF.

NOTE

Installation of the ExD AQ-25x Option is reversible. Contact your Agilent representative to revert the instrument to its default configuration.

Magnets and lenses

Inside the ExD cell, permanent ring magnets and electrostatic lenses flank the filament.

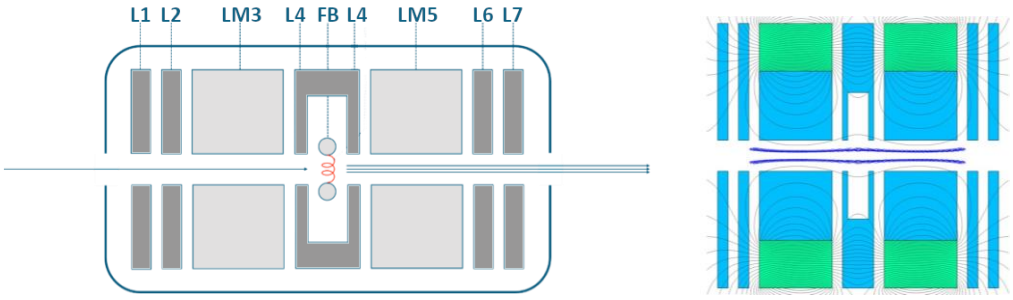


Figure 4. ExD cell cross-sectional views. Left: lenses are dark grey and lens magnets are light grey. Right: Magnetic field lines with calculated electron trajectories along the central horizontal axis.

- A negative filament bias (FB) relative to the filament holder lens (L4) draws electrons away from the filament.

- Positive electrical potentials on the magnet pole pieces (LM3, LM5) also draw electrons away from the filament. Magnetic field lines confine electrons to an electron cloud near the central axis of the ion flight path.
- Electrostatic lenses (L1, L2, L6, L7) shape the electron cloud and guide ions through the cell. Negative electrical potentials at the entrance and exit lenses keep electrons inside the cell.

NOTE

ExD cell L7 is the collision cell entrance lens. When the ExD cell is installed, this lens voltage is now set by ExDControl, instead of MassHunter.

Filament insert and cassette

The *filament insert* is the electron source for ExD. It holds a rhenium alloy wire suspended between posts. Thermionic emission is achieved by resistive heating.

The *filament cassette* houses the filament insert and plugs into a slot in the ExD cell.

Figure 5. Filament cassette with filament insert.

The filament will slowly thin with regular use. Repeated heating and cooling stresses the material and may accelerate filament failure. While the ExD cell may be tuned to transmit ions with a burned-out filament, ExD will not be possible until the filament is replaced. See **Maintenance**.

CAUTION

Heated rhenium is susceptible to oxidation. To preserve the filament lifetime, collision cell gas must adhere to the standard of 99.999% purity and the instrument must remain under vacuum while the filament is ON.

ExD Controller and ExDControl software

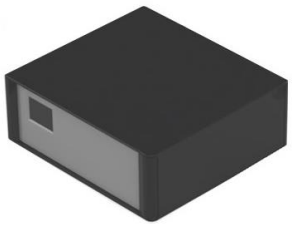


Figure 6. The ExD Controller.

The *ExD Controller* supplies DC voltages to the ExD cell lenses and current to the filament according to values set in the *ExDControl software*.

ExDControl is a separate application from MassHunter instrument control software. You will use both ExDControl and MassHunter to tune your ExD-enabled instrument.

NOTE

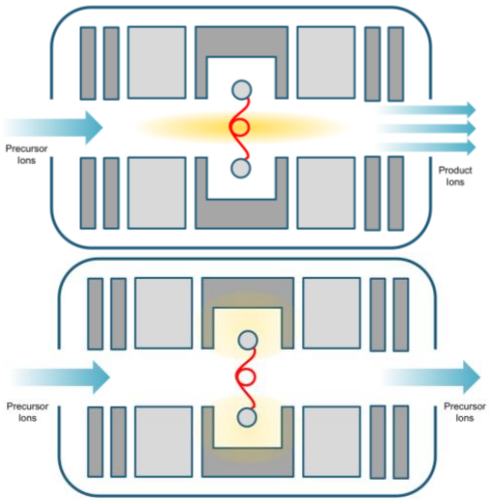
Refer to the ExD Controller User Guide and ExDControl Software User Guide for more information.

2. Operation

Basics of Operation

ExD cell operating modes

The ExD cell may be tuned to operate in two modes:



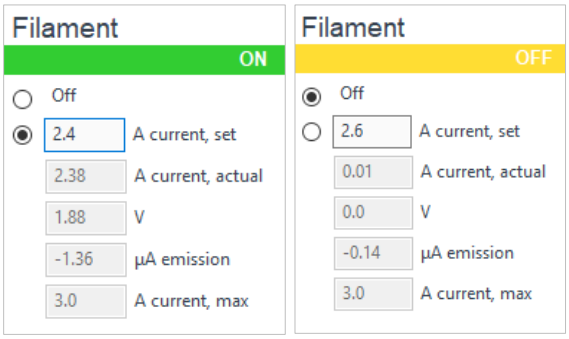
ExD ("Fragment")

The ExD cell is tuned to perform ExD, fragmenting a percentage of the ions that pass through the cell.

Transmission ("Fly-Through")

The ExD cell is tuned to maximize transmission of ions through the cell while minimizing the creation of ExD fragment ions.

Filament states



The ExD cell filament should be ON or OFF depending on the instrument state. See **Table 2** below.

The user sets the current amperage when the filament is ON. See **To optimize the filament current**.

Figure 7. ExDControl **Filament** panel, with filament ON (left) and OFF (right).

Table 2. Turn the ExD cell filament ON/OFF depending on the instrument state.

Instrument State	Filament State	
Shut Down, Standby	**OFF**	Always ensure the filament is OFF prior to venting and shutting down the instrument. Turn the filament OFF while the instrument is not in use to extend the filament lifetime.
On, Positive Mode	**ON**	While the ExD cell may be tuned for transmission in Positive Mode with filament OFF, sensitivity is higher with filament ON.
On, Negative Mode	**OFF**	Electron emission interferes with negative ion transmission in Negative Mode.

Lens profiles

A *lens profile* or *profile* is the set of electrical potentials for the eight lenses in the ExD cell. Each profile saves as an ExD tunefile (*.exd).

Tune the lens profile for either transmission or ExD in the ExDControl **Tuning** tab, either using autotune, or by manually adjusting the lens voltages.

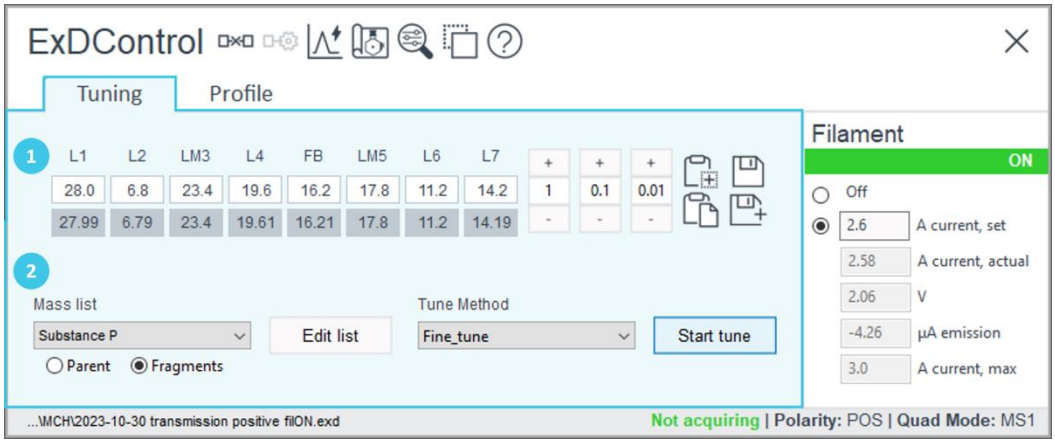


Figure 8. A profile in the ExDControl **Tuning** tab. Fields for (1) manual tune and (2) autotune.

Two tunefiles are always open in ExDControl **Profiles** tab: one selected for use when the instrument is in **Total Ion Mode** (MS1) and one for **Isolation Mode** (MS2). As the instrument changes quadrupole states, the ExD cell will automatically switch between profiles, enabling different experiments.

Table 3. Experiments may use different ExD cell operating modes in MS1 and MS2.

Experiment	MS1 Profile Tuned For	MS2 Profile Tuned For
MS/MS ECD	Transmission	ECD
Intact Mass, MS/MS CID, etc.	Transmission	Transmission

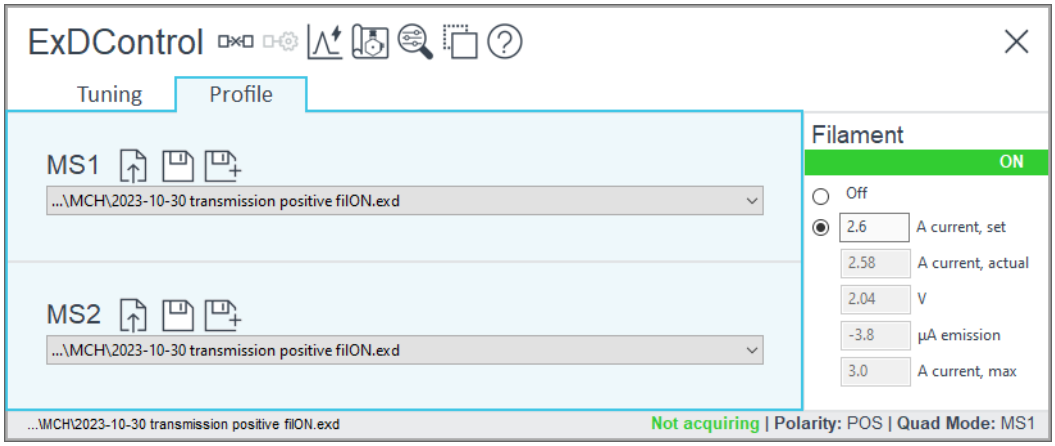


Figure 9. Tunefiles selected for use in MS1 and MS2 in the ExDControl **Profile** tab.

Before Operation

CAUTION

All components must be installed by trained personnel prior to their use.

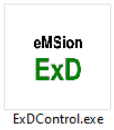
1. Ensure that the ExD Controller is ON. The ExD Controller should always be ON, except during maintenance.

   To turn power ON, hold down the **ON/OFF** button on the back panel of the ExD Controller until the front LCD lights up.

Figure 10. The ExD Controller turned ON.

Figure 11. The ExD Controller turned OFF.

2. Open the ExDControl software. In the Windows Start Menu of the instrument PC, click **E-MSION35** > **ExDControl36**.



   Or go to **C:\Program Files\ExDControl36** and double-click **ExDControl36.exe**.

   ExDControl will automatically connect to the instrument and ExD Controller. When ExDControl is connected, the connection status indicator will display readouts of the instrument state.

   If the software is disconnected, click the  **Connect** menu icon. If the connection fails, see **To resolve ExDControl connection issues.**

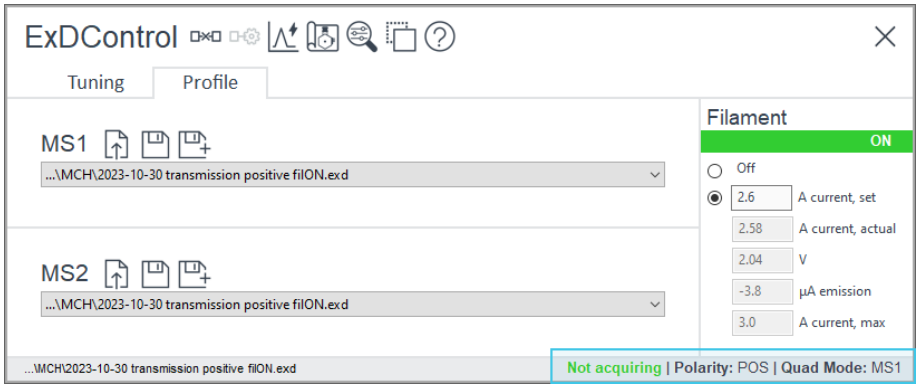


Figure 12. Connection status indicator in the bottom right when ExDControl is connected.

Shutting Down

WARNING

Even when the instrument is disconnected from power, if the ExD Controller is ON, hazardous voltages can exist in the wiring between the ExD Controller D-sub cable and the ExD cell.

WARNING

When the ExD Controller is plugged into a power supply, even if the power switch is OFF, hazardous voltages can exist in wiring between the ExD Controller, the power supply, and the power switch.

Before shutting down the instrument,

1. Ensure the filament is OFF in the ExDControl **Filament** panel. Wait until the **act** readout is 0 A.

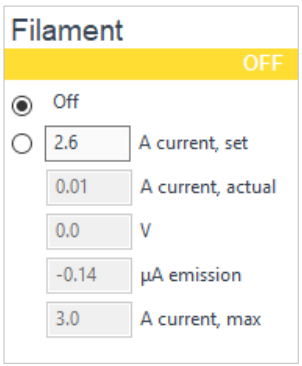


Figure 23. Filament OFF.

CAUTION

Ensure that the ExD cell filament current is 0 A before venting the instrument. The heated filament will fail abruptly after exposure to oxygen and may contaminate neighboring parts.

CAUTION

Turn the filament OFF before turning the ExD Controller OFF. The ExD Controller uses a current ramp rate to prevent damage to the filament from rapid cooling.

2. Turn ExD Controller OFF. Press the power button on the back of the Controller until the front LCD turns black.
3. Disconnect the ExD Controller power cord.
4. Continue with the standard instrument shut down process. See the Agilent LC/Q-TOF Maintenance App for guidance.

Daily Use

CAUTION

The ExD cell must always be tuned properly to avoid a loss in sensitivity.

At the start of each day,

1. Set the filament appropriately for the instrument state. Refer to **Table 2**. Allow about 20 minutes for thermal stabilization.
2. In the ExDControl Profile tab, load profiles for MS1 and MS2. See **To set default profiles** for a starting point for instrument and ExD cell settings.

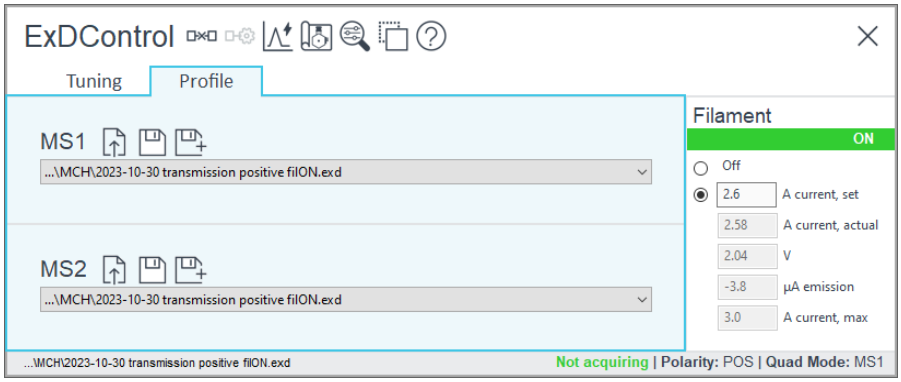


Figure 13. Tunefiles selected for use in MS1 and MS2 in the ExDControl **Profile** tab.

NOTE

For best performance, profiles must be used in the instrument quadrupole state they were tuned in. A profile tuned for transmission in MS1 will not yield optimal transmission in MS2.

3. Check performance of the profiles. Use standard samples to compare performance day-to-day. See **Tuning Standards** for recommended standards.
4. If necessary, retune ExD cell profiles. For best performance, profiles should be retuned daily, whenever instrument ion optics preceding the ExD cell change, or whenever performance drifts. See **To autotune the ExD cell**.
5. For guidance on running MassHunter instrument tunes (e.g., Transmission Tune, System Tune, etc.) see **To run MassHunter instrument tunes with the ExD cell installed**.
6. Review guidance in **Acquisition** before running an ExD experiment.

To set default profiles

Use the instrument and ExD cell settings in **Table 4** and **Table 5** as a "default" starting point for ExD autotunes.

Table 4. Default instrument ion optics settings.

MH Parameter	Setpoint	
Oct1DC	36	V
Lens1	34.5	V
QuadDC	33	V
PostFilterDC	32.5	V
HexDC	21	V
HexDelta	-6	V
Hex2DC	13.5	V
Hex2DV	-1	V
Collison Cell Gas Pressure	24	psi

Table 5. Default ExD cell profiles for Transmission (MS1), Transmission (MS2), and ECD (MS2).

MH Parameter	L1	L2	LM3	L4	FB	LM5	L6	L7
Transmission (MS1):	18	26.5	26.5	29	23.5	26	24	22
Transmission (MS2):	34	0	32	33	28	32	15	26
ECD (MS2)	35	5	38	40	33.8	36.4	20	26

To autotune the ExD cell

Autotuning in ExDControl adjusts the ExD cell lens profile to maximize abundances of a user-selected mass list. Selecting a **Parent** mass list will tune the cell for transmission while selecting a **Fragments** mass list will tune the cell for ECD.

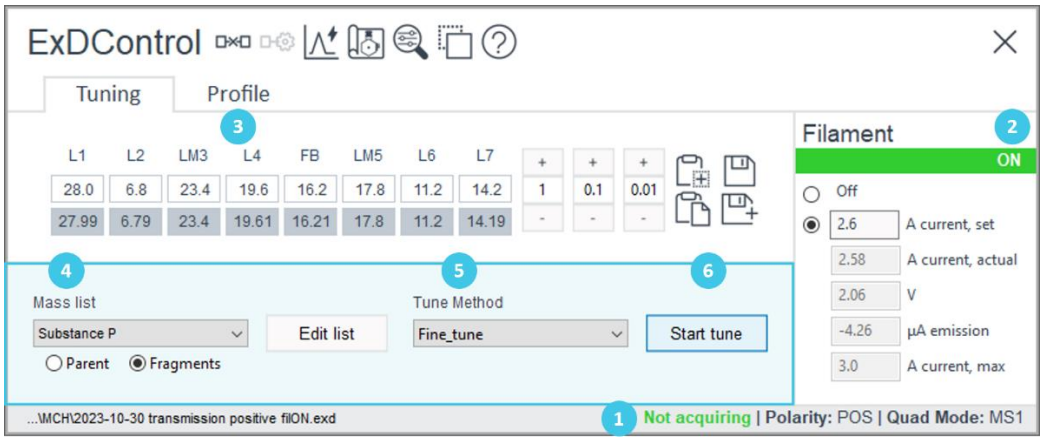


Figure 15. ExDControl Autotune field.

1. Ensure the instrument is in the appropriate quadrupole state. To tune the lens profile for use in MS1, put the instrument in **Total Ion Mode**. To tune the lens profile for use in MS2, put the instrument in **Isolation Mode** and isolate the precursor *m/z*. Use a **Wide** window, if possible, to increase signal.
2. Ensure the filament is in the appropriate state. Refer to **Table 2**.
3. Choose which ExD tunefile to autotune in the ExDControl **Profiles** tab. Autotune will use the chosen lens profile as a starting point. To begin from a default lens profile, see **To set default profiles**.
4. Infuse the sample and ensure the signal is stable. See **Tuning Standards** for guidance on choosing a standard to use to tune the ExD cell.

   In ExDControl, select the sample and the mass list to tune for:

   - **Parent** — **Transmission.** Optimizes parent ion intensities.
   - **Fragments** — **ECD.** Optimizes fragment ion intensities. Can only be selected if the filament is ON and the selected mass list has fragment ions defined.

NOTE

You will need to create a custom autotune sample to use to tune for transmission in MS2. Include only the *m/z* precursor being isolated in the parent mass list (e.g., *m/z* 922 for Agilent tuning mix in Positive Mode). See To add/edit autotune samples for guidance.

5. Select the autotune method:
   - **Coarse** — Ramps lens voltages over a wide range centered around the profile that is active when autotune begins.

- **Fine** Ramps lens voltages over a narrow range, centered around the profile that is active when autotune begins.
- **Extended** A coarse tune followed by a fine tune.
- **Filament** Applies a special profile while adjusting filament current to find the threshold for emission.

4. Click **Start Tune**. A window will appear to show progress.

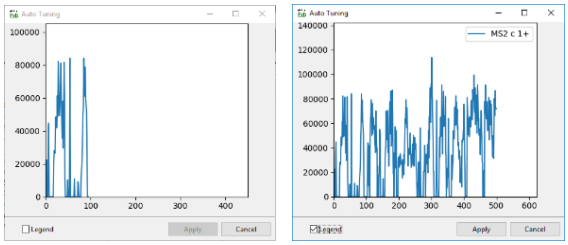
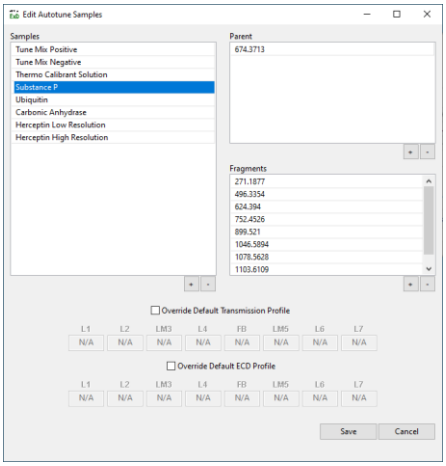


Figure 16. The **Auto Tuning** window plots a graph of the selected mass list *m/z* intensities vs. scans.

5. Once the tune is complete (5-10 minutes), click **Apply** to accept the profile generated. Click **Cancel** to revert all lens voltages to their previous values.

To add/edit autotune samples



Autotune samples in ExDControl have user-defined mass lists for parent and/or fragment ions. Autotune adjusts ExD cell lens voltages to maximize the intensity of either the parent or fragment mass list *m/z* values, depending on which option is selected.

Figure 17. ExDControl **Edit Autotune Samples** window. Several tuning standards are included by default.

To add a new sample or edit a sample mass list:

1. In the ExDControl **Tuning** tab, click **Edit list** to open the **Edit Autotune Samples** window.
2. Use the **+/-** buttons below the panels to add or remove items from the **Samples** list or the **Parent** and **Fragments** mass lists for each sample.

   The parent mass list should only include precursor *m/z* values. The fragment mass list should only include *m/z* values for ECD fragment ions (*c*- or *z*-ions).

NOTE

For best results, only include ECD fragment ions that are produced efficiently in the Fragments mass list.

To optimize the filament current

CAUTION

Rapid changes to the current (heat-cycling) will shorten filament lifespan.

The current applied to the filament must heat the wire to produce a sufficient level of electron emission for ExD to occur. As the filament ages, the current will need to be raised to compensate for changes in resistance due to material evaporation.

Table 6. Filament insert models with recommended starting and maximum heating current.

Filament Insert (p/n)	Starting Current	Will Not Typically Exceed
11146	2.4 A	2.7 A

To adjust the filament current to increase ECD efficiency,

1. Infuse your sample, or a tuning standard with known ECD fragment ions, e.g., **Substance P**.
2. Ensure a working ECD lens profile is open in ExDControl.
3. Beginning from the recommended starting current (Table 6), increase the **set** current in the **Filament** panel in small (0.05 A) steps while monitoring signal intensity of ECD fragment ions. When returns in signal intensity begin to diminish, stop increasing the current. Use the lowest current setpoint that yields satisfactory ECD to extend the filament lifespan.

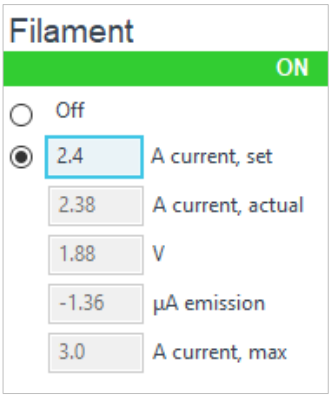


Figure 18. ExDControl **Filament** panel with the filament **On** and set to 2.4 A.

NOTE

A refine autotune is recommended after changing the filament current. Lenses FB and L4 are particularly sensitive to changes in electron emission.

4. Check that the filament is not overheated. Rhenium evaporating from the filament (*m/z* 184.9530 and 186.9558) should not exceed ~2.5e5 counts.
5. Check for filament exposure to oxygen. A high proportion of rhenium oxide (*m/z* 200.9479, 202.9507; 216.9428, 218.9456; 232.9378, 234.9405; 248.9326, 250.9354) relative to rhenium in the spectrum indicates accelerated filament aging. See **Troubleshooting Table**.

To run MassHunter instrument tunes with the ExD cell installed

MassHunter instrument tunes may still be used with the ExD cell installed. However, the ExD cell is tuned independently from MassHunter, in ExDControl.

Before beginning a MassHunter instrument tune:

1. Ensure that the ExD cell is properly tuned for transmission in MS1 and MS2. Low sensitivity due to poor transmission through the ExD cell will prevent the MassHunter tune from converging on optimized settings.
2. Select the **Tune using current parameters** checkbox in the MassHunter **Tune** context **Preferences** tab and click **Apply**.

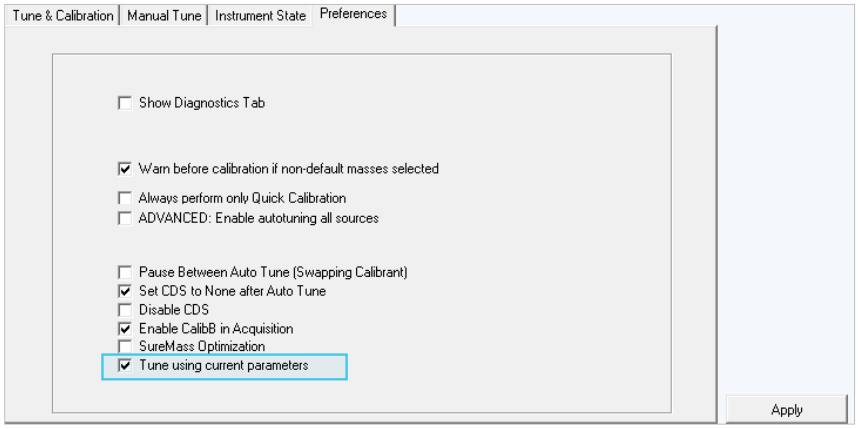
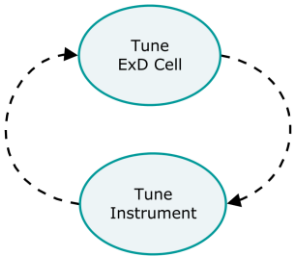


Figure 14. Tune using current parameters selected in the MassHunter **Preferences** tab.

By selecting **Tune using current parameters**, the MassHunter tune will begin from current instrument settings instead of from default settings; this is important to do because the ExD cell profiles are tuned to the current instrument settings, not the default ones.

NOTE



Both the instrument tune and the ExD cell tune affect sensitivity. Tuning to increase signal intensity with one while the other is poorly tuned will produce a local maximum rather than a global maximum.

The instrument optics parameters in the MassHunter Manual Tune tab define important characteristics of the ion beam. Retune the ExD cell after these settings change.

Advanced Use

To manually tune the ExD cell

The ExD cell lens profile can be manually adjusted in the ExDControl **Tuning** tab.

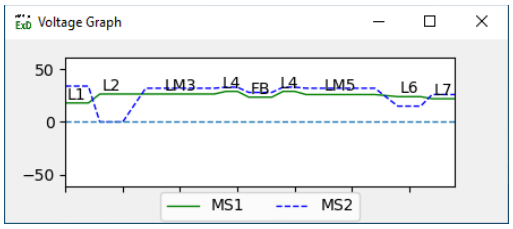


Figure 19. ExDControl **Voltage Graph** shows selected profiles tuned for transmission in MS1 (green line) and transmission in MS2 (blue line).

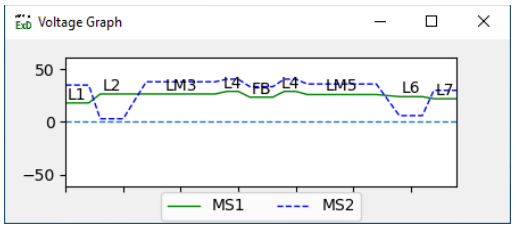


Figure 20. ExDControl **Voltage Graph** shows selected profiles tuned for transmission in MS1 (green line) and ECD in MS2 (blue line).

Use large steps (1 V) to find the limits of working voltages for a lens followed by small steps (0.1 V) to find the optimum within the range. Inner lenses (FB, L4, LM3, LM5) are more sensitive to changes in voltage than outer lenses (L1, L2, L6, L7).

Follow the sequence below to manually tune the lens profile for transmission or ECD on an infused tuning standard or analyte. Use the MassHunter **Tune** context to view *m/z* signal intensities for precursor or product ions.

1. Ensure source parameters are optimized for the infused tuning standard or analyte and signal is stable.
2. Hold **Shift** and click to select L2, LM3, L4, FB, LM5, L6, and L7 together. Adjust these lenses in unison to maximize signal intensity.

   For Q-TOF models excluding the 6560, **Oct1DC** voltage defines ion energy. With the filament OFF, the ExD cell lens profile will not exceed **Oct1DC**. With the filament ON, the lens profile will not exceed **Oct1DC** + ~10 V. Voltages should be set higher with the filament ON to compensate for negative electron charge.
3. Adjust FB and L4. For transmission profiles, L4 is usually greater than FB by 0.5-5 V. For ECD profiles, L4 is usually greater than FB by 5-10 V.
4. Adjust LM3 and LM5 separately and then in unison. LM3 and LM5 are usually greater than FB.
5. Adjust L2 and L6 separately. For profiles tuned in MS2, lowering L2 tends to improve transmission of the isolated precursor.
6. Adjust L1 and L7 separately in large steps.
7. If tuning for ECD, do the following to maximize ECD fragment ion intensity:
   a. Adjust LM3, L4, FB, and LM5 in unison in small steps.

b. Optimize the difference between L4 and FB. Increasing the ΔV roughly corresponds to increasing electron energy.

8. Repeat the steps above, making fine adjustments until satisfied with transmission or ECD performance.

NOTE **To test the effectiveness of a transmission tune profile, add 5-10 V of collision energy in MassHunter. If transmission dramatically increases with the addition of collision energy, then the ExD profile is not fully optimized.**

NOTE **When tuning for ECD on amidated substance P, first tune the ExD cell in MS2 to achieve signal intensity of roughly 1 million counts for the isolated *m/z* 674.3713 (M+2H)2+ precursor. Then, tune to maximize intensity of the *m/z* 624.3940 c5+ product ion.**

To add collision energy to an ECD profile

When characterizing peptides or proteins by top or middle-down ECD MS/MS methods, sequence coverage may be limited by noncovalent interactions in the gas-phase ion structure, which prevent backbone fragments from dissociating. This phenomenon is known as electron capture without dissociation (EC-noD).

Adding supplemental collision energy (CE) can disrupt noncovalent interactions, reducing EC-noD and increasing sequence coverage. To add CE to a ECD lens profile,

1. Set **Collision Energy** in the MassHunter **Tune** context **Manual Tune** > **Quad** tab and click **Apply**.

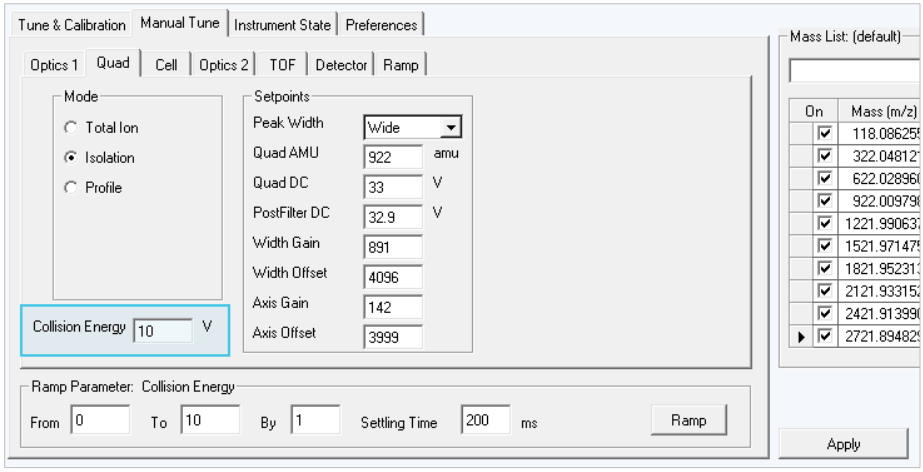


Figure 21. Above, 10 V of collision energy set in MassHunter Tune context.

2. Adjust the ECD lens profile by adding the same amount of voltage to each lens. In the ExDControl **Tuning** tab, hold **Shift** and click to select all lenses together before using the + buttons to raise the entire lens profile.

3. It may be worth refining the ExD profile after raising all voltages.

4. After adjusting the ExD profile for use with collision energy in the **Tune Context**, you will need ensure the methods used with this ExD profile while in the **Acquisition Context** use a similar amount of collision energy.

NOTE

Q-TOF instruments add collision energy by raising the voltages of the instrument optics preceding the collision cell. Electron capture is most efficient when electron energy relative to ions is low. By also raising the ExD cell lens profile voltages, the drop in potential that accelerates ions into the collision cell happens after the ExD cell, rather than before.

To alter the collision cell gas pressure

During installation, the instrument collision cell gas is set to produce typical CID MS/MS fragmentation. When the ExD Option is added, the pressure is adjusted to account for the shortened length of the collision cell. See **Table 7** for typical pressure ranges.

For certain analytes, raising or lowering the collision cell gas pressure may increase ECD efficiency. To alter the collision cell gas pressure,

1. In the MassHunter **Tune** context **Manual Tune** > **Cell** tab, enter a new psi value in the **Collision Cell Gas Set** field, and press **Enter** to apply.

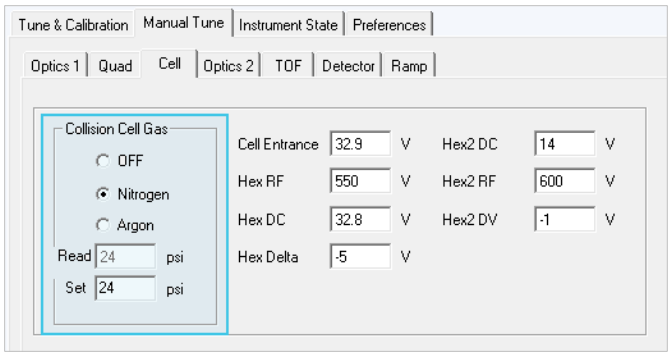


Figure 22. Above, 24 psi collision cell gas pressure set in MassHunter Tune context.

Table 7. Typical collision cell nitrogen gas pressures for LC/Q-TOF instruments without the ExD Option and with the ExD Option. The pressure is set during installation and adjusted as needed.

Instrument without ExD Option	Instrument with ExD Option
20-22 psi	22-24 psi

CAUTION

Collision cell gas pressure affects CID fragmentation patterns. Reset the collision cell gas pressure to the setting established during ExD cell installation before resuming CID MS/MS work.

Tuning Standards

WARNING

Wear personal protective equipment when handling chemicals.

Use standards to tune ExD cell profiles when experimental samples are quantity-limited or low-concentration. Profiles tuned on a standard may be used for experimental samples with similar mass, charge, and composition.

Table 8. Recommended ExD cell tuning standards.

Standard	Use to Tune the ExD Cell For	CAS or Catalog Number
Agilent Tuning Mix	Transmission	G1969-85000
Substance P, amidated	ECD of peptides and small proteins (< ~8 kDa)	33507-63-0
Ubiquitin, cytochrome *c*, carbonic anhydrase	ECD of proteins (> ~8 kDa)	multiple

To prepare substance P

Amidated substance P (1.3 kDa) is used to tune the ExD cell for ECD of peptides and small proteins.

The peptide sequence is **R P K P Q Q F F G L M - NH_2**.

Materials

- Substance P, amidated
- Water, LCMS-grade
- Methanol, LCMS-grade
- Formic acid, high purity

1. Allow the closed container to equilibrate to room temperature before weighing to reduce moisture uptake.
2. Prepare in 50/50/0.1 (% v/v/v) methanol/water/formic acid to a final concentration of 10 µg/mL, appropriate for Agilent Dual AJS ESI. When dissolving in solution, *gently* mix to avoid oxidation.
3. Avoid repeated freeze-thaw cycles in solution.

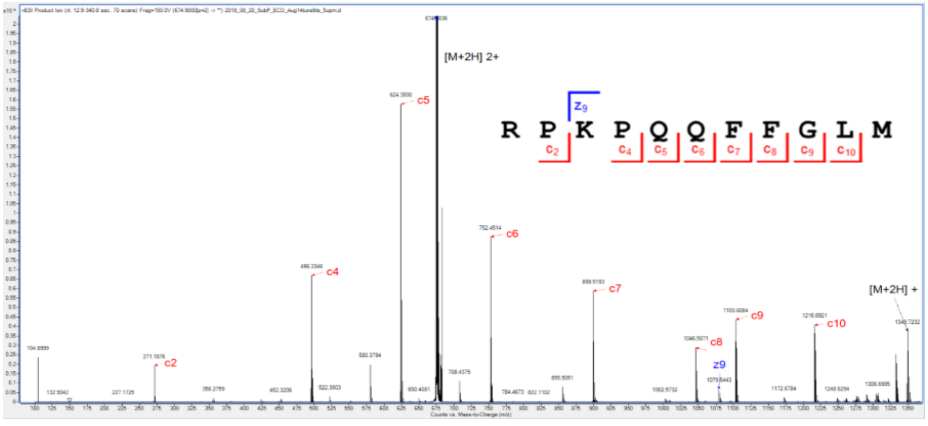


Figure 24. Substance P (amidated) product ion spectrum collected from the $[M+2H]2^+$ precursor ion at *m/z* 674 during a targeted MS/MS-ECD experiment.

To set up direct infusion

CAUTION

The nebulizer needle and tip may require cleaning after infusing large amounts of tuning standards.

Parts

- Syringe pump
- Clean syringe (250 µL minimum volume)
- Clean PEEK™ tubing (~2 ft, 1/16” OD x 0.005” ID) or similar for infusion line
- Needle sheath
- Three compatible fingertight fittings
- Compatible zero dead volume LC union

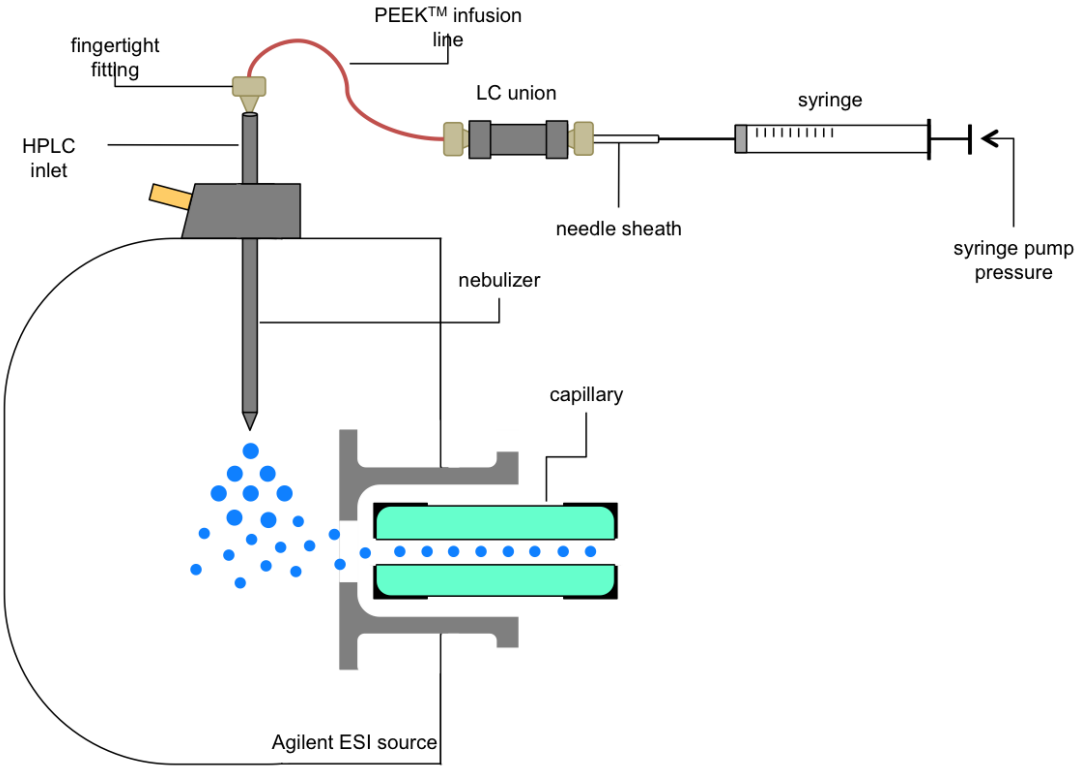


Figure 25. Direct infusion set up.

1. Fill a clean syringe with tuning standard and install in the syringe pump.
2. Assemble the infusion line as shown above. Use parts from the loop infusion kit, if needed.
3. Begin infusion. For substance P at 10 µg/mL concentration, infuse at a rate of 300 µL/hr.

PRO	Stable sample flow. Flow rate can be changed.
CON	The syringe will require refills. Before starting an autotune, ensure the syringe is full.

To set up loop infusion

The loop infusion method uses back-pressure from Calibrant Delivery System (CDS) Bottle A to flow a pre-loaded amount of substance P through loops of PEEK tubing inserted between the line to Bottle A and the reference nebulizer.

NOTE

If using a Dual ESI Source with gas splitter, make sure the Reference Nebulizer Gas valve is open when infusing from Bottle A.

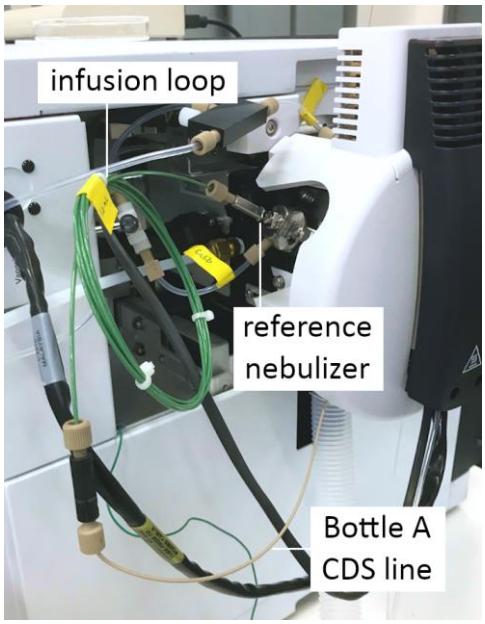


Figure 26. Loop infusion set up.

Parts

- Clean syringe (22 gauge, minimum 1 mL volume)
- Loop infusion kit (ships with the ExD Option):
  - 1 mL volume infusion loop (PEEK™, 1/16” OD x 0.030” ID)
  - Three fingertight fittings (natural)
  - Needle sheath (PTFE, 1/16” OD x 0.030 ID)
  - Zero dead volume LC union (black)

1. Prepare a clean CDS bottle of 50 mL of 50/50 (v/v) acetonitrile/water and install on port A of the CDS.
2. Use the syringe and the needle sheath to fill the infusion loop with substance P solution until it overflows (~1.2 mL).
3. Remove the needle sheath fitting from the infusion loop.
4. Disconnect the CDS line to the reference nebulizer and reconnect the fingertight fitting to the LC union on the infusion loop.
5. Connect the fingertight fitting on the other end of the infusion loop to the reference nebulizer.
6. In the MassHunter Tune Context, click the button for **Calibrant Bottle A** in the lower left corner of the page to start infusion.

PRO	Stable sample flow over a long period of time.
CON	The loop will require a refill after ~1 hr of use at the Bottle A flow rate.

3. Data Acquisition and Analysis

Acquisition

Follow the guidance below when setting up an ECD experiment:

- Document the ExD cell settings used for your sample runs and worklists. ExD cell settings are not recorded by MassHunter acquisition software.
- ECD requires a minimum precursor charge state of 2+ in positive ion mode because electron capture neutralizes one charge. Fragmentation efficiency will roughly increase with the square of precursor charge state.

NOTE

Electron Induced Dissociation (EID) may be used on singly charged precursors. See Concepts. However, the ExD cell can only be tuned for EID manually.

- The addition of collision energy after ExD may improve ECD of tightly-folded structures. See **To add collision energy to an ExD profile**.
- Plan to average ECD spectra to improve data quality. Prioritize high-value peaks with the **Auto MS/MS Preferred List.** Or, add duplicate targets to a **Targeted MS/MS** isolation list.
- Because the **Auto MS/MS** decision engine was developed for use with CID, it often cycles through the isolation list too quickly to obtain high quality ECD data. Use **Targeted MS/MS** if retention times and/or masses are known.
- Work around the quadrupole isolation range limits by acquiring pseudo-MS1 ECD spectra with the **Quad amu** set to just below the high *m/z* precursor of interest acting as a filter. This enables performing ECD on multiple precursor charge states, improving product ion signal.

NOTE

The Quad amu setting must be saved as part of the MassHunter tune file before switching to the Acquisition context.

Analysis

ExD data recorded by Agilent LC/Q-TOF instruments will be in *.d format. When analyzing ExD data, keep in mind the following:

- Data files will not identify the ion activation method used as ExD.
- ExD product ion peaks are usually lower in intensity than CID peaks. Peaks with only a few hundred or thousand counts are common and can be considered legitimate if mass error, isotopic envelope shape, and signal-to-noise are reasonable.
- ExD is often accompanied by hydrogen rearrangement to/from product ions.

ExDViewer

ExDViewer enables rapid and easy processing of ExD and CID data. The software:

- Accepts multiple MS vendor and vendor-agnostic data file formats.
- Deconvolutes isotopically resolved ions.
- For defined targets, annotates all protein fragment ion types — b, y, c, z, a, x, including side chain fragmentation which distinguishes isobaric amino acids leucine/isoleucine and aspartate/isoaspartate.
- Displays raw and deconvoluted spectral views, sequence coverage map, and peaks tables for investigating assignments.

Visit e-msion.com/exdviewer/ for more information. Access the user guide at e-msion.com/exdviewer-learning/.

Third-party tools for top- and middle-down analysis

ExD is uniquely suited to top and middle-down targeted characterization of peptides, proteins, and protein complexes. Bioinformatics tools for these workflows are still evolving, but several options are currently available.

Table 5. Suggested software options for ExD data processing and analysis.

Software	Notes	Availability	Source
ProSite Lite *Northwestern University*	• Well-known industry standard for analyzing electron-based fragmentation data sets • Requires deconvoluted data	Free	Fellers et. al., 2015.
UniDec *University of Oxford; Arizona*	• Universal deconvolution of mass and ion mobility spectra	Free	Marty et. al., 2015.
LcMsSpectator *Pacific Northwest National Laboratories*	• Spectrum annotation for a wide range of fragment types • Not meant for complex spectra analysis	Free	Park et. al., 2017.
MASH Explorer *University of Wisconsin-Madison*	• Under development • Profile data deconvolution and fragment assignment	Free	Cai et. al., 2016.
ProteinProspector MS-Product *UCSF MS Facility*	• Use to generate theoretical fragment ion *m/z* values from an amino acid sequence	Free	Baker, P.R. and Clauser, K.R.
Protein Metrics Product Suite *Protein Metrics Inc.*	• Comprehensive • Well-known industry standard	Commercial	

4. Maintenance

WARNING **Apart from replacing the filament, the ExD cell is not user-serviceable. Powerful magnets inside the ExD cell can disrupt medical pacemakers and create a crush hazard by attracting small objects. Contact your Agilent representative for assistance.**

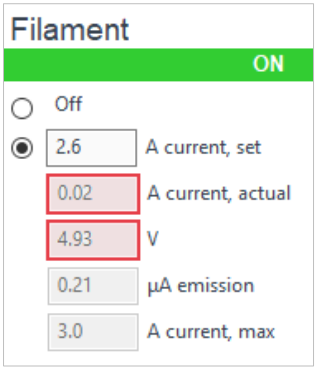
WARNING **The ExD Controller is not user-serviceable. Do not remove covers. Hazardous voltages may be present. Contact your Agilent representative for assistance.**

CAUTION **Damages to ExD Option or instrument components sustained during user maintenance are not covered under warranty.**

NOTE **Contact e-MSion with questions or to order replacement parts. See Contact Us.**

To check for filament burnout

CAUTION **If multiple filaments rapidly fail, check for exposure to oxygen. See Troubleshooting Table.**



Eventually the filament will fail or "burnout" from routine use. When the filament current setpoint is > 0 A, symptoms of burnout are:

- Filament current actual approaches 0 A.
- Filament voltage drop approaches 5 V maximum.

Ensure that the D-sub cable is connected to the ExD Controller and to the vacuum feedthrough to the ExD cell. D-sub cable damage or disconnection will mimic symptoms of filament failure.

Figure 27. ExDControl **Filament** panel showing symptoms of filament burnout.

To resolve, replace the filament insert. See **To replace the filament**.

To check for filament current leakage

Occasionally, material deposited in the ExD cell may cause current leakage between the filament insert and the protective cassette housing. To avoid this issue, always clean the filament cassette before reusing.

The symptom of conductivity caused by material deposition is:

- With the filament OFF, setting the voltage difference between FB and L4 to ~40 V causes a change in the emission current readout in the ExDControl **Filament** panel.

To resolve, replace the filament cassette and insert. See **To replace the filament.**

To replace the filament

CAUTION

Filament replacement should only be performed by trained individuals. Contact your Agilent representative for assistance if needed.

The following steps describe how to replace the filament insert in the ExD cell after burnout. This is the most common maintenance task for the ExD cell.

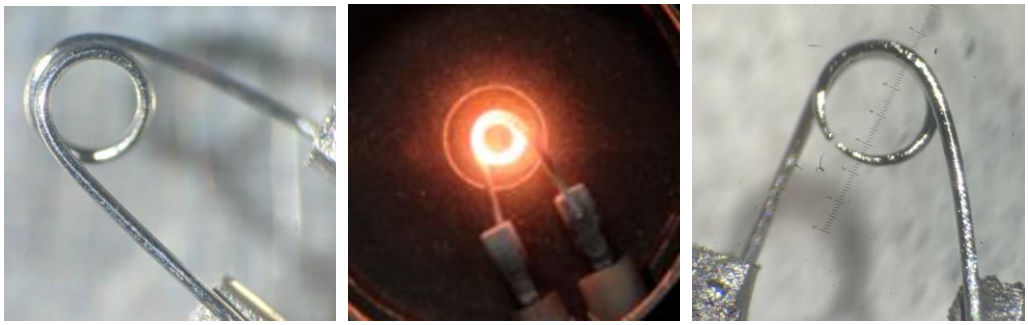


Figure 28. Close-ups of a new filament (left), a filament heated to the threshold of thermionic emission (middle), and a filament that has burned out after routine use (right).

Parts

- Filament insert (e-MSion p/n 11146)
- Filament cassette (e-MSion p/n 10966)

Tools

- Screwdriver, Phillips, 00
- Screwdriver, TORX, T6
- Lint-free cloth (Agilent p/n 05980-60051)
- Digital multimeter

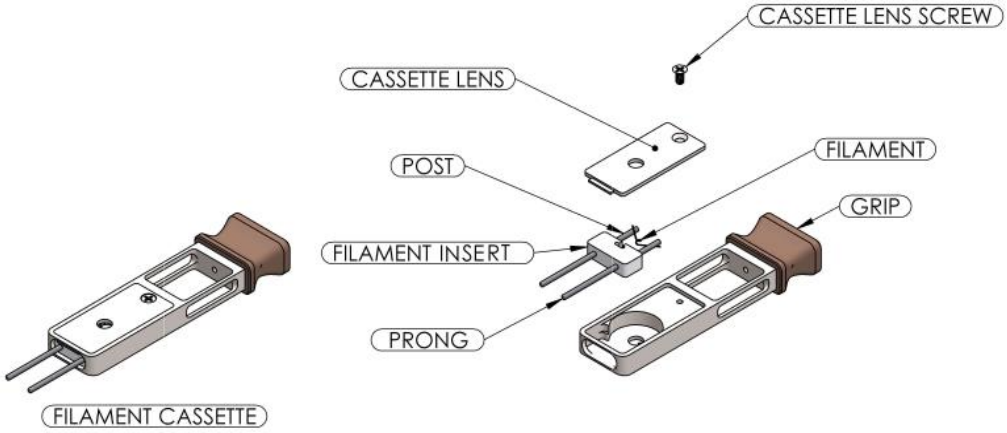


Figure 29. Filament cassette assembly (e-MSion p/n 10960) includes filament insert housed inside the filament cassette.

Step 1. Inspect the replacement filament insert

CAUTION

To avoid damage or contamination to the filament wire, hold the filament insert by its long metal contact prongs or ceramic body and always wear gloves. Avoid touching the wire loop and legs.

Replacement filament inserts are delivered in packaging that protects the components from physical damage or moisture intrusion.

1. Unpack the replacement filament insert.
2. Use a magnifier to inspect the filament wire loop.

   - The filament wire surface should appear smooth (no pitting).
   - The wire should form an unbroken loop securely attached on either end to the filament posts.
   - The wire loop should be centered between the filament posts.

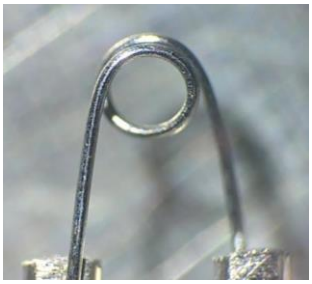


Figure 30. Filament wire loop.

Step 2. Prepare the replacement filament cassette

1. Obtain a spare filament cassette.
2. Use the 00 Phillips screwdriver to remove the cassette lens screw and the cassette lens.

   Check the interior and exterior of the cassette for dirtiness. If necessary, clean the cassette by swabbing with aluminum oxide or sonicating in 50% methanol. If the cassette is still dirty, use a micro fiberglass brush to scrape inner surfaces clean then sonicate in 50% methanol.

NOTE

Heat discoloration on the filament cassette does not impact function. Carbonization or other contamination, however, must be removed before use.

3. Without touching the filament wire, slide the filament insert prongs through the opening in the base of the cassette.

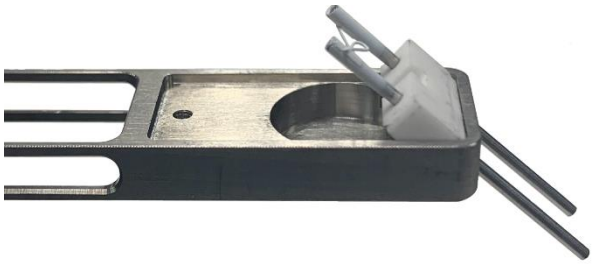


Figure 31. Sliding the filament insert into the filament cassette.

4. Use the filament prongs to maneuver the filament insert into place in the cassette. The ceramic body of the filament insert should be flush with the cassette and the wire loop should be concentric with the cassette aperture.

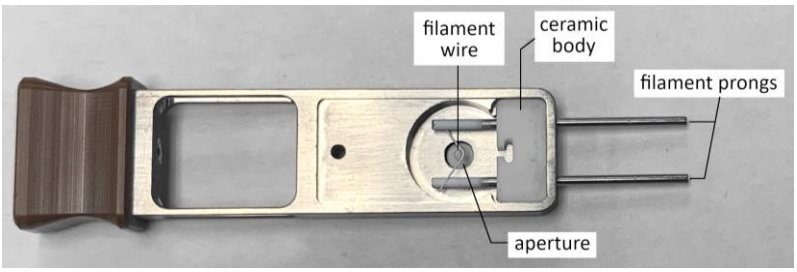


Figure 32. The filament insert inside the filament cassette.

5. Replace the cassette lens and cassette lens screw.

Step 2. Shut down the system

WARNING

The system is not safe for filament replacement until all power cords are disconnected from the instrument and ExD Controller.

1. Follow instructions in **Shutting Down**.
2. To access the vacuum manifold cover, remove the front and top cosmetic covers on the instrument. Instrument-specific instructions may be found in the Agilent Maintenance Guide.
3. Unplug the D-Sub cable from the manifold cover vacuum feedthrough.

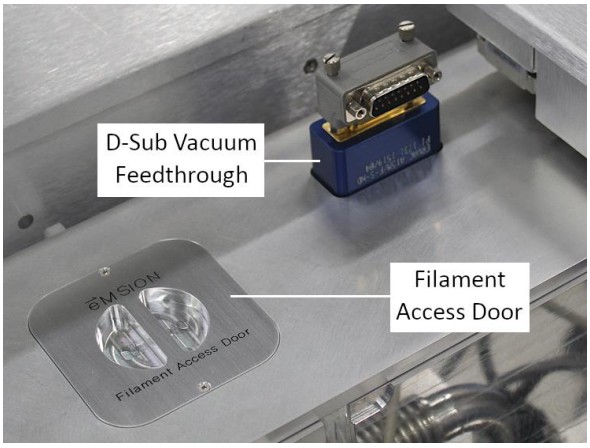


Figure 33. The manifold cover with filament access door.

Step 3. Replace the filament cassette assembly

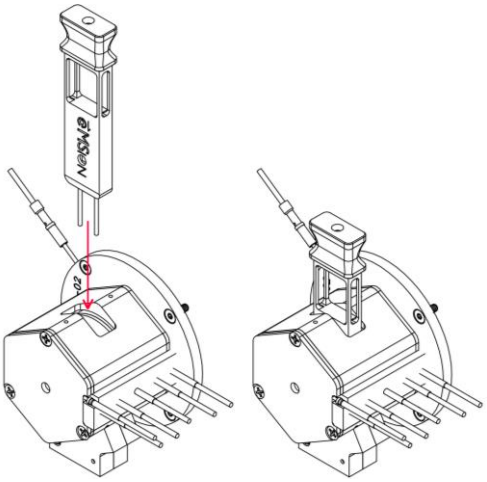
WARNING

The ExD cell filament operates at temperatures high enough to cause serious burns. Allow parts to cool and use the insulated grip when handling the filament cassette.

WARNING

While the filament access door is open, keep surrounding area clear. Loose parts that enter the instrument can obstruct the turbopumps and create ejected material and smoke inhalation hazards.

1. Once the instrument has vented, use a T6 screwdriver to remove the two filament access door screws. Lift the door and set aside. If the door does not lift easily, wait for the system to finish venting. Do not pry.



2. Remove the filament cassette assembly from the ExD cell by gently pulling the insulated grip upward. Place on a dust-free, heat-resistant surface.
3. Insert the replacement filament cassette assembly into the ExD cell. Hold the insulated grip and slide the prongs into the slot until you hear a click. The direction that the filament cassette faces has no impact.

Figure 34. The filament cassette assembly inserts into a slot in the ExD cell.

CAUTION

Do not remove or insert the filament cassette at an angle.

4. Test the resistance of the filament circuit across the D-Sub vacuum feedthrough with a multimeter. Resistance should read 0.1-1.0 Ω.

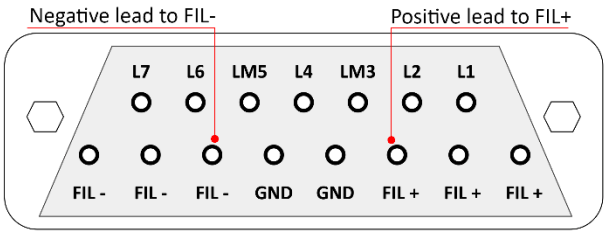


Figure 35. Male D-Sub pinout. Pins labeled with corresponding ExD Cell elements.

5. Reattach the D-Sub cable to the vacuum feedthrough.
6. Clean the filament access door O-ring with a lint-free wipe. If the O-ring is damaged, replace the O-ring.
7. Replace the filament access door and screws.

NOTE

Tighten the filament access door screws evenly, alternating between screws to promote even compression of the O-ring when the vacuum is reestablished.

Step 4. Restart the system

1. Replace the instrument covers.
2. Reconnect power cords to the instrument and ExD Controller.
3. Press the instrument manual power switch to restart the instrument.
4. Turn on the ExD Controller. Press and hold the **ON/OFF** button on the back panel of the Controller until the front LCD lights up.

Step 5. Turn the filament ON

CAUTION

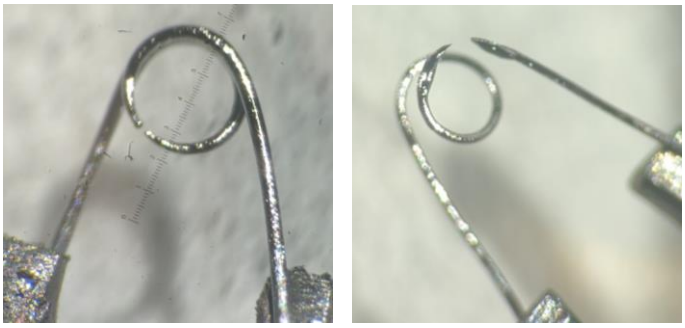
Keep the filament OFF until instrument vacuum is reestablished.

1. In the ExDControl software **Filament** panel, select ON.
2. Enter the recommended starting heating current (Table 6) and press **Enter** to apply.
3. Open the most recent profiles tuned for transmission in MS1 and MS2 in ExDControl. If signal intensity of Agilent tuning mix is not satisfactory, retune these profiles. See **To autotune the ExD cell**.
4. See **To optimize the filament current** to optimize the filament current setting for ECD.

NOTE

After you turn the filament ON, you may notice an atypical disparity between set and actual voltage readouts for FB and L4, as well as an unusually large 'Emission' readout caused by contaminants on the surface of the heated filament. After about 30 minutes of heating, these should burn off and all voltage and current readouts should return to normal.

To evaluate the cause of filament failure

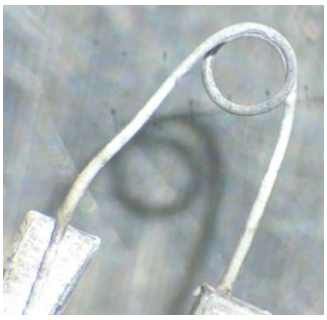


At the end of its design life, the filament will burn out from repeated heat-cycling and material loss. Overheating will cause the filament to burn out more quickly.

Figure 36. (Left) A filament that failed at the end of its design life from routine use. Note the slight thinning around the failure site. **(Right)** A relatively new filament that failed due to overheating; considerably less thinning around the failure site is observed.

If a filament rapidly fails after installation, check the wire for pitting and corrosion indicative of rapid oxidation.

Figure 37. Two filaments that failed because of impurities in the gas supply near the ExD Cell. The white residue is an experimental coating.



If ECD efficiency is consistently sub-standard after tuning, however, and none of the indicators of burn-out are present, the filament may have bent during installation or become warped after repeated use, causing the electron trajectories to no longer align with the ion flight path.

Figure 38. Example of a mechanically-damaged filament. The bent left leg pushes the loop off-center.

To resolve ExDControl connection issues

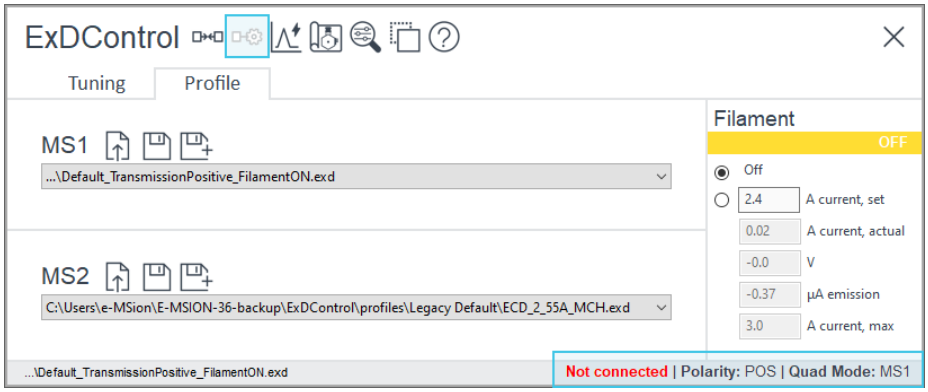
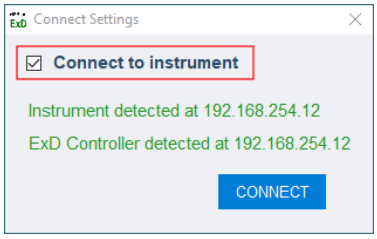


Figure 39. ExDControl connection status indicator showing **Not connected**. The connection settings menu icon is highlighted.



If ExDControl is unable to connect, click the **Connection Settings** menu icon. Connection settings should be the same as **Figure 40**.

Figure 40. ExDControl connection settings.

If connection settings are not the same as **Figure 40,**

1. Hold **Shift** + click the **Connect** button to open the connection settings configuration window (**Figure 41**).

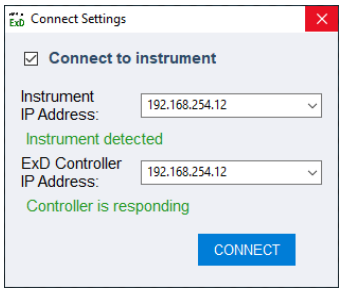


Figure 41. Configuring connection settings.

2. Enter **192.168.254.12** in both IP address fields. Be sure to press **Enter** after typing in each address.
3. Once the statuses update to “Instrument detected” and “Controller is responding,” click **Connect**.

To reboot the instrument SmartCard

1. Close ExDControl.
2. Put the instrument in **Standby** and close MassHunter.
3. In the Windows Start Menu, click **Agilent MassHunter LCMS Acq** > **Acq Tools**.
4. Double-click the **Remove MassHunter Processes** tool to end all MassHunter background processes.
5. Double-click the **Shutdown SmartCard** tool to run.
6. When prompted by the tool, press the power button on the instrument to turn OFF. Wait a few seconds, then press the power button again to turn the instrument ON.

CAUTION **Be sure to press the power button to turn the instrument ON again within the allotted time.**

7. Reopen MassHunter. Wait for the SmartCard to reestablish communication with the instrument PC. At this point, the instrument should "beep" and the MassHunter window will finish opening.
8. After MassHunter reopens, wait for any insufficient high vacuum errors to clear. During this time, the instrument should remain in **Standby**.
9. Reopen ExDControl. If ExDControl does not automatically connect, click the **Connect** menu icon. If the connection fails, see **To resolve ExDControl connection issues****.**

Troubleshooting Table

Use the table below to search for possible causes and corrective actions for issues with the ExD AQ-25x Option.

NOTE

Questions or need support? Contact Us.

Table 8. Guidance for troubleshooting issues with the ExD AQ-25x Option.

Problem	Potential Cause	Resolving Action
ExDControl cannot connect to instrument / ExD Controller or connection drops repeatedly during use.	Poor cable connection(s).	Ensure that ExD Controller cable connections are secure and correctly configured. Refer to the *ExD Controller User Guide*.
	Network issue.	Ensure instrument Ethernet switch is powered on and network is operational.
		See **To reboot the instrument SmartCard**.
	ExD Controller issue.	Verify ExD Controller is powered ON. Restart ExD Controller. See **Before Operation**.
	Software issue.	Restart ExDControl software. See **To resolve ExDControl connection issues**.
		Uninstall and reinstall ExDControl software. Check https://e-msion.com/downloads for a new version.
ExD cell lens voltage setpoints do not match actuals.	ExDControl software not connected.	Verify ExD Controller is powered ON and ExDControl software is connected.
	Poor cable connection(s).	Verify connectivity of all cables between ExD cell, ExD Controller, network switch, and instrument PC.
	ExD cell shorting or other hardware malfunction.	Another symptom of shorting within the ExD cell is increased ExD cell tune instability. Neither the ExD cell nor ExD Controller are user-serviceable. Contact your Agilent representative for assistance.
ExD cell filament is burning out rapidly.	Exposure to oxygen from gas supply.	Check collision cell gas purity (99.999%). N2 gas from an N2 generator may not be sufficiently pure.
		Consider adding an oxygen scrubber to the gas supply line. If oxygen scrubber is present, check its condition.
	Exposure to oxygen from atmosphere.	Ensure that instrument vacuum levels are normal. Filament must always be OFF when instrument vents.
CAUTION	If the filament fails abruptly due to exposure to oxygen, then the ExD cell may be contaminated with conductive debris. See **ExD cell shorting** above.	
	Filament is overheated.	Decrease filament current. See **To optimize the filament current.**
Filament current setpoint does not match actual.	Filament burnout.	See **To check for filament burnout**.
	D-sub cable disconnected.	Ensure D-sub cable is connected.
MassHunter Error: “Mainboard 2: collision cell hexapole DC 1 fault [162].”	ExD Cell tune is allowing electrons to escape and cause electrical shorts within the instrument.	Decrease L2 and L6 voltages to confine electrons within the ExD cell.

MassHunter Error: "Medusa" faults [39] and [40] and/or other errors.	Instrument vacuum not sufficient.	Following venting for maintenance, wait until instrument vacuum pressures to return to normal levels. If errors persist, contact your Agilent representative.
Poor sensitivity in MS1 and/or MS2.	Sample preparation.	Verify purity and concentration of all reagents.
	Acquisition method.	Verify source settings. If using LC, check flow rates, mobile phase composition, injection volumes, etc.
	LC or ion source needs maintenance.	Check for leaks/clogs.
		Verify temperature, flow actuals match setpoints.
		Ensure source parts are clean and positioned correctly.
	Instrument tune not compatible with ExD cell tune or not optimized for mass range of interest.	Load or reload a previously-working MassHunter tune file and ExD tunefiles. See **To set default profiles**.
		Retune the instrument. See **To run MassHunter instrument tunes with the ExD cell installed**.
	ExD cell tune is poor.	Retune the ExD cell for transmission. See **To autotune the ExD cell**.
	Incorrect ExD cell filament state.	For example, filament is OFF when the ExD cell lens profile was tuned with filament ON.
	Charge buildup on ExD cell or other internal components.	To diagnose, switch to negative mode and back to positive. If signal is briefly restored but then decreases again, internal surfaces may be charging. Contact your Agilent representative.
No ECD or poor ECD efficiency.	ExD cell lens tune is poor.	Retune the ExD cell for transmission. See **To autotune the ExD cell**.
	Filament current is low.	See **To optimize the filament current**.
	Current leakage in filament circuit.	See **To check for filament current leakage**.
	Filament is damaged.	Mechanical damage to the filament shape can limit ECD efficiency. Vent and inspect filament.
	Filament burnout.	See **To check for filament burnout**.
	Filament circuit open.	If filament circuit resistance is infinite, check for the source of the open circuit. See **Step 3. Replace the filament cassette assembly** for guidance on measuring circuit resistance.

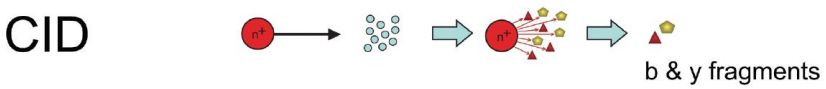
5. Concepts: Gas-Phase Fragmentation

Since the mass of an intact ion is often insufficient for unambiguous characterization, mass spectrometry methods often include the gas-phase fragmentation of precursor ions into characteristic product ions to provide more structural information.

Several methods for ion activation exist, each producing a distinct fragmentation pattern.

Collision Induced Dissociation

Collision induced dissociation (CID), the most common method of ion fragmentation in mass spectrometry, uses vibrational ion activation. Collisions between ions and inert gas molecules in the instrument result in the build-up of internal energy until the weakest bonds in the ion break, generating characteristic *b*- and *y*-ion fragments from polypeptides.



While CID is a robust and well-understood technique, it has limited utility for the study of large proteins and fragile molecules.

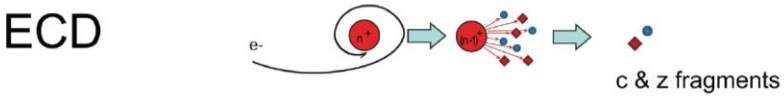
For protein characterization applications, CID removes labile motifs such as post-translational modifications (PTMs) as neutral losses, precluding PTM localization. Additionally, as protein size increases, sequence coverage using only CID decreases.

For glycoprotein characterization applications, CID typically generates product ions derived from glycosidic cleavages, which provide only sequence information without indicating linkage types or branching.

Electron-Based Dissociation

In contrast to CID, electron-based dissociation ("ExD") utilizes ion-electron reactions to achieve a range of fragmentation mechanisms.

Electron Capture Dissociation (ECD) involves the capture of low-energy electrons (< 1 eV) by multiply charged cation analytes. ECD is the principal fragmentation technique enabled by the ExD Cell.



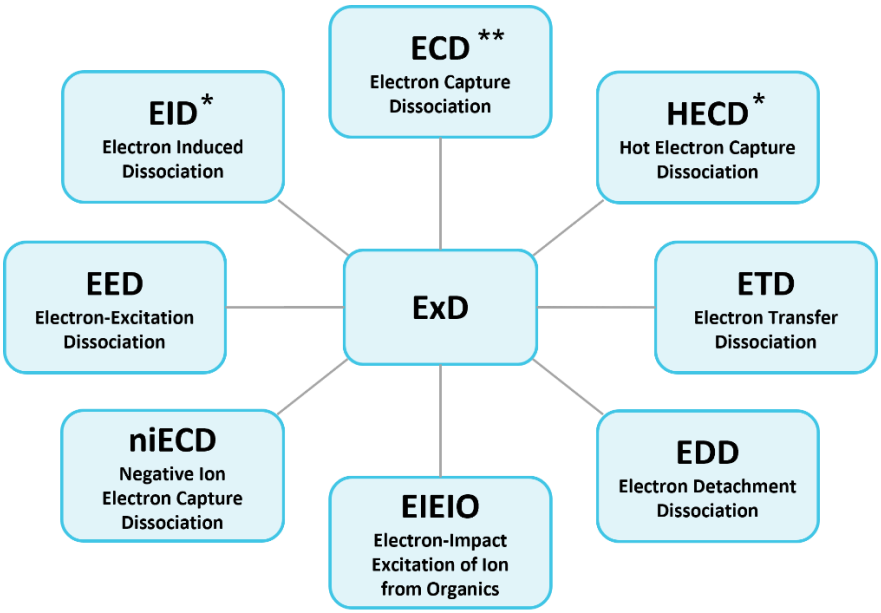


Figure 42. The ExD family of electron-based fragmentation methods. The primary fragmentation method for the ExD AQ-25x Option is ECD (**). The ExD Option is also capable of EID and HECD (*) although with lower efficiency.

ECD uniquely complements the existing CID capabilities of Agilent LC/Q-TOF mass spectrometers. Where CID preferentially cleaves C-N bonds in the peptide backbone to yield *b*- and *y*-ion fragments, ECD cleaves N-C_α bonds, yielding *c*- and *z*-ion fragments via the capture of low-energy electrons.

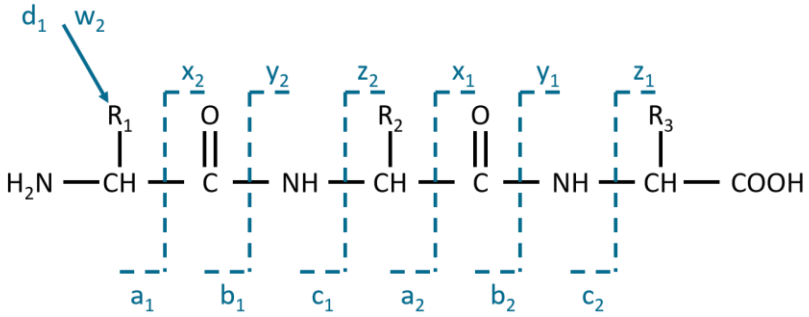


Figure 43. Product ions from peptide backbone and side-chain fragmentation. Peptide fragmentation nomenclature proposed by Roepstroff and Fohlman [Roepstroff, 1984] with adaptations from Biemann [Biemann, 1990].

In addition, ECD can produce secondary fragmentation of ions. *d* and *w* ions generated from side-chain losses are useful for confirming sequence assignment and distinguishing isobaric residues leucine/isoleucine. Another secondary fragmentation pathway can be used to distinguish aspartate/isoaspartate. The yield of secondary fragment ions can be increased with **hot ECD** (HECD), which uses higher-energy electrons than ECD.

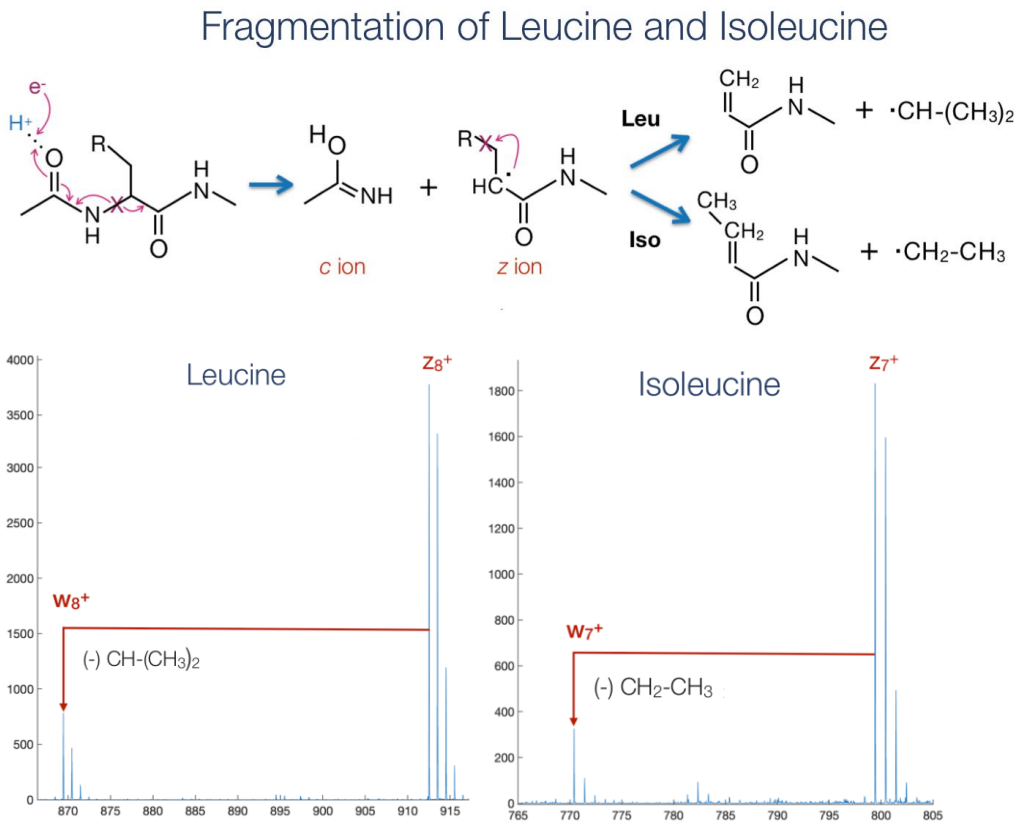


Figure 44. Secondary fragmentation of a *z*-ion produces diagnostic *w*-ions for distinguishing L8 from I7 in synthetic peptide ECDDisoDELIGHTFLK.

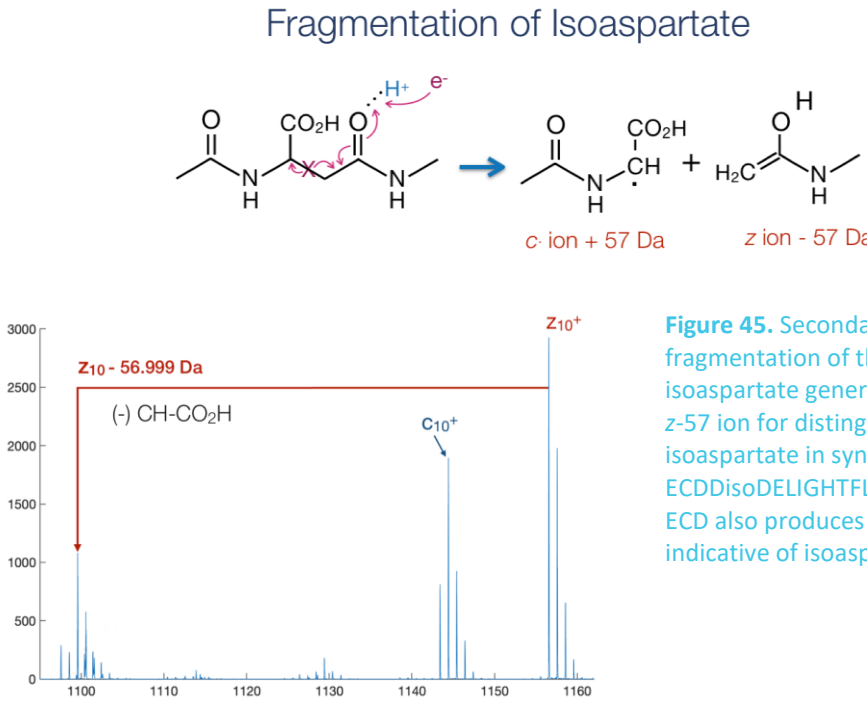


Figure 45. Secondary fragmentation of the C_α-C_β bond in isoaspartate generates a diagnostic *z*-57 ion for distinguishing isoaspartate in synthetic peptide ECDDisoDELIGHTFLK. Not shown: ECD also produces c+57 ions indicative of isoaspartate.

Electron induced dissociation (EID) is another electron-based fragmentation method. EID can fragment singly-charged precursors without neutralizing their charge, unlike ECD. This makes EID especially useful for glycan, glycoprotein, metabolite, and lipid characterization applications.

Both EID and CID produce glycosidic cleavages useful for glycan sequencing, with CID contributing *B* and *Y* ion fragments and EID contributing *C* and *Z* fragments. Unlike CID, however, EID can also produce *A* and *X* cross-ring cleavages, which are critical for determining linkages and branching in sugars.

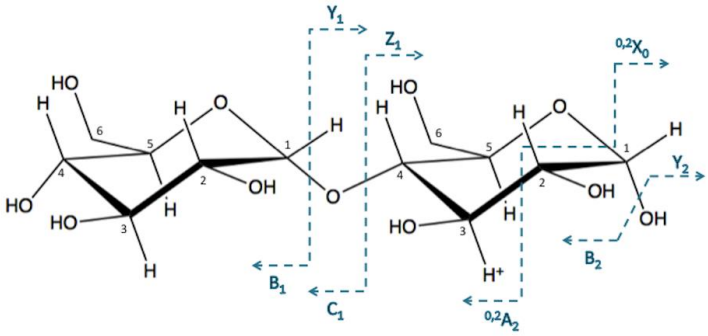


Figure 46. Product ions from glycosidic linkage and cross-ring fragmentation. Nomenclature proposed by Domon and Costello [Domon, 1980].

ExD Efficiency

ExD efficiency can be expressed as

$$\boldsymbol{ExD\ efficiency = \frac{Total\ abundance\ of\ fragment\ ions}{Total\ abundance\ of\ isolated\ precursor\ ions}}$$

It is influenced by the following factors:

- Electron energy
- Physical alignment of ions and electrons
- Analyte ion charge
- Ion-electron interaction period

With the ExD cell, the first two factors can be adjusted (via tuning) by the user to improve efficiency. Since the ExD cell does not use ion trapping and the length of the cell is fixed, the ion-electron interaction period is dependent on analyte ion kinetic energy.

The ExD cell is most efficient at producing low-energy electrons suitable for ECD. The efficiency with which the cell facilitates higher-energy electron-based fragmentation techniques (e.g., EID) is lower.

It is important to note that ECD efficiency for peptides tends to increase proportionally to the square of the precursor charge state. For a 2+ precursor ion, ECD efficiency of ~1-5% is reasonable. For a 20+ multiply-charged protein, the efficiency will be greater, although intensity of the ECD product ions will be distributed across a larger number of fragments and isotopes.

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Until its next release, this guide is valid for the 3.6.0 version or higher of the ExDControl software.