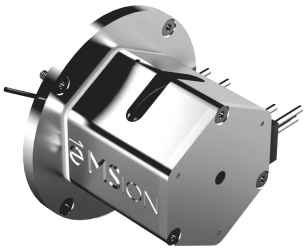
ECD for the Masses

ExD AQ-250 Option

AQ-251 • AQ-252 • AQ-253

for Agilent LC/Q-TOF



User Guide

R010 • March 2021

Notices

Patents

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Customer Feedback

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

You can reach us at Support@e-MSion.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 Option for Agilent LC/Q-TOF.
- Instructions for maintenance tasks and troubleshooting.
- An introduction to the fundamental concepts of electron-based fragmentation.

Related Documentation

- *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)

Terms Used

In this document:

- *ExD* refers to a family of electron-based gas-phase molecular ion dissociation techniques.
- *ExD tunefile* is a file documenting parameters that the ExDControl Software uses to set ExD Cell voltages.
- *MassHunter* refers to the Agilent MassHunter Data Acquisition program.
- *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 Information

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.**

General Safety Precautions

- Always shut down the instrument and disconnect the instrument power cord(s) and the ExD Cell D-Sub cable before attempting any maintenance.
- Always set the ExD Cell filament current to 0 A and turn OFF the ExD Controller before shutting down the instrument.
- 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.
- The ExD Controller is **not user-serviceable**. Do not attempt to open.

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

Contents

This chapter describes the design and function of the principal components of the ExD AQ-250 Options.

General Description

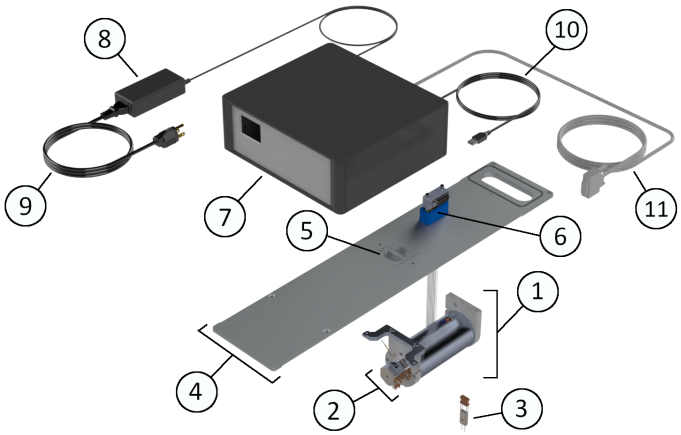


Figure 1. Main components of the ExD AQ-250 Option.

Table 1. Components.

Label	Part
1	ExD-Collision Cell Assembly **AQ-251:** with 6545XT IBC **AQ-252:** with IBC
2	ExD Cell
3	Filament cassette with filament insert
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-250 Options (-251, -252) are hardware and software packages that equip Agilent LC/Q-TOF mass spectrometers with the ability to perform electron-based fragmentation.

Key components include:

- The **ExD Cell**
- The **filament insert and cassette**
- The **ExD Controller and ExDControl software**

"ExD" describes a family of electron-based gas-phase molecular ion dissociation techniques. The techniques available for use with the ExD AQ-250 Option are summarized below. See **Concepts** for more information.

Table 2. Electron-based fragmentation techniques available for use with the ExD AQ-250 Option.

Technique	Ion Mode	Approximate Electron Energy	Fragment Ion Types
ECD*	Positive	<1 eV	c, z, y d, w (peptide side-chain)
HECD	Positive	<10 eV	a, y, c, z increased d, w (peptide side-chain)
EID	Positive	6-20 eV	a, y, x, c, z d, w (peptide side-chain)

*ECD is the principal fragmentation technique used with the ExD Cell.

The ExD Cell

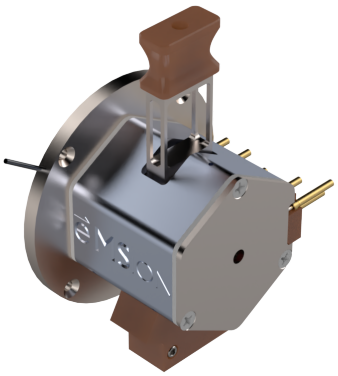


Figure 2. The ExD Cell.

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 ions, RF potentials or ion trapping.

During installation, the original Agilent collision cell is exchanged for the ExD-Collision cell assembly. The manifold cover is replaced by a custom cover, with a vacuum feedthrough for ExD Cell wiring and an access door for filament replacement.

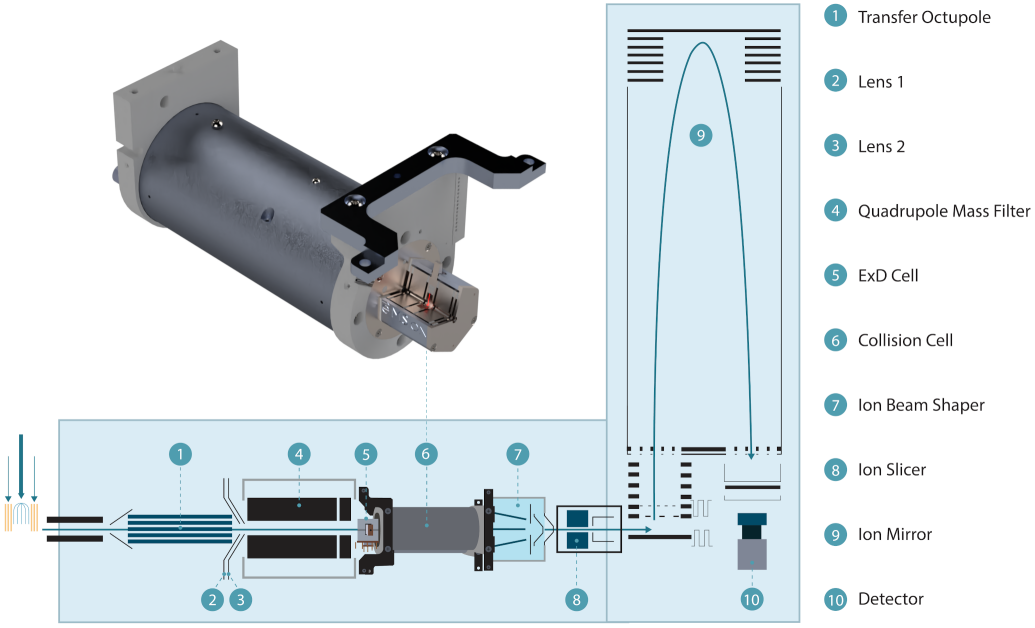


Figure 3. A modified 6545XT LC/Q-TOF. In Agilent LC/Q-TOF instruments, the ExD Cell mounts to the entrance of a shortened collision cell. Models AQ-251 and AQ-252 include an ion beam compressor for 6545XT and for other high-resolution 6500 Series Q-TOFs respectively pre-attached to the ExD-Collision cell assembly.

NOTE

***Installation of the ExD AQ-250 Option is a reversible process*. A trained Field Service Engineer can revert the instrument to its default configuration using the original parts removed during installation.**

Filament insert and cassette

The *filament insert* is the electron source for ExD. It holds a rhenium alloy wire suspended between posts. The *filament cassette* houses the filament insert and plugs into a slot in the ExD Cell.

Figure 4. Filament cassette with filament insert.

Thermionic emission is achieved by resistively heating the filament. Depending on the current amperage and the Cell lens profile, the ExD Cell can either perform electron-based fragmentation or transmit ions without performing electron-based fragmentation.

The filament is a consumable part. Stress from repeated heating and cooling will slowly thin the wire until it breaks. While the instrument can still be used with a burned-out filament, the ExD Cell cannot perform electron-based fragmentation until the filament is replaced. See **Maintenance and Troubleshooting**.

CAUTION

***When heated, the filament is sensitive to the presence of oxygen*. If the gas flow through the Cell contains trace amounts of oxygen, the filament will quickly burn out. To preserve the filament lifetime, collision cell gas must adhere to the standard of 99.999% purity and the manifold vacuum quality must be high.**

Magnets and lenses

Inside the ExD Cell, a set of permanent ring magnets and electrostatic lenses flanks the filament.

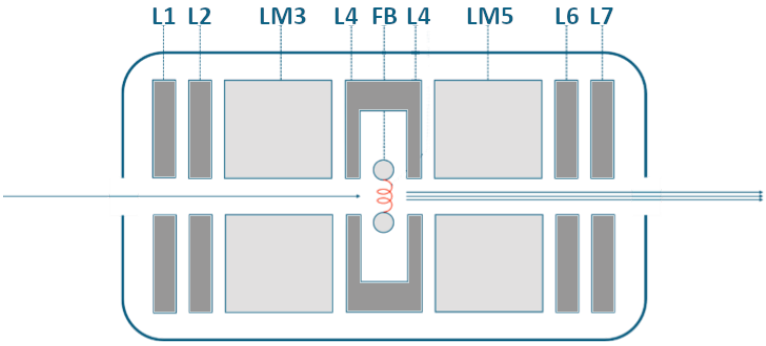


Figure 5. Cross-sectional view of the ExD Cell. The filament is the red coil, lenses are dark grey, and lens magnets are light grey.

The filament bias (FB) voltage applied to the filament wire is biased negative relative to the filament holder lens (L4) to draw electrons away from the filament.

Positive electrical potentials on the magnet pole pieces (LM3 and LM5) also help draw electrons away from the filament. Magnetic field lines then collect and confine electrons emitted by the filament to an “electron cloud” near the central axis of the ion flight path.

Electrostatic lenses (L1, L2, and L6) shape the electron cloud and guide ions through the Cell. Negative electrical potentials at the entrance and exit lenses (usually L2 and L6) keep electrons inside the Cell. On Agilent instruments, L7 is the collision cell entrance lens.

All lens voltages, including voltages applied to the magnet poles are set through the ExDControl software.

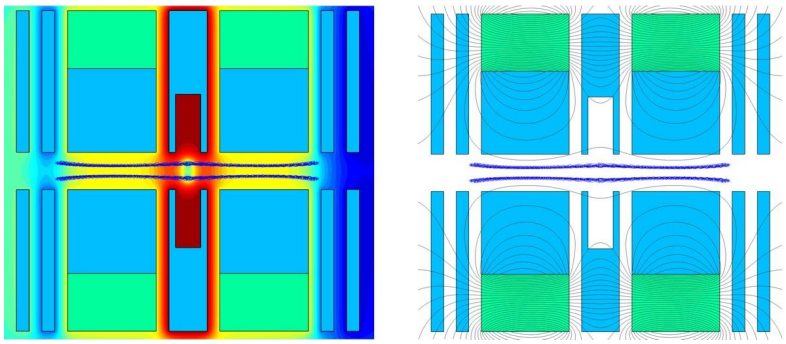


Figure 6. Cross-sectional view of the ExD Cell electromagnetic fields. Electrical potentials, with blue as negative and red as positive (left). Magnetic fields (right). Calculated electron trajectories are shown in dark blue along the central horizontal axis.

The ExD Controller and ExDControl software

NOTE

See the *ExD Controller User Guide* and *ExDControl Software User Guide* for more information.

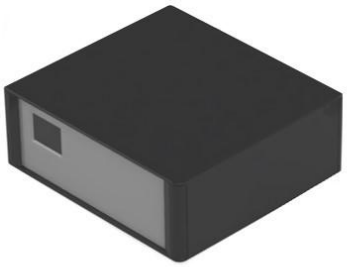


Figure 7. The ExD Controller.

The ExD Controller supplies DC voltages to the ExD Cell lenses and current to the filament according to values set by the user in the ExDControl software.

After installing the ExD Cell, using the ExDControl software will become part of your daily interaction with the instrument. Like other elements in the ion flight path, the ExD Cell has a significant effect on ion transmission, even when not being used to perform ExD.

CAUTION

***The ExD Controller is not user-serviceable*. Tampering with or self-repair of the ExD Controller will void its warranty, if existing. Contact e-MSion or supported distributor to replace the ExD Controller in case of failure.**

2. Operation

Contents

This chapter provides basic instructions for operating the ExD Cell.

Before Operation

CAUTION

All product component parts must be installed and configured by trained personnel prior to their use.

1. Check that the ExD Controller is ON. The ExD Controller *should always be on* while the ExD Cell is installed, 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 screen lights up.

Figure 8. The ExD Controller turned ON.

Figure 9. The ExD Controller turned OFF.

1. Open the ExDControl software. In the Windows Start Menu of your instrument PC, click **E-MSION** > **ExDControl**.

   Or, go to **C:\Program Files\E-MSION** (or an alternate installation location) and double-click **ExDControl.exe**.

Figure 10. ExDControl.exe icon.

2. Click **Connect** > **Connect** to connect to the ExD Controller.

   If the connection attempt fails, click **Connect** > **Connection Settings**. If the connection settings window does not match **Figure 11**,

   a. Hold **Shift** + click the **Connect** button to open the connection settings configuration window (**Figure 12**).

   b. Enter **192.168.254.12** in both IP address fields. Be sure to press **Enter** after typing in each address.

   c. Once the statuses update to “Instrument detected” and “Controller is responding,” click **Connect**.

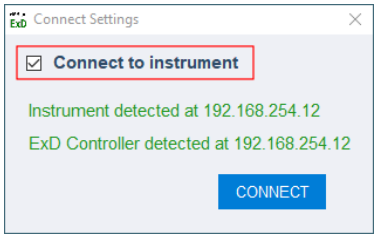


Figure 11. ExDControl connection settings for ExD AQ-250 Option.

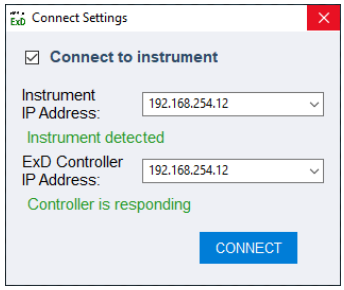


Figure 12. Configuring connection settings.

3. Check that the connection status indicator in the ExDControl software main window shows readouts of the instrument state. If the connection is unsuccessful, see the *ExDControl Software User Guide*.

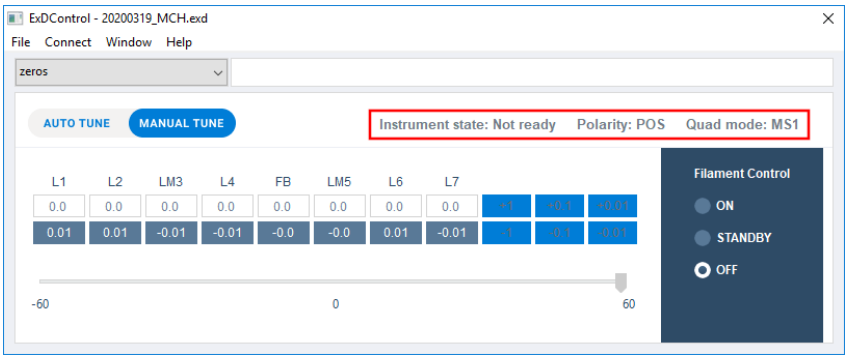


Figure 13. ExDControl software main window, with connection status indicator boxed in red.

Basics of Operation

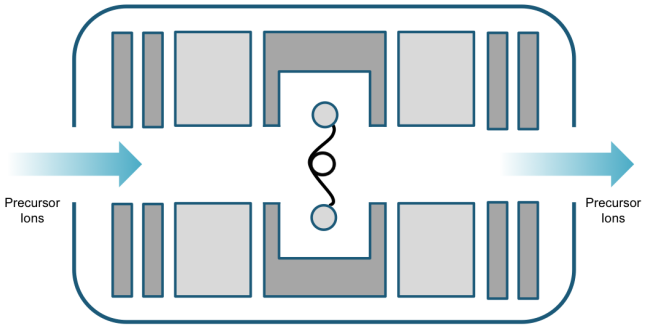
ExD Cell operating modes

The ExD Cell is controlled from the instrument control PC via the ExDControl software, which relays commands to the Cell through the ExD Controller.

The two main components affecting ExD Cell function are:

- Filament heating current
- Lens profile

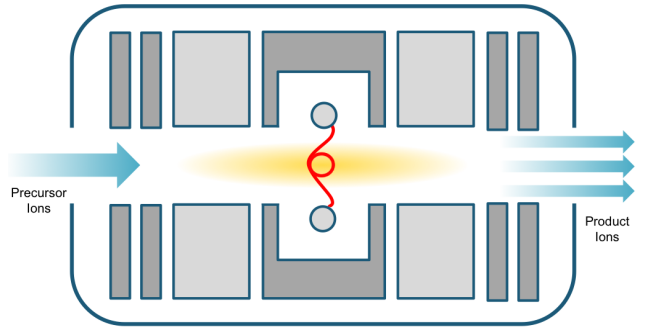
Through the systematic adjustment of these components, the ExD Cell can be tuned to operate in one of three basic modes:



Fil = Off, Standby / ExD = Off

The ExD Cell is tuned to transmit ions while the filament is not heated sufficiently for ExD.

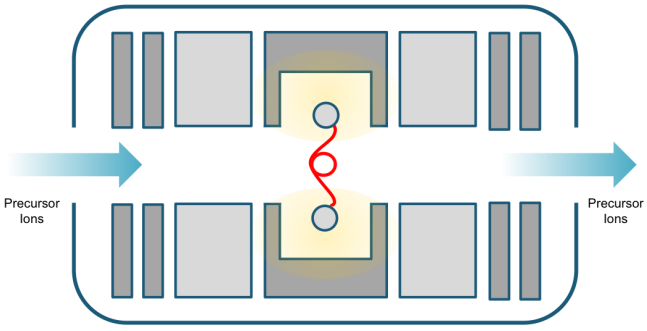
Used for normal operation of the Q-TOF instrument when electron-based fragmentation is *not* desired.



Fil = On / ExD = On

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

Requires that the filament be sufficiently heated for ExD.



Fil = On / ExD = Off

With the filament sufficiently heated for ExD, the ExD Cell is tuned to minimize the creation of ExD fragment ions while maximizing transmission.

Used for Targeted and Auto MS/MS-ExD experiments, so that rapid switching between the ExD=Off MS1 profile and ExD=On MS2 profile occurs without heat-cycling the filament.

CAUTION **Rapid changes to the filament heating current (“heat-cycling”) shortens filament lifespan.**

Filament settings

There are three possible settings for the filament:

- **Off** (0 A)
- **Standby**

  Standby current is below the level required for electron emission.
- **On**

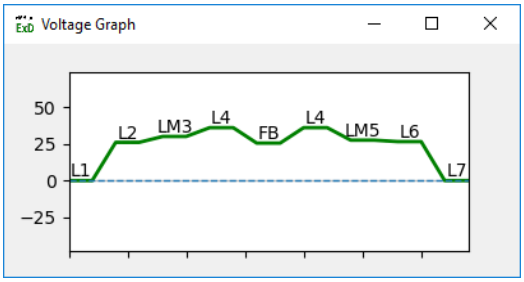
  Adjust the **On** current amperage in the **Filament Parameters** window of the ExDControl software. See **To optimize the filament heating current**.

Profiles

NOTE

The lens profile can be tuned to produce different types of electron-based fragmentation. See Concepts.

A set of electrical potentials for the eight lenses in the ExD Cell is referred to as a *lens profile* or *profile*. The lens profile can be tuned by adjusting the voltages applied to each lens to optimize either:



- **Transmission**, where ions pass through the ExD Cell without fragmenting, or
- **ExD**, where ions undergo electron-based fragmentation inside the ExD Cell.

Figure 14. Voltage graph of a profile tuned for ECD.

Profiles can be created as needed in the ExDControl software **Profiles Table**. The contents of the table save in an ExD tunefile (*.exd).

As the instrument changes scan types, the ExDControl software will automatically switch between profiles flagged for MS1 and MS2 in the **Profiles Table**.

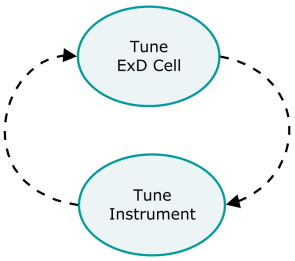
Profiles Table

MS1	MS2	Last tuned for	Profile	Description	L1	L2	LM3	L4	FB	LM5	L6	L7
☑	☐	MS1	MS1 hot trans	F=2.55x2.38 P=15psi 540K12	-20.0	18.0	22.5	23.0	20.0	22.5	16.0	0.0
☐	☐	MS2	MS2 hot trans	F=2.6x2.49 P=15psi 1.0M674	-20.0	18.0	22.0	21.5	19.0	22.0	0.0	0.0
☐	☑	MS2	MS2 ECD	F=2.65x2.54 P=17psi 12K624	-25.0	24.0	30.5	34.5	26.0	29.5	18.0	0.0
☐	☐	MS1	MS1 standby	F=0.5x0.29 P=15psi 470K122	10.0	12.0	20.0	19.0	18.0	21.0	10.0	0.0
☐	☐	MS1	xMS1 ECD	x F=2.6x2.49 P=15psi 10K624	-25.0	22.0	28.7	32.8	22.0	27.0	10.0	0.0

Figure 15. The **Profiles Table** above is set up for a Targeted MS/MS-ECD experiment, where ECD only occurs while a precursor is being isolated. The ExDControl software will apply the “MS1 hot trans” profile when the instrument scans in MS1 and the “MS2 ECD” profile in MS2.

Tuning

General principles



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

NOTE

For best results, wait at least 20 minutes for the ExD Cell to equilibrate after turning the filament On from Off/Standby before using the Cell.

- Regular fine-tuning of the ExD Cell lens profiles and filament heating current is recommended.
- The instrument settings found in the MassHunter **Manual Tune** tab define ion beam kinetic energy and trajectory. Since ion energy affects ExD Cell tuning, lens profiles tend to work best with the instrument tune file used when the profile was created.
- For Q-TOF models excluding the 6560, the **Oct1 DC** voltage setting defines ion energy. Optimal ExD Cell lens voltages with the filament **Off/Standby** will not exceed **Oct1 DC**. With the filament **On**, voltages will not exceed **Oct1 DC** + ~10 V.

  When electrons are being emitted from the filament, the voltages on the ExD Cell lenses tend to be slightly higher to compensate for the negative charge of the electrons.
- Keep L7 at 0 V in the ExDControl software. L7 is the collision cell entrance lens, and is controlled using the MassHunter **Cell Entrance** parameter.
- When collision energy is added, all lenses in the ExD profile (excluding L7) should be raised by approximately the same amount.
- The lens profile for an optimized ExD tune should be nearly symmetric with a slight downward slope from the entrances lenses (L1, L2, LM3) to the exit lenses (LM5, L6).
- FB will typically always be set slightly below L4, LM3, and LM5.

  The voltage difference between FB and L4 is the primary determinant of electron emission. Calculating electron energy is complicated by interactions with the negative potential of the electron cloud.

Following a standard process to tune the ExD Cell will result in consistent and reproducible results. The tuning procedures described below are meant to help you to standardize tuning of the ExD Cell.

To autotune the ExD Cell

Use autotune to automatically optimize ExD Cell lens voltages for transmission or ECD. Autotuning uses the abundance of known tuning standard peaks to guide the process of creating a profile that can be used for experimental samples.

If the instrument is in **Total Ion Mode** when the autotune starts, the results will overwrite the selected MS1 profile in the **Profiles Table**. If the instrument is in **Isolation Mode**, the results will overwrite the selected MS2 profile.

1. Open the **Auto Tune** tab in the ExDControl software.

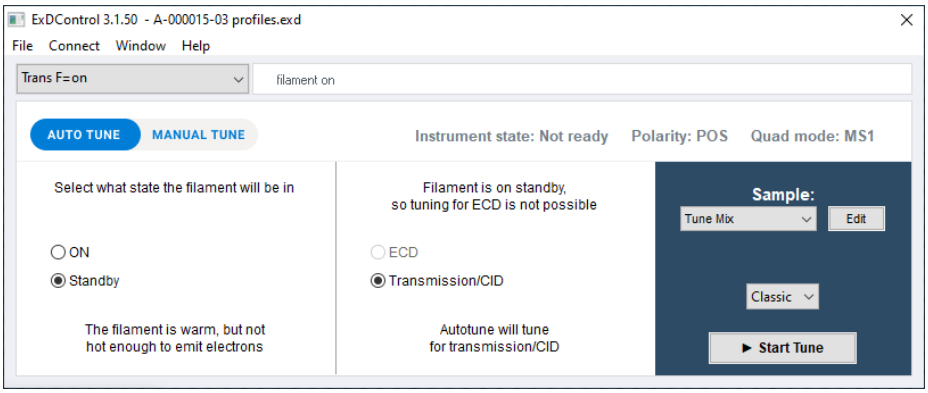


Figure 16. The ExDControl **Auto Tune** tab.

2. Select the appropriate filament setting (**On**, **Standby**) for your experiment.
3. Select what to tune for:

   - **Transmission/CID** Optimizes transmission of ions through the ExD Cell.
   - **ECD** Optimizes ECD.

   The **ECD** option may only be selected if the filament **On** setting is selected and an ECD-compatible sample is selected in the **Sample** menu.

4. Select a tuning standard from the **Sample** dropdown menu. To add a sample to the list, see **To add a new autotune sample**.
5. Select an autotune method from the dropdown menu:

   - **Classic** Starting from a default profile, tunes for transmission or ECD by individually adjusting each lens element of the Cell.
   - **Refine** Starts from a previously tuned profile and refines to increase transmission or ECD. To be used for small adjustments.

6. Click **Start Tune** to begin the autotune procedure. The **Auto Tuning** window will appear to illustrate the tune progress.

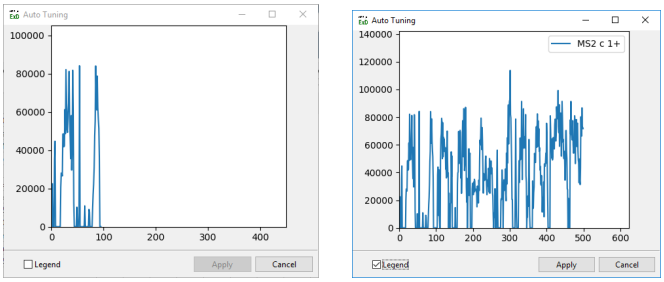


Figure 17. The **Auto Tuning** window plots a graph (*m/z* intensity vs. scans) while an ECD autotune for substance P is in progress (left) and finished (right).

7. Once the tune is complete (5-10 minutes), click **Apply** to accept the profile generated. Click **Cancel** to revert all lens voltages to their original values. Intensities and lens voltages recorded during autotune are saved in a log file in **E-MSION > ExDControl > logs > autotune**.

To add a new autotune sample

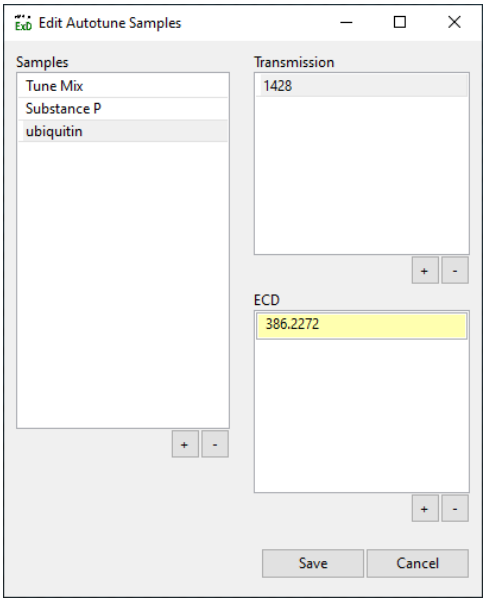
To add a new sample to the list of options in the ExDControl **Auto Tune** tab,

1. Click **Edit** to open the **Edit Autotune Samples** window.
2. Below the **Samples** panel, click **+** to add a new sample to the list.
3. Click to select the new sample and fill in the sample name.
4. Below the **Transmission** panel, click **+** to add an ion to the list and fill in its *m/z* value.

   The autotune algorithm will seek to maximize transmission of all ions listed in this panel.

5. If you intend to autotune for ECD, click **+** below the **ECD** panel to add an ECD fragment ion (*c*- or *z*-ion) to the list and fill in its *m/z* value.

   If ion(s) are added to the **ECD** panel, the sample will be treated as an ECD tuning standard, and any ion(s) in the **Transmission** panel will be treated as precursor ion(s).



NOTE

If possible, choose an ECD fragment ion that the sample is known to produce efficiently.

Figure 18. Add/remove custom ExD Cell tuning standards from the **Edit Autotune Samples** window. To access, click **Edit** in the ExDControl **Auto Tune** tab.

To manually tune the ExD Cell for transmission

The procedure below maximizes transmission of tuning mix without fragmentation. The same procedure may also be used on other analytes.

Use large steps (1 V) to find the limits of the range of working voltages for a lens followed by small steps (0.1 V) to find the optimum within the range. Note that *inner lenses* (FB, L4, LM3, LM5) are more sensitive and will respond to small adjustments (0.1 V) more so than outer lenses (L1, L2, L6).

1. Infuse Agilent tuning mix through Calibrant Delivery System Bottle B.
2. In the ExDControl **Manual Tune** tab, select the profile to tune from the drop-down menu.
3. Set Filament to **On**, **Standby** or **Off**, depending on the experiment.
4. Hold **Shift** and click to select L2, LM3, L4, FB, LM5, and L6 together. Adjust these lenses in unison until tuning mix peak abundance is maximized. The optimal potential for these lenses should be near the **Cell Entrance** lens voltage, and if the filament is **Off** or in **Standby**, less than **Oct 1 DC**.
5. Adjust FB and L4 in a range of ± 5 V. FB is typically lower than LM3 and LM5. L4 is typically greater than FB by 0.5-5 V.
6. Adjust LM3 and LM5 separately and then in unison.
7. Adjust L2 and L6 separately. Optimal values should be less than **PostFilter DC**. If tuning in MS2, note that lowering L2 may increase transmission.
8. Adjust L1 in 5 V steps. The optimal value should be less than **PostFilter DC**.
9. Iterate steps 5-8, making fine adjustments until satisfied with the level of transmission produced.

NOTE

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

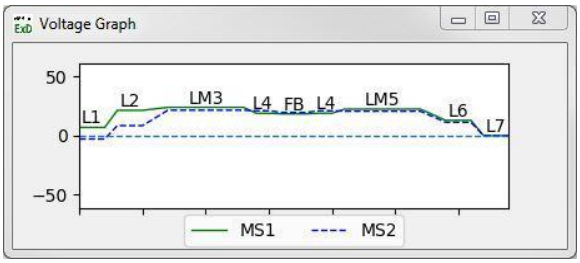


Figure 19. A pair of profiles for transmission in MS1 and MS2 displayed in the **Voltage Graph** window.

CAUTION

If you intend to add collision energy (CE), you will need to increase the ExD Cell lens voltages by approximately the same amount to optimize transmission. Since adding CE raises the voltages of the instrument optics preceding the ExD Cell, the Cell lens profile must adjust to match.

To manually tune the ExD Cell for ECD

ECD tuning maximizes the signal intensity of ECD product ion peaks. The procedure below focuses on the c_5^+ product ion at *m/z* 624.3940 produced from the *m/z* 674.3713 $(M+2H)^{2+}$ precursor of amidated substance P.

1. Infuse substance P. See **Tuning Standard Preparation and Infusion Methods** for guidance. Make sure source parameters are optimized.

   With the ExD Cell optimized for transmission, a signal intensity of roughly 1 million counts for *m/z* 674 in MS1 is suitable to begin tuning for ECD.

2. In the MassHunter **Manual Tune** tab, isolate *m/z* 674 using a wide window.
3. In the ExDControl software, set Filament to **On.**
4. In the ExDControl **Profiles Table,** check the MS2 box next to the profile to tune for ECD. If no such profile exists, add a new profile to the table and copy the lens voltages from an MS2 profile optimized for transmission with the filament **On** into the new profile.
5. In the ExDControl **Manual Tune** tab, increase L4 by ~5 V.
6. Hold **Shift** and click to select LM3, FB, L4, and LM5 together. Adjust in unison to maximize *m/z* 624 intensity.

   Note that small adjustments (0.1 V) to lens voltages, especially to inner lenses (LM3, L4, FB, LM5) can significantly affect ECD efficiency.
7. Optimize the difference between L4 and FB. Increasing the difference between these lenses roughly corresponds to increasing electron energy.
8. Adjust LM3 and LM5 separately, then in unison in 0.1 V steps.
9. Adjust L2 and L6 separately.
10. Adjust L1. Setting L1 or L2 relatively negative to the rest of the lens profile may increase isolation efficiency.
11. See **To optimize the filament heating current**.
12. Iterate steps 7-10, making fine adjustments until satisfied with the level of ECD produced.

NOTE

If not sample-limited, you may further optimize an ECD profile developed using substance P by tuning the ExD Cell on your sample of interest to maximize its known ECD product peaks.

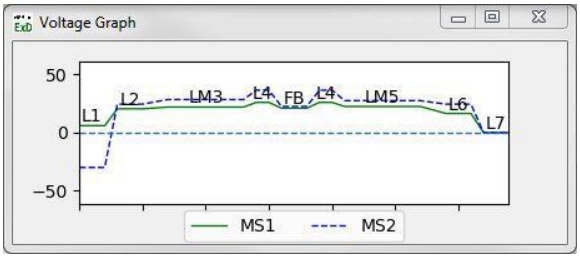


Figure 20. A pair of profiles for transmission in MS1 and ECD in MS2 displayed in the **Voltage Graph** window.

To optimize the filament heating current

CAUTION

Making rapid changes to the filament heating current (heat-cycling) shortens filament lifespan.

NOTE

For best results, wait at least 20 minutes for the ExD Cell to equilibrate after turning the filament On from Off/Standby before using the Cell.

The filament **On** current must heat the filament wire to a sufficient level of emission for ExD to occur. Over time, you will need to adjust the **On** current to compensate for thinning of the filament wire.

Table 3. Recommended starting and maximum heating current by filament insert model.

Filament Insert (p/n)	Starting Heating Current (A)	Does not Typically Exceed (A)
11146	2.3	2.6

1. If ECD efficiency of a profile tuned for ECD is low, increase the "Set" current in the **Filament Parameters** window in 0.05 A steps while monitoring signal intensity of analyte ECD fragment ions. If you begin to observe diminishing returns in signal intensity, stop increasing the current and decrease to the point of highest fragmentation for current input.

   Note that significant changes to the filament current will require retuning of any ExD profiles, particularly FB and L4.

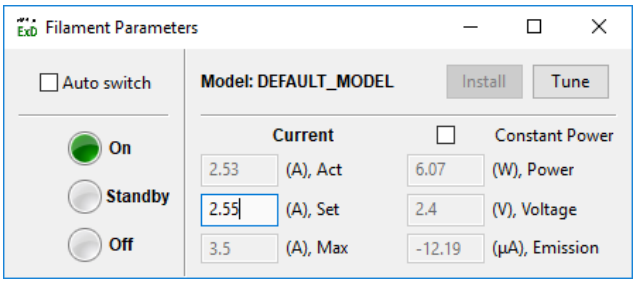


Figure 21. The **Filament Parameters** window with the filament **On** and set at 2.55 A.

2. Rhenium will evaporate from the heated filament wire. If rhenium peaks at *m/z* 184.9530 and 186.9558 are greater than ~2.5e5 counts, the filament may be overheated. Consider decreasing the current.
3. Look for rhenium oxide peaks (*m/z* 200.9479 and 202.9507, 216.9428 and 218.9456, 232.9378 and 234.9405, 248.9326 and 250.9354, etc.). A high proportion of rhenium oxide relative to rhenium indicates that enough oxygen is present to quickly degrade the filament. See **Oxygen**.

NOTE

Use the lowest current which provides satisfactory ECD to extend the filament lifespan.

To set up the ion chromatogram as a manual tuning aid

Scan-to-scan variation in signal makes tracking gains in signal intensity while manually tuning the ExD Cell challenging. Instead of watching the fluctuation of peaks in the spectrum window of the MassHunter Tune context, use the ion chromatogram to keep track of changes in signal intensity over time.

- When tuning for transmission, use the TIC chromatogram.
- When tuning for ECD, set up an EIC chromatogram to track changes in intensity of a prominent ECD product ion. To do so,
    1. In the Acquisition context, select the **Chromatogram** tab in the **Q-TOF** page of the **Method Editor** panel.
    2. Add a new row to the **Chromatograms** table. Enter 'EIC' as the chromatogram type and extract an *m/z* range encompassing an ECD product ion. The experiment type must be 'Both' or 'MS/MS'.

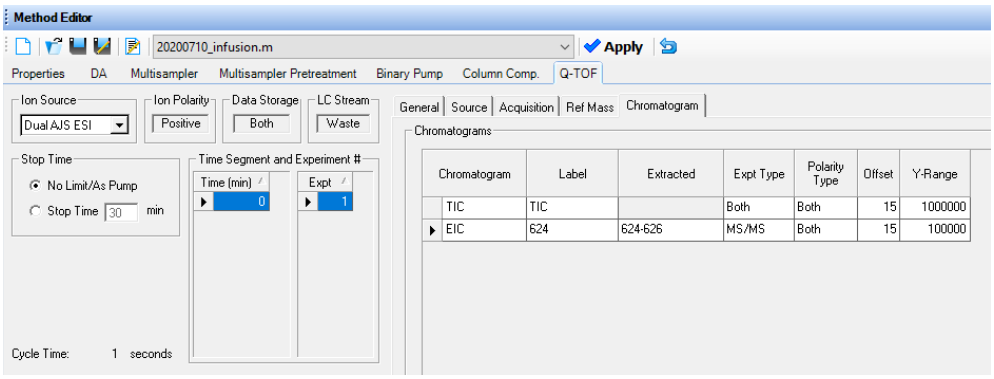


Figure 22. The **Chromatograms** table, set up to display an EIC of the *m/z* 624 c_5^+ ECD product ion of amidated substance P.

To tune the instrument with the ExD Cell installed

With the ExD Cell installed, MassHunter automatic tuning can still be used to tune the instrument. Keep in mind that ExD Cell components *are not adjusted* during MassHunter automatic tuning.

Before running an instrument automatic tune with the ExD Cell installed, make sure the ExD Cell is tuned to allow sufficient ion transmission through the instrument.

The following instrument parameters in the MassHunter **Manual Tune** tab can influence ExD Cell function:

- Oct1 DC
- Lens 1 DC
- Cell Entrance

- Hex DC and Hex Delta
- Collision Cell Gas Pressure

  Experiment with the effect of changing the collision cell gas pressure from its default value (22 psi) on transmission and ECD.

  If the MassHunter version has disabled changes to the collision cell gas pressure (e.g. B.08 and B.09), use the ExDControl software to override:

  a. Click **Window** > **Show Collision Cell Gas Pressure**.

  b. Enter the desired pressure in the box and press **Enter**. Wait until the actual pressure reading matches the set pressure.

NOTE

Changes to the collision cell gas pressure made by ExDControl will not be visible in the MassHunter. Any method report generated from MassHunter will show the MassHunter pressure setting, not the actual pressure set by ExDControl.

Tuning Standard Preparation and Infusion Methods

WARNING **Wear a protective lab coat, gloves, and eyewear when handling acids and solvents.**

CAUTION **Special attention should be given to the cleanliness of the nebulizer needle and tip when infusing large amounts of tuning standards.**

NOTE **A lens profile developed using a tuning standard can be used to perform ECD on other experimental samples.**

Table 4. ExD Cell tuning standards.

Tune	Sample(s)	Description
Transmission/CID	Agilent tuning mix	Optimizes transmission of ions through the ExD Cell.
ECD	Substance P	To optimize ECD fragmentation of ions.

To prepare substance P

Materials

- Substance P, amidated (CAS no. 33507-63-0)
- Water, LCMS-grade
- Methanol, LCMS-grade
- Formic acid, high-purity

Amidated substance P (~1.3 kDa) is the peptide standard used for tuning the ExD Cell for ECD.

Prepare in 50/50/0.1 (% v/v/v) methanol/water/formic acid dilution buffer. Using the Agilent Dual AJS ESI source, a final concentration of 10 µg/mL is typically suitable for tuning the ExD Cell for ECD.

Keep in mind the following:

- Before weighing out substance P, allow the closed container to equilibrate to room temperature to reduce moisture uptake.
- Avoid repeated freeze-thaw cycles of substance P in solution.
- When dissolving substance P in solution, *gently* mix to avoid oxidation during sample preparation.

ECD of substance P

The amidated substance P amino acid sequence is **R P K P Q Q F F G L M - NH_2**. Use ProteinProspector MS-Product (UCSF MS Facility) to generate a list of ECD product ions for the tuning standard.

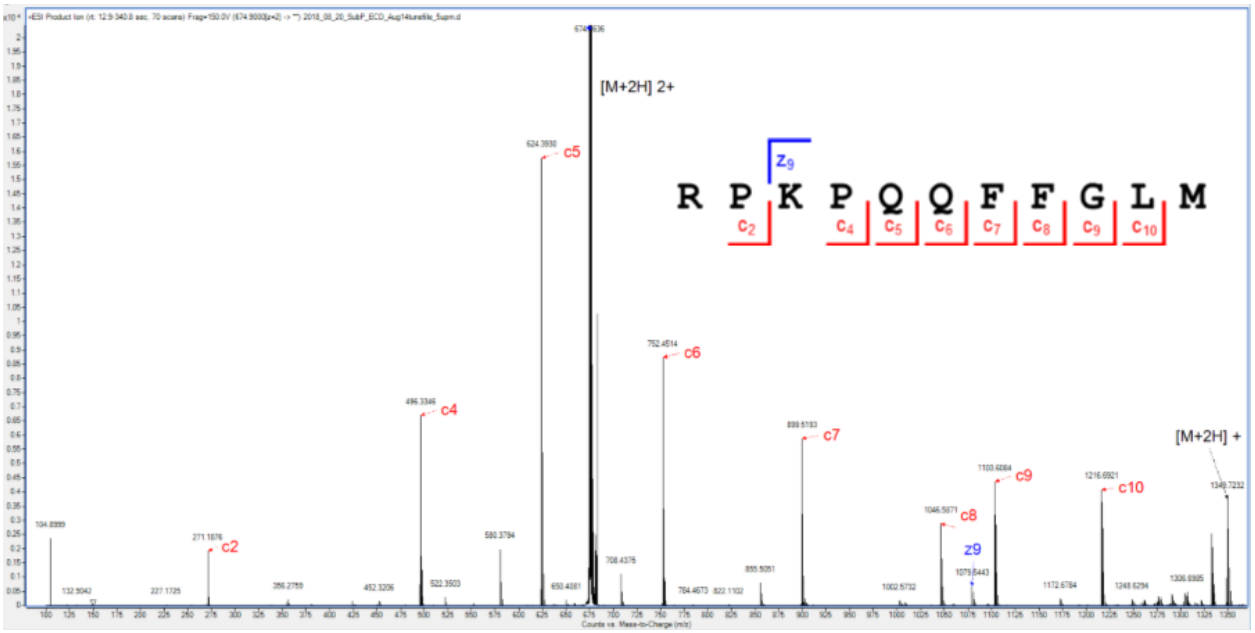


Figure 24. Substance P (amidated) product ion spectrum collected from the $[M+2H]2^+$ precursor ion at *m/z* 674 during a targeted MS/MS-ECD experiment.

To set up direct infusion

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

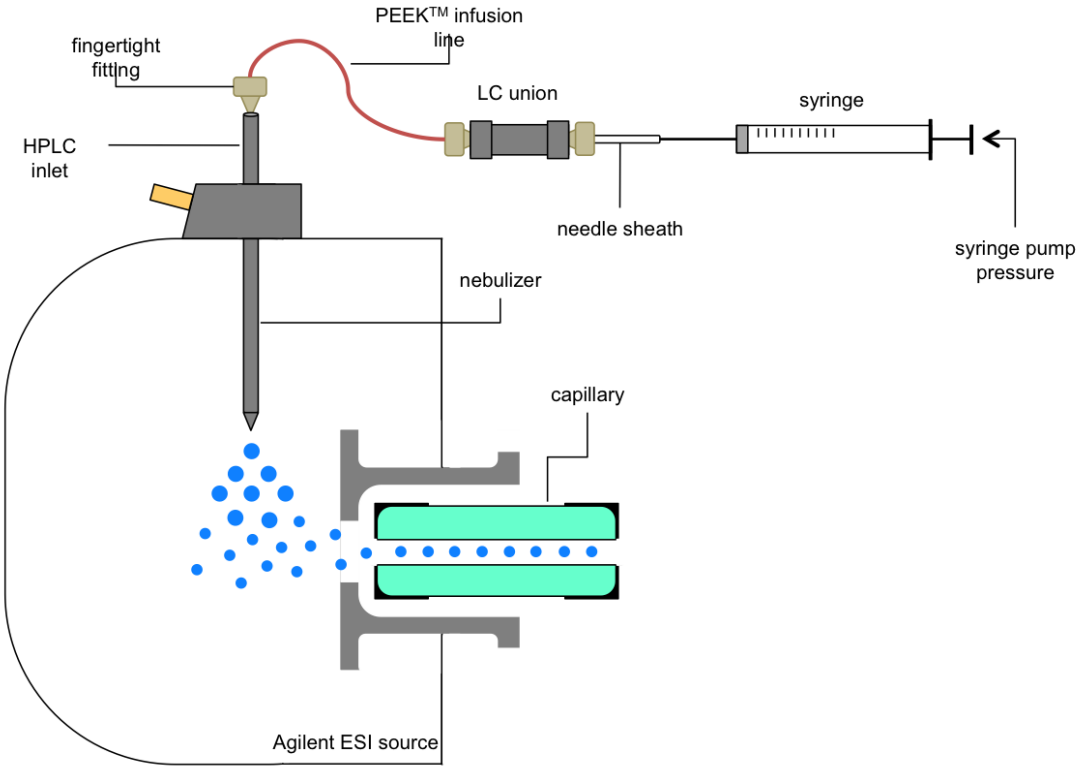


Figure 25. Direct infusion set up.

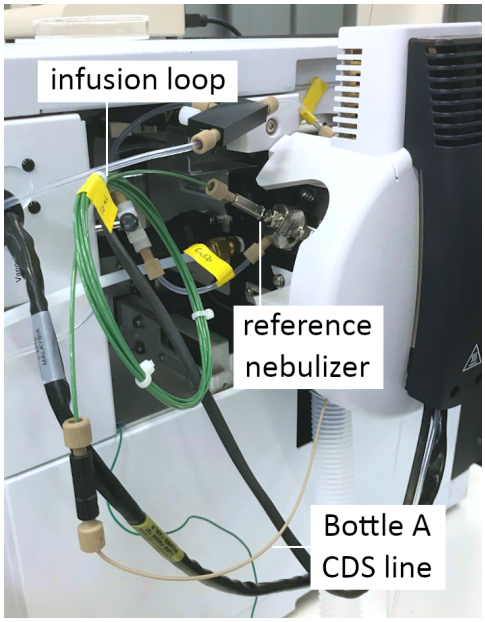
1. Fill a clean syringe with substance P and install in the syringe pump.
2. Assemble the infusion line as shown in **Figure 25**. Use parts from the loop infusion kit shipped with the ExD Cell if needed.
3. Infuse at an initial rate of 300 µL/hr.

PRO	Stable sample flow. Flow rate can be changed.
CON	If you are tuning the ExD Cell, the syringe will require frequent refills. Before starting an autotune, make sure the syringe is full.

To set up loop infusion

NOTE

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



Parts

- Clean syringe (22 gauge needle, 1 mL minimum volume)
- Loop infusion kit (ships with the ExD Cell):
  - 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)

Figure 26. Loop infusion set up.

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.

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.

Shutting Down

To put the system in Standby

1. In the instrument control application, put the mass spectrometer in Standby mode.
2. Set the filament to **Standby** or **Off** in either the ExDControl software **Manual Tune** tab or the **Filament Parameters** window.

To shut down the system

Turn off the ExD Cell before shutting down the instrument. The ExD Cell is considered "off" when all Cell voltages and currents are zero.

1. Turn the filament **Off** in the ExDControl software **Filament Parameters** window. Check that the actual current readout decreases to 0 A.
2. Turn ExD Controller off. Press the power button on the back of the Controller until the front LCD screen turns black.
3. Unplug the ExD Controller power cord.
4. Continue with the standard instrument shut down process. See the Agilent LC/Q-TOF Maintenance App for guidance.

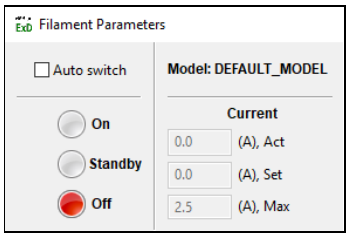


Figure 27. The **Filament Parameters** window, with the filament **Off**.

CAUTION

Make sure the filament is OFF before venting. Venting the mass spectrometer without first turning the filament off may expose the heated filament to high levels of oxygen, causing the filament to burn out.

Turning the ExD Controller off without first turning the filament OFF will set all Cell voltages and currents to zero, but at a risk of damaging the filament due to rapid cooling.

3. Data Acquisition and Analysis

Contents

This chapter provides an overview of ExD data acquisition and analysis.

Acquisition

Keep in mind the following tips when setting up a method for ECD:

- 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 charge state of 2+ in positive ion mode because electron capture neutralizes one charge. EID can be used on singly-charged precursors because it does not result in charge reduction.
- ECD efficiency will roughly increase with the square of charge state.
- The addition of CID energy after ExD may improve ECD of tightly-folded compounds by helping to separate dissociated fragments.
- Averaging more scans may help to achieve adequate signal-to-noise for low-intensity ECD peaks. A slower scan rate, if possible, will also help.
- While **Auto MS/MS** should still work with ECD, the Agilent decision engine is intended for use with CID, and will cycle through the isolation list quicker than is ideal for obtaining good ECD data. For this reason, e-MSion recommends using **Targeted MS/MS** if retention times and/or masses are known. Filling the isolation list with the same precursor repeated over and over is one way of ensuring that your precursor of interest is isolated.
- When performing ECD on a high *m/z* precursor, low isolation efficiency combined with low ECD efficiency may prohibit using **Targeted MS/MS**. A work-around is to record spectra in MS1 with the **Quad amu** set to just below the precursor of interest while using an ExD profile developed for ECD in MS1. *The Quad amu setting must be saved as part of the MassHunter tune file before switching to the Acquisition context.*

As the instrument switches between MS and MS/MS scans during an acquisition run, the ExDControl software will automatically apply the two profiles designated for MS1 and MS2 from the **Profiles Table**.

Analysis

Data generated by Agilent Q-TOF mass spectrometers with the ExD Cell installed will still be in *.d format. From here, many analysis workflows are possible.

When analyzing ExD data, keep in mind the following:

- Data files will not identify the ion activation method used as ExD.
- ExD product peaks are typically lower in intensity than CID peaks. Peaks with only a few hundred or thousand counts are not uncommon, and can be

considered legitimate as long as mass error, isotopic envelope shape, and signal-to-noise are reasonable.

- ExD fragmentation is often accompanied by hydrogen rearrangement to/from the product ions.

Third-party tools for top-down data processing and analysis

Electron-based fragmentation is uniquely suited to the top-down characterization of proteins and protein complexes. This is because:

- Bonds cleave adjacent to the site of electron capture during ECD. Unlike CID, where the most labile bonds are cleaved first, ECD is less dependent on amino acid sequence, leading to greater sequence coverage of long polypeptides compared to CID.
- ECD efficiency tends to increase with the square of charge state.
- ECD is capable of preserving labile post-translational modifications that are often scrambled or lost during bottom-up workflows.

Bioinformatics tools for top-down data analysis are still evolving, but several free and commercial options are available to assist with the analysis of ECD data:

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 • Simple to use • Not meant for complex spectra analysis	Free	Park et. al., 2017.
mmass *Strolham et. al.*	• Basic assignment of *c* and *z* ions • Open source • No longer in development	Free	Strolham et. al., 2010.
MASH Explorer *University of Wisconsin-Madison*	• Under development • Profile data deconvolution and fragment assignment	Free	Cai et. al., 2016.
Protein Metrics Product Suite *Protein Metrics Inc.*	• Comprehensive • Well-known industry standard	Commercial	

4. Maintenance and Troubleshooting

Contents

This chapter provides instructions for performing routine maintenance of the ExD AQ-250 Option hardware as well as information for troubleshooting issues that may occur during operation of the ExD Cell.

NOTE **Contact e-MSion or a supported distributor to order replacement parts.**

CAUTION **Damages to the ExD Cell, filament, or filament cassette caused by the user during maintenance are not covered under warranty.**

CAUTION **Outside of the filament, internal ExD Cell parts *are not user-serviceable*. The ExD Cell requires a jig to reassemble.**

CAUTION **The ExD Controller *is not user-serviceable*.**

To check for filament burn-out

CAUTION

If your filaments are rapidly burning out, oxygen in the manifold may be the cause. To check, see Oxygen in Troubleshooting.

Eventually, stress from heating will cause the filament to fail, known as "burn-out." Symptoms of burn-out include:

- Filament current actual "(A), Act" approaches zero while the current setpoint, "(A), Set", is > 0 A.
- Voltage drop across the filament "(V), Voltage" approaches the maximum value of 5 V while the current setpoint is > 0 A.
- Power consumption for the filament circuit "(W), Power" approaches zero while the current setpoint is > 0 A.

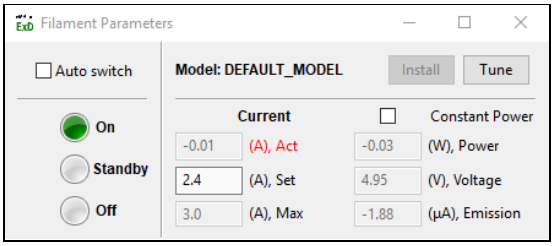


Figure 28. The appearance of the **Filament Parameters** window when either the filament is burned out or the D-sub cable is disconnected.

Make sure 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.

See **To replace the filament** for instructions on replacing the filament.

NOTE

The ExD Cell can still be tuned for transmission when the filament is burned out, but cannot perform electron-based fragmentation until the filament is replaced.

To check for filament current leakage

Occasionally, carbon buildup 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 carbonization is:

- With the filament **Off**, setting the voltage difference between FB and L4 to ~40 V causes a permanent change in the "Emission" current readout in the **Filament Parameters** window.

To fix, vent and replace the filament cassette and insert.

To replace the filament

CAUTION

Filament replacement should only be performed by trained individuals. If you have not yet watched the filament replacement process, submit a request for a Field Service Engineer to replace your filament.

The most common maintenance task associated with the ExD Cell is replacing the filament insert after burn-out from repeated heat-cycling and material loss.

Figure 29. Close-ups of a new filament (left), a filament heated to the threshold of thermionic emission (middle), and a filament that has burnt out after routine use (right).

The following steps describe how to replace the filament insert in the ExD Cell.

Parts

- Replacement filament
- Spare filament cassette, ships with the ExD Cell

Tools

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

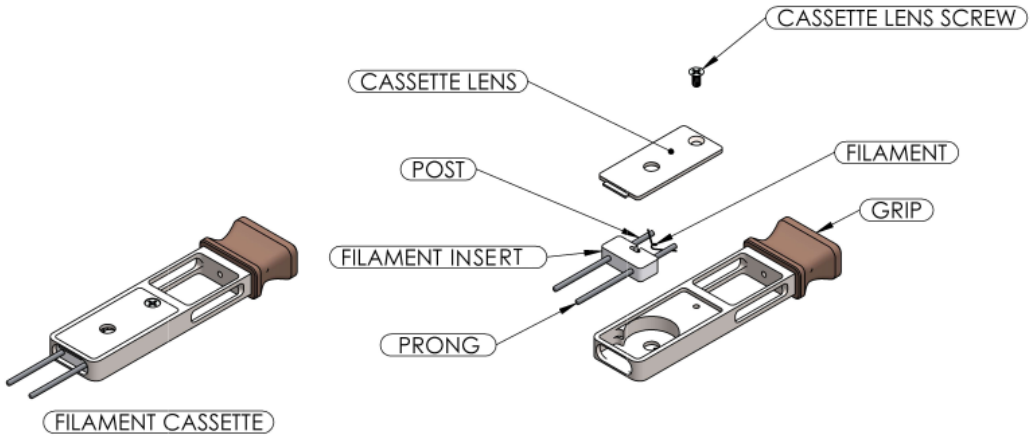


Figure 30. Assembly of the filament cassette with the filament insert.

Step 1. Prepare the replacement filament

CAUTION

Do not touch the filament wire! Hold the filament insert by its prongs or by the filament holder. Always wear gloves.

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.

   - ✓ If coated, the filament wire surface should not show large areas with exposed metal.
   - ✓ If uncoated, 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.

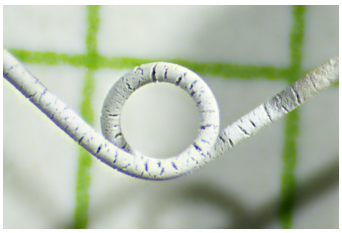
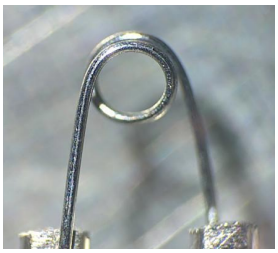


Figure 31. Coated and conditioned filament wire loop (**Top**). Uncoated filament (**Bottom**).



3. Obtain a spare filament cassette.
4. Use the 00 phillips screwdriver to remove the cassette lens screw and the cassette lens.
5. Check the interior and exterior of the cassette for dirtiness.

NOTE

Heat discoloration on the filament cassette is acceptable. Carbonization or other contamination on the filament cassette should be cleaned off before use.

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.

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

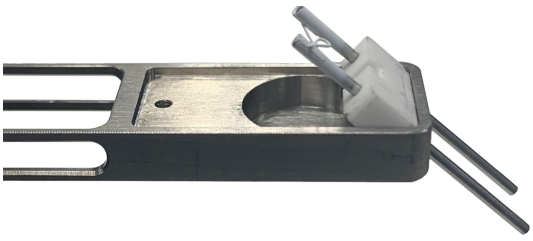


Figure 32. Sliding the filament insert into the filament cassette.

7. 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.

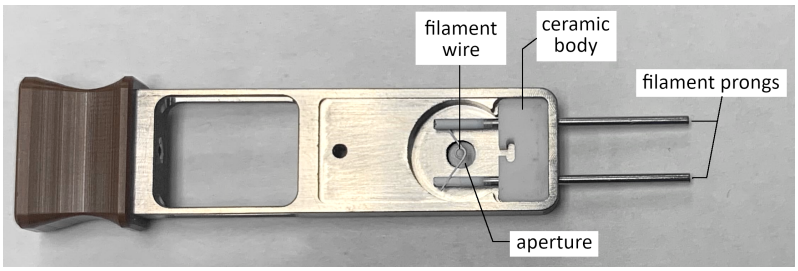


Figure 33. The filament insert inside the filament cassette.

8. Replace the cassette lens and cassette lens screw.

Step 2. Shut down the system

WARNING

The instrument is not safe for filament replacement if the power cord(s) is not disconnected from the instrument.

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

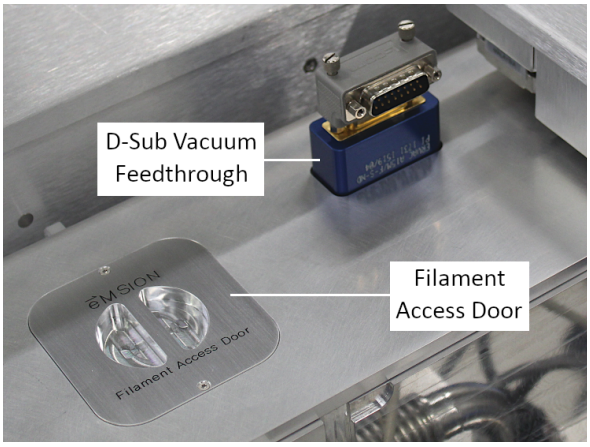


Figure 34. The manifold cover with filament access door.

CAUTION

Take care to avoid debris (e.g. dust or fibers) falling into the instrument or the ExD Cell. Contamination of the instrument, filament, or filament slot will impair performance.

Step 3. Replace the filament cassette

WARNING **When removing the filament cassette from the instrument, only touch the insulated grip. Other components may still be very hot.**

CAUTION **To avoid damaging the ExD Cell, do not remove or insert the filament cassette at an angle.**

1. 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 from the ExD Cell by gently pulling the insulated grip straight upward until the filament prongs clear the access door. Place on a dust-free, heat-resistant surface.

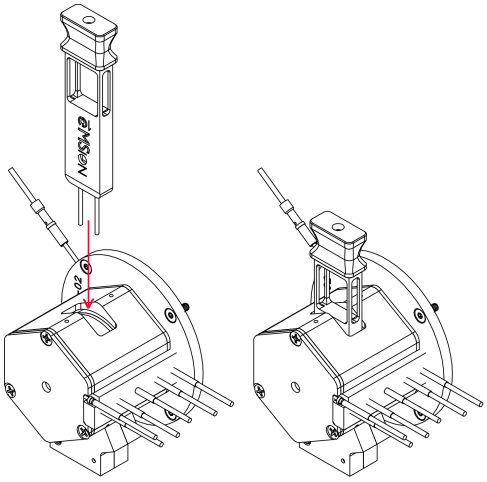
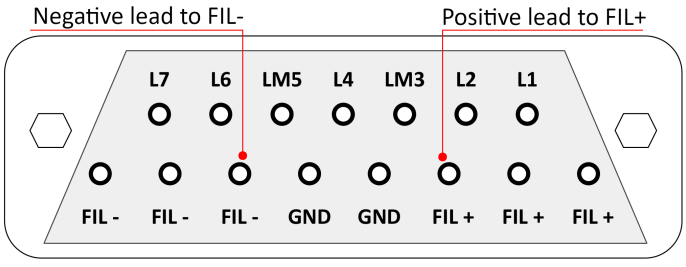


Figure 35. Insertion point of the filament cassette on the ExD Cell.

3. Insert the replacement filament cassette into the ExD Cell. Hold the insulated grip and slide the prongs into the filament slot until you hear a click. The direction the filament cassette faces does not matter.
4. Test the resistance of the filament circuit across the D-Sub vacuum feedthrough with a multimeter. Connect the leads as shown in **Figure 36**.



If the circuit is complete, resistance should read about 0.1-1.0 Ω. If not, see **Filament Circuit**.

Figure 36. 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 re-established.**

Step 4. Restart the system

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

Step 5. Set the initial filament On current

CAUTION

To avoid burning out the new filament, be sure the filament current remains Off (0 A) in the ExDControl software until vacuum is reestablished.

1. Reconnect the ExDControl software by clicking **Connect** > **Connect**.
2. In the ExDControl software, apply the last profiles tuned for transmission in MS1 and MS2 with the filament in **Standby**. If signal intensity of Agilent tuning mix is not satisfactory, re-tune these profiles. See **Tuning**.
3. Open the **Filament Parameters** window. Turn the filament **On** and set the heating current to the recommended starting value for your filament model:

Table 6. Recommended starting and maximum heating current by filament insert model.

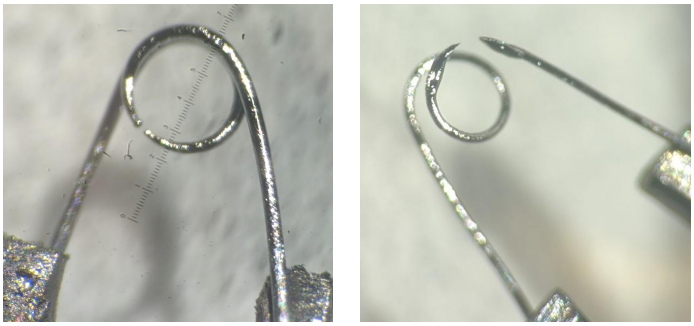
Filament Insert (p/n)	Starting Heating Current (A)	Does not Typically Exceed (A)
11146	2.3	2.6

4. Tune for ECD in MS2 by running a classic ExD autotune followed by a refine ExD autotune on an ExD tuning standard.
5. See **To optimize the filament heating current** to optimize the initial **On** current for ECD.
6. In the ExDControl software, apply the last profiles tuned for transmission in MS1 and MS2 with the filament **On**. If signal intensity of Agilent tuning mix is not satisfactory, re-tune these profiles. See **Tuning**.

NOTE

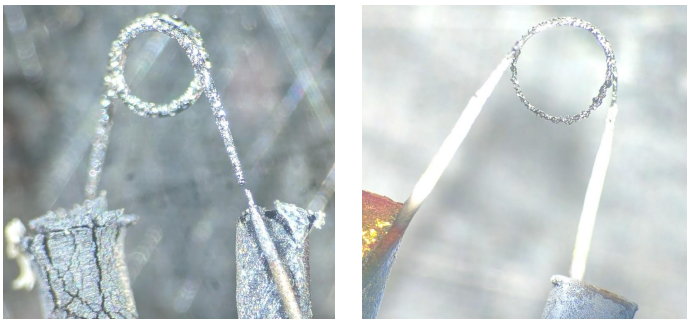
As you raise the heating current, you may notice an atypical disparity between the set and actual voltage readouts for FB and L4, as well as an unusually large 'Emission' current readout. The likely cause is contaminants on the surface of the heated filament. After about 20 minutes of heating, these should burn off and all voltage and current readouts should return to normal.

To evaluate filament failure



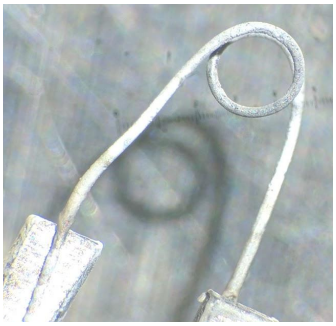
At the end of its design life, the filament will burn out from repeated heat-cycling and material loss. Overheating will cause the filament to burn out earlier.

Figure 37. (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 38. 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 39. Example of a mechanically-damaged filament. The bent left leg pushes the loop out-of-center.

To check for ExD hardware malfunction

First, check whether instrument and ExD Cell tuning is optimized. Load a previously working ExD Cell tunefile and its corresponding MassHunter tune file.

Next, perform a basic check for ExD hardware malfunction: comparing the lens profile "set" values to the "actual" values in the ExDControl **Manual Tune** tab.

- If a constant mismatch between these values for one or more lenses exists, use the decision tree in **Figure 40** to attempt to pinpoint the cause.
- If no mismatch exists but transmission still cannot be rescued through tuning, there may be a physical obstacle within the Cell from improper installation or maintenance. Please contact e-MSion to determine whether an instrument vent by trained personnel is required to investigate.

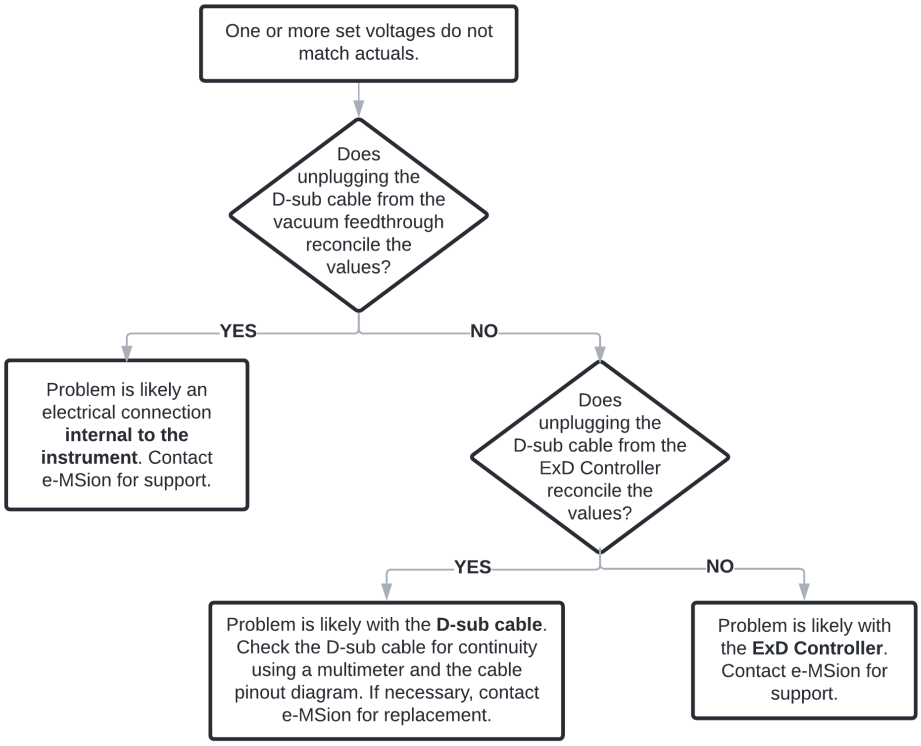


Figure 40. Decision tree for checking ExD AQ-250 Option for hardware malfunction.

If the issue remains undiagnosed and/or unresolved, contact e-MSion for further assistance at Support@e-msion.com.

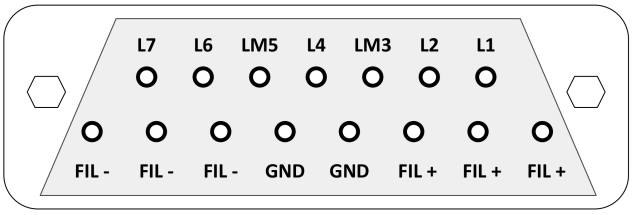


Figure 41. Top view of a male D-sub pinout.

Troubleshooting Table

If problems occur with operation of the instrument with the ExD Cell installed, use the table below to search for possible causes and corrective actions.

NOTE

Questions or need support? Contact e-MSion at Support@e-MSion.com.

Table 7. Troubleshooting issues with the ExD Cell, ExD Controller, and ExDControl software.

Problem	Potential Causes	Suggested Course of Action
ExD Controller not connecting to instrument firmware addon.	Poor cable connection(s).	Check that cable connections are secure and correct. See **Installation** in the *ExD Controller User Guide*.
	ExD Controller issue.	Restart the ExD Controller. See **To power ON/OFF the ExD Controller** in the *ExD Controller User Guide*.
	Software Issue.	Restart the ExDControl software.
ExD Controller connection repeatedly drops during use.	Network or firmware issue.	• Ensure Ethernet switch (or equivalent) is powered on and network is operational. • Restart the ExD Controller. • Check https://e-msion.com/downloads for a software update that fixes the bug.
ExD Cell lens voltage actuals not matching setpoints.	ExDControl software not connected, poor ExD Cell or Controller cable connection(s), or hardware malfunction.	• Verify ExD Controller is powered ON and ExDControl software is connected. See **Connection Settings** in the *ExDControl Software User Guide*. • Verify connectivity of all cables between ExD Cell, ExD Controller, network switch, and PC. • Restart ExD Controller. See **To power ON/OFF the ExD Controller** in the *ExD Controller User Guide*. • See **To check for ExD hardware malfunction**. • If no change, contact e-MSion.
ExD Cell filament current actual not matching setpoint.	Filament burn-out, D-sub cable disconnected.	• Ensure D-sub cable is connected. • Check for filament burn-out. See **To check for filament burn-out**.
MassHunter Errors:		
"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 trap electrons in the ExD Cell.
"Medusa" faults [39] and [40] and/or other errors.	-	Wait until vacuum pressures are closer to pre-install levels. If errors persist, contact your local service engineer or e-MSion.
Poor sensitivity and/or ion transmission in MS1 and/or MS2.	Sample preparation.	Verify purity and concentration of all reagents.

	Acquisition method.	Verify source parameter settings. If using LC, check flow rates, mobile phase composition, injection volumes, divert to waste times.
	LC or ion source needs maintenance.	• Check for leaks/clogs. • Verify temperature, flow actuals match setpoints. • Ensure source parts are clean and in position.
	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 tunefile. Copies created during installation should be saved in the **Verification** folder in **C:\Users\"username"\E-MSION**. • See **To tune the instrument with the ExD Cell installed**.
	ExD Cell lens profile(s) not optimized.	• Load a previously-working ExD tunefile and its corresponding MassHunter tune file. • Retune the ExD Cell. See **Tuning**. The ExD Cell will transmit ions best using profiles tuned separately for MS1 and MS2.
	Incorrect filament setting (e.g. **On** when lens profile was tuned in **Standby**).	Verify that filament heating current is set to the value used when the profile was created.
	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, charging may be building up on internal surfaces. Contact e-MSion for support.
Oxygen • Rhenium oxide observed in the mass spectrum. • Rapid filament burn- out.	Oxygen contamination from gas supply.	• Check collision cell gas purity (99.999%). N_2 gas from an N_2 generator may not be sufficiently pure. • Replace any plastic or teflon tubing or connectors with metal substitutes. • Consider using an oxygen scrubber. If oxygen scrubber is present, check its condition.
	Vacuum leak.	Check all sealing surfaces.
No ECD or poor ECD efficiency.	ExD Cell lens profile is not optimized for ECD.	See **ExD Cell lens profile not optimized** above.
	Insufficient filament heating current.	• See **To optimize the filament heating current**. • There may be current leakage. See **To check for filament current leakage**.
	Filament is deformed.	Mechanical damage to the filament shape may be limiting ECD efficiency. Vent and inspect filament. If necessary, replace.
	Filament has burned out.	To diagnose, see **To check for filament burn-out**. Vent and replace the filament.

Filament circuit.	If filament circuit resistance is infinite, check for the source of the open circuit. See **Step 3. Replace the filament** for instructions on measuring circuit resistance.

5. Concepts

Contents

This chapter provides information on the underlying concepts of the ExD Cell.

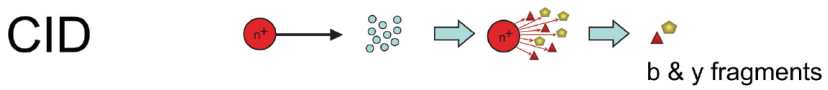
What is electron-based fragmentation?

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.

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.

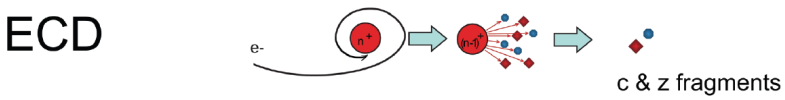
For proteomics applications, CID removes labile motifs such as post-translational modifications (PTMs) as neutral losses, precluding PTM localization. Additionally, as protein size increases, sequence coverage using only CID decreases.

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

ExD: Electron-Activated Dissociation

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

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



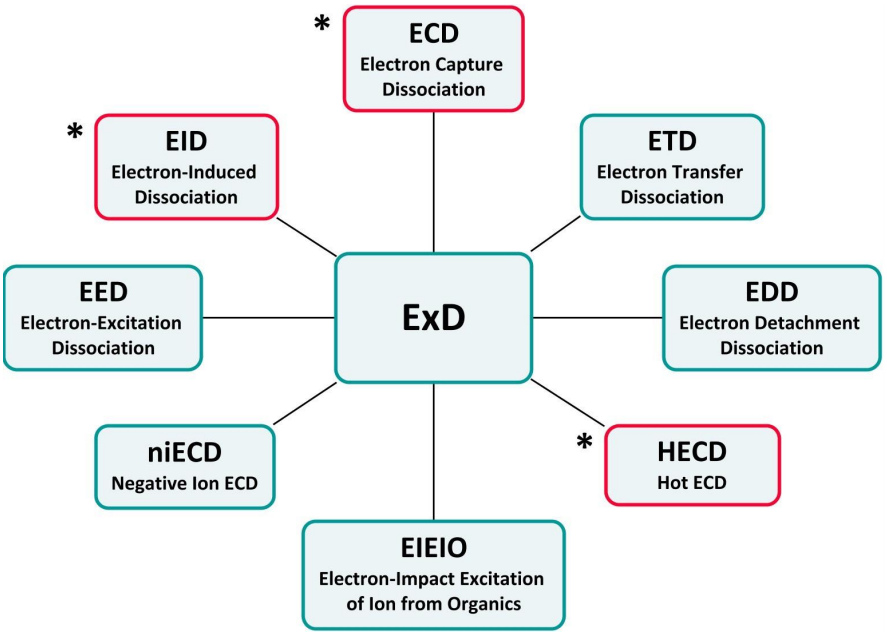


Figure 42. The ExD family of electron-based fragmentation techniques. The techniques that the ExD Cell has been used to produce are starred "*" and outlined in red.

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.

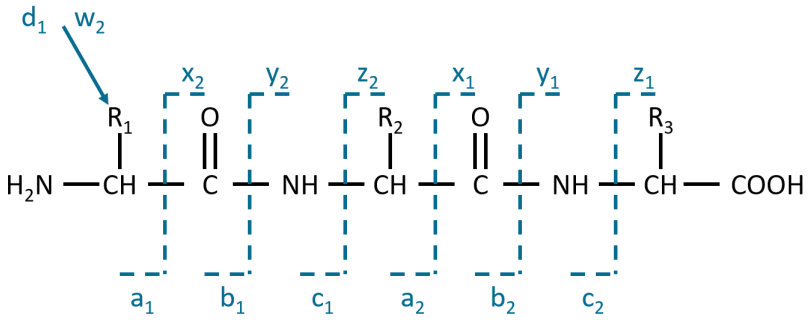


Figure 43. Product ions from peptide backbone and side-chain fragmentation. Peptide fragmentation nomenclature proposed by Roepstroff and Fohlman [Roepstroff, 1984] with adaptations from Biemann [Biemann, 1990].

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.

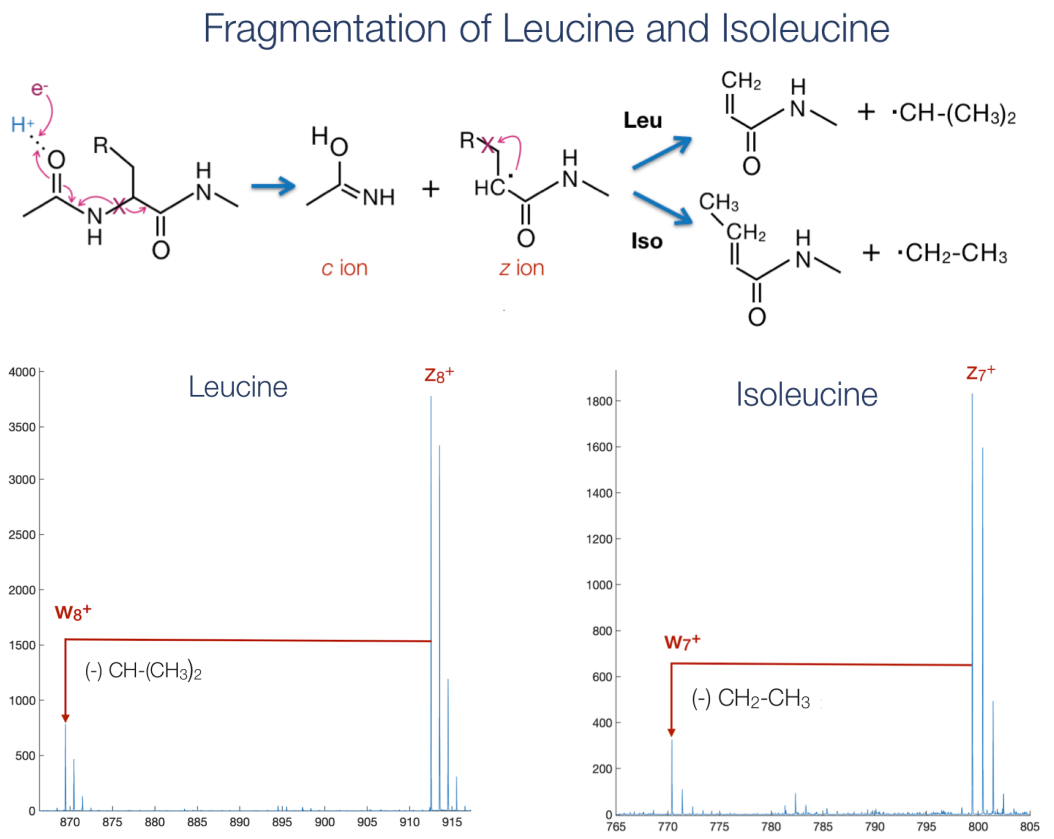


Figure 44. Secondary fragmentation of a *z* ion produces diagnostic *w* ions for distinguishing L8 from I7 in synthetic peptide ECDDisoDELIGHTFLK on an Agilent 6545XT LC/Q-TOF.

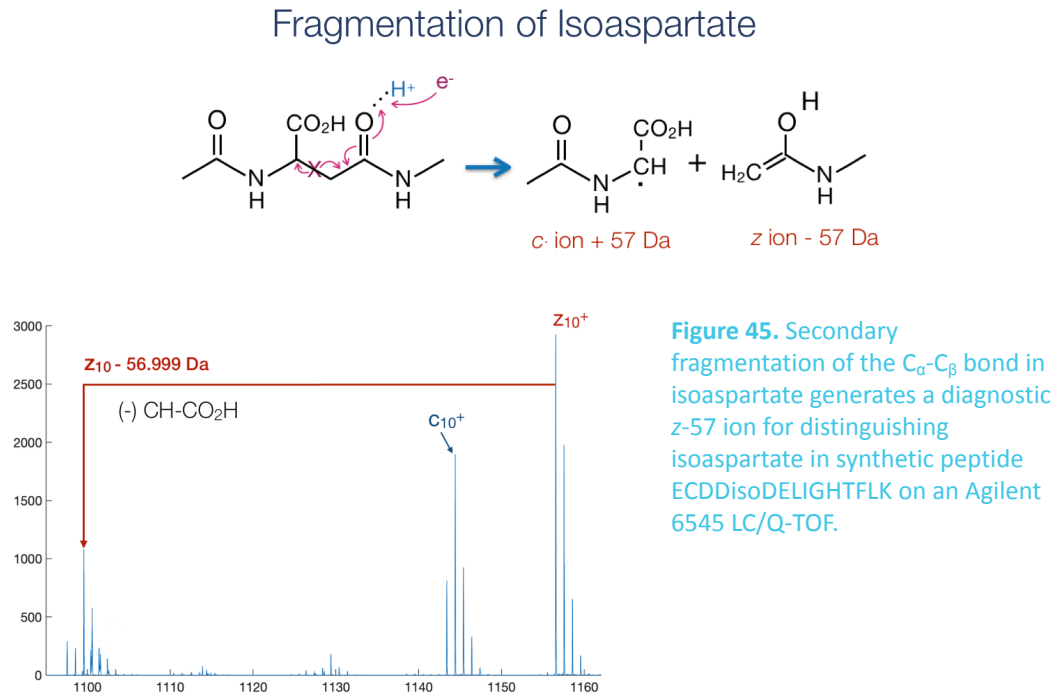


Figure 45. Secondary fragmentation of the C_α-C_β bond in isoaspartate generates a diagnostic *z*-57 ion for distinguishing isoaspartate in synthetic peptide ECDDisoDELIGHTFLK on an Agilent 6545 LC/Q-TOF.

Electron induced dissociation (EID) is another powerful electron-based fragmentation technique. It can be used to fragment singly-charged precursors without neutralizing their charge, unlike ECD. This and its unique fragmentation make EID especially useful for glycomics and metabolomics 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.

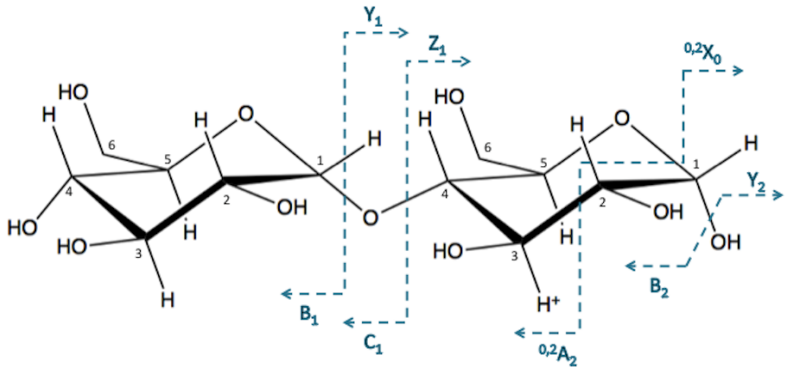


Figure 46. Product ions from glycosidic linkage and cross-ring fragmentation. Nomenclature proposed by Domon and Costello [Domon, 1980].

ExD Efficiency

ExD efficiency can be expressed as

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

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 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 (i.e. more slowly moving ions will capture electrons more efficiently).

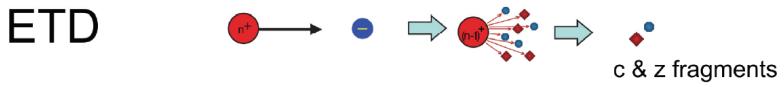
The ExD Cell is most efficient at producing low-energy electrons suitable for ECD. The efficiency with which the Cell facilitates higher-energy electron-based fragmentation techniques (EID and HECD) is lower.

It is important to note that ECD efficiency for peptides generally increases proportionally to the square of the charge state. For a 2+ precursor ion like m/z 674, ECD efficiency of ~1-5% is reasonable. For a 20+ multiply-charged protein, the

efficiency will be greater, although intensity of the ECD products will be distributed across a larger number of fragments and isotopes.

Comparison to Electron Transfer Dissociation

Electron transfer dissociation (ETD) technologies provide similar electron-based fragmentation to ECD. Where ECD uses the capture of free electrons, ETD uses electron transfer from reagent anions to analyte cations to generate fragment ions.



Because the ExD Cell can be tuned for different electron energies, it can provide types of fragmentation beyond ECD that are not readily possible with ETD. In addition, the free electrons used for ExD can more easily penetrate folds of a protein than ETD anions, potentially leading to greater sequence coverage of tightly-folded proteins.

ExD Cell function also does not affect the duty cycle of the mass spectrometer. Where ETD requires ion trapping, the ExD Cell fragments ions within the length of time it takes ions to traverse the Cell. As a result, the ExD Cell functions on a microsecond timescale, making it possible to implement electron-based fragmentation after IM separation. The ExD Cell also avoids any collisional cooling incidental to ion trapping - an important factor for maximizing dissociation.

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MAN-Ag-002

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Until its next release, this guide is valid for the 3.1.0 version or higher of the ExDControl software.