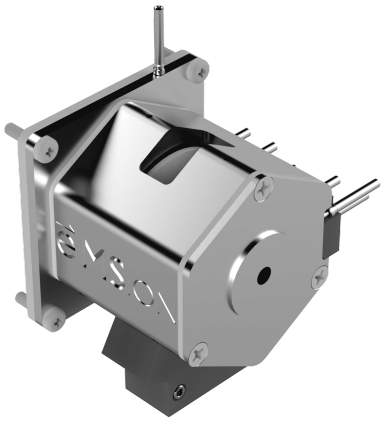
eMSION

ECD for the Masses

ExD WS-250 Option

WS-251 • WS-252 • WS-253

for Waters SYNAPT MS



User Guide

R001 • December 2020

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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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Mailing address	e-MSion, Inc. 2121 NE Jack London Corvallis, OR 97330 USA

About this Guide

The purpose of this document is to provide:

- A functional description of the ExD WS-250 Option for Waters SYNAPT 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.

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 fully shut down the instrument according to Waters Corporation user instructions and disconnect 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 WS-250 hardware to avoid contamination.
- Handle all ExD WS-250 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.

Contents

1. Parts and Function

Contents

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

General Description

The ExD WS-250 Options (WS-251, WS-252, and WS-253) are hardware and software packages that equip Waters SYNAPT mass spectrometers with the ability to perform electron capture dissociation.

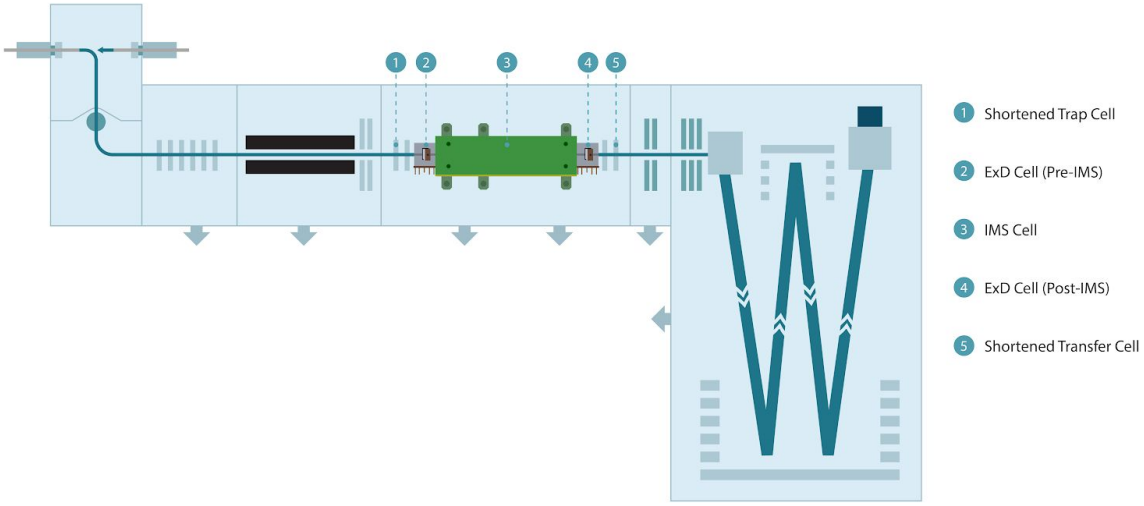


Figure 1. Diagram of ExD Cell placement in a Waters SYNAPT G2-Si mass spectrometer.

Table 1. Description of ExD WS-250 Options.

ExD Option	Configuration	Label	Description
WS-251	ExD-IM	②	The ExD Cell attaches to the entrance of the instrument IMS cell. It sits between a shortened Trap cell, which replaces the original instrument Trap cell, and the IMS chamber.
WS-252	IM-ExD	④	The ExD Cell attaches to the exit of the instrument IMS cell. It sits between the IMS chamber and a shortened Transfer cell, which replaces the original instrument Transfer cell.
WS-253	ExD-IM-ExD	② + ④	Two ExD Cells attach to the entrance and exit of the instrument IMS cell, as described above.

Key components of each Option 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 Cell are summarized in the table below. For more information, see **Concepts**.

Table 2. Electron-based fragmentation techniques available for use with ExD WS-250 Options.

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

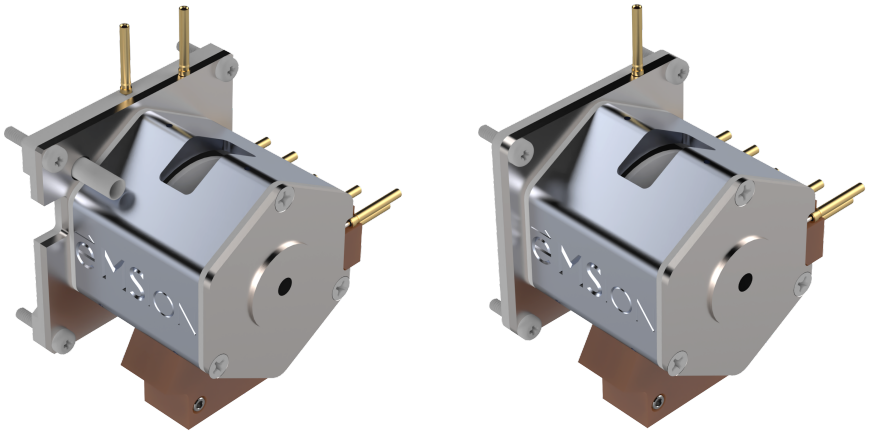


Figure 2. The ExD Cell, IMS entrance position (left) and exit position (right).

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 reagent anions, RF potentials or ion trapping.

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 3. Filament cassette with filament insert.

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 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 even trace amounts of oxygen, the filament will quickly burn out. To preserve the filament lifetime, IMS gas must adhere to a 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 flank the filament.

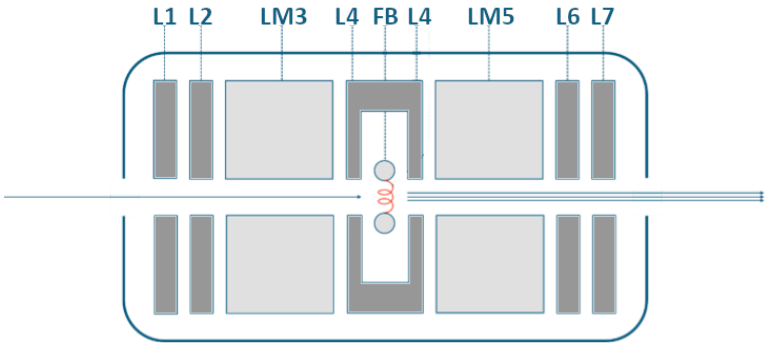


Figure 4. Cross-sectional view of the ExD Cell. The filament is the red coil, lenses are dark grey, and lens magnets are light grey.

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.

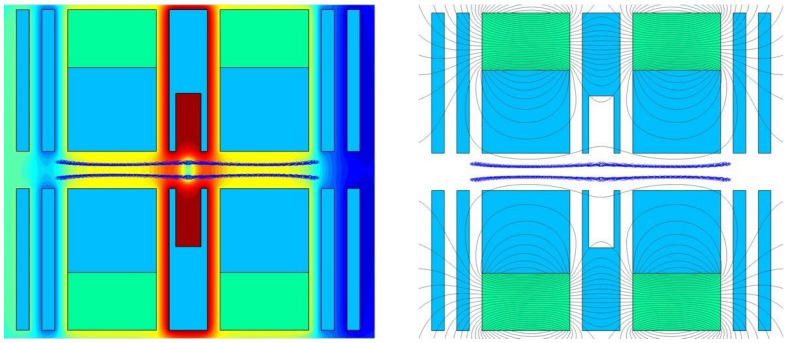


Figure 5. Cross-sectional view of the ExD Cell electromagnetic fields. Electrical potentials, with blue as negative and red as positive (left). Magnetic fields (right). Calculated electron trajectories are shown in dark blue along the central horizontal axis.

ExD Controller and ExDControl software

NOTE

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

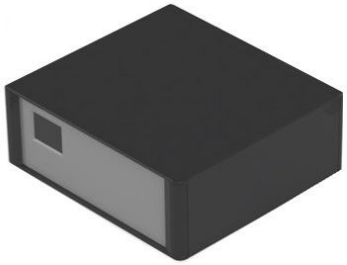


Figure 6. The ExD Controller.

The ExD Controller supplies DC voltages to the ExD Cell lenses and current to the filament according to values set 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 7. The ExD Controller turned ON.

Figure 8. 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 9. ExDControl.exe icon.

NOTE

If both IM entrance and exit cells are installed (ExD WS-253 Option), and you would like to use them both simultaneously, you will need two ExD Controllers set up and two instances of the ExDControl software open, one for each ExD Cell/Controller pair.

3. Click **Connect** > **Connect** to connect to the ExD Controller. If the connection attempt fails, click **Connect** > **Connection Settings.**

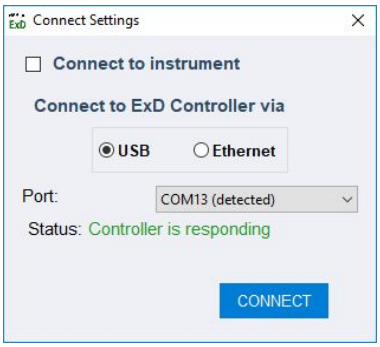


Figure 10. ExDControl connection settings configured for USB network connection to the ExD Controller.

   a. If checked, uncheck "Connect to instrument."

   b. Select **USB**.

   c. In the drop-down menu, select the appropriate COM port for the ExD Controller.

   d. Once the status updates to "Controller is responding," click **Connect**.

   e. Ensure **Auto Connect** is checked in the **Connect** menu so that current connection settings will be reused if software is restarted.

NOTE

If connecting two instances of the ExDControl software, be sure to note which instance is connected to which ExD Controller, and by extension, which ExD Cell.

4. Check that the connection status indicator in the ExDControl software main window reads “Connected to Controller.” If the connection is unsuccessful, see the *ExDControl Software User Guide*.

Figure 11. ExDControl software main window, with connection status indicator boxed in red.

Basics of Operation

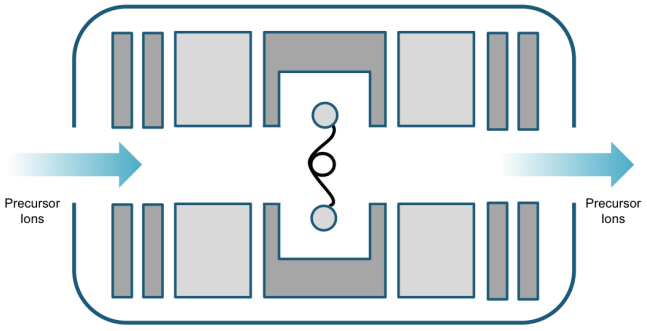
ExD Cell operating modes

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

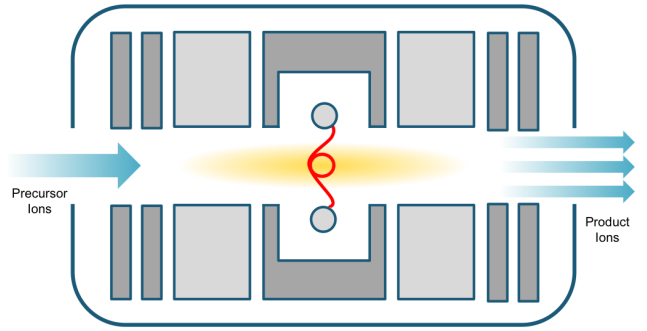
Through the systematic adjustment of these components, the ExD Cell can be tuned to operate in one of three basic modes:



Fil = Off, Standby / ExD = Off

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

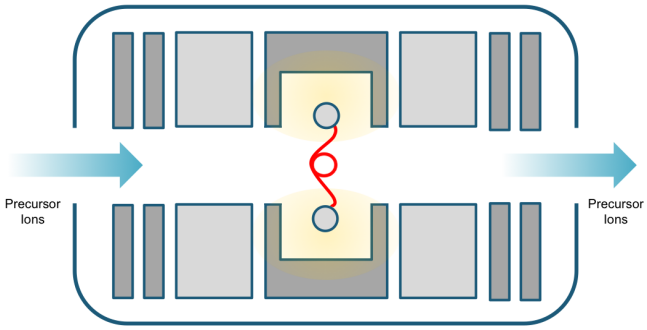
Used for normal operation of the instrument when electron-based fragmentation is *not* desired.



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

CAUTION

Put the filament in Standby instead of Off whenever possible in order to avoid the gradual process of deposit formation on the surface of a cold filament ("filament poisoning").

There are three possible settings for the filament:

- **Off** (0 A)
- **Standby** (0.5 A)
- **On**

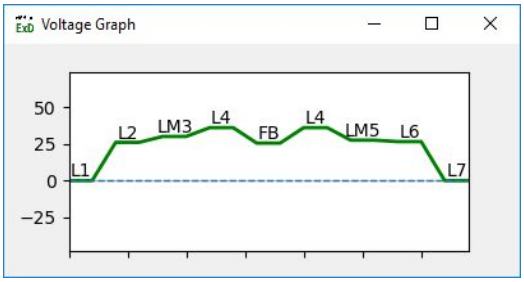
  Adjust the **On** current amperage in the **Filament Parameters** window of the ExDControl software. See **To optimize the filament heating current**.

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 12. 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).

Profiles Table

Active	Profile	Description	L1	L2	LM3	L4	FB	LM5	L6	L7
☐	Trans #1	V-Mode, ToF, MS1, F=OFF, substance P	0.6	0.0	2.0	1.0	-3.0	1.0	1.1	1.1
☐	Trans #2	V-Mode, ToF, MS1, F=2.45A, substance P	4.0	0.0	2.0	1.0	-3.0	1.0	8.0	8.0
☐	ECD #1	V-Mode, ToF, MS2, F=2.45A, substance P	14.0	22.0	25.0	22.5	18.0	22.5	24.0	20.0
☐	Trans #3	V-Mode, IM, MS1, F=OFF, ubiquitin	-40.6	-35.6	3.2	11.7	12.3	19.3	35.3	35.7
☐	Trans #4	V-Mode, IM, MS2, F=2.45A, ubiquitin	-32.0	-26.6	3.2	11.7	12.3	19.3	35.3	35.7
☑	ECD #2	V-Mode, IM, MS2, F=2.45A, substance P	-35.0	-19.5	1.5	15.2	5.3	19.4	38.0	39.5

Add Remove

Figure 13. The ExDControl software **Profiles Table**.

To put the system in Standby

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

1. First set the filament to **Standby** in the ExDControl software.

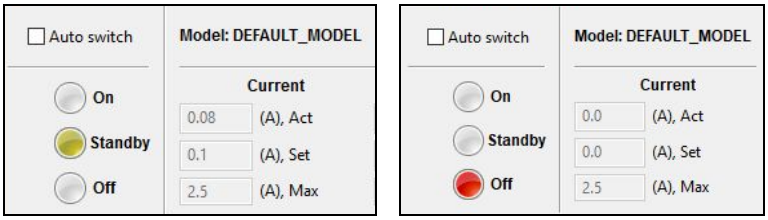


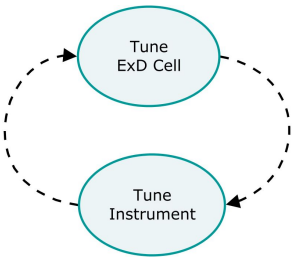
Figure 14. The **Filament Parameters** window, with the filament set to **Standby** (left) and **Off** (right).

2. Follow standard procedure to put the instrument in **Standby**.

CAUTION **Do not vent the system without first turning the filament OFF. See To shut down the system.**

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 MassLynx Tune page define ion beam kinetic energy and trajectory. Since ion energy affects ExD Cell tuning, lens profiles tend to work best with the instrument settings file used when the profile was created.
- When collision energy is added, all lenses in the ExD profile should be raised by approximately the same amount.
- For an ExD Cell in the **IM entrance** position, the lens profile for an optimized ExD tune should be nearly symmetric in both TOF mode and IM mode.
- For an ExD Cell in the **IM exit** position, the lens profile for an optimized ExD tune should be nearly symmetric in TOF mode. In IM mode, the lens profile voltages may increase moving from L1 to L7.
- 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.
- FB will typically be set below L4.

  The voltage difference between FB and L4 is the primary determinant of electron energy, although calculation of electron energy is complicated by interactions with the negative potential created by 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 tune the ExD Cell for transmission

To tune the ExD cell to transmit ions,

1. Infuse the chosen tuning standard.
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 peak abundance is maximized and peak distribution appears normal.
5. Adjust FB in +/- 0.1-1 V increments.
6. Adjust L4 +/- 1-10 V from FB. L4 will typically be greater than FB. The optimal voltage difference between L4 and FB will increase as the filament current increases.
7. Moving from inner to outer lenses, adjust each lens. Use large steps (1 V) to find the limits of the range of working voltages and then small steps (0.1 V) to find the optimum within the range.
    - Inner lenses are more sensitive and will respond to small adjustments (0.1 V), whereas outer lenses (L1, L7) typically respond to larger adjustments.
    - FB, L4, LM3, and LM5 are interdependent. Changing one of these lenses may require you to adjust the others.

NOTE

In IM Mode, the instrument settings for parameters in close proximity to the ExD Cell affect ExD Cell function.

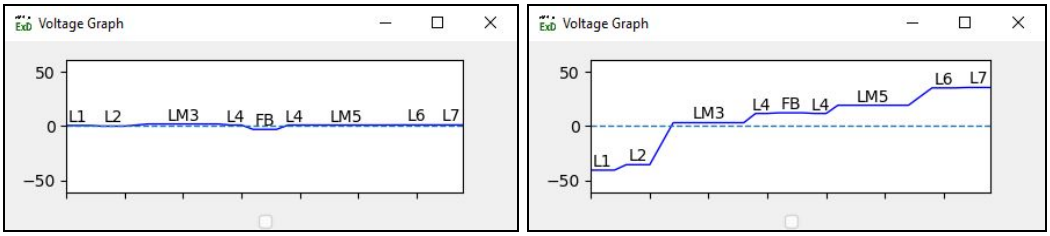


Figure 15. Examples of ToF transmission (left) and IM transmission (right) of substance P with an ExD cell in the IM **exit** position on a SYNAPT G2-Si. Filament is **Off**.

To 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, a tuning standard.

1. Infuse substance P.
2. In MS2, isolate *m/z* 674 with a 4-5 *m/z* range isolation window.
3. Set the filament to **On.** See **To optimize the filament heating current**.
4. Open the ExDControl software **Manual Tune** tab and select the profile to tune for ECD from the drop-down profiles menu.

   If no such profile exists, add a new profile to the **Profiles Table** and copy the lens voltages from an MS2 profile optimized for transmission with the filament **On** into the new profile.
5. Adjust the difference between FB and L4 to maximize *m/z* 624 (c_5^+) intensity. The difference will typically increase as filament current increases.
6. Re-adjust individual lenses until ECD is optimized.
   - Make sure to finely tune the difference between FB and L4, since it has the greatest effect on electron energy.
   - FB, L4, LM3, and LM5 are interdependent. Changing one of these lenses may require you to adjust the others.

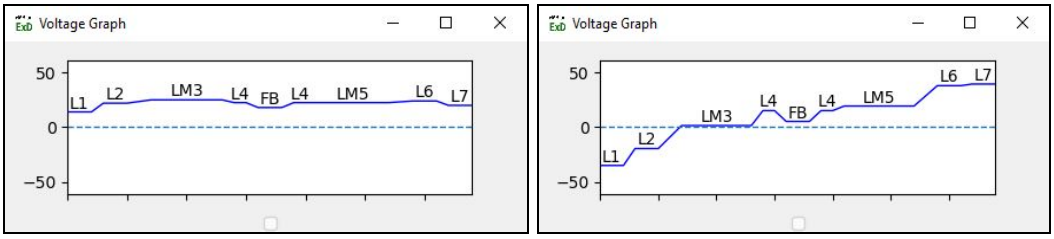


Figure 16. Examples of ToF ECD (left) and IM ECD (right) of ubiquitin with an ExD cell in the IM **exit** position on a SYNAPT G2-Si. Filament is **On**.

NOTE

If not sample limited, you may further tune an ECD profile developed on substance P on your sample of interest (or a similar sample) to maximize the intensity of its known ECD products.

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.

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 in the filament current will require retuning of any ExD profiles, particularly FB and L4.

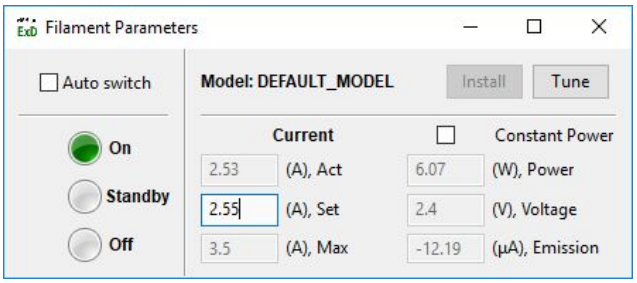


Figure 17. The **Filament Parameters** window with the filament **On** and set at 2.55 A.

2. Rhenium will evaporate from the heated filament wire. If rhenium peaks at *m/z* 184.9530 and 186.9558 are greater than 250,000 counts in intensity, 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.
3. Once satisfied with the **On** current level, check the **Constant Power** box to operate the filament in constant power mode. In this mode, the ExDControl software will adjust the current to keep power constant, extending the filament lifespan.

   If you do not use constant power mode, then you will need to adjust the **On** current over time to compensate for thinning of the filament wire.

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

To prepare substance P

WARNING

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

Materials

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

Amidated substance P (~1.3 kDa) is the peptide standard used for tuning the ExD Cell for ECD. A lens profile developed for ECD of substance P can be used to perform ECD on other experimental samples.

Prepare in 50/50/0.1 (% v/v/v) methanol/water/formic acid dilution buffer to an appropriate concentration for the source in use.

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.

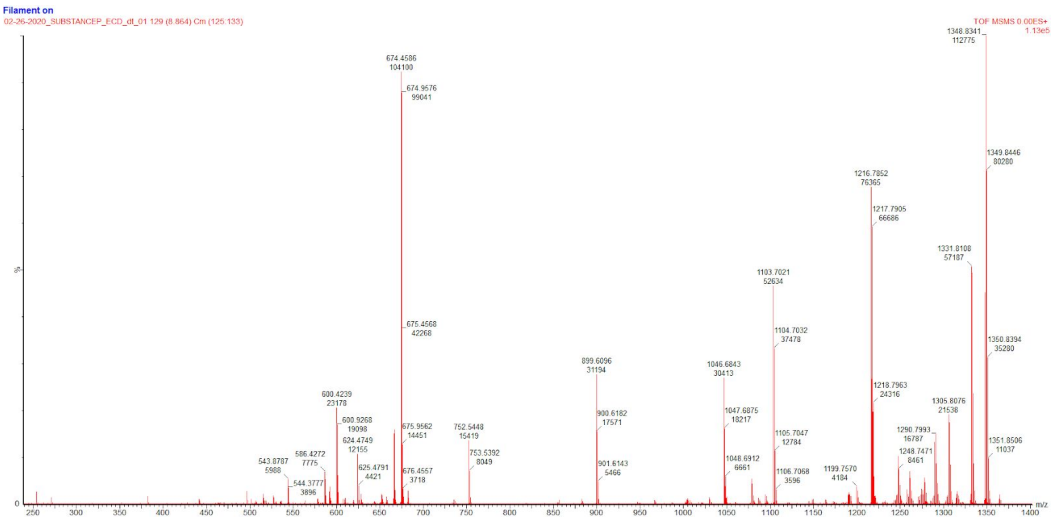


Figure 18. An IM-ECD spectrum of the $[M+2H]^{2+}$ precursor of amidated substance P (*m/z* 674.3713), 9 scans combined in MassLynx.

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.

3. Data Acquisition and Analysis

Contents

This chapter provides tips for 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 in MassLynx.
- 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 the ExD Cell 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.

Analysis

To analyze ExD data

Data generated by a Waters mass spectrometer with the ExD Cell installed will still be in *.RAW 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 3. Suggested software options for ExD data 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. 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 of the ExD WS-250 Series 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 **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 shut down the system

Before shutting down the system for a maintenance or service procedure,

1. Turn the filament **Off** in the ExDControl software **Filament Parameters** window.
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.
4. Continue with the standard instrument shut down process.

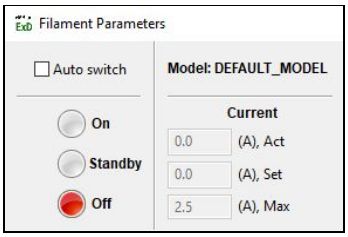


Figure 19. The **Filament Parameters** window, with the filament **Off**.

CAUTION

Make sure the filament is OFF before venting. Venting 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.

To check for filament burn-out

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.

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

- Current actual "(A), Act" approaches zero while setpoint, "(A), Set", is > 1 A.
- Voltage drop across the filament "(V), Voltage" approaches the maximum value of 5 V while the current setpoint is > 1 A.
- Power consumption for the filament circuit "(W), Power" approaches zero while the current setpoint is > 1 A.

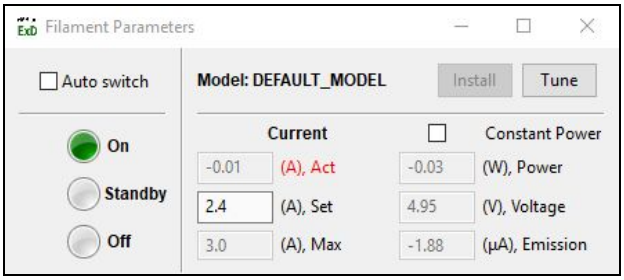


Figure 20. Appearance of **Filament Parameters** window when either filament is burned out or D-sub cable is disconnected.

1. Make sure 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.

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, always clean the filament cassette before reusing.

To check for conductivity caused by carbonization:

- 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 personnel.**

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.

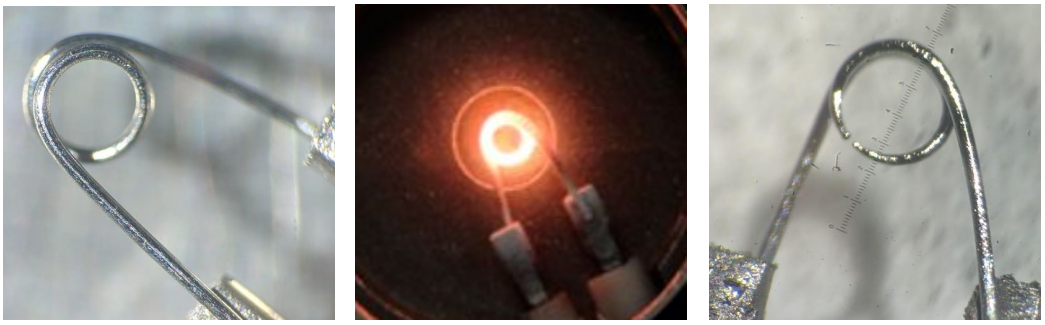


Figure 21. Close-ups of a new filament (left), a filament heated to the threshold of thermionic emission (middle), and a filament that has burnt out after routine use (right).

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
- Digital multimeter

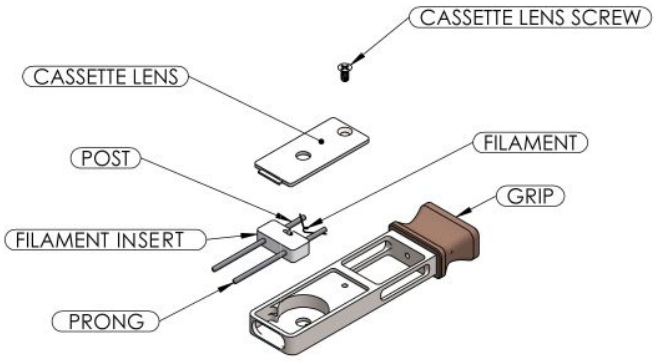


Figure 22. Assembly of the filament cassette with the filament insert.

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.

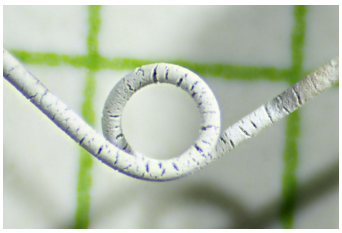
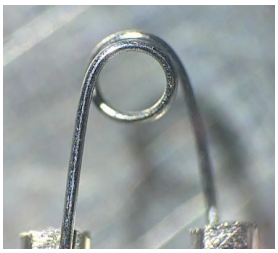


Figure 23. Coated and conditioned filament wire loop (**Top**). Uncoated filament (**Bottom**).



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.

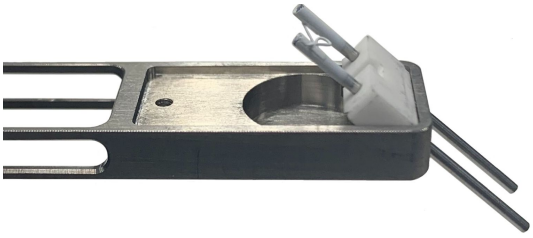


Figure 24. Sliding the filament insert into the filament cassette.

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.

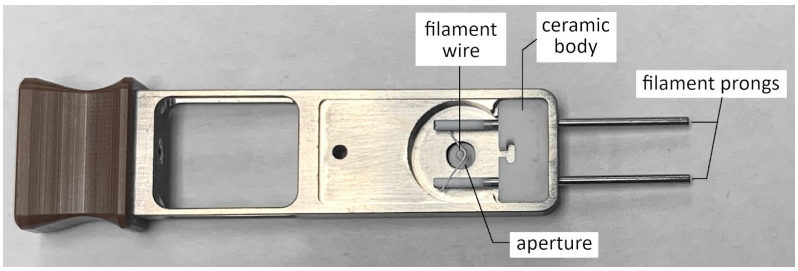


Figure 25. The filament insert inside the filament cassette.

8. Replace the cassette lens and cassette lens screw.

Step 2. Shut down the system

WARNING

The instrument is not safe for installation if the main power switches on the back of the instrument are not OFF.

1. Turn the filament **Off** in the ExDControl software. Check that the actual current readout decreases to 0 A.
2. Turn off the ExD Controller. Press the power button on the back of the Controller until the front LCD screen turns black.
3. Follow standard procedure to shut down the instrument.
4. Once the system is vented and powered down, remove any instrument covers necessary to access the triwave region manifold cover plate.
5. Unplug the D-Sub cable from the cover plate vacuum feedthrough.

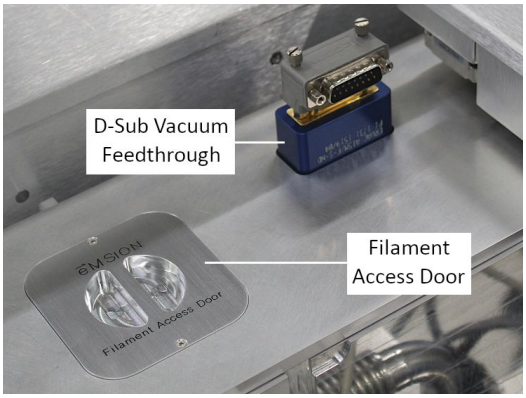


Figure 26. The manifold cover plate with filament access door.

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.

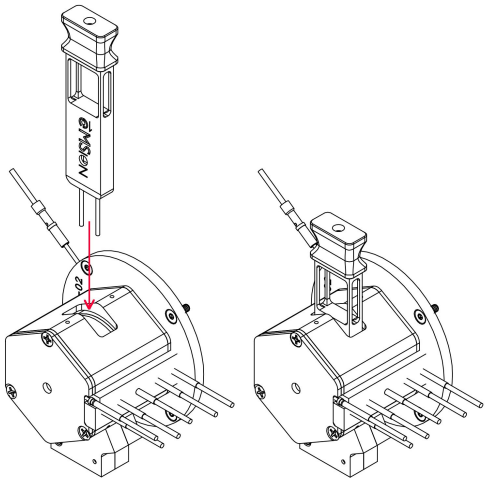
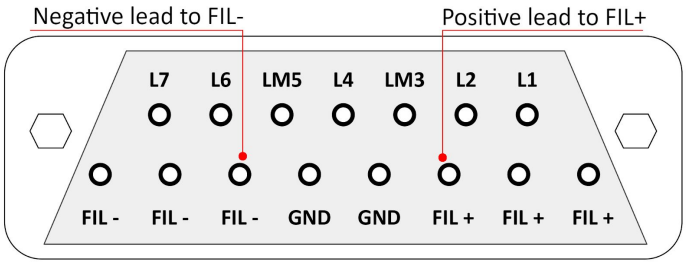


Figure 27. Insertion point of the filament cassette on the ExD Cell.

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 28**.



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

Figure 28. 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 instrument covers.
2. Follow standard procedure to restart the instrument.
3. Once the instrument has pumped down, 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.

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.

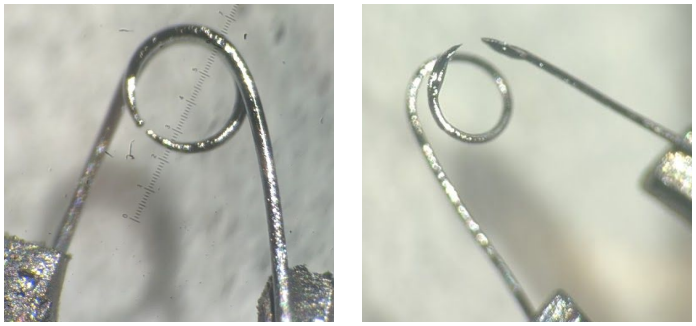
Step 5. Set the initial filament On current

1. Reconnect the ExDControl software by clicking **Connect** > **Connect**.
2. In the ExDControl software, apply the last profile tuned for transmission with the filament in **Standby**. If signal intensity is not satisfactory, see **Tuning**.
3. When satisfactory transmission is achieved, click **Window** > **Filament Parameters** to open the ExDControl **Filament Parameters** window.
4. Click the filament **On** button and set the heating current to the initial value recommended by e-MSion for your filament insert model.
5. See **To optimize the filament heating current** to optimize the initial filament **On** current for ECD.

NOTE

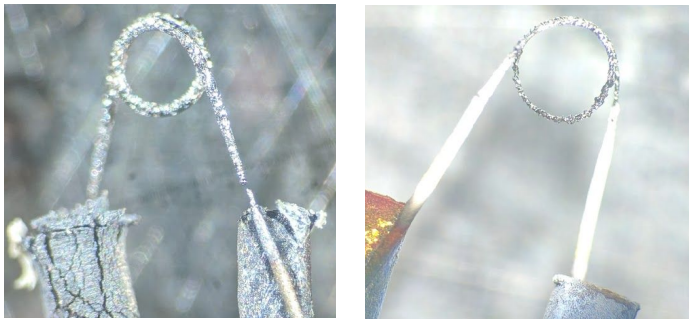
As you raise the heating current, you may notice an atypical disparity between the set and actual voltage readouts for FB and L4, as well as an unusually large 'Emission' current readout. The likely cause is contaminants on the surface of the heated filament. After about 20 minutes of heating, these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

To evaluate filament failure



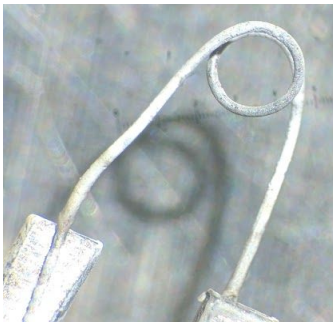
At the end of its design life, the filament will burn out from repeated heat-cycling and material loss. Overheating will cause the filament to burn out earlier.

Figure 29. (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 30. 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 31. 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 MassLynx 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 32** 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.

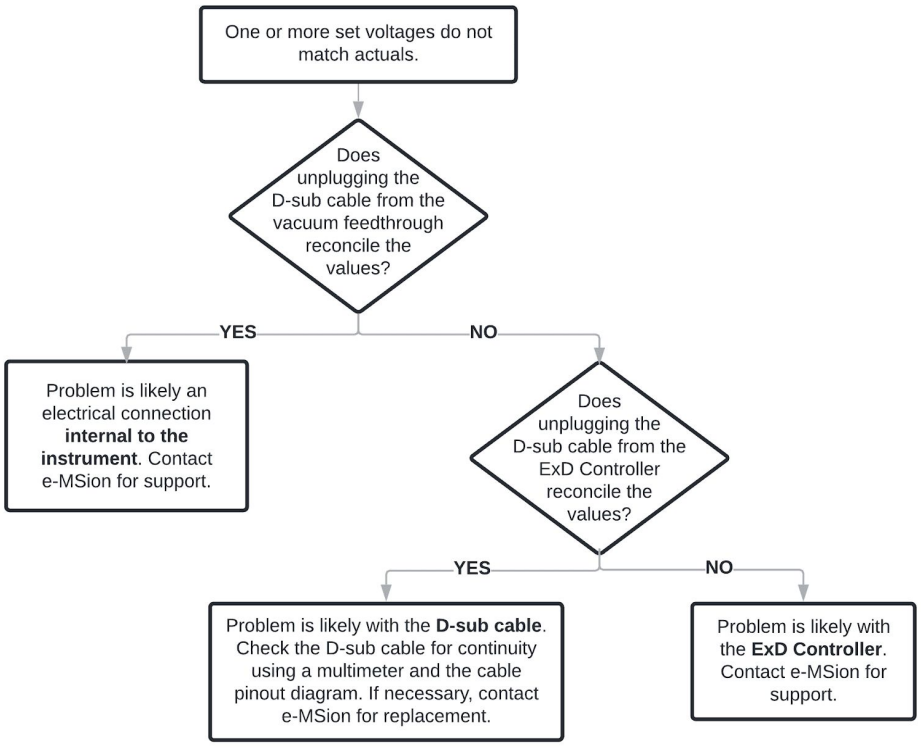


Figure 32. Decision tree for checking ExD Option for hardware malfunction.

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

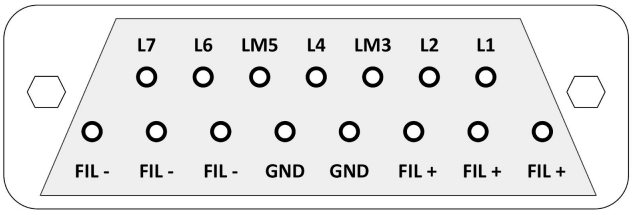


Figure 33. Top view of a male D-sub pinout.

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 for further assistance and/or replacement of parts.

Table 4. Troubleshooting issues with the ExD Cell, ExD Controller, and ExDControl software.

Problem	Potential Causes	Suggested Course of Action
Unsuccessful ExDControl software network connection to ExD Controller.	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. See **Connection Settings** in the *ExDControl Software User Guide*.
ExD Controller connection repeatedly drops during use.	Network issue or software bug.	• Ensure USB cable is connected to ExD Controller and to instrument control PC. • 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, and instrument control PC. • Restart the 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**.
Poor sensitivity and/or ion transmission in MS1 and/or MS2.	Sample preparation.	Verify purity and concentration of sample and buffer components.
	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 all temperature and flow actuals match setpoints. • Ensure source components are clean and properly positioned.
	Instrument tune incompatible with ExD Cell tune or not optimized for mass range of interest.	• Load or reload a previously-working MassLynx settings file and ExD tunefile.
	ExD Cell lens profile(s) not optimized.	• Load a previously-working ExD tunefile and its corresponding MassLynx settings 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 IMS gas purity. • Consider replacing plastic or teflon tubing or connectors with metal substitutes to prevent slow diffusion of O_2 into the supply line. • 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.	Retune the ExD Cell. See **Tuning**.
	Insufficient filament heating current.	• See **To optimize the filament heating current**. • 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, there is a problem with the filament circuit. See **Step 3. Replace the filament cassette** for instructions on measuring circuit resistance.

5. Concepts

Contents

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

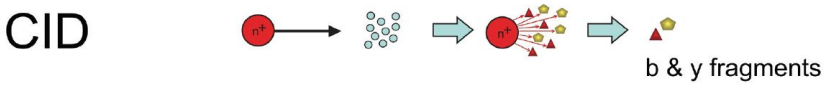
What is electron-based fragmentation?

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.

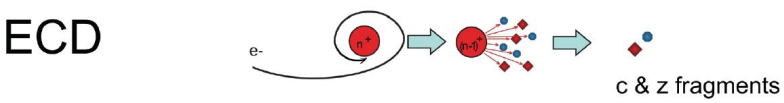
For proteomics applications, CID removes labile motifs such as post-translational modifications (PTMs) as neutral losses, precluding PTM localization. Additionally, as protein size increases, sequence coverage using only CID decreases.

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.



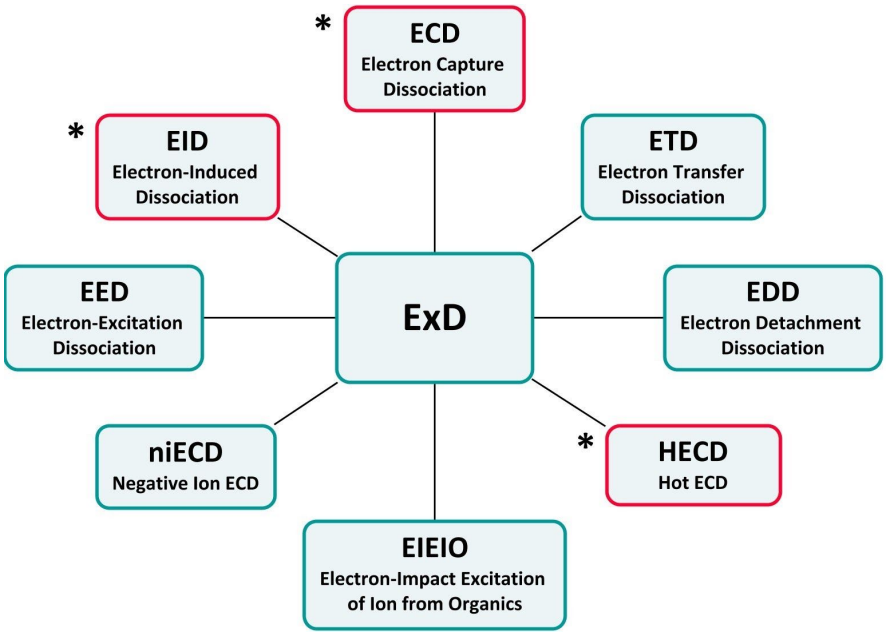


Figure 40. The ExD family of electron-based fragmentation techniques. The techniques that the ExD Cell has been used to produce are starred "*" and outlined in red.

ECD uniquely complements the existing CID capabilities of Waters 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.

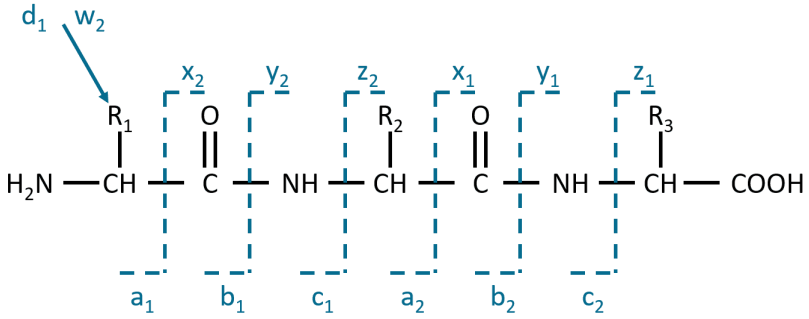


Figure 41. Product ions from peptide backbone and side-chain fragmentation. Peptide fragmentation nomenclature proposed by Roepstroff and Fohlman [Roepstroff, 1984] with adaptations from Biemann [Biemann, 1990].

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.

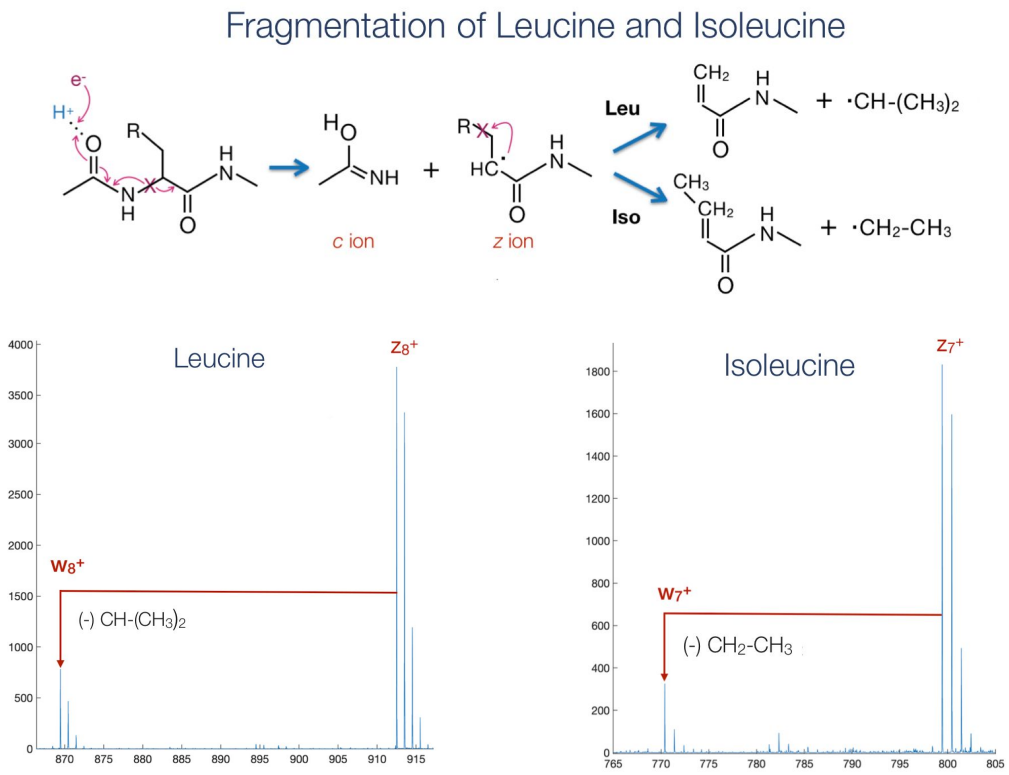


Figure 42. Secondary fragmentation of a *z* ion produces diagnostic *w* ions\ for distinguishing L7 from I8 in synthetic peptide ECDDisoDELIGHTFLK.

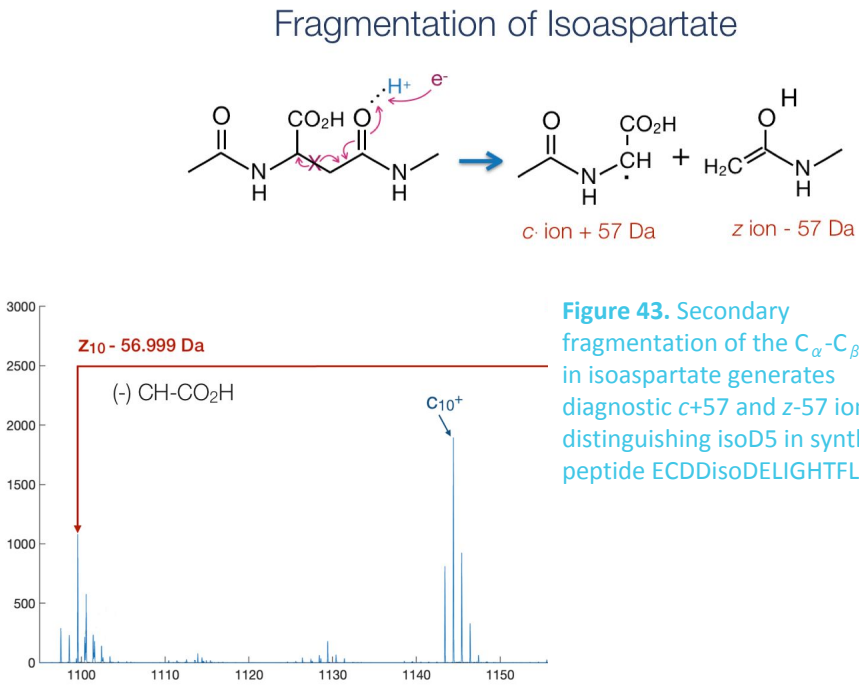


Figure 43. Secondary fragmentation of the C_α-C_β bond in isoaspartate generates diagnostic *c*+57 and *z*-57 ions for distinguishing isoD5 in synthetic peptide ECDDisoDELIGHTFLK.

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.

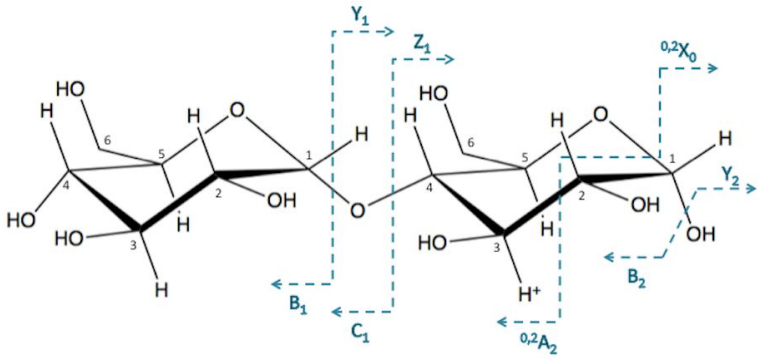


Figure 44. Product ions from glycosidic linkage and cross-ring fragmentation. Nomenclature proposed by Domon and Costello [Domon, 1980].

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).

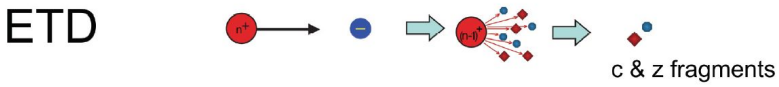
The ExD Cell is most efficient at producing low-energy electrons suitable for ECD. The efficiency with which the Cell facilitates higher-energy electron-based fragmentation techniques (EID and HECD) is lower.

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 ExD Controller Software.