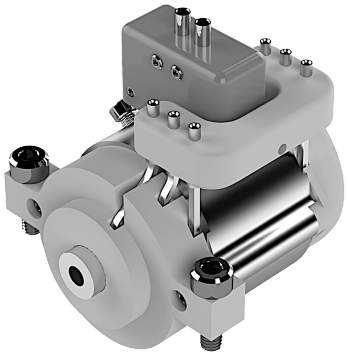
ECD for the Masses

ExD TQ-160 Option

for Thermo Scientific Q Exactive Orbitrap MS



User Guide

R003 • July 2023

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 Thermo Scientific Q Exactive Orbitrap MS.
- 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*

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.
- *Tune* refers to the Thermo Scientific Tune software.
- *MS1* corresponds to **Full Scan** in Tune.
- *MS2* corresponds to **All-Ion Fragmentation (AIF)** with or without precursor mass selection in Tune.

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 ensure the ExD cell filament is OFF before shutting down the instrument.
- Always shut down the instrument, turn OFF the ExD Controller, and disconnect all instrument and Controller power cord(s) before attempting maintenance.
- 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.

Safety and Regulatory Certifications

For products with ExD Controller model ExD19 ONLY:

The e-MSion Electron-based Fragmentation Option for Thermo Scientific Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometers complies to the following regulations on Electromagnetic Compatibility (EMC):

- AS/NZS CISPR 11: 2011 Group 2 Class A
- EN 61326-1:2013
- ICES-001: 2006 Updated 2014
- FCC 18:305:2019
- FCC 18: 307: 2019

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 TQ-160 Option.

General Description

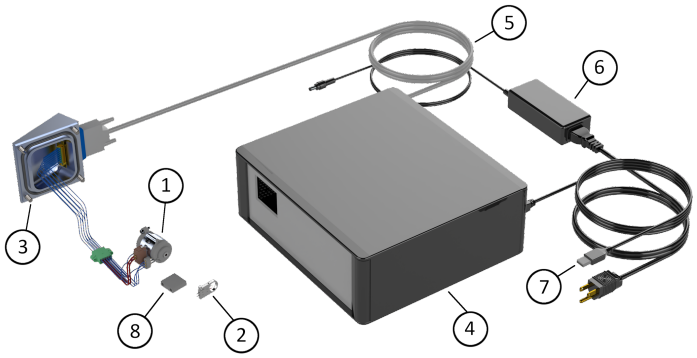


Figure 1. Main components of the ExD TQ-160 Option.

Table 1. Components.

Label	Part
1	ExD Cell
2	Filament insert
3	HCD cover assembly
4	ExD Controller
5	D-Sub cable
6	Power adapter and power cord
7	USB cable
-	Ethernet cable
-	Trigger cable
8	PEEK retaining block
-	Toolkit

The ExD TQ-160 Option is a hardware and software package that equips Q Exactive Orbitrap mass spectrometers with the ability to perform electron-based fragmentation.

Key components include:

- **ExD cell**
- **Filament insert**
- **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 TQ-160 Option are summarized below. See **Concepts** for more information.

Table 2. Electron-based fragmentation techniques available for use with the ExD TQ-160 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.

ExD cell

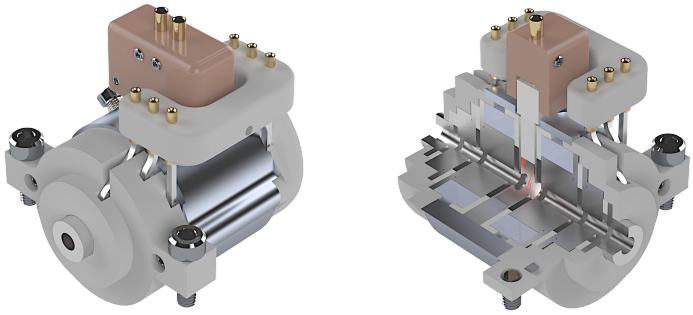


Figure 2. The ExD Cell (left), with cutout (right).

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

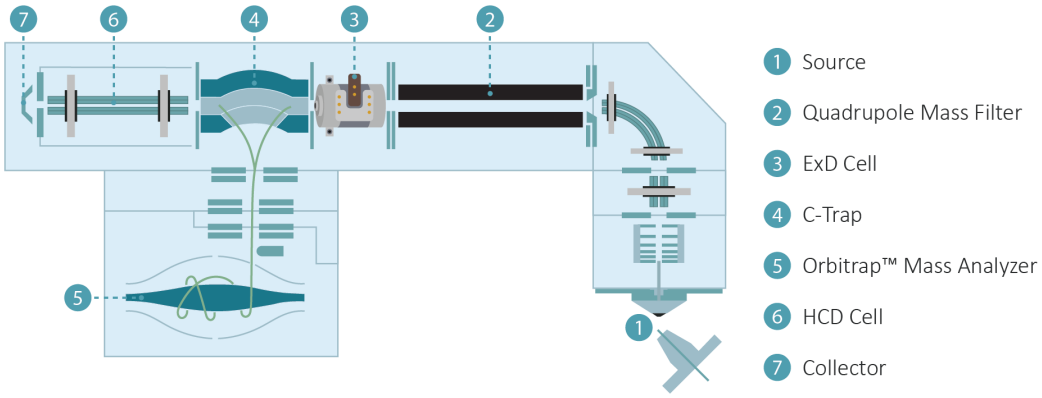


Figure 3. On Thermo Scientific Q Exactive Orbitrap instruments, the ExD Cell installs between the C-trap and the isolating quadrupole, replacing the transfer octupole.

NOTE

***Installation of the ExD Cell is reversible*. Parts removed during installation should be stored intact so that trained personnel can revert the instrument to its factory configuration.**

Magnets and lenses

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

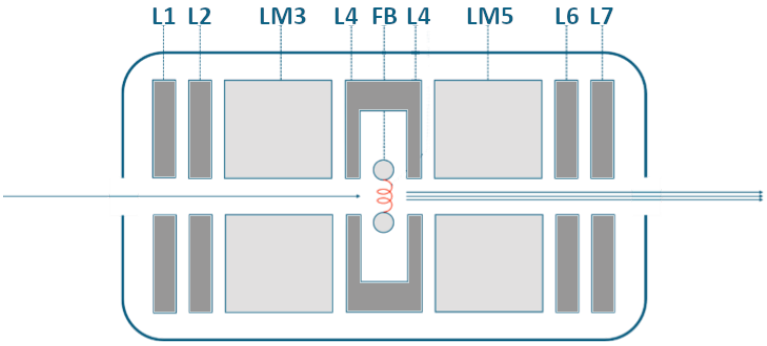


Figure 4. Cross-sectional view of the ExD Cell. The filament is the red coil, lenses are dark grey, and lens magnets are light grey.

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, L6, and L7) 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.

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

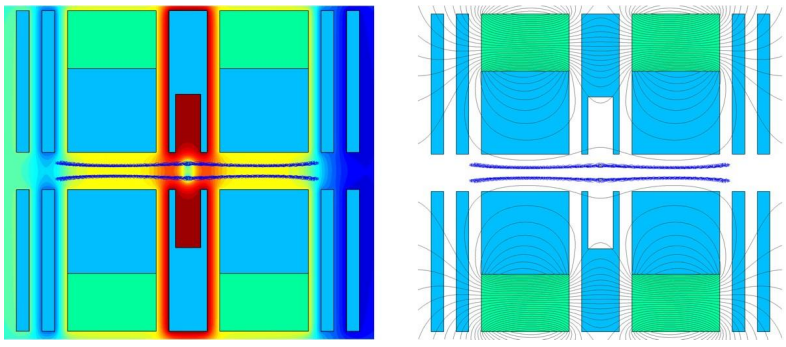


Figure 5. Cross-sectional view of the ExD Cell electromagnetic fields. Electrical potentials, with blue as negative and red as positive (left). Magnetic fields (right). Calculated electron trajectories are shown in dark blue along the central horizontal axis.

Filament insert

The *filament insert* is the electron source for ExD. It holds a rhenium alloy wire suspended between posts.

Thermionic emission is achieved by passing a current through 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 electron interaction and therefore without fragmentation.

The filament is a consumable part. Stress from continual 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**.

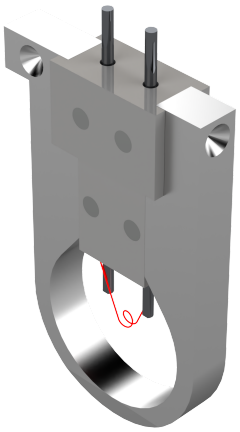


Figure 6. Filament insert.

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, HCD cell gas must adhere to the standard of 99.999% purity and the manifold vacuum quality must be high.**

ExD Controller and ExDControl software

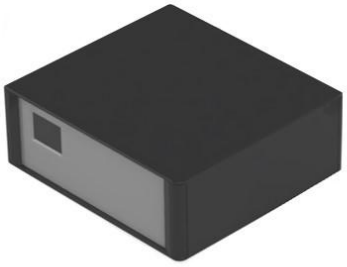


Figure 7. The ExD Controller.

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.**

NOTE

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

2. Operation

Contents

This chapter provides 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 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.

2. 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

3. If the ExD Controller does not automatically connect after the ExDControl software launches, click **Connect** > **Connect**.

   Check for the instrument state readouts that indicate that the ExDControl software is connected to the ExD Controller and the instrument. If no connection, see the *ExDControl Software User Guide*.

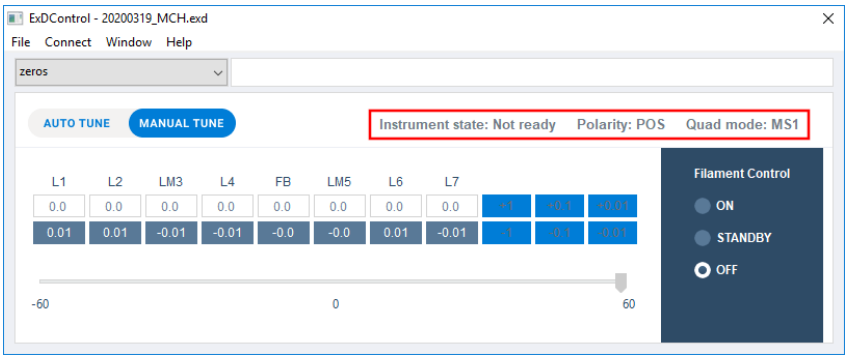


Figure 11. ExDControl main window, with instrument state readouts boxed in red.

Basics of Operation

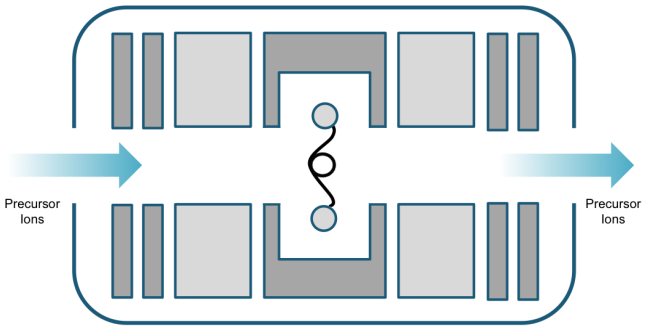
ExD Cell operating modes

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

The two main factors affecting ExD Cell function are:

- Filament heating current
- Lens profile

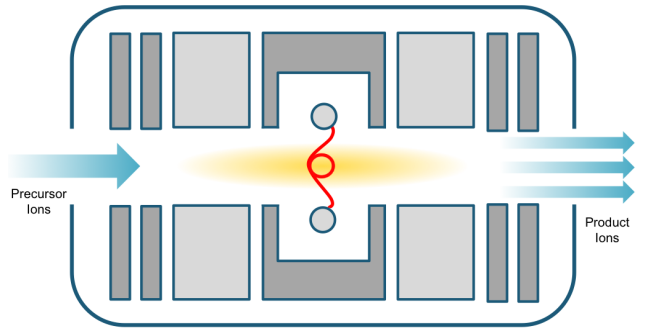
Through the systematic adjustment of these components, the ExD Cell can be tuned to operate in one of three basic modes:



Fil = Off, Standby / ExD = Off

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

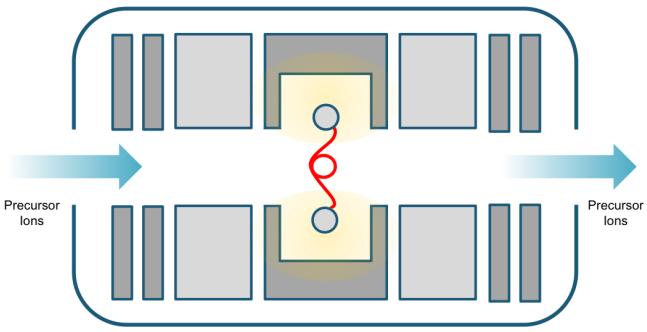
Used for normal operation of the instrument when electron-based fragmentation is *not* desired.



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 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.

Filament settings

- **Off** (0 A)
- **Standby** (0.1 A)
- **On**

  While in use, ExDControl will automatically adjust the **On** current to maintain constant voltage output as the filament wire thins from wear.

  You may also adjust the **On** current in the ExDControl **Filament Parameters** window. See **To optimize the filament heating current**.

Profiles

NOTE

Profiles may be tuned to produce different types of ExD. See Concepts.

Lens profile or *ExD profile* refers to a set of electrical potentials for the eight lenses in the ExD cell. Profiles are tuned by adjusting each lens voltage to optimize either:

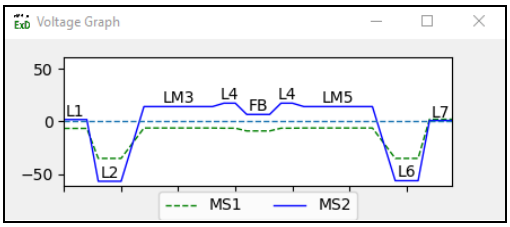


Figure 12. Voltage graph of a profile tuned for ECD.

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

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

Profiles Table

MS1	MS2	Tuned For	Profile	Description	L1	L2	LM3	L4	FB	LM5	L6	L7
☐	☐	MS1	Transmission MS1 Calibrant Solution	Filament current 0 Amp	0.5	-16.7	1.2	0.7	-2.5	1.2	-16.7	1.6
☐	☐	MS1	Transmision MS1 Calibrant Solution	Filament current 2.2 Amp	-1.1	-18.0	-2.7	0.2	-5.4	-2.7	-18.0	1.1
☐	☐	MS1	Transmission MS1 Sub P	Filament current 0 Amp	-1.1	-18.0	-2.7	0.2	-5.4	-2.7	-18.0	1.1
☑	☐	MS1	Transmission MS1 Sub P	Filament current 2.2 Amp	-0.3	-19.0	-1.9	0.5	-4.1	-1.9	-19.0	1.0
☐	☑	MS2	ECD MS2 Sub P	Filament current 2.2 Amp	-0.77	-60.0	9.8	13.9	4.0	9.8	-60.0	-1.77
☐	☐	MS1	Transmissionm MS1 Ubiqutin Denatured ESI	Filament current 2.2 Amp	0.7	-32.0	0.6	1.8	-3.0	0.6	-32.0	1.8
☐	☐	MS2	ECD MS2 Ubiquitin Denatured ESI	Filament current 2.2 Amp	0.23	-59.9	8.8	12.4	4.3	8.8	-59.9	-1.57
☐	☐	MS1	Transmision MS1 Ubiquitin native nanospray	Filament current 2.2 Amp	2.4	-32.0	3.3	6.1	0.5	3.3	-32.0	1.2
☐	☐	MS2	ECD MS2 Ubiquitin native nanospray	Filament current 2.2 Amp	-0.77	-59.9	8.5	13.3	5.2	8.5	-59.9	-1.07
☐	☐	MS1	Transmision MS1 CA native nanospray	Filament current 2.2 Amp	0.7	-32.0	0.7	1.9	-2.9	0.7	-32.0	1.8
☐	☐	MS2	ECD MS2 CVA native nanospray #1	Filament current 2.2 Amp	-0.77	-59.9	8.2	13.0	4.9	8.2	-59.9	-1.07
☐	☐	MS2	ECD MS2 CVA native nanospray #2	Filament current 2.2 Amp	1.7	-46.0	9.5	10.4	3.5	9.5	-46.0	2.0
☐	☐	MS2	NIST mAB Transmission in MS2 Low Res High Pressure	Filament current 2.2 Amp	1.6	-59.6	2.6	4.4	-1.5	2.6	-59.6	1.5
☐	☐	MS2	NIST mAB ECD Low Res High Pressure #1	Filament current 2.2 Amp	1.6	-57.6	3.4	5.2	-0.7	3.4	-59.6	1.5
☐	☐	MS2	NIST mAB ECD Low Res High Pressure #2	Filament current 2.2 Amp	0.5	-58.0	7.1	5.5	1.4	7.1	-58.0	0.6
☐	☐	MS2	NIST mAB ECD Low Res High Pressure #3	Filament current 2.2 Amp	0.5	-58.0	8.8	7.2	3.1	8.8	-58.0	0.6

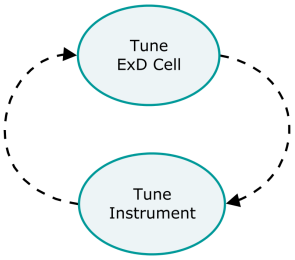
Add Remove

Figure 13. In the **Profiles Table** above, the "Transmission MS1 Sub P" profile will be applied when the instrument is in **Full Scan Mode** and the "ECD MS2 Sub P" profile in **AIF-MS/MS Mode**.

As the instrument alternates between **Full Scan Mode** and **AIF Mode**, ExDControl will apply profiles flagged for MS1 and MS2 respectively in the **Profiles Table**.

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.

CAUTION

When filament is ON and current is above 1.5 A, ExD cell lenses L2 and L6 must ALWAYS be set at or below -20 V to prevent electrons emitted from the heated filament from exiting the ExD cell and causing instrument electronics failure.

- Like the Tune Calibration procedure, regular fine-tuning of ExD Cell lens profiles and filament heating current is recommended.
- ExD Cell lens voltages are dependent on QE flatapole voltages, which determine ion beam kinetic energy.
- The voltage on L1 and L7 should never exceed flatapole DC voltages; otherwise, they will prevent ion transmission.
- When the filament is **Off/Standby**, voltages of lenses L4, LM5, and L6 should not exceed the flatapole DC voltages. When the filament is **On** and emitting electrons, however, L4, LM5 and L6 voltages may exceed the flatapole DC voltages by 5-15 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.
- The lens profile for an optimized ExD tune should be nearly symmetric with lens pairs L1-L7, L2-L6 and LM3-LM5 typically (but not always) very close to their pair partner in value.
- FB should be more negative than L4, LM3, and LM5. The voltage difference between FB and L4 is the primary determinant of electron emission.
- Adjust ExD Cell lens voltages from the inside outwards, beginning with FB. The inner lenses (FB, L4, LM3, and LM5) and outer lenses (L1 and L7) are more sensitive and will respond to small adjustments of 0.1 V, while L2 and L6 are less sensitive and may be adjusted in increments of 0.5-1 V.

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

To autotune the ExD Cell

NOTE

Autotune may only be used to tune for transmission or ECD.

Use autotune to automatically optimize ExD Cell lens voltages for transmission or ECD. The algorithm uses the abundance of known standard peaks to guide the process of tuning the ExD profile.

If the instrument is in **Full Scan** mode when the autotune starts, the results will overwrite the selected MS1 profile in the **Profiles Table**. If the instrument is in **AIF** mode, the results will overwrite the selected MS2 profile.

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

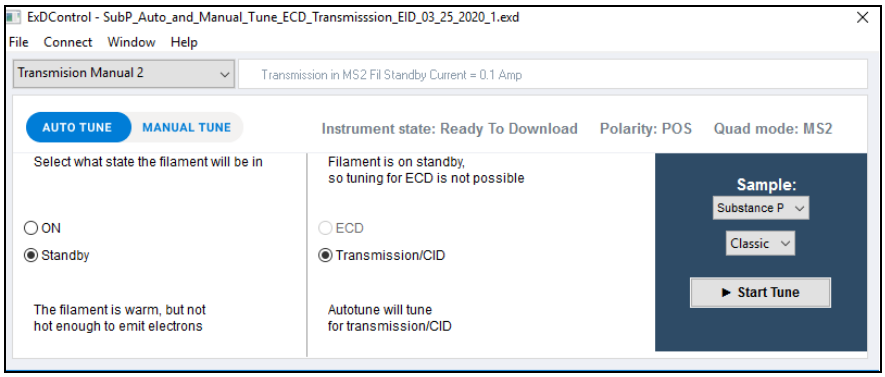


Figure 14. The ExDControl **Auto Tune** tab.

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.

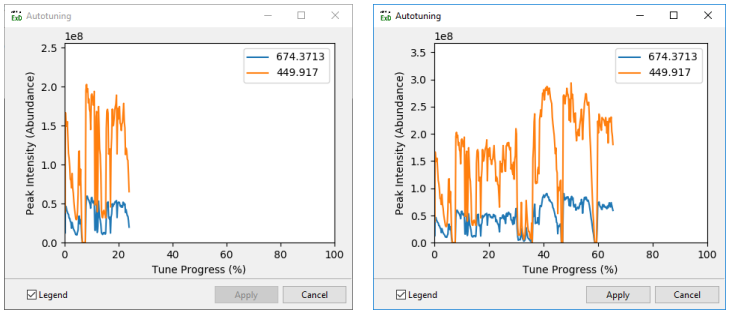


Figure 15. The **Auto Tuning** window plots a graph (*m/z* intensity vs. % progress) while an ECD autotune is in progress (left) and finished (right).

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.

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(s).

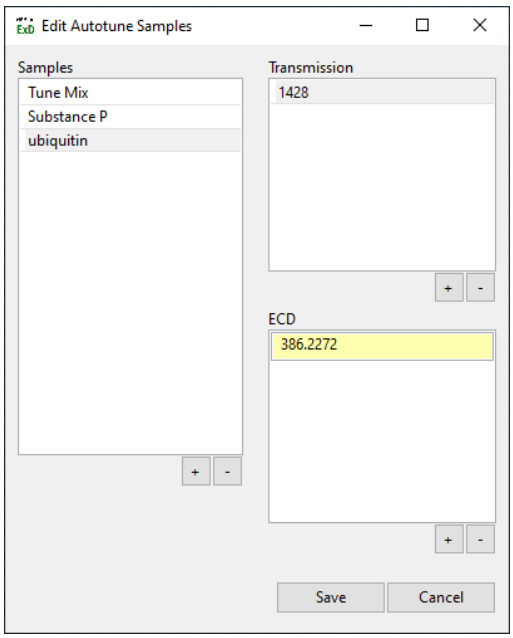


Figure 16. The **Edit Autotune Samples** window.

NOTE

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

To manually tune the ExD Cell for transmission

Tuning for transmission seeks to optimize ion transmission without ECD fragmentation. Follow the steps below to tune the ExD cell to transmit ions with the filament **Off**, **Standby**, or **On**.

1. Infuse a tuning standard. Ensure signal is stable.
2. Set up **Mass Traces** in Tune to plot TIC if tuning in MS1 or the sample precursor *m/z* of interest if tuning in MS2.
3. Open the ExDControl software **Manual Tune** tab. Select a profile to tune for transmission from the drop-down menu.
4. Set Filament to either **Off**, **Standby,** or **On**, depending on the experiment.
5. If tuning with Filament **Standby**/**Off**, set L2 and L6 to -30 V, FB to -1 V, and all other lenses to 0 V.

   If tuning with Filament **On** and heated to emit electrons, set L2 and L6 to -50 V, FB to -2 V, and all other lenses to 0 V.
6. Hold **Shift** and click to select LM3, L4, FB, and LM5 together. Adjust in unison in ± 0.1 V steps within the range of [-6, 6] V until sample peak abundance is maximized and the peak distribution appears standard.
7. Adjust FB in ± 0.1 V steps to maximize peak intensities. FB should always be less than LM3, L4, and LM5.
8. Adjust L4 in ± 0.1 V steps to maximize peak intensities. L4 should be greater than FB by about 0.5-5 V.
9. Adjust LM3 and LM5 in ± 0.1 V steps. Values should differ from L4 by 0.5-5 V.
10. Adjust L1 in ± 0.1 V steps.
11. Adjust L7 in ± 0.1 V steps.
12. Adjust L2 and L6 in unison in ± 1 V steps. Values should be within [-40, -20] V.

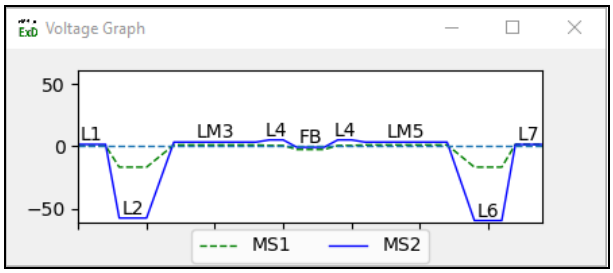


Figure 17. Profiles for transmission in MS1 and MS2 displayed in the **Voltage Graph** window.

To manually tune the ExD Cell for ECD

1. Infuse an ECD tuning standard. Ensure signal is stable. A profile tuned on a standard will work well for other samples of similar size and structure.
2. In Tune, set the scan type to **AIF - MS/MS** and isolate a precursor ion with a minimum charge of 2+ using a 4-5 *m/z* range isolation window. Set HCD collision energy to 10 eV (default).

NOTE

For QE models excluding UHMR, if working with peptides or small to mid-sized proteins and capable of adjusting Ion Transfer parameters, set Source DC Offset to about 50 V (default 25 V), Bent Flatapole DC slightly higher than default (default 6 V), and C-Trap Entrance Lens – Inject slightly lower than default (default 5.8 V). If working with large proteins, use default settings.

3. Set up **Mass Traces** in Tune to trace a dominant ECD fragment ion *m/z*.
4. Open the ExDControl software **Manual Tune** tab. Select the profile to tune for ECD from the drop-down menu.
5. Set the filament to **On.** See **To optimize the filament heating current**.
6. Set L2 and L6 to -50 V, FB to -3 V, and all other lenses to 0 V.
7. Hold **Shift** and click to select LM3, L4, FB, and LM5 together. Raise in unison in 0.1 V steps until target ECD fragment ions appear.
8. Adjust FB in ± 0.1 V steps to maximize ECD fragment ion intensity. FB should always be less than LM3, L4, and LM5.
9. Adjust L4 in ± 0.1 V steps. L4 should be greater than FB by about 10 V.
10. Adjust LM3 and LM5 in ± 0.1 V steps.
11. Adjust LM3, L4, FB, and LM5 in unison in ± 0.1 V steps.
12. Repeat steps 8, 9 and 10. Final values for L4 should be greater than FB by about 10 V and LM3 and LM5 by about 5 V.
13. Adjust L1 in ± 0.1 V steps.
14. Adjust L7 in ± 0.1 V steps.
15. Adjust L2 and L6 in unison in ± 1 V steps. Values should be within [-60, -20] V.

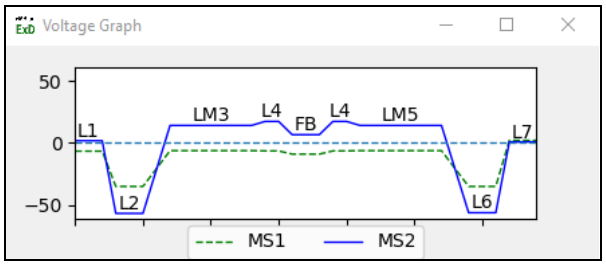


Figure 18. Profiles for transmission in MS1 and ECD in MS2 displayed in the **Voltage Graph** window.

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 10 minutes for the ExD Cell to equilibrate after turning the filament On from Off/Standby before using the Cell.

For ExD to occur, the filament **On** current must provide a sufficient level of electron emission.

Table 3. Recommended **On** current range, by filament model.

Filament (part no.)	On Current Range (A)
F2001 (11478)	2.0 - 2.3

If ECD efficiency using a profile tuned for ECD is low,

1. Slowly increase the **On** current in 0.01 A steps.

   If returns in ECD fragment ion signal intensity begin to diminish, decrease the current to the point of highest fragmentation for current input.

NOTE

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

Changes to the filament current may require retuning of any ExD profiles meant for use with the filament On, particularly FB and L4.

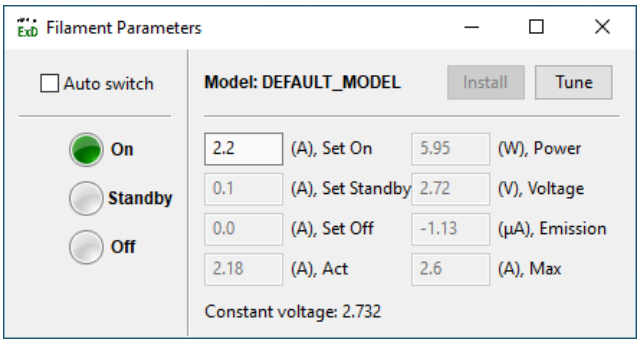


Figure 19. The **Filament Parameters** window with the filament **On** and set at 2.2 A.

2. After adjusting the filament **On** current, check the following:

   a. Rhenium evaporates from the heated filament wire. If rhenium ion intensity is greater than ~1e4, then the filament may be overheated. Decrease the filament heating current.

   b. A high proportion of rhenium oxide relative to rhenium indicates that oxygen is quickly degrading the filament. See **Oxygen**.

Table 4. Rhenium and rhenium oxide *m/z* values.

Rhenium Ions	Rhenium Oxide Ions
m/z 184.9530 and 186.9558	*m/z* 200.9479 and 202.9507, 216.9428 and 218.9456, 232.9378 and 234.9405, 248.9326 and 250.9354, etc.

To tune the instrument with the ExD Cell installed

With the ExD Cell installed, the Tune automatic tuning procedures can still be used. Keep in mind that ExD Cell components *are not adjusted* by the Tune software.

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

To prepare ECD tuning standards

WARNING

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

NOTE

A lens profile developed using a tuning standard can be used to perform ECD on other experimental samples with similar charge states.

Table 5. ECD tuning standards.

ECD Tuning Standard	Instrument	Preparation
Carbonic Anhydrase (CAS no. 9001-03-0)	UHMR	**Native:** 10 µM in 100 mM ammonium acetate (nanospray).
NISTmAb (NIST RM no. 8671)	UHMR	**Native:** 1 mg/mL in 100 mM ammonium acetate (nanospray).
Ubiquitin (CAS no. 79586-22-4)	Other QE	**Native:** 10 µM in 100 mM ammonium acetate (nanospray). **Denatured:** 1 µM in 50/50/0.1 (v/v/v) ACN/water/formic acid (ESI).

Keep in mind the following:

- Unless sample is high-purity, use a clean-up step before directly infusing.
- Use LCMS-grade solvents.
- Choose the precursor selected for ECD by its abundance and charge state.

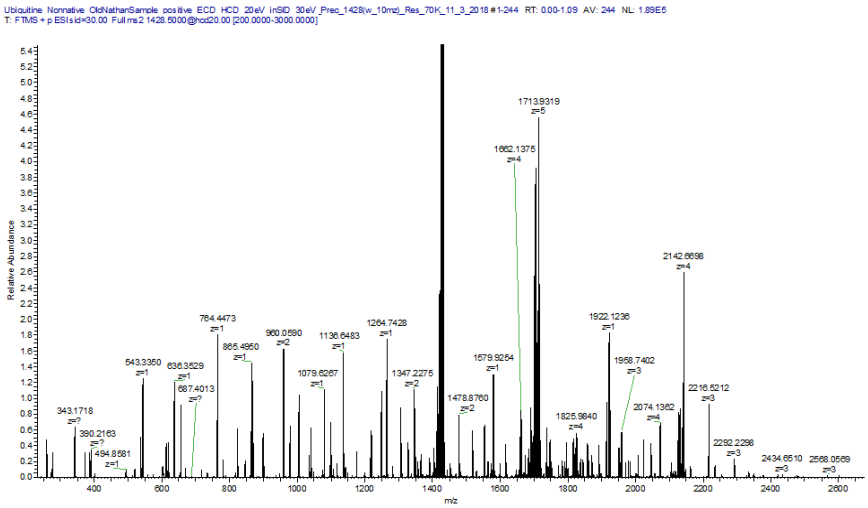


Figure 20. Averaged ECD spectrum of 6+ precursor of native ubiquitin.

To put the system in Standby/Off condition

If planning to leave the instrument in **Standby/Off** while not in use,

1. Set the filament to **Standby** or **Off** in ExDControl.

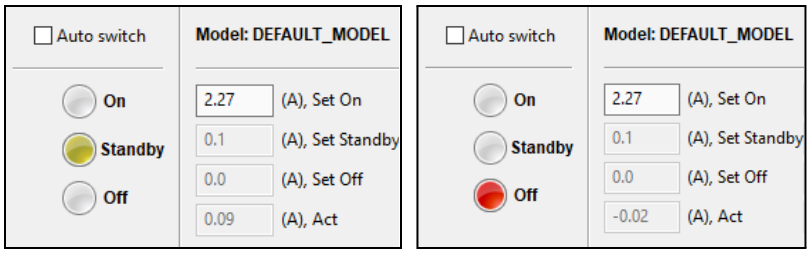


Figure 21. The **Filament Parameters** window, with the filament set to **Standby** (left) and **Off** (right).

2. In the Tune software, put the instrument in the **Standby** or **Off** condition.

CAUTION

Putting the instrument in the Off condition will shut off high voltages, as well as the sheath and auxiliary gas, increasing the likelihood of atmosphere gases being introduced to the filament. Be sure to set the filament to Off to prevent damage to the heated filament wire.

To shut down the system

CAUTION

The filament current should ALWAYS be set to zero ("Off") before venting to prevent damage to the heated filament wire.

1. Set the filament to **Off** in ExDControl.
2. Turn the ExD Controller off. Press and hold the power button on the back panel until the front LCD screen turns black.
3. Unplug the ExD Controller power cord.

CAUTION

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.

4. In the Tune software, put the instrument in the **Off** condition.
5. Put the electronics power switch in the **Service Mode** position.
6. Put the main power circuit breaker switch in the **Off** position to vent the system
7. Unplug the instrument power cord from the rear panel.

WARNING

Before performing maintenance, ensure the system has vented and power to the instrument and ExD Controller has been disconnected. Wait for a sufficient time before removing any protective housings, as capacitors inside the instrument may still be charged.

3. Data Acquisition and Analysis

Contents

This chapter provides an overview of ExD data analysis.

Acquisition

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

- ECD requires a minimum charge state of 2+ (since electron capture neutralizes one charge), but efficiency will roughly increase with the square of charge state. EID can be used on singly-charged precursors.
- Averaging more scans will help to achieve adequate signal-to-noise for low-intensity ECD peaks. A slower scan rate will also help, if the experiment allows.
- The addition of CID energy after ExD may help separate dissociated fragments in tightly folded compounds from one another.
- Document the ExD Cell settings used for your sample runs and worklists.

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

Analysis

Data generated by a Thermo Scientific mass spectrometer with the ExD Cell installed will be in the standard .raw format. From here, many possible workflows are available for data analysis.

When analyzing ECD data, keep in mind the following:

- Data files will not identify the ion activation method used as ExD.
- Analysis software capable of identifying ETD-type fragments will also work for analyzing ECD data, as the fragment ion types produced are similar.
- ECD product peaks are typically lower in intensity than fragment ions produced by collisional activation (CID, HCD, etc.). 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.
- ECD is often accompanied by hydrogen transfer between product ions (e.g. from c- to z-ions and vice versa if the lifetime of the intermediate complex holding them together is long enough).

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:

- ECD fragments polypeptides with less bias than CID, resulting in greater sequence coverage.
- ECD efficiency tends to increase with 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, in addition to Thermo Scientific ProSightPC software, are available.

In addition to Thermo Scientific ProSightPC, e-MSion recommends several third-party software packages to assist with the analysis of ECD data:

Table 6. Suggested software options for ExD data analysis.

Software	Notes	Availability	Source
ExD Viewer *e-MSion, Inc.*	• Interactive spectrum annotation for a wide range of fragment types • Option for deconvolution of raw data	Free	https://e-msion.com/exdviewer/
ProSight Lite *Northwestern University*	• Well-known industry standard for analyzing electron-based fragmentation data sets • Requires deconvoluted data	Free	Fellers et. al., 2015. http://prosightlite.northwestern.edu
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. https://omics.pnl.gov/software/lcmsspectator
mmass *Strolham et. al.*	• Basic assignment of *c* and *z* ions • Open source • No longer in development	Free	Strolham et. al., 2010. http://www.mmass.org
MASH Explorer *University of Wisconsin-Madison*	• Under development • Profile data deconvolution and fragment assignment	Free	Cai et. al., 2016. http://ge.crb.wisc.edu/MASH_Explorer/index.htm
Protein Metrics Product Suite *Protein Metrics Inc.*	• Comprehensive • Well-known industry standard	Commercial	https://www.proteinmetrics.com

4. Maintenance and Troubleshooting

Contents

This chapter provides instructions for performing routine maintenance and guidance for troubleshooting issues that may occur during operation.

NOTE **Contact e-MSion at support@e-msion.com or a supported distributor to order replacement parts.**

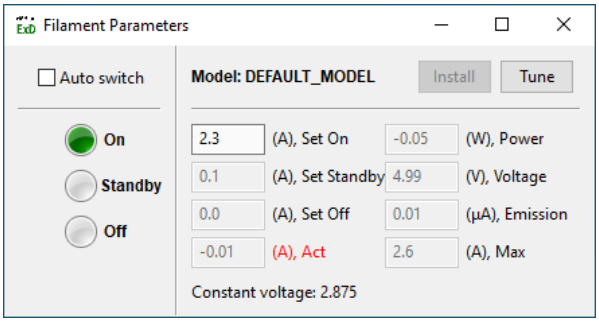
CAUTION **Damages to the ExD Cell, filament, or filament cassette caused by the user during maintenance are not covered under warranty. If you feel you need assistance with filament replacement, contact e-MSion.**

CAUTION **Outside of the removing and replacing 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

Eventually, stress from heating will cause the filament to fail, or "burn-out". Symptoms of burn-out are visible when the current setpoint is > 1 A:



- Current actual **(A), Act** approaches 0 A.
- Voltage drop **(V), Voltage** approaches maximum of 5 V.
- Power consumption **(W), Power** approaches 0 W.

Figure 22. Filament Parameters if either filament is burned out or D-sub cable is disconnected.

1. Ensure the D-sub cable is properly connected. D-sub cable damage or disconnection will mimic symptoms of filament failure.
2. See **To replace the filament** for instructions on replacing the filament.

NOTE

The instrument may continue to operate with a burned-out filament, however, the filament must be replaced in order to perform electron-based fragmentation.

To replace the filament

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

Figure 23. Close-ups of a new filament (left), a filament heated to the threshold of thermionic emission (middle), and a filament that has burned out (right).

Parts

- Replacement filament insert

Tools

- Screwdriver, flat blade
- Hex key, 4 mm
- Hex key, 6 mm
- Hex key, 2.5 mm
- Hex key, 1.5 mm
- Hex key, 0.9 mm

4.1. Inspect replacement filament insert

CAUTION

Do not touch the filament wire! Hold the filament by the filament holder. Always wear gloves.

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

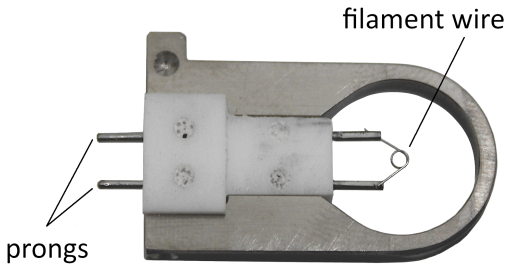


Figure 24. ExD Cell filament.

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

4.2. Uninstall ExD cell

1. Shut down the system. See **To shut down the system.**

WARNING

Before continuing, ensure the system has vented and power to the instrument and ExD Controller has been disconnected. Wait for a sufficient time before removing protective housings, as capacitors inside the instrument may still be charged.

2. **Detector-side.** Loosen three screws on the back panel and slide forward to remove detector-side instrument housing.
3. Use a 4 mm hex key to remove the three screws from the hinge and bottom right corner of the C-Trap RF power supply enclosure (**Figure 25**).
4. Use a flat-blade screwdriver to release the captive screw in the bottom right corner behind the enclosure.

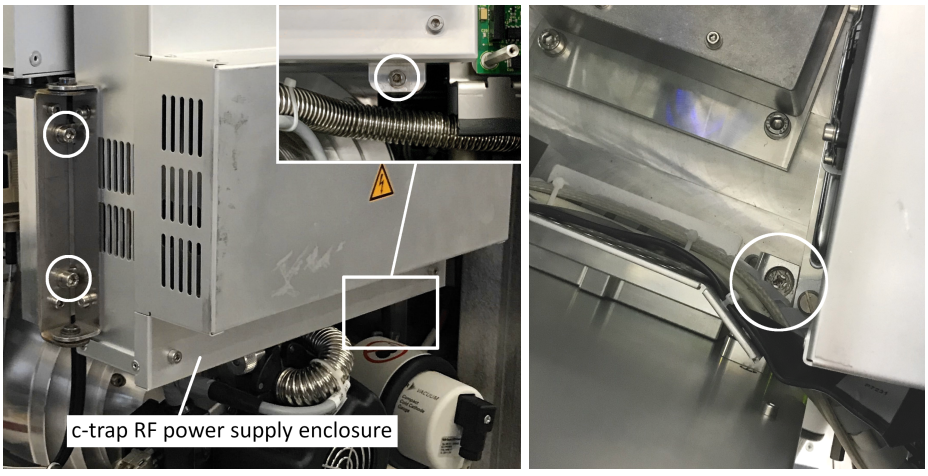


Figure 25. C-Trap RF power supply enclosure screws, front (left) and rear (right).

5. Swing open the enclosure to access the C-Trap flange.
6. Use a 6mm hex key to remove the four C-Trap flange screws (**Figure 26**).
7. Carefully pull the C-Trap assembly from the instrument (**Figure 27**).

Figure 26. C-Trap flange.

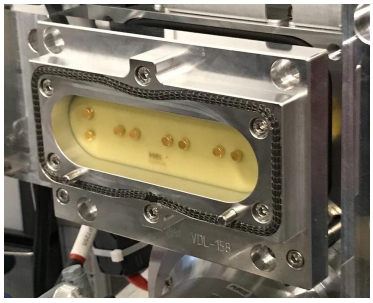


Figure 27. Removing the C-Trap assembly.

8. Set the C-Trap aside on a clean, dust-free surface. Cover with aluminum foil to protect from exposure to dust.

4.3. Replace filament insert and reinstall cell

WARNING **Parts inside the instrument will be hot after venting. Allow parts to cool before touching.**

1. Disconnect the green wiring harness connectors.

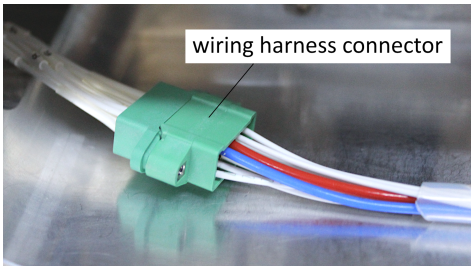


Figure 28. Wiring harness connectors.

1. Use a 2.5 mm hex key to remove ExD cell mounting screws.

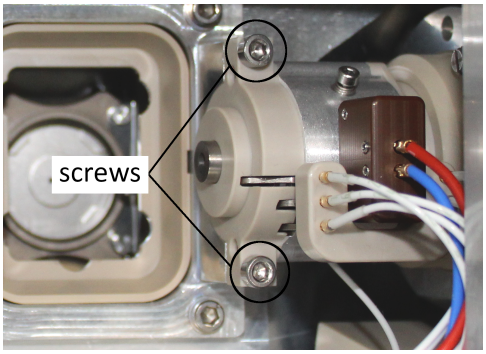


Figure 29. The ExD cell replaces the transfer multipole in the C-Trap chamber.

2. Place ExD cell on a clean, dust-free surface.

3. Use a 1.5 mm hex key to loosen the shroud screw.

CAUTION **Do not fully remove shroud screw. Screw is difficult to replace in Cell.**

4. Grasp the filament insert by the base (brown) and pull upwards to remove from the ExD cell.

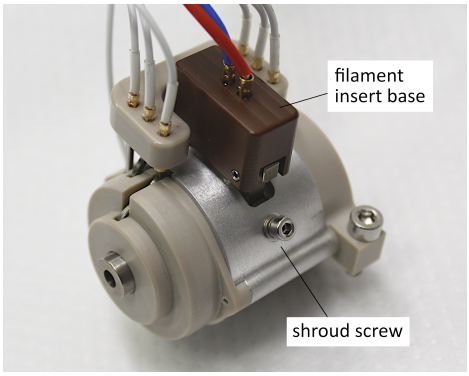


Figure 30. ExD cell with filament insert.

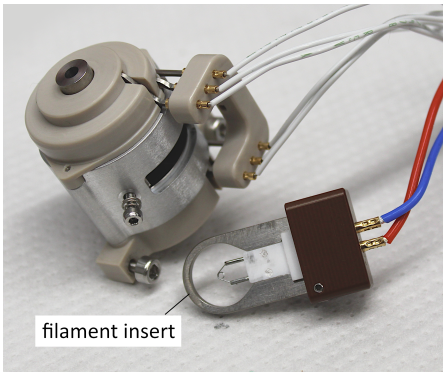
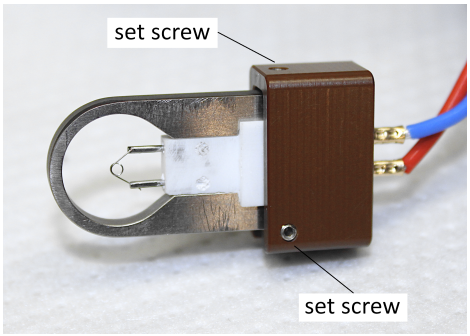


Figure 31. Filament slot.

5. Use a 0.9 mm hex key to loosen the three set screws in the filament insert base.
6. Slide the filament insert prongs out of the base to remove. Set the failed filament insert aside.



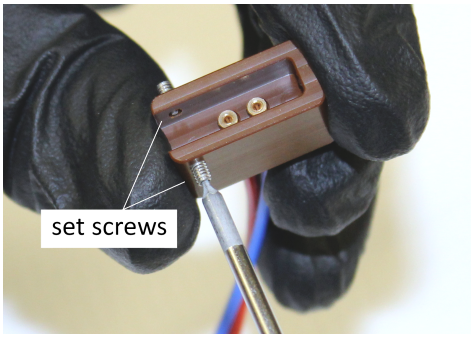


Figure 32. Filament insert set screws, side view (left) and top view (right).

7. Slide the replacement filament insert prongs into the base so that the set screw bores align.
8. Tighten the three set screws, alternating between screws, to secure the filament insert in the base.
9. Slide the replacement filament insert into the ExD cell headfirst so that the screw bore in the filament insert edge aligns with the shroud screw.
10. Tighten the shroud screw.

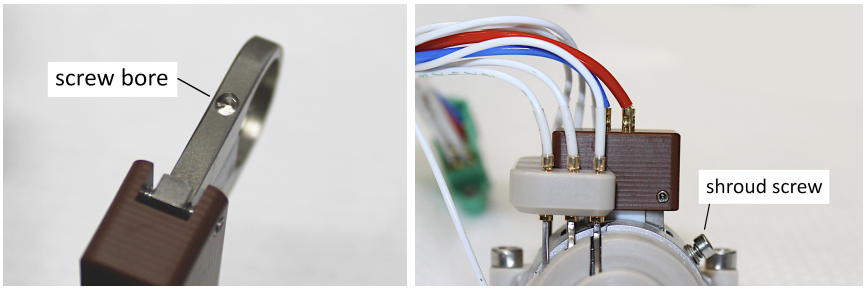


Figure 33. Shroud screw in the filament insert edge.

11. Ensure the ExD cell wiring harness is still attached correctly.

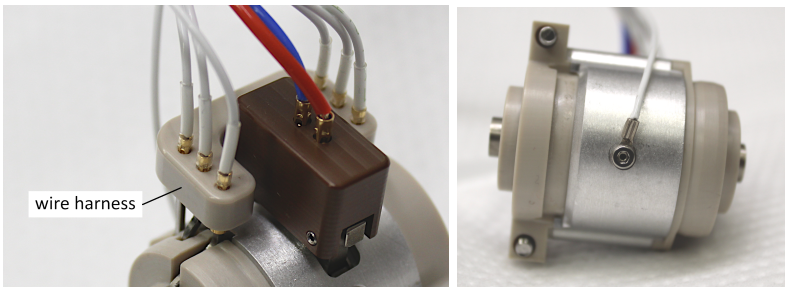


Figure 34. ExD Cell wiring harness, top view (left) and bottom view (right).

4.4. Check filament circuit

1. Reconnect the green wiring harness connectors. See **Figure 28**.
2. Use a multimeter to test the filament circuit resistance across the D-Sub vacuum feedthrough in the HCD cover. A completed circuit will have a resistance of 0.1-1.0 Ω. If the circuit is open, check the integrity of the filament insert and wiring harness connections.

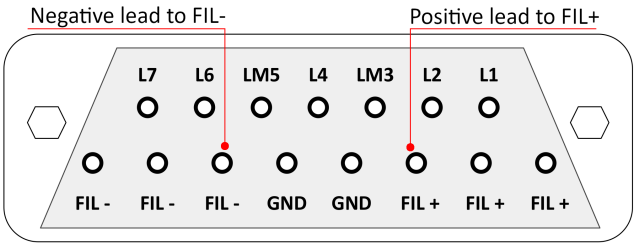


Figure 35. Male D-Sub pinout.

4.5. Reinstall ExD cell

1. Reinstall the ExD Cell in the C-Trap chamber. Hold the cell in place while tightening the two mounting screws to secure the cell.
2. Clean the C-Trap flange o-ring and mating surface to ensure proper seal.

3. Carefully slide the C-Trap assembly into the C-Trap chamber and tighten the original flange screws evenly.

CAUTION **Ensure ExD cell wires are routed appropriately. Avoid catching wires on the C-Trap.**

4. Close the C-Trap RF power supply enclosure and secure with original screws.
5. Replace detector-side instrument housing.
6. Plug in the instrument power cord and ExD Controller power cord.
7. Put the main power circuit breaker switch in the **On** position.
8. After five minutes of pumping down, put the electronics switch in the **Operating Mode** position.
9. Turn the ExD Controller on. Press and hold the **ON/OFF** button on the back panel of the Controller until the front LCD screen lights up.

CAUTION **To avoid burning out the filament, keep filament Off (0 A) until vacuum is re-established.**

4.6. Set initial filament On current

1. When the instrument vacuum is sufficient for operation, reconnect to the ExD Controller by clicking menu item **Connect** > **Connect**.
2. Infuse your preferred ECD tuning standard.
3. In ExDControl, select the last profile tuned for transmission with filament **Off/Standby** profile for MS1. Set filament current to **Off** or **Standby** to match. If signal intensity is not satisfactory, re-tune the profile. See **Tuning**.
4. In the **Filament Parameters** window, turn filament **On** and set the heating current to 0.3 A.
5. With the instrument in **Full Scan** mode, gradually increase the "Set" current to 2.2 A in 0.1 A steps. Monitor TIC or NL intensity, high vacuum, and ultra-high vacuum readbacks in Tune.

CAUTION **If high vacuum readbacks increase significantly due to outgassing from the new filament, wait for a return to baseline before continuing to increase the filament heating current.**

NOTE **A decrease in TIC or NL intensity indicates the start of electron emission from the filament. This usually occurs around 1.7-1.8 A, however, this level of emission is often insufficient for ECD.**

6. Put the instrument in **AIF - MS/MS** mode. Isolate an abundant, multiply charged precursor using a 4-5 *m/z* range isolation window. Set HCD collision energy to 10 eV (default).
7. In ExDControl, select the last profile tuned for ECD.
8. Check for key ECD fragment ions visible in the spectrum. For example:

- $\mathbf{z_{17}}^{2+}$ at *m/z* 960.0570 from native ubiquitin $[M+6H]^{6+}$ precursor
- $\mathbf{c_9}^{+}$ at *m/z* 1149. 5337 from native carbonic anhydrase $[M+10H]^{10+}$ precursor

If visible, tune to optimize ECD. See **Tuning**. If not visible, try increasing filament current further in 0.1 A steps.

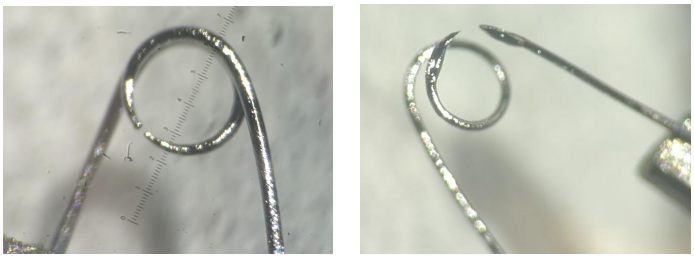
9. Optimal current is generally in the range of 2.15 - 2.25 A. Improve the filament longevity by decreasing the heating current to the point of highest fragmentation for current input.

NOTE

As current increases, outgassing of the heated filament may cause a disparity between set and actual L4 and FB voltage readbacks as well as a large 'Emission' current readout. After around 20 minutes of heating, readouts should return to normal.

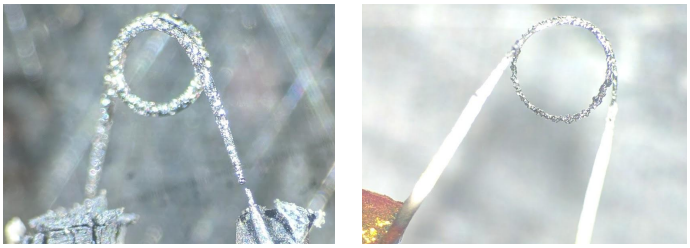
4.7. Evaluate cause of filament failure

1. Once the replacement filament insert is successfully installed, inspect the failed filament insert to determine the cause of failure.



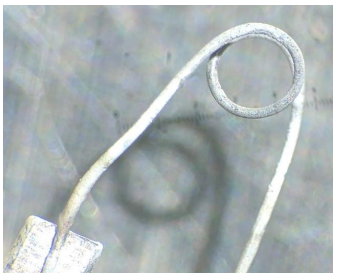
At the end of its life, the filament will burn out from repeated heat-cycling and material loss. Overheating will cause the filament to burn out earlier.

Figure 36. (Left) A filament that failed from routine use, with slight thinning around the failure site. **(Right)** A relatively new filament that failed due to overheating, with much less thinning around the failure site.



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.



If ECD efficiency is consistently low and none of the indicators of burn-out are present, the filament may have bent during installation or 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 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 instrument tune settings 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 39** 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.

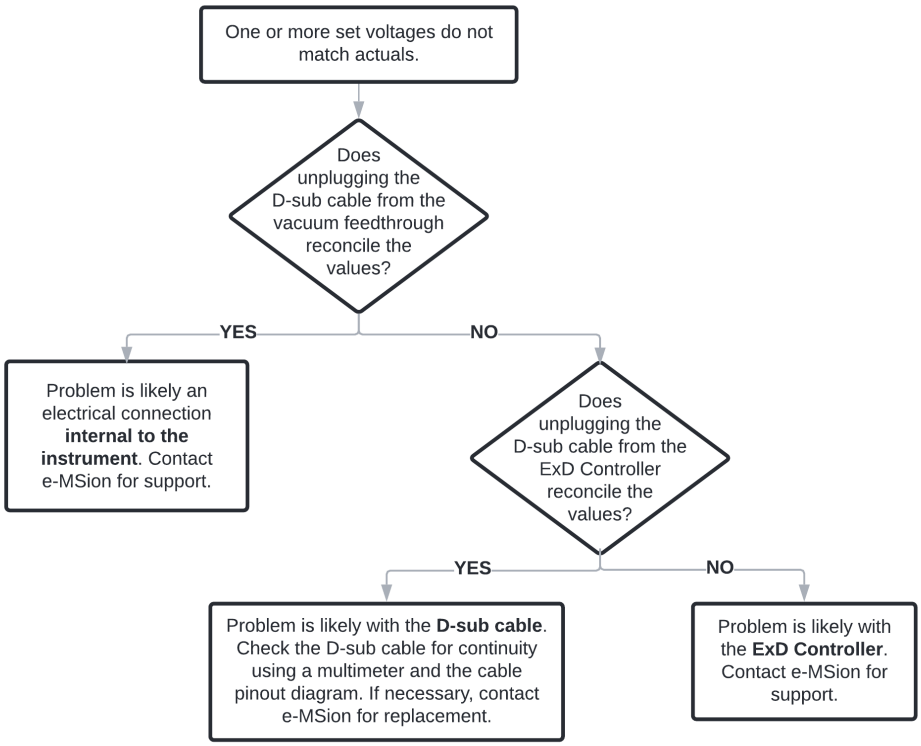


Figure 39. Decision tree for checking for ExD hardware malfunction.

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

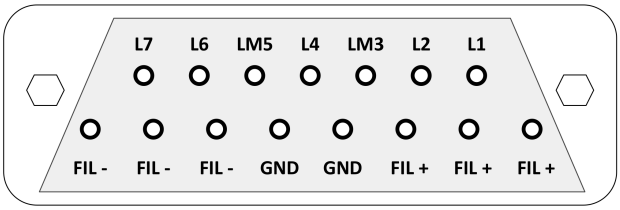


Figure 40. Top view of a male D-sub pinout.

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.	Poor cable connection(s).	Check that cable connections are secure and correct. See **Installation** in the *ExD Controller User Guide*.
	Firmware 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 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. • 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*. • If no change, contact e-MSion.
ExD Cell filament current actual not matching setpoint.	ExDControl software not connected, poor ExD Cell or Controller cable connection(s), or hardware malfunction.	• Check for filament burn-out. See **Maintenance**. • Verify ExD Controller is powered ON and ExDControl software is connected. • 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*. • If no change, contact 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, verify 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 tune file and ExD tunefile. • See **Tuning**.
	ExD Cell lens profile(s) not optimized.	• Load a previously-working ExD tunefile. • 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 HCD cell gas purity (99.999%). N_2 gas from an N_2 generator may not be sufficiently pure. • Check supply line and fittings for leaks.
	Vacuum leak.	Check all sealing surfaces.
No ECD or poor ECD efficiency.	ExD Cell lens profile is not optimized for ECD.	See **Tuning**.
	Insufficient filament heating current.	Optimize filament heating current. See **To optimize the filament heating current**.
	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.
	Faulty filament circuit.	If filament circuit resistance is infinite, check for the source of the open circuit. Measure filament circuit resistance using **Figure 40** pinout. Resistance should be between 0.1 - 1 Ω.

6. Concepts

Contents

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

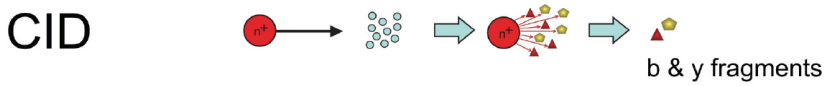
What is electron-based fragmentation?

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.

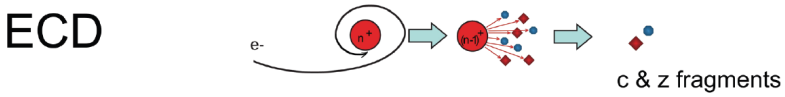
For proteomics applications, CID removes labile motifs such as post-translational modifications (PTMs) as neutral losses, precluding PTM localization. Additionally, as protein size increases, sequence coverage using only CID decreases.

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.



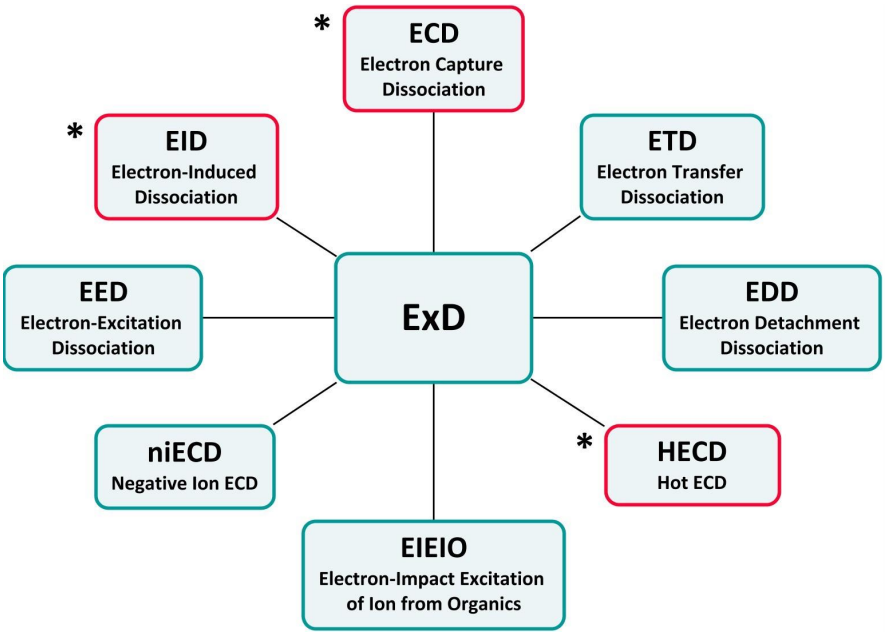


Figure 41. The ExD family of electron-based fragmentation techniques. The techniques that the ExD Cell has been used to produce are starred "*" and outlined in red.

ECD uniquely complements the existing CID capabilities of Thermo Q Exactive 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.

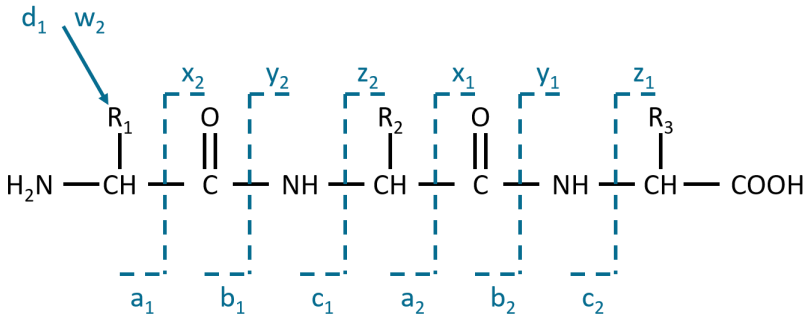


Figure 42. Product ions from peptide backbone and side-chain fragmentation. Peptide fragmentation nomenclature proposed by Roepstroff and Fohlman [Roepstroff, 1984] with adaptations from Biemann [Biemann, 1990].

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.

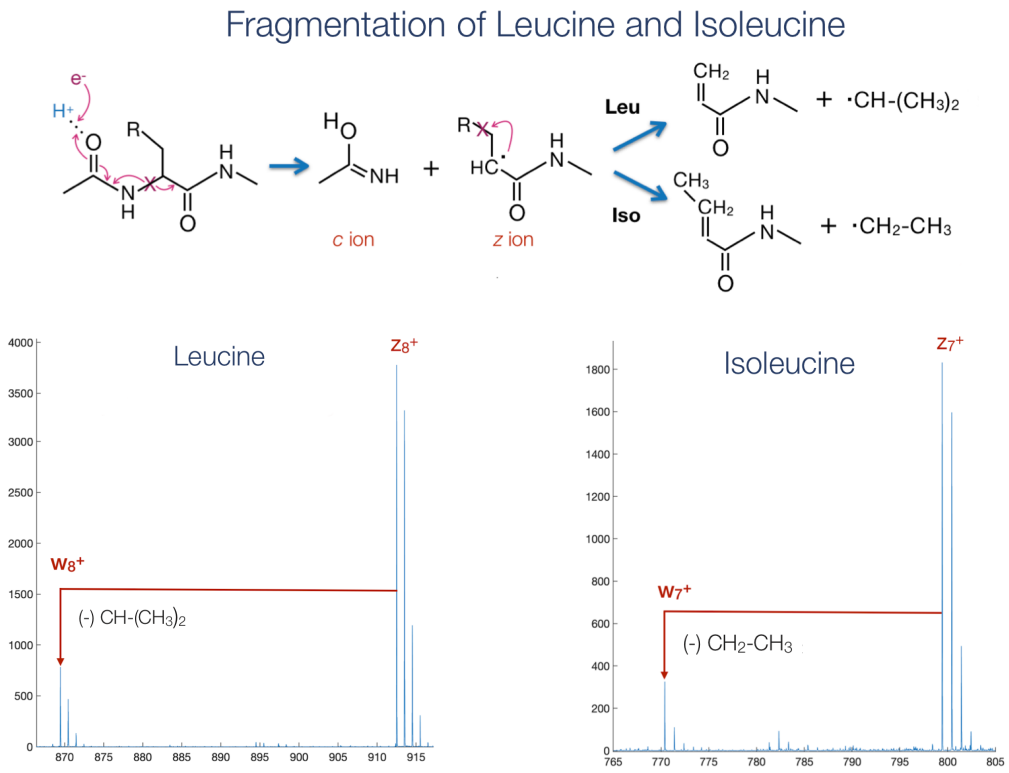


Figure 43. Secondary fragmentation of a *z* ion produces diagnostic *w* ions\ for distinguishing L7 from I8 in synthetic peptide ECDDisoDELIGHTFLK.

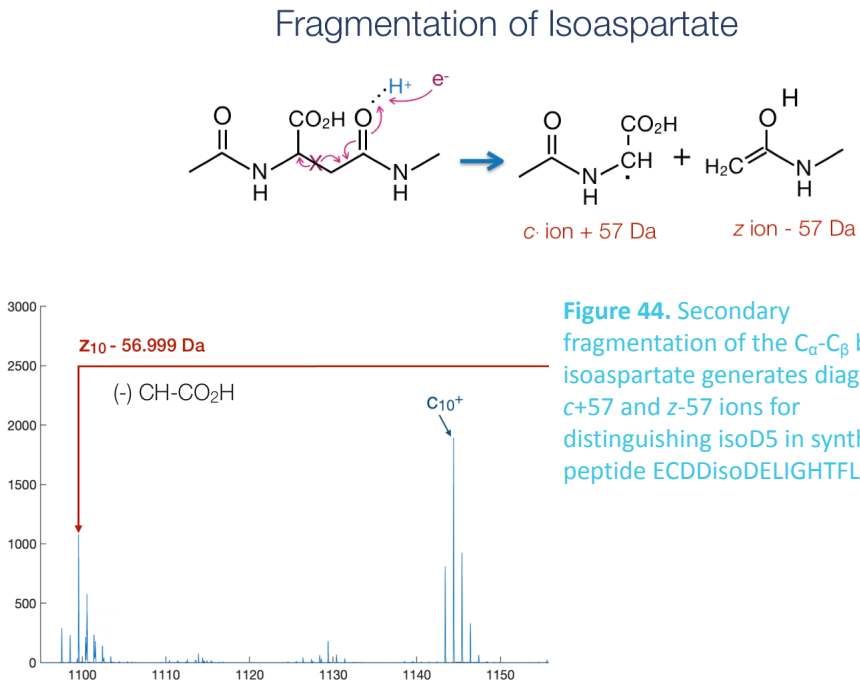


Figure 44. Secondary fragmentation of the C_α-C_β bond in isoaspartate generates diagnostic *c*+57 and *z*-57 ions for distinguishing isoD5 in synthetic peptide ECDDisoDELIGHTFLK.

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.

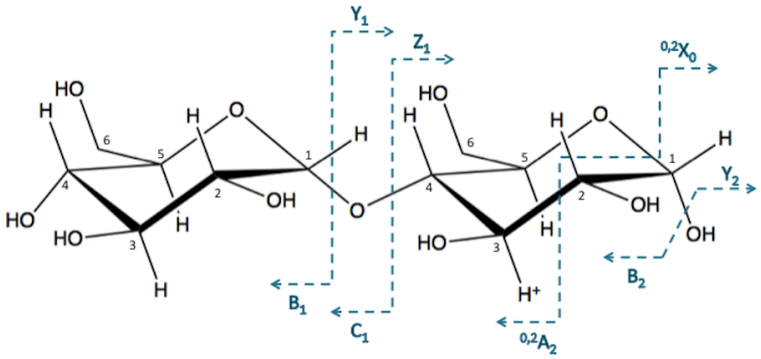


Figure 45. Product ions from glycosidic linkage and cross-ring fragmentation. Nomenclature proposed by Domon and Costello [Domon, 1980].

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).

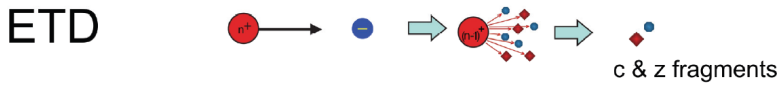
The ExD Cell is most efficient at producing low-energy electrons suitable for ECD. The efficiency with which the Cell facilitates higher-energy electron-based fragmentation techniques (EID and HECD) is lower.

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. The ExD Cell also avoids any collisional cooling incidental to ion trapping - an important factor for maximizing dissociation.

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