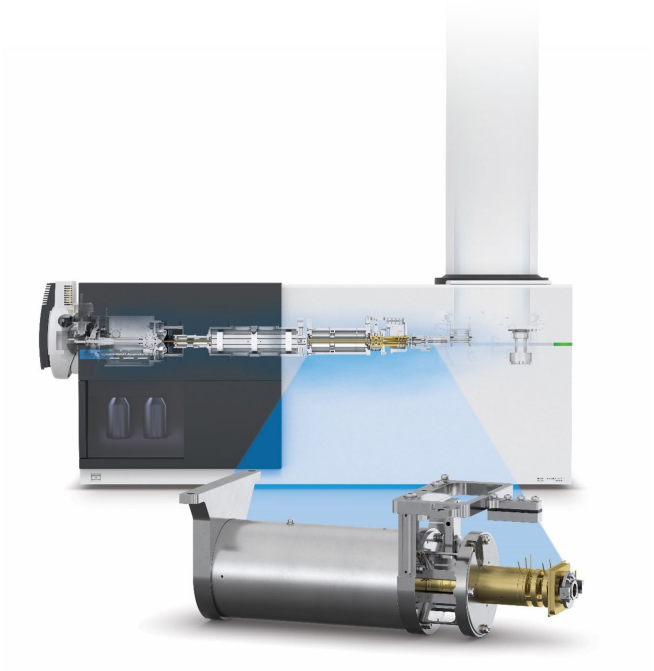
Agilent

ExD Cell

User Guide



Notices

Document Identification

D0117564 Revision A.01
January 2025

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Agilent Technologies, Inc.
5301 Stevens Creek Blvd.
Santa Clara, CA 95051

Hardware and Software Revision

This guide is valid for the G1997A ExD cell, MassHunter 11.0, and ExDControl 3.6 until superseded.

Instrument Manufacturing

Manufactured by Agilent Technologies Singapore Pte. Ltd.
No. 1 Yishun Avenue 7, Singapore 768923

Operating Temperature

Operating Temperature: 15°C to 35°C
Storage Temperature: -40°C to 70°C

Software Manufacturing

Manufactured for Agilent Technologies
5301 Stevens Creek Blvd
Santa Clara, CA 95051

Warranty

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CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

In This Guide

This guide provides information on the Agilent G1997A ExD cell system running ExDControl 3.6 or higher.

Additional Resources

User Documentation

Instrument documentation, step by step videos, and more can be found by scanning the code or navigating to **https://aglt.co/LCMSUserDocs.**

Data analysis and library management documentation can be found by scanning the code or navigating to **https://aglt.co/DALibMgmtDocs**.

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Contents

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1 Hardware and Software Overview

ExD-Enabled MS System Overview

The ExD cell upgrades Agilent 6545XT AdvanceBio LC/Q-TOF mass spectrometer (MS) instruments with the ability to perform electron-based dissociation (ExD) on analytes.

Key components include:

- ExD cell
- Filament assembly
- ExD Controller
- ExDControl software

NOTE

If the instrument is used in a manner not specified by the manufacturer, the protection provided by the instrument may be impaired.

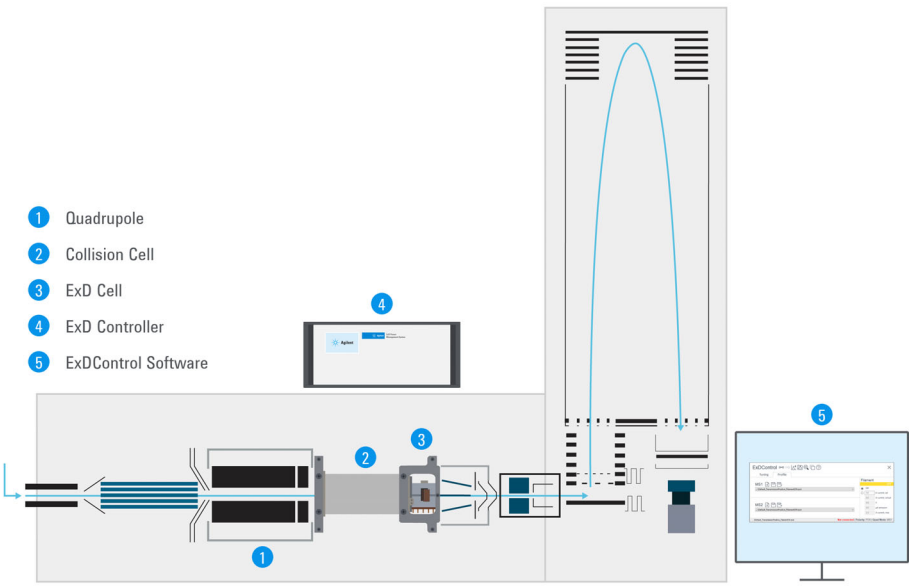


Figure 1. Schematic of Agilent 6545XT AdvanceBio LC/Q-TOF with ExD cell installed.

Electron Capture Dissociation

Electron capture dissociation (ECD) is the principal method of electron-based dissociation enabled by the ExD cell. During ECD, low-energy electrons emitted by the filament react with multiply charged peptide and protein cations to produce sequence-informative c/z-type product ions.

On anAgilent LC/Q-TOF, ECD complements traditional collision induced dissociation (CID) by yielding increased sequence coverage of large peptides and proteins while preserving labile post-translational modifications, such as phosphorylation and glycosylation.

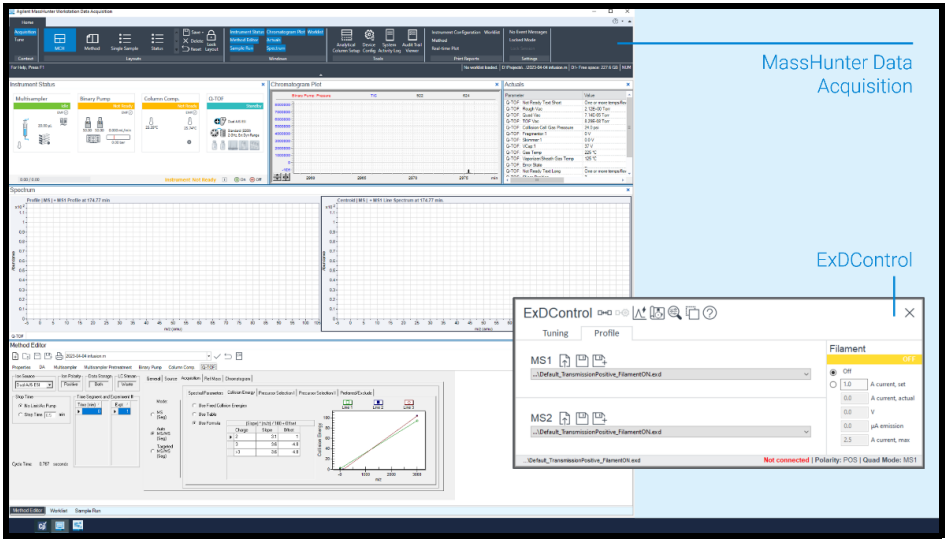


Figure 2. Instrument control PC monitor with MassHunter Data Acquisition and ExDControl software applications running concurrently.

Hardware

ExD cell

The ExD cell uses a compact arrangement of permanent magnets and electrostatic lenses around an electron-emitting filament to facilitate the ion-electron interactions. This setup produces ECD on a microsecond timescale, without ion trapping.

Hardware Components

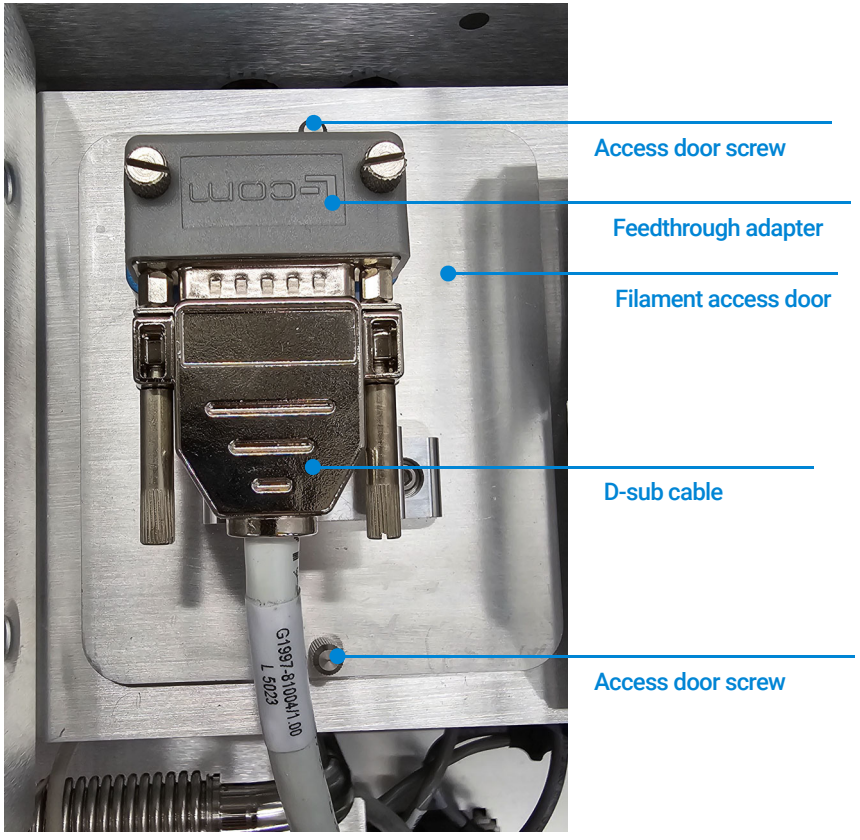


Figure 3. ExD cell installed in an Agilent 6545XT AdvanceBio LC/Q-TOF with filament access door secured.

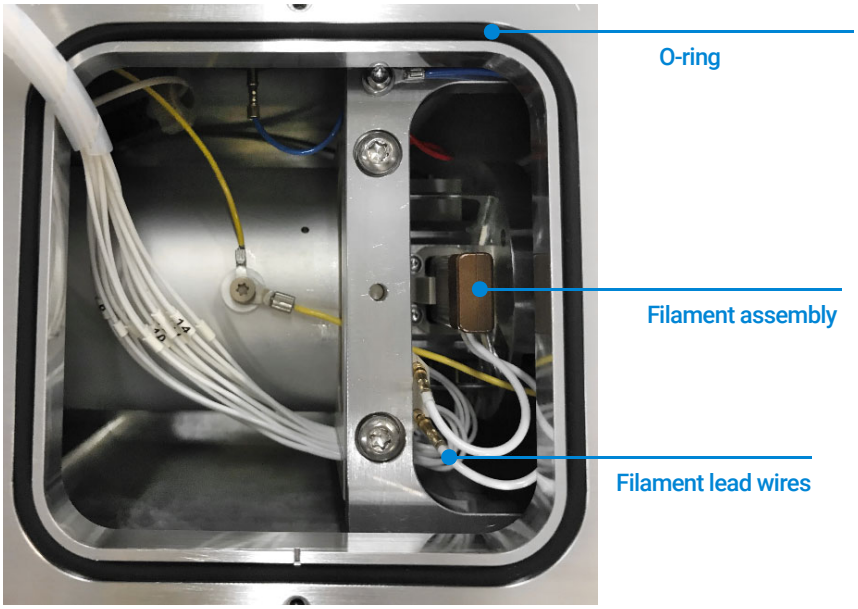


Figure 4. ExD cell installed in an Agilent 6545XT AdvanceBio LC/Q-TOF with the filament access door open.

Filament Assembly

The filament insert is the electron source for ExD. The filament is housed inside the filament assembly, which plugs into the slot in the ExD cell.

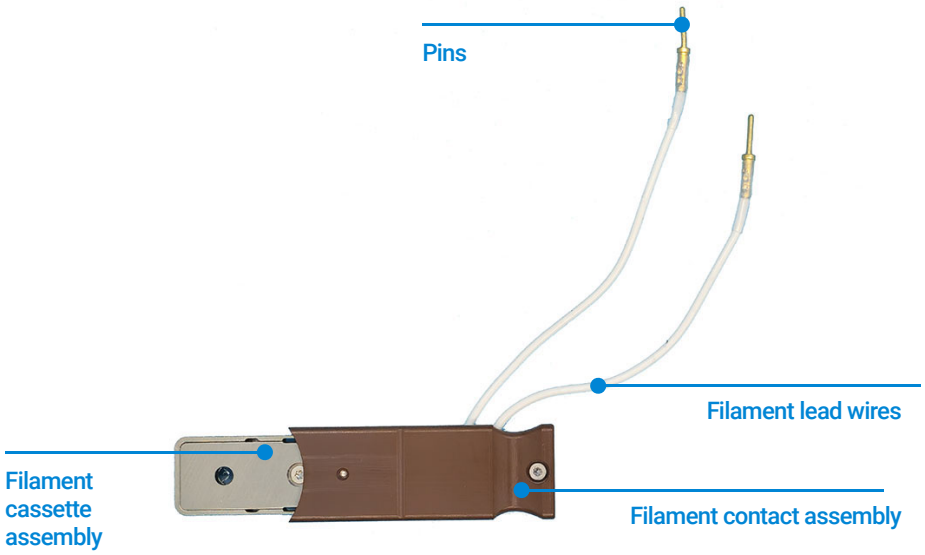


Figure 5. The filament assembly.

Filament Insert and Filament

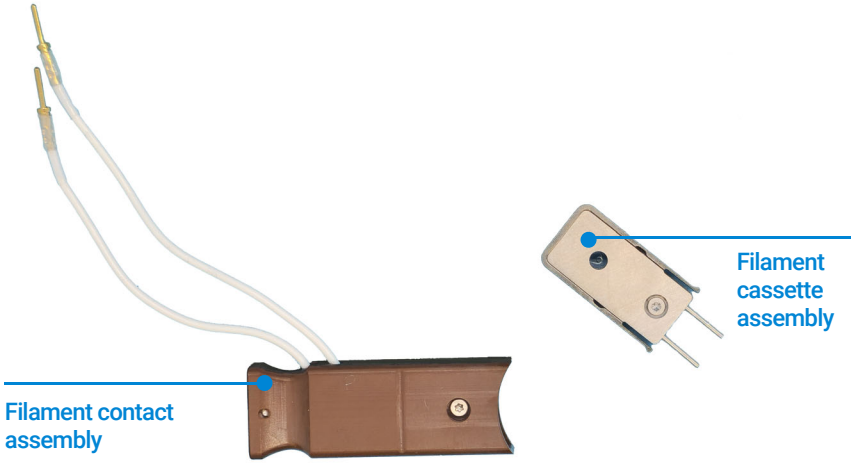


Figure 6. Filament Cassette with Filament cassette assembly detached.

The filament insert is a consumable part. Thermal stress from routine use causes the filament to burn out over time. While the ExD cell can operate when the filament has burned out, sensitivity is impacted. See **"Diagnosing Filament Failure"** on page 90 for guidance on how to diagnose filament failure and replace the filament.

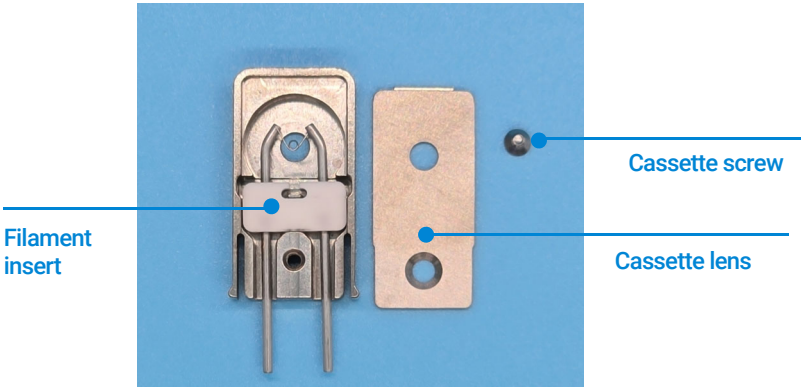


Figure 7. Open Filament cassette assembly showing the filament insert.

CAUTION **The heated rhenium filament wire is susceptible to oxidation. To protect against failure, the instrument must remain under vacuum while the filament is on.**

CAUTION **The heated rhenium filament wire is susceptible to oxidation. To protect against failure, the nitrogen gas supply must be Ultra High Purity Grade (99.999%). Use of an oxygen scrubber in the collision cell gas supply line is highly recommended. See "Oxygen Scrubber Set Up" on page 88.**

ExD Controller

The ExD Controller is an external power management system that supplies DC voltages to ExD cell lenses, and DC current and voltage to the filament according to values set in the ExDControl software.

Figure 8. ExD controller front panel

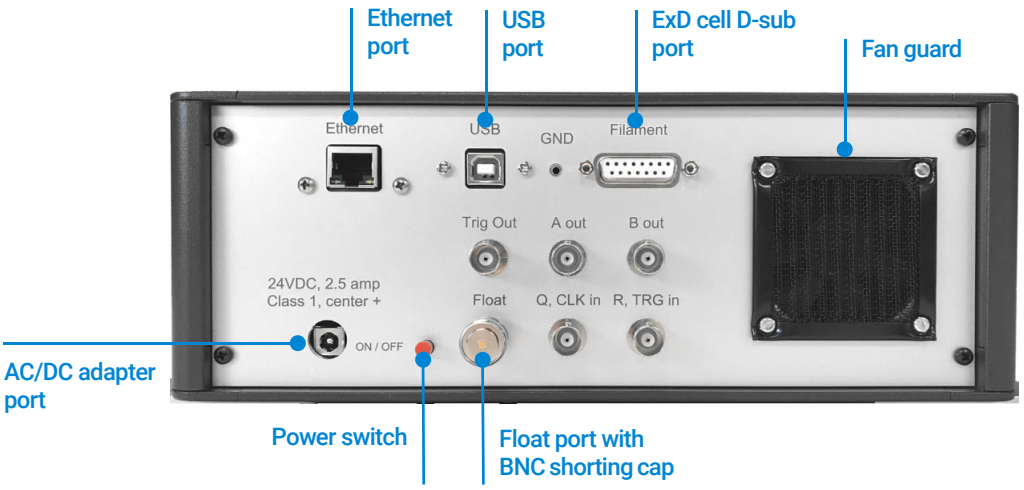


Figure 9. ExD Controller back panel

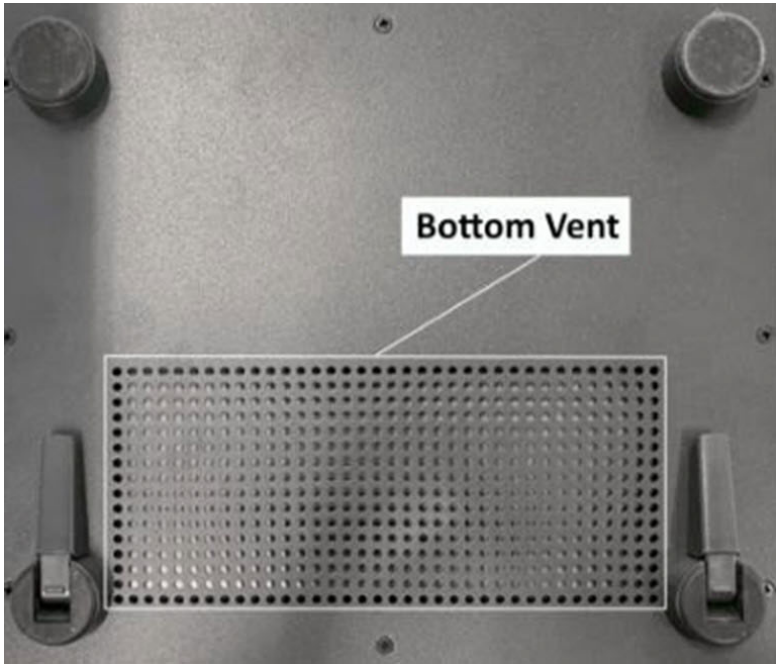


Figure 10. ExD Controller bottom panel with vent.

Software

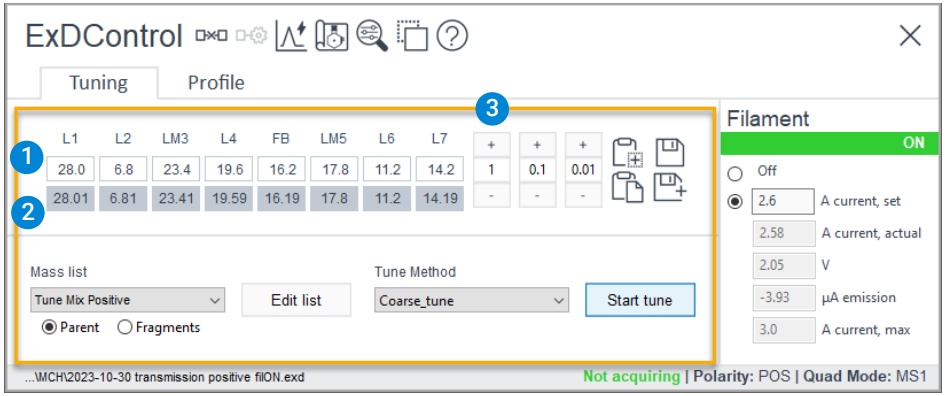
ExDControl is the software application used to operate the ExD cell. Use ExDControl to:

- Tune the ExD cell lens voltages.
- Open and save ExD cell lens profile voltage settings as *.exd files.
- Select which lens profile is active while the system is in MS1 (Total Ion Mode) and MS2 (Isolation Mode).
- Tune the filament heating current.
- Monitor filament status.

ExDControl User Interface

Tuning Tab

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



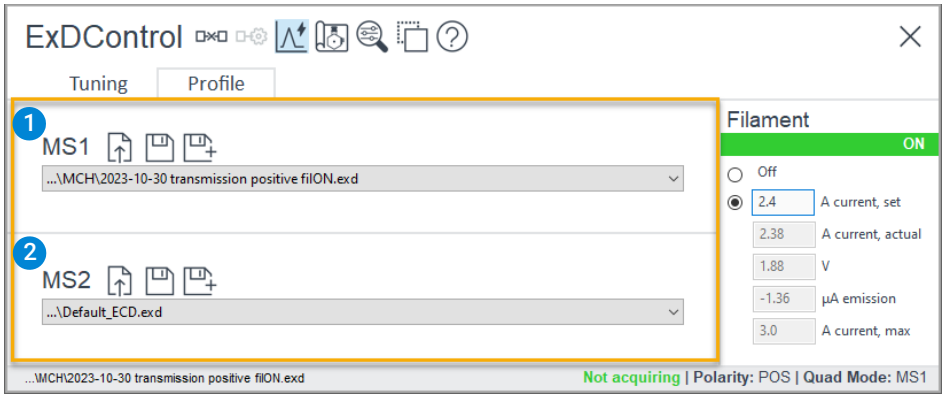
Label/Icon	Description
1	User-adjustable voltage settings for ExD cell lenses. To adjust a lens, select the field, edit the value, and press **Enter**. To adjust multiple lenses at the same time, press **Shift** and select each field, then use the **step control** buttons to raise or lower the lenses in unison.
2	The ExD Controller measures voltage readbacks for ExD cell lenses.
3	Step control buttons. Click **+** to add or **–** to subtract 1, 0.1, or 0.01 V from one or more selected lenses.
	Copy voltage settings.
	Paste voltage settings.
	Save *.exd file.
	Save As *.exd file.
Mass List	Displays installed or created mass lists.
Mass List Type - Parent	If selected, only the parent ion *m/z* values from the mass list are used by the autotune method.
Mass List Type - Fragments	If selected, only the fragment ion *m/z* values from the mass list are used by the autotune method.

Label/Icon	Description
Edit list	Click to add/edit/remove mass lists.
Tune Method	Drop-down menu displays the name of the selected autotune method.
Start tune	Click to run the autotune method.

Profile Tab

The Profile tab allows the selection of lens profiles. A lens profile is the set of electrical potentials for the eight lenses in the ExD cell. Each profile saves as an ExD file (*.exd).

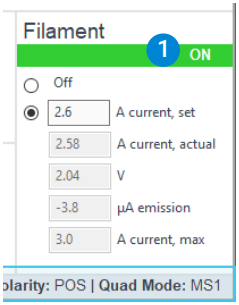
Two lens profiles are always open in the ExDControl Profile tab: one selected for use when the instrument is in Total Ion Mode (MS1) and one for Isolation Mode (MS2).



Label/Icon	Description
1	MS1 drop-down menu displays the *.exd file that is active while the instrument is in MS1 (Total Ion Mode)
2	MS2 drop-down menu displays the *.exd file that is active while the instrument is in MS2 (Isolation Mode)
	Open *.exd file
	Save *.exd file
	Save As *.exd file

Filament Panel

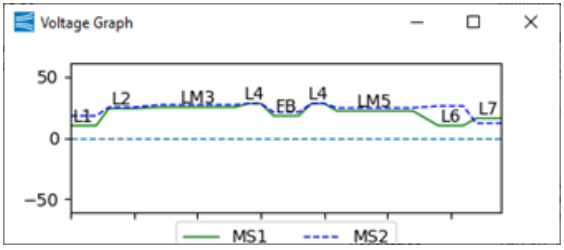
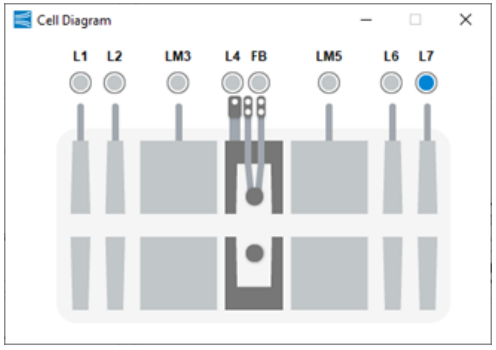
Displays the instrument state as ON or OFF as well as providing settings for current amperage when the filament is On.



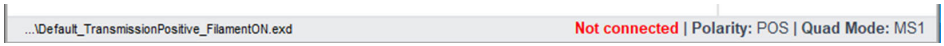
Label/Icon	Description
1	Filament status bar.
Off	Turns the filament Off by setting the current to 0 A.
A Current, set	Turns the filament on by setting current to the user-adjustable value displayed. To adjust, select the field, edit the value, and press **Enter**.
A Current, actual	Filament current read back.
V	Filament circuit voltage drop.
µA emission	Electron emission from the filament.
A Current, max	Maximum current setting.

Menu Bar

Icon	Tool	Description
/	Connect/ Disconnect	Connects ExDControl to the instrument. When connected, the disconnect displays.
	Connection Settings	Opens the Connect Settings window. See **"Resolve Connection Issues"** on page 84. When ExDControl is connected, this functionality is unavailable.

Icon	Tool	Description
	Voltage Graph	Opens the Voltage Graph window, which displays step plots of the two ExD cell lens profiles selected for MS1 and MS2 in the ExDControl Profile tab. 
	Cell Diagram	Opens the Cell Diagram window, an interactive schematic of the ExD cell lenses and lens magnets. 
	Tracked Parameters	Opens the Tracked Parameters window, which displays a comparison of previously saved values for instrument and ExD cell tune settings and current values. To overwrite the set of saved tune settings, click the **Save Current Set Values** button.
	Always On Top	Pins ExDControl window above all other windows.
	About	View ExDControl and ExD Controller firmware version numbers and contact information for product support.

Status Bar



Parameter	Status	Description
System Status	Not connected	ExDControl is not connected to the instrument.
	Not acquiring	ExDControl is connected to the instrument.
	Acquiring	ExDControl is connected to the instrument.
	Armed	ExDControl is connected to the instrument.
	Connected to Controller	ExDControl is connected directly to the ExD Controller, not the instrument.
Polarity	POS	Instrument polarity is positive.
	NEG	Instrument polarity is negative.
Quad Mode	MS1	Instrument quadrupole is in Total Ion Mode.
	MS2	Instrument quadrupole is in Isolation Mode.

LCD Status Indicators

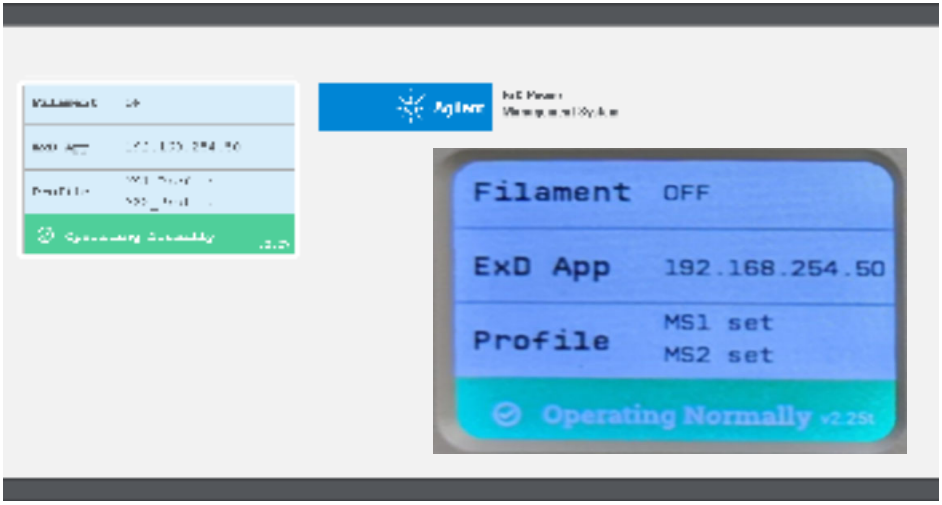


Figure 11. LCD when ExDControl is communicating with the ExD Controller, and the filament is on.

Table 1. Status Indicators on the ExD Controller LCD.

Parameter	Status	Description
Filament	ON	Filament state = ON, in ExDControl.
	OFF	Filament state = OFF, in ExDControl.
ExD App/Connection	192.168.254.50	Ethernet connection to ExDControl.
	COM Port Number	USB connection to ExDControl.
	None	No connection to ExDControl.
Profile	MS1 set MS2 set	.exd files open as MS1 and MS2 profiles in ExDControl Profile tab.
Status	Operating Normally	Communication between ExDControl and ExD Controller.
	Disconnected	No communication between the instrument add on and ExD Controller.
Firmware	vX.X	ExD Controller firmware version.

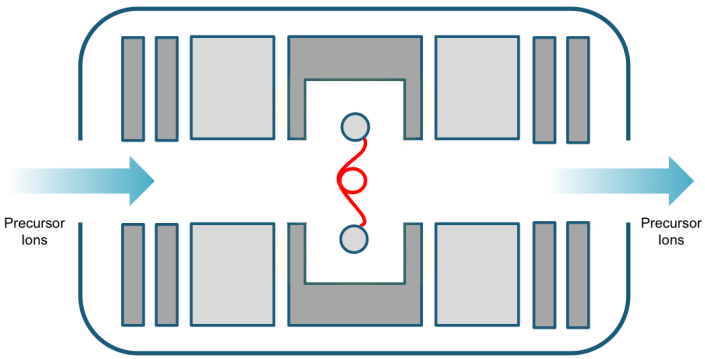
2 Operation

Overview

The ExD cell is tuned to operate in two modes:

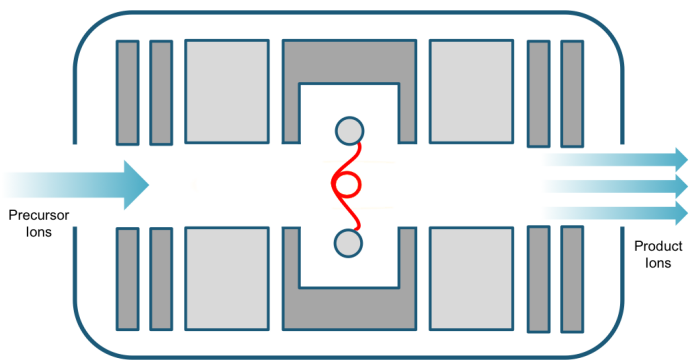
Transmission

Maximizes transmission of the ions through the cell while minimizing the creation of ECD fragment ions.



ECD

Performs ECD, fragmenting some of the ions that pass through the ExD cell.



Before you begin

These instructions include the following assumptions:

- The instrument is configured. For information on configuring the instrument, see the MassHunter Control Panel help.
- The LC modules and the LC/Q-TOF are powered on, but the LC pump is in standby.
- MassHunter Acquisition software is installed and running.
- ExDControl is installed and running.

Start ExDControl

1 Verify that the ExD Controller is on. The ExD Controller remains on while the instrument is operating. To turn the ExD Controller on, press and hold the ON/OFF power switch on the back panel until the front panel LCD lights up.

2 Open the ExDControl software by double-clicking the ExDControl icon or browsing to **Start > ExDControl36**.

The software splash screen shows the software initialization.

3 Confirm the Status Bar reads **Not acquiring** in the lower right before continuing.

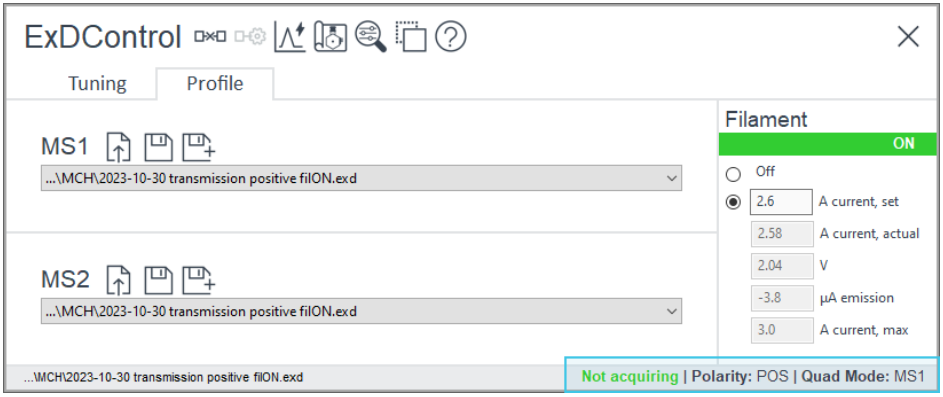


Figure 12. The ExDControl status bar is on the bottom of the main window.

NOTE

If ExDControl is not connected, click **Connect** in the menu bar. If the connection attempt fails, see **"Resolve Connection Issues"** on page 84.

4 Click **Always On Top** in the menu bar to pin the ExDControl window above all others to facilitate use of ExDControl alongside MassHunter Acquistion.

Daily Operation

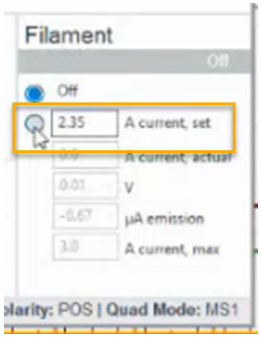
Turning On the Filament

1 In the MassHunter Acquisition software for LC/MS Tune context, set the Ion Polarity to **Positive**.

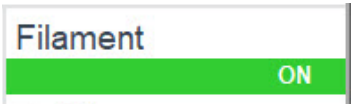
NOTE

To operate the instrument in Negative Ion Polarity, see **"Filament Off Transmission (Positive)"** on page 55.

2 In ExDControl, turn the filament on by selecting the radio button next to the filament **A current set point** from the prior day. Allow 20 minutes for thermal stabilization.



The Filament Status Bar turns green.

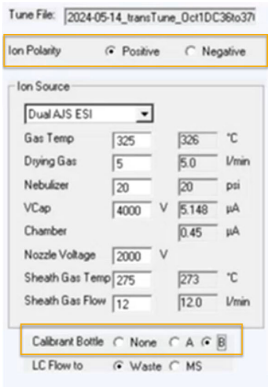


NOTE

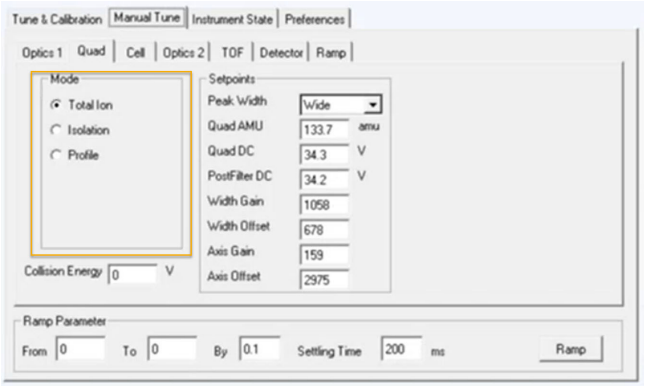
Allow 20 minutes for thermal stabilization after periods of noncontinuous use.

Calibrating the Instrument

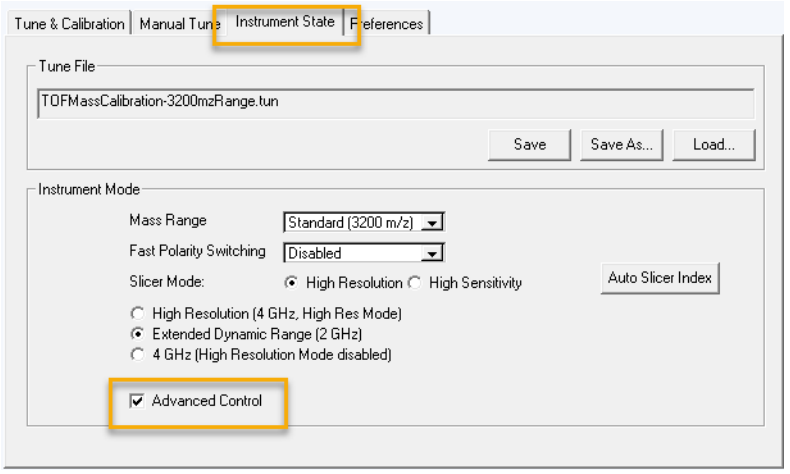
1 Select **CDS bottle B** to infuse tuning mix.



2 Review the **Manual Tune > Quad** tab and select **Total Ion** for mode.



If necessary, click the **Instrument State** tab, then select **Advanced Control** to display the Manual Tune tab.



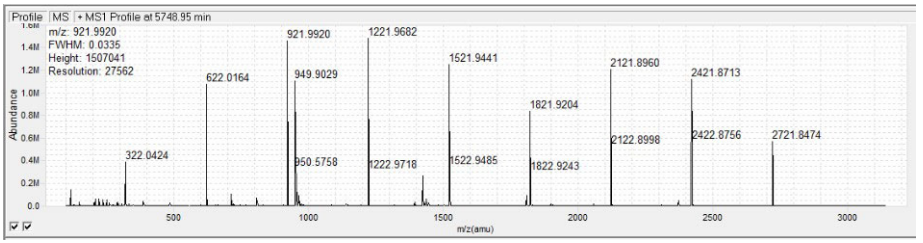
3 Select the Profile tab, then click **Open MS1** to load an optimized lens profile for transmission.

4 Browse to the most recent saved transmission lens profile or select a default profile from C:\Users\<username >\E-MSION-36\ExDControl\profiles and click **Open**. The profile loads into the MS1 field.

5 Click **Open MS2**.

6 Browse to the most recent saved transmission lens profile or select a default profile from C:\Users\<username >\E-MSION-36\ExDControl\profiles and click **Open**. The profile loads into the MS2 field.

7 Check that the signal intensity and distribution of the tuning mix ions are similar to the prior day or the post installation report.



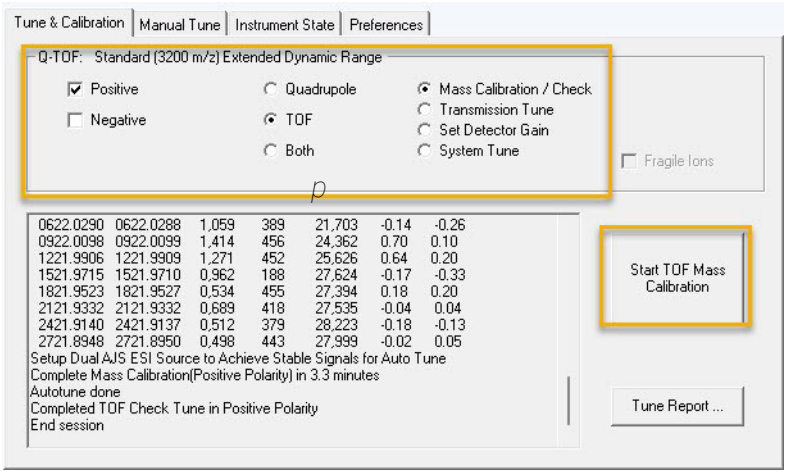
NOTE

If signal is low, run a **"Transmission of Small Molecules and Peptides (Positive)"** on page 46. If signal is absent, see **"Default Tune Parameters"** on page 89 to restore the signal.

8 Run a Mass Calibration in MassHunter Acquisition software. In the Tune context, select **Tune & Calibration.**

9 Set the Q-TOF settings to **Positive**, **TOF**, and **Mass Calibration/Check**.

10 Click **Start TOF Mass Calibration**.



Review the report for passing results, then continue the daily operation tasks.

NOTE

If calibration fails, **"Resolve Tuning Mix Peak Imbalance"** on page 96.

Tuning the ExD Cell for Daily Operation

Follow the appropriate tuning process to tune the ExD cell for a specific experiment type. See **“Tuning the ExD cell for specific experiment types”** on page 46 for more information.

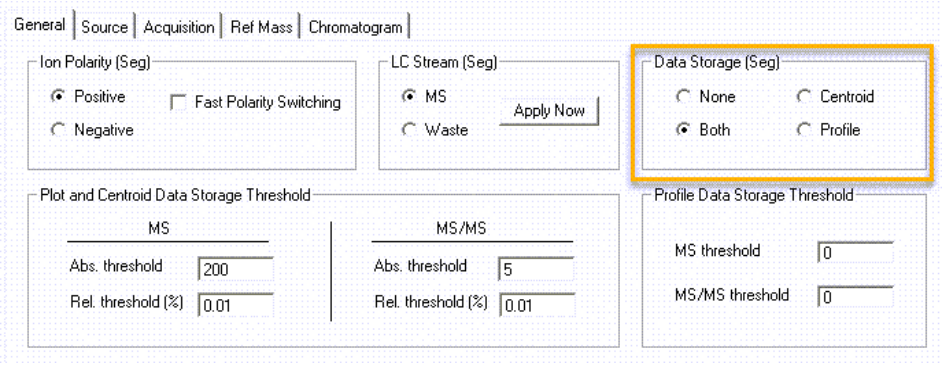
Acquiring ECD data

With the addition of the ExD cell to the 6545XT AdvanceBio LC/Q-TOF, the following additional acquisition modes are available:

- **Targeted ECD-MS/MS**: Use to fragment specific, known precursor m/z values of peptides or proteins with ECD. Optionally, add supplemental collision energy to promote dissociation after electron capture.
- **Auto ECD-MS/MS**: Use to perform ECD when peptide or protein precursor m/z values are unknown.
- **MS-Only + ECD**: Use to perform "all ions" ECD on an analyte without isolation, or with the quadrupole amu setting as an upper m/z threshold. For disulfide-bonded complexes, ECD may be used to cleave subunits.

Use the following guidance when acquiring ECD data:

- When setting up an ECD experiment in MassHunter Acquisition software for LC/MS systems, document the ExD cell settings used for sample runs and worklists. ExD cell settings are not recorded in the Agilent *.d data file.
- ECD requires a minimum precursor charge state of 2+ in positive ion mode because electron capture neutralizes one charge.
- Average ECD spectra to improve data quality. Prioritize high-value peaks with the Auto MS/MS Preferred List or add duplicate targets to a Targeted MS/MS isolation list.
- Use Targeted MS/MS if retention times and/or masses are known.
- Select whether to acquire profile or centroid data in the MassHunter Acquisition software context General tab based on downstream analysis software compatibility.



- ExDViewer requires Profile data. Choose **Profile** or **Both** as data storage options for compatible analysis in ExDViewer.
- MassHunter BioConfirm software requires Centroid data. Choose **Centroid** as the data storage option for compatible analysis in BioConfirm.

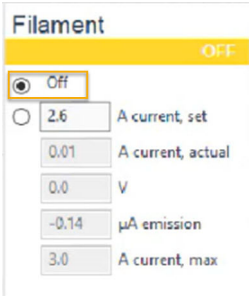
NOTE

When using MassHunter Bioconfirm software, if Both is selected, profile data must be hidden using the TOF Reprocessor tool included with MassHunter Qualitative Analysis in C:\Program Files\Agilent\MassHunter\Workstation\Qual\X.X\Bin.

Standby Procedure

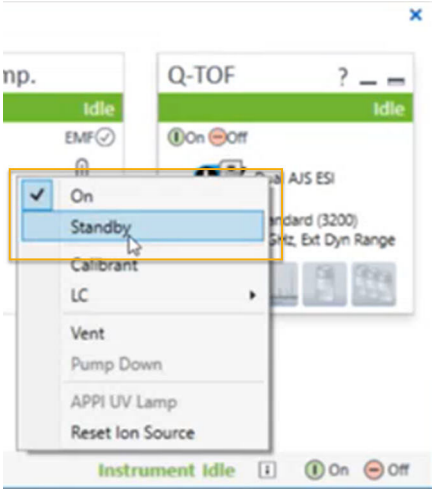
After acquiring data, turn the filament off and set the system to standby. To use worklist scripts, see **"Using ExDControl scripts"** on page 94.

1 Select **Off** in the ExDControl Filament panel.



The filament status changes to OFF.

2 In the MassHunter Acquisition Instrument context, set the instrument to Standby.



Analyzing ECD data

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

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

Table 2. Software used for analysis

Workflow	Software	For more information
Bottom-up Peptide Analysis	BioConfirm 12.1	**https://aglt.co/DALibMgmtDocs**
Top/Middle-Down Protein Analysis	ExDViewer 4.6	**https://exdviewer.agilent.com/**

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3 Tuning

Overview

Tuning optimizes instrument parameters to maximize sensitivity, maintain resolution, and ensure mass accuracy for a particular type of experiment or analyte.

There are two separate software applications to tune and operate the ExD-enabled MS system:

- MassHunter Acquisition software, which is used to tune instrument parameters. See the **6500 Series Q-TOF LC/MS Tuning Guide** for guidance.
- ExDControl, which is used to tune the ExD cell optics and filament current.

NOTE

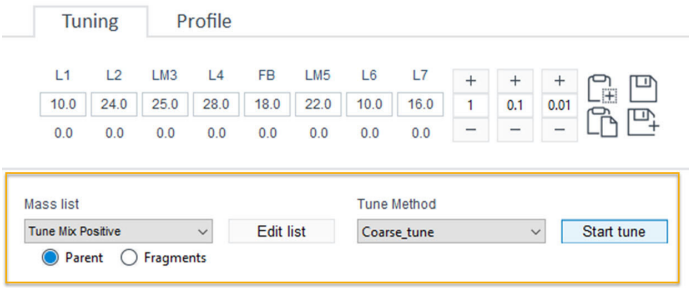
Fast Polarity Switching must remain disabled on ExD-enabled MS systems. The ExDControl software does not switch profiles automatically in response to changes in instrument polarity.

Tuning the ExD cell with ExDControl

NOTE

Before proceeding with tuning of the ExD cell, ensure that the Daily Operation procedures are completed. See **Chapter 2**, “Daily Operation” for more information.

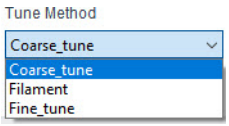
The Tuning tab in ExDControl allows for using autotune method options or manually adjusting the lens voltages. For more information on Manual tune, see **“Manual Tune”** on page 92.



Optimal ExD cell tune parameter settings vary depending on the type of experiment or analyte. For best results, use the experimental analyte or a tuning standard that resembles the physical properties (size, charge) of the experimental analyte to tune the ExD cell using the autotune settings.

Autotune Methods

Autotune methods in ExDControl adjust the ExD cell lens profiles to maximize abundances of the selected mass list.



Coarse and Fine autotune methods adjust the ExD cell lens profile voltages to maximize abundances of either parent or fragment ions for a tuning standard, defined in a user-selected mass list.

The Filament autotune method uses a standard lens profile to adjust the ExD cell filament current to a setting at the threshold of electron emission. No tuning standard is required.

Table 3. Tune Methods Types and Purpose

	Description	Purpose
Coarse_tune	Has a large step size	Use after making instrument changes.
Filament	Optimizes filament emission	Use after exchanging a filament.
Fine_tune	Has a small step size	For daily use or similar samples.

ExDControl Autotune Steps

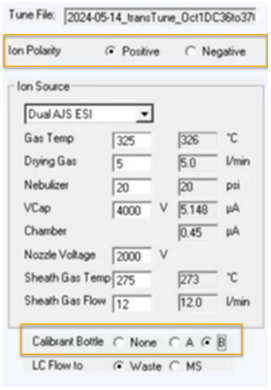
Using the Profile and Tuning tabs in ExDControl, set up autotune methods for specific experiment types using the following steps. See **"Tuning the ExD cell for specific experiment types"** on page 46.

NOTE

Autotune procedure steps remain the same, but selections and configuration of the tunes differ based on the experiment type.

1 Determine the infusion method for the experiment type and in MassHunter Acquisition software, set in the Tune context.

- CDS bottle B
- Direct Infusion (None)

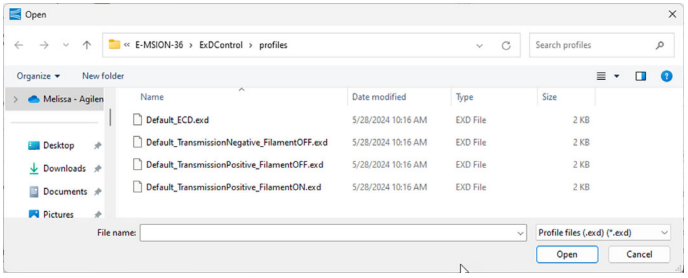


2 Set the quadrupole mode in MassHunter Acquisition software, which determines which of the loaded profiles is active in ExD Control.

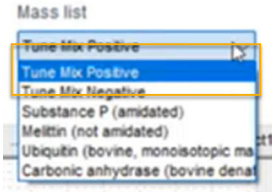
- **Total Ion Mode** sets the MS1 profile as active.
- **Isolation Mode** sets the MS2 profile as active. After entering the isolation mode, isolate the precursor m/z. Use a Wide window, if possible, to increase the signal.

3 In ExDControl, on the Profile tab, click **Open MS1** or **Open MS2** based on the experiment type.

4 Browse to the appropriate directory and select a *.exd file to use as a starting point, then click **Open**. If an appropriate file does not exist, use a default profile.



5 From the Tuning tab, select a **Mass List** option from the list.



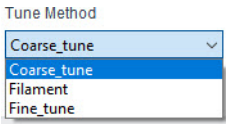
6 Select a **Mass List Type**.

- **Parent:** Tunes the cell for transmission by maximizing parent ion intensities.
- **Fragments:** Tunes the cell for ECD by maximizing fragment ion intensities.

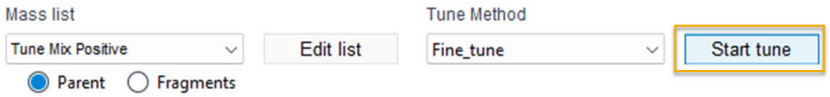
NOTE

Selecting a Parent mass list type tunes the cell for transmission while selecting a Fragments mass list type tunes the cell for ECD.

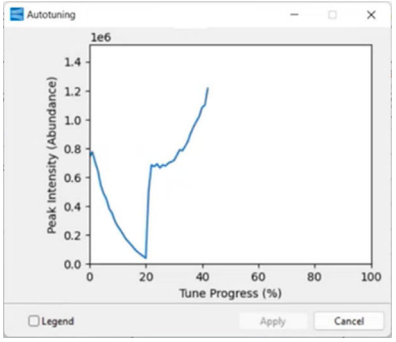
7 Click **Tune Method** and select a tune option from the list.



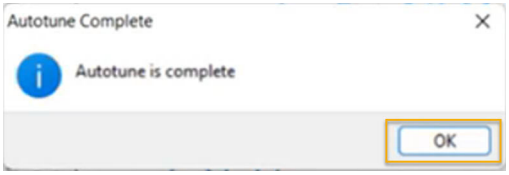
8 Click **Start tune** to begin the autotune.



9 Monitor the progression in the Autotuning window.



10 Once the autotune completes, click **OK**.



11 Click **Apply** to commit the autotune settings to ExDControl.

12 To save the lens profile settings, in the Tuning tab, click **Save As** to save the lens profile.

13 In the Save As dialog box, browse to an appropriate location.

14 Enter a descriptive name for File Name and click **Save**.

NOTE

As a best practice for file organization, use a consistent naming convention. Include the date (YYYY-MM-DD format), the instrument polarity, the tuning standard, the ExD cell operating mode (transmission or ECD), the filament current and avoid special characters.

Tuning the ExD cell for specific experiment types

Transmission of Small Molecules and Peptides (Positive)

Tuning Standard	Polarity	Filament State	Quadrupole Mode	Mass List	Mass List Type	Tune Method
Tuning Mix with Melittin, ESI-X, 2-Fold or 10-Fold Dilution (See page 58)	Positive	ON	Total Ion	Tune Mix Positive	Parent	Fine_Tune

1 Prepare the **Tuning Standard**.
2 In the MassHunter Acquisition software Tune context, set the **Ion Polarity** to Positive.
3 On the Manual Tune tab, set the Mode to **Total Ion Mode**.
4 In the Tune context, set the **CDS bottle B** to infuse tuning mix.
5 In ExDControl on the Profile tab, click **Open MS1** and select a lens profile previously tuned for transmission.
6 On the Tuning tab, select **Tune Mix Positive** for Mass List.
7 Select **Parent** for Mass List type**.**
8 Select **Fine_tune** for Tune Method.
9 Click **Start tune**. Monitor the progression in the Autotuning window.
10 Once the autotune completes, click **OK**.
11 Click **Apply** to commit the settings to ExDControl.
12 On the Tuning tab, click **Save As** to save the lens profile with a descriptive name and transmission designation.
13 In the Profiles tab, **Open** the saved lens profile into the MS2 field.

Minimize peptide electron capture products

Because the filament is on, low-abundance products of electron capture charge reduction (ECCR) and electron ECD might be present in the spectrum.

To minimize peptide electron capture products, tune the ExD lens profile to maximize transmission of a peptide precursor of similar size and charge to the experimental sample.

For example, use the following autotune method settings to maximize transmission of the melittin MH^{3+} precursor at m/z 949.9.

Tuning Standard	Polarity	Filament State	Quadrupole Mode	Mass List	Mass List Type	Tune Method
Tuning Mix with Melittin, ESI-X, 2-Fold Dilution (See **page 58**)	Positive	ON	Total Ion	Melittin (not amidated)	Parent	Fine_Tune

To eliminate peptide electron capture products, tune the ExD cell for transmission with filament off. See **"Filament Off Transmission (Positive)"** on page 55.

Peptide ECD-MS/MS

Tuning Standard	Polarity	Filament State	Quadrupole Mode	Mass List	Mass List Type	Tune Method
Tuning Mix with Melittin, ESI-X, 2-Fold Dilution (See page 58)	Positive	ON	Isolation	Melittin (not amidated)	Fragments	Fine_tune Coarse_tune

1 Prepare the **Tuning Standard**.
2 In the MassHunter Acquisition software Tune context, set the Ion Polarity to **Positive**.
3 On the Manual Tune tab, set Mode to **Isolation**.
4 In the Tune context, set the **CDS bottle B** to infuse the tuning mix. Ensure that the signal is stable before continuing.
5 Isolate the [M+3H]3+ melittin precursor at m/z 949.9.
6 In ExDControl on the Profile tab, click **Open MS2** and select a lens profile last tuned for peptide ECD. If none are saved, select the Default_ECD.exd profile.
7 On the Tuning tab, select **Melittin (not amidated)** for Mass List.
8 Select **Fragments** for Mass List type.
9 Select **Coarse_tune** for Tune Method.
10 Click **Start tune**.
11 Monitor the progression in the Autotuning window. Observe the intensity of ECD fragment ions in the spectrum. The melittin c_7^{1+} fragment ion is at m/z 656.4454.
12 Once the autotune completes, click **OK**.
13 Click **Apply** to commit the settings to ExDControl.
14 In the ExDControl Tuning tab, repeat fine autotunes for transmission until intensities of tuning standard peaks no longer increase in the spectrum.
15 Click **Apply** to commit the settings to ExDControl.
16 On the Tuning tab, click **Save As** to save the lens profile with a descriptive name and transmission designation.

17 In the ExDControl Profiles tab, open a lens profile for transmission in MS1 field and verify that the newly tuned lens profile for peptide ECD is opened in the MS2 field.

Using other peptide tuning standards

For best results, the peptide tuning standard should resemble the size and charge of the experimental sample. Use a tuning standard concentration and infusion flow rate that produces a minimum signal of 200k.

Isolate the highest abundant charge state as the precursor and create a custom mass list. See **"Modifying a Mass List"** on page 60 for more information on adding ECD fragment ion values to custom mass lists.

Protein Intact MS-Only

Tuning Standard	Polarity	Filament State	Quadrupole Mode	Mass List	Mass List Type	Tuning Methods
Protein standard specific for experiment	Positive	ON	Total Ion	Depends on protein standard	Parent	Coarse_Tune Fine_Tune

1 Prepare a protein tuning standard specific for the experiment. For best results, the protein tuning standard should resemble the size and charge of the experimental sample.
2 In the MassHunter Acquisition software Tune context, set the Ion Polarity to **Positive**.
3 On the Manual Tune tab, set the Mode to **Total Ion Mode.**
4 Infuse the protein tuning standard via direct infusion. Ensure that the signal is stable before continuing.

NOTE

To set up direct infusion tuning standard, see **"Set up Direct Infusion"** on page 86.

5 In ExDControl on the Profile tab, click **Open MS1** and select a lens profile previously tuned for transmission on a protein tuning standard. If none are saved, select one tuned for transmission on tuning mix.
6 On the Tuning tab, select a **Mass List** appropriate for the protein standard.
7 Select **Parent** for Mass List type.
8 Click **Save As** to save the lens profile in ExDControl. Give the lens profile a descriptive name.
9 In the ExDControl Profiles tab, open a lens profile for transmission in MS1 field and verify that the newly tuned lens profile for protein ECD is opened in the MS2 field.

Minimize protein electron capture products

Because the filament is on, low-abundance products of ECCR and ECD may be present in the spectrum.

To minimize protein electron capture products, do the following:

1 Manually adjust LM3, L4, L5 to maximize precursor and minimize products in 0.1 V steps, within +/- 3 V, iteratively. See **“Manual Tune”** on page 92.

2 Reduce the filament current by 0.05 A. Repeat steps 5–8 to tune the ExD cell lens profile using the lowered filament current.

To eliminate protein electron capture products, tune the ExD cell for transmission with filament off. See**“Filament Off Transmission (Positive)”** on page 55.

Protein ECD-MS/MS

Tuning Standard	Polarity	Filament State	Quadrupole Mode	Mass List	Mass List Type	Tuning Method
Protein standard specific for experiment	Positive	ON	Isolation	Depends on protein standard	Fragments	Fine_tune

1 Prepare a protein tuning standard specific for the experiment. For best results, the protein tuning standard should resemble the size and charge of the experimental sample.

2 In the MassHunter Acquisition software Tune context, set the Ion Polarity to **Positive**.

3 Infuse the protein tuning standard via direct infusion. Ensure that the signal is stable before continuing.

NOTE

To set up direct infusion tuning standard, see **"Set up Direct Infusion"** on page 86.

4 On the Manual Tune tab, set Mode to **Isolation.**

5 Isolate a multiply charged protein precursor.

6 In ExDControl on the Profile tab, click **Open MS2** and select a lens profile previously tuned for ECD on a protein tuning standard of similar size and charge.

If none are saved, select a profile previously tuned for ECD on a peptide tuning standard.

7 On the Tuning tab, select Fragments for **Mass List** appropriate for the protein standard.

8 Select **Fragments** for Mass List type.

9 Select **Fine_tune** for Tune Method.

10 Click **Start tune.**

11 Monitor the progression in the Autotuning window.

12 Once the autotune completes, click **OK**.

13 Click **Apply** to commit the settings to ExDControl.

14 Repeat with **Fine_tune** for transmission until intensities of ECD fragment ions no longer increase in the spectrum.

15 Click **Apply** to commit the settings to ExDControl.

16 On the Tuning tab, click **Save As** save the lens profile with a descriptive name.

17 In the ExDControl Profiles tab, open a lens profile for transmission in MS1 field and verify that the newly tuned lens profile for protein ECD is opened in the MS2 field.

Transmission of Small Molecules (Negative)

Tuning Standard	Polarity	Filament State	Quadrupole Mode	Mass List	Mass List Type	Tuning Method
Tuning Mix, ESI-X, 10-Fold Dilution	Negative	OFF	Total Ion Mode	Tune Mix Negative	Parent	Coarse_tune Fine_Tune

1 Prepare the **Tuning Standard**.

2 In the MassHunter Acquisition software Tune context, set the **Ion Polarity** to Negative.

3 On the Manual Tune tab, set the Mode to **Total Ion Mode**.

4 In the Tune context, set the **CDS bottle B** to infuse tuning mix.

5 In ExDControl on the Profile tab, click **Open MS1** and select a lens profile previously tuned for negative ion transmission with the filament off. If no such profile exists, open Default_TransmissionNegative_FilamentOFF.exd and update instrument tune settings to the values in negative mode values in Default Settings. See **"Default Tune Parameters"** on page 89.

6 On the Tuning tab, select **Tune Mix Negative** for Mass List.

7 Select **Parent** for Mass List type**.**

8 Select **Coarse_tune** for Tune Method.

9 Click **Start tune**. Monitor the progression in the Autotuning window.

10 Once the autotune completes, click **OK**.

11 Click **Apply** to commit the settings to ExDControl.

12 In the ExDControl Tuning tab, repeat fine autotunes for transmission until intensities of tuning mix peaks no longer increase in the spectrum.

13 Click **Apply** to commit the settings to ExDControl.

14 On the Tuning tab, click **Save As** save the lens profile with a descriptive name.

15 In the Profiles tab, **Open** the saved lens profile in MS2 field.

Filament Off Transmission (Positive)

Tuning Standard	Polarity	Filament State	Quadrupole Mode	Mass List	Mass List Type	Tuning Method
Tuning Mix, ESI-X, 10-Fold Dilution	Positive	OFF	Total Ion Mode	Tuning Mix Postive	Parent	Coarse_tune Fine_Tune

1 Prepare the **Tuning Standard**.
2 In the MassHunter Acquisition software Tune context, set the **Ion Polarity** to Positive.
3 On the Manual Tune tab, set the Mode to **Total Ion Mode**.
4 In the Tune context, set the **CDS bottle B** to infuse tuning mix.
5 In ExDControl on the Profile tab, click **Open MS1** and select a lens profile previously tuned for positive ion transmission with the filament off.

NOTE

If no such profile exists, open Default_TransmissionPositive_FilamentOFF.exd and update instrument tune settings to the values in Default Settings. See **"Default Tune Parameters"** on page 89.

6 On the Tuning tab, select **Tuning Mix Positive** for Mass List.
7 Select **Parent** for Mass List type**.**
8 Select **Coarse_tune** for Tune Method.
9 Click **Start tune**. Monitor the progression in the Autotuning window.
10 Once the autotune completes, click **OK**.
11 Click **Apply** to commit the settings to ExDControl.
12 In the ExDControl Tuning tab, repeat fine autotunes for transmission until intensities of tuning standard peaks no longer increase in the spectrum.
13 Click **Apply** to commit the settings to ExDControl.
14 On the Tuning tab, click **Save As** save the lens profile with a descriptive name.
15 In the Profiles tab, **Open** the saved lens profile in MS2 field.

After a filament off positive lens profile has been optimized, it can be used as a starting point for autotuning the lens profile for transmission of peptides, proteins, and other molecules.

Tuning Standards

NOTE

Make sure that there is a tight seal when placing the bottle on the LC/MS system. If ripples are seen in the bottle during tuning, the bottle may have a poor seal which causes problems with tuning. If the problem does not resolve, contact Agilent Support for additional assistance.

NOTE

Instrument source components may require cleaning after infusing large volumes of tuning standards. <<add qr code>>

Preparing 10-Fold Tuning Mix

To prepare an LC/Q-TOF tuning/calibration mix of 1:10 dilution for use with the ExD cell:

1 Add the following components, in the order listed, to a clean Calibration Delivery System bottle (CDS) (9300-2576).
   - 85.5 mL LC/MS-grade acetonitrile (5191-5100)
   - 4.5 mL LC/MS-grade water (5191-5121)
   - 10 mL ESI-X Low Concentration Tuning Mix (5191-6449)

Make up fresh tuning solution at least once every month to ensure best performance.

NOTE

ESI-L Low Concentration Tuning Mix (G1969-85000) with Biopolymer Reference Mass Kit (G1969-85003) may also be used.

Preparing Tuning Mix with Melittin, ESI-X, 2-Fold Dilution

To prepare 200 mL 1:2 dilution LC/Q-TOF tuning/calibration mix for with melittin for tuning peptide ECD:

1 Add the following components, in the order listed, to a 250 mL or larger amber glass bottle with cap (9301-6525).
   - 100 mL ESI-X (5191-6449)
   - 95 mL LC/MS Grade acetonitrile (5191-5100)
   - 4.0 mL LC/MS Grade water (5191-5121)
   - 0.5 mg melittin (G1997-85001), dissolved in 1.0 mL LC/MS Grade Water
2 Mix contents thoroughly.
3 Pour half the prepared mix into a CDS (Calibration Delivery System) bottle (9300-2576).

   Store the remainder at 4 °C to prolong shelf life to inhibit the formation of adducts.

CAUTION

Vials of lyophilized melittin should be stored properly until expiration date at -20 °C.

Peptide and Protein Tuning Standards

Use a tuning standard resembling the size and charge of the experimental sample to tune the ExD cell. If not sample limited, use the experimental sample to tune the ExD cell. See **"Modifying a Mass List"** on page 60 for guidance on how to create a custom mass list for ExDControl autotune.

NOTE

Prepare ubiquitin and carbonic anhydrase tuning standards in 15% acetonitrile, 0.01% formic acid to a final concentration of 10 µM.

Table 4. Peptide and protein tuning standard mass lists included in ExDControl

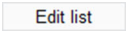
Tuning Standard	Type	Size	Source	Part Number	Sequence
Melittin Standard (nonamidated)	Peptide	2.8 kDa	Agilent	G1969-85001	GIGAVLKVLTTGLPALISWIKRKRQQ
Ubiquitin from Bovine Erythrocytes	Protein	8.4 kDa	Sigma Aldrich	U6253	MQIFVKTLTGKTITLEVEPSDTIENVKAKIQ DKEGIPPDQQRLIFAGKQLEDGRTLSDYNI QKESTLHLVLRLR
Carbonic Anhydrase from Bovine Erythrocytes	Protein	29 kDa	Sigma Aldrich	C2624	(Acetyl)SHHWGYGKHNGPEHWHKDFPIA NGERQSPVDIDTKAVVQDPALKPLALVYG EATSRRMVNNGHSFNVEYDDSQDKAVL KDGPLTGTYRLVQFHFHWGSSDDQGSE HTVDRKKYAAELHLVHWNTKYGDFGTAA QQPDGLAVVGVFLKVGDANPALQKVLDA LDSIKTKGKSTDFPNFDPGSLLPNVLDYW TYPGSLTTPPLLESVTWIVLKEPISVSSQQ MLKFRTLNFNAEGEPELLMLANWRPAQP LKNRQVRGFPK

Modifying a Mass List

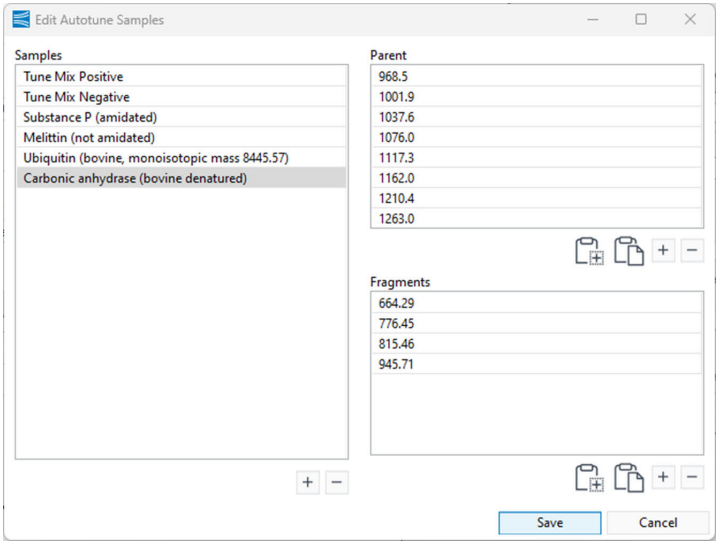
Edit or create a mass list, add/remove ions from a current or new list to use for different tuning standards.

Editing a Mass List

1 Click **Edit List**.



2 Select a Mass List in the Samples box.



3 Modify Parent or Fragment data.

- **a** Click **+** to enter single m/z values.
- **b** Click **-** to delete single m/z values.
- **c** Click  to copy lists of Parent or Fragment values from the screen (window)
- **d** Click  to paste lists of Parent or Fragment values from the screen or Excel.

NOTE

Pasting Parent or Fragment lists overwrites the existing lists, it does not append to the end.

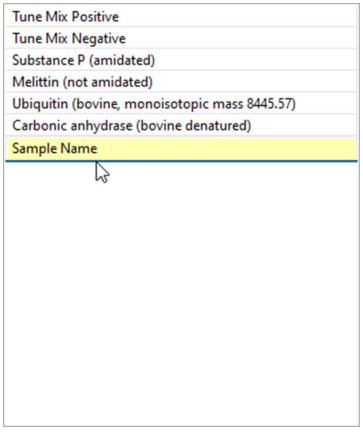
4 Click **Save** when finished.

Adding a Mass List

1 Click **Edit List**.

2 Click the **+** button under the Samples box.

3 Enter a Sample Name in the text field.



4 Add Parent or Fragment data.

- **a** Click **+** to enter single m/z values.
- **b** Click **-** to delete single m/z values.
- **c** Click  to copy lists of Parent or Fragment values from the screen (window)
- **d** Click  to paste lists of Parent or Fragment values from the screen or Excel.

5 Click **Save** when finished.

The new list appears in the Mass List drop-down menu.

CAUTION

Do not delete the default Mass Lists. These files are used as templates to create lists. Contact your Agilent Support representative for further support.

Tuning the Instrument

- In MassHunter Acquisition software, enable **Tune using current parameters** in the Tune Context Preferences tab. With this option enabled, system tunes begin from current instrument settings instead of from default settings. The MassHunter Acquisition default settings are not compatible with the ExD cell profile voltages, and MassHunter Acquisition tunes will fail.

CAUTION

Do not concurrently tune the instrument in positive and negative mode. ExDControl cannot automatically switch between tune settings for positive ion mode and negative ion mode.

- Lens profiles tuned for transmission must be selected in the MS1 and MS2 fields in the ExDControl Profiles tab. Not selecting lens profiles tuned for transmission causes low sensitivity due to poor transmission through the ExD cell.
- After a MassHunter Acquisition software System or Transmission tune, retune the ExD cell to compensate for changes in MassHunter Acquisition parameters.

4 Maintenance

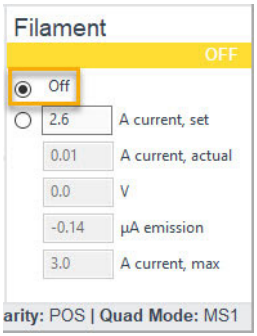
Shutting down

WARNING **The instrument, the ExD cell, and ExD Controller must be powered down completely before proceeding with any maintenance procedures.**

WARNING **Turn the filament off before turning the ExD Controller off and venting the instrument. The heated filament can fail abruptly after exposure to atmosphere and contaminate nearby parts.**

Before shutting down the instrument, perform the following to disconnect the ExD cell from power:

1 Turn the filament off in the ExDControl Filament panel. Wait for 0 A actual readout.



2 On the ExD Controller, press and hold the ON/OFF power switch on the back panel until the front panel LCD goes dark.

3 Disconnect the ExD Controller power cord from line power.

Proceed with the instrument shutdown process.

Periodic Tasks

Replacing the ExD cell Filament

Over time, thermal stress from routine use causes the ExD cell filament to thin until it fails ("burnout"). While the ExD cell may be tuned to transmit ions after the filament has failed, instrument sensitivity is impacted.

Schedule

Once every four to six months (recommended) or when a loss of instrument sensitivity is noted. See **"Diagnosing Filament Failure"** on page 90 for more information.

Equipment List

- Screwdriver, Torx, T5 (Wiha SKU: 26617)
- Lint-free cloth (05980-60051)
- Powder-free, nitrile gloves
- Digital multimeter
- Magnifier

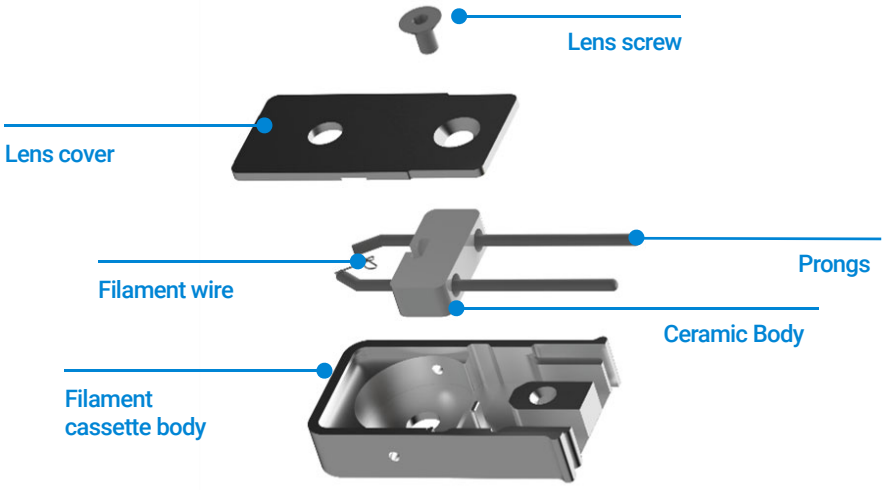
Replacement Parts

- Filament Insert (G1997-67011)
- Filament Cassette Assembly (G1997-67003)

Preparation of Filament Cassette Assembly

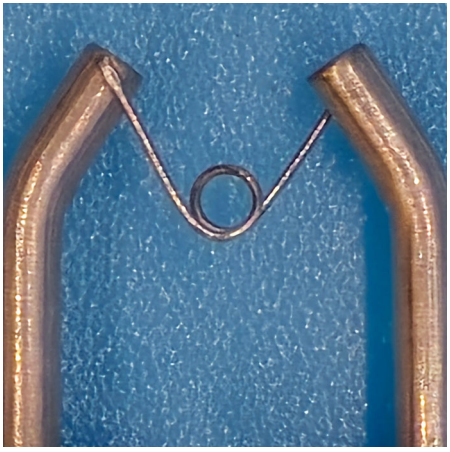
1. Put on clean, powder-free nitrile gloves.
2. Prepare a clean, dust-free work surface.
3. Put on a fresh pair of clean, powder-free nitrile gloves.
4. Unpack the new filament in the clean, dust-free work area.

CAUTION **Avoid touching the wire. Displacing the wire loop can affect the formation of the electron cloud in the ExD cell, causing low ECD efficiency.**



5 Use a magnifier to inspect the filament wire loop.

- The 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 is centered between the filament posts.

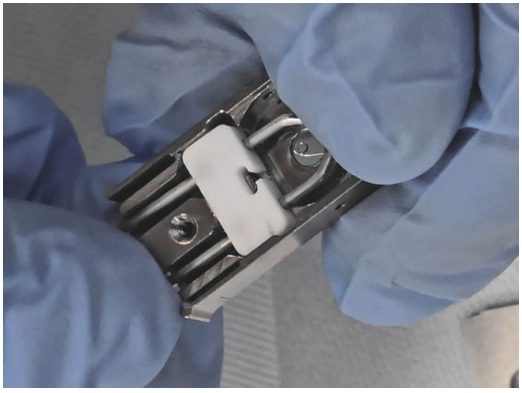


6 Use the T5 Torx Driver to remove the screw from the replacement filament cassette body.

7 Remove the cassette lens cover from the filament cassette body.

8 Inspect the interior and exterior of the filament cassette body and lens cover for dirtiness. See **"Cleaning the Filament Cassette"** on page 73.

9 Hold the filament insert by the prongs and place into the replacement cassette body.



10 Firmly seat the filament into the cassette.

11 Inspect the alignment of the filament with the replacement cassette body. The ceramic body of the filament should be flush with the cassette body and the wire loop should be concentric with the cassette aperture.

12 Replace the cassette lens and use the T5 Torx driver to fasten the included screw.

13 Place the replacement filament cassette assembly in a dust-free location.

Filament Cassette Assembly Swap

1 Vent the instrument.

2 Power down and disconnect the ExD Controller from all power supplies. See **"Shutting down"** on page 64.

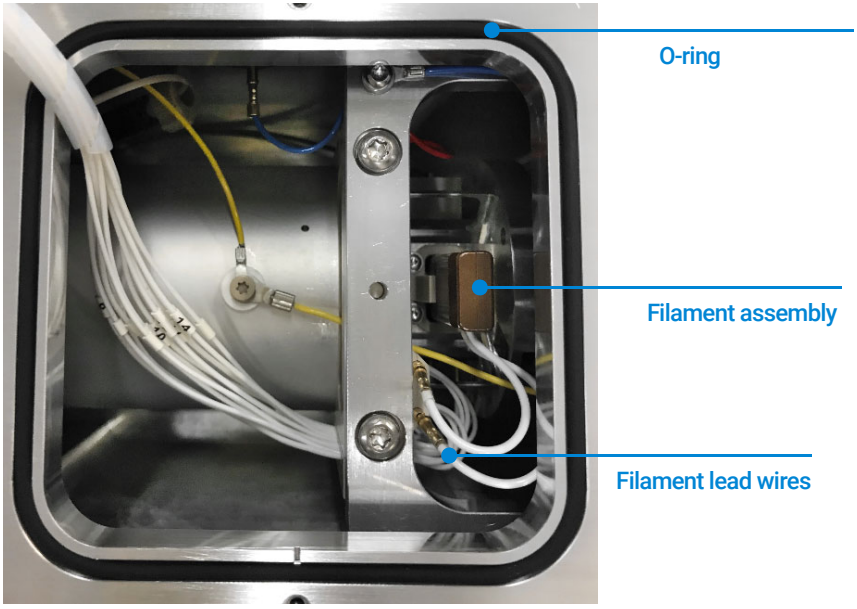
3 Check and clean debris from fan guard and bottom vents as necessary. See **"Cleaning the ExD Controller Fan Guard and Vent"** on page 74.

4 Ensure that the manifold has reached atmospheric pressure. Remove the front and top cosmetic covers from the instrument.

5 Locate and disconnect the D-sub cable from the feed through adapter on top of the filament access door.

6 Loosen the two screws in the filament access door and lift by the handle to remove the door.

7 Set the door off to the side, face up, leaving the internal D-sub cable connected to the instrument.



8 Gently disconnect the two white filament lead wire pins.

9 Gently pull upwards on the brown insulated sheath to remove the filament cassette assembly up and out from the ExD cell.

CAUTION

Do not rock the handle while removing the filament assembly. Rocking may separate the filament contact assembly from the cassette assembly during removal, leaving the cassette assembly still seated in the ExD cell.

10 Place the filament cassette assembly on a dust-free, heat-resistant surface.

11 Grip the metal component in one hand and the brown plastic component in the other.

WARNING **Metal component can be very hot. Do not touch the metal components or related parts until they are cool.**

12 Pull while gently wiggling the filament cassette side-to-side to remove the filament cassette from the filament contact assembly.

NOTE Clean the filament cassette assembly and store in a dust-free location when the filament swap is complete. See **"Cleaning the Filament Cassette"** on page 73.

13 Insert the replacement filament cassette assembly prongs into the receptacles in the brown plastic contact assembly sheath. The direction does not matter.

14 Press the two components together until the cassette clicks into place.

15 Examine the filament cassette to ensure that the filament wire is in the center of the aperture.

16 With the white contact wires facing toward the front of the instrument, slide the combined filament cassette assembly into the slot of the ExD cell.

17 Press downwards until the assembly clicks into place.

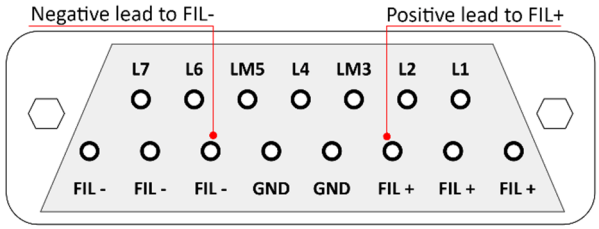
18 Reconnect the two filament wire pins.

19 Examine the filament access door O-ring. Clean the O-ring with a lint-free wipe or replace if the O-ring is damaged.

CAUTION **Do not use vacuum grease, or solvents.**

20 Using the handle, replace the filament access door and tighten the two screws.

21 Use a digital multimeter to measure the resistance of the filament circuit across the electrical feed through. Resistance should read between 0.1 and 1.0 Ω.



22 Reconnect the D-Sub cable to the electrical feed through.

23 Reconnect and turn on the ExD Controller.

24 Restart the instrument to begin pump down.

25 Once the pump down is complete, launch the MassHunter Acquisition and ExDControl software.

Restore System Performance

CAUTION

Wait to turn the filament on until the Quad vacuum pressure is below 9.0e-5 Torr.

Equilibrate and tune the filament:

1. Ensure the instrument is on and in positive mode.
2. In the ExDControl Filament Panel, turn the filament on.
3. Gradually increase the filament current to 2.4 A in 0.5 A steps. Check that filament readbacks match the setpoints.
4. Allow the filament to equilibrate for 30 minutes at 2.4 A.

NOTE

An atypical disparity between voltage setpoints and readbacks for FB and L4 or unusually large 'Emission' readout may be seen, caused by contaminants on the surface of the heated filament. After equilibration, the contaminants burn off and all voltage and current readouts should return to normal.

5. In ExDControl, select **Filament** for Tune Method and click **Start tune**.

NOTE

The filament tune determines the minimum current for electron emission. Typically, this current is too low for optimal ion transmission or ECD.

Manually optimize the filament current:

1. Prepare ESI-X Tuning Mix with melittin.
2. In the MassHunter Acquisition software Tune context, set the Ion Polarity to **Positive**.
3. Reset the **CDS bottle B** to infuse the tuning mix. Ensure that the signal is stable before continuing.
4. On the Manual Tune tab, set Mode to **Isolation**.
5. Isolate the $[M+3H]^{3+}$ melittin precursor at m/z 949.9.
6. In ExDControl on the Profile tab, click **Open MS2** and select a lens profile previously tuned for peptide ECD. If none are saved, select one tuned for transmission on tuning mix.
7. In the Filament Panel, increase the filament current by 0.05 A.
8. On the Tuning tab, select **Melittin (not amidated)** for Mass List.

9 Select **Fragments** for Mass List type.

10 Select **Fine_tune** for Tune Method.

11 Click **Start tune**.

12 Check whether the intensity of the melittin ECD c_7^{1+} fragment ion at m/z 656.4454 has increased.

13 Repeat steps 7 through 12 until ECD fragment ion intensities no longer increase. Do not exceed 0.2 A above the Filament Tune current setting.

14 Check that the filament is not overheated. Rhenium evaporating from the filament (m/z 184.9530 and 186.9558) should not exceed ~10,000 counts in the spectrum.

15 Check for filament exposure to oxygen. A high proportion of rhenium oxide (m/z 200.9479, 202.9507; 216.9428, 218.9456; 232.9378, 234.9405; 248.9326, 250.9354) relative to rhenium in the spectrum indicates accelerated filament aging. See **"Gas Delivery"** on page 88.

16 Retune ExD Cell profiles for use with the new filament. See **"Tuning the ExD cell for specific experiment types"** on page 46.

17 In MassHunter Acquisition software, run a Mass Calibration again.

Cleaning the Filament Cassette

Schedule

After replacing the filament.

Equipment List

- Screwdriver, Torx, T5 (Wiha SKU: 26617)
- Lint-free cloth (05980-60051)
- Powder-free, nitrile gloves
- Micro fiberglass brush (Micro-Mark 14259)

1. Place the filament cassette to be cleaned in a clean, dust-free area.
2. Use the T5 Torx Driver to remove the screw from the filament cassette body.
3. Remove the cassette lens cover from the filament cassette body.
4. Sonicate metal components of the filament cassette in 50% methanol.
5. Dry with nitrogen gas.
6. Inspect metal components for residual dirtiness.

   If necessary, clean the metal cassette body by swabbing with aluminum oxide. If the cassette body is still dirty, use a micro-fiberglass brush to scrape inner metal surfaces clean, then repeat sonication in 50% methanol and drying with nitrogen gas.
7. Replace the cassette lens and use the T5 Torx driver to fasten the replacement screw.
8. Store the clean filament assembly spare in a clean, dust-free container and store safely for future use.

Cleaning the ExD Controller Fan Guard and Vent

Schedule

Monthly

1 Check and clean debris or blockage from the fan guard and vents as necessary.

5 Compliance

Agilent Regulatory Compliance Statement

CE Compliance

Your Agilent instrument has been designed to comply with the requirements of the applicable directives of the European Union, such as Electromagnetic Compatibility (EMC) Directive, Low Voltage Directive (LVD), Machinery Directive (MD), RoHS Directive, etc.

Agilent has confirmed that each product complies with the relevant Directives by testing samples against the harmonized EN (European Norm) standards published on the Official Journal of the European Union (OJEU).

Proof that a product complies with these directives is indicated by:

- the CE Marking appearing on the rear of the product, and
- the documentation package that accompanies the product containing a copy of the Declaration of Conformity. The Declaration of Conformity is the legal declaration by Agilent that the product complies with the relevant directives listed above, and shows the EN standards to which the product was tested to demonstrate compliance.

UK Compliance

Your Agilent instrument has been designed to comply with the requirements of the applicable regulations of the United Kingdom, such as The Electromagnetic Compatibility Regulations 2016, The Electrical Equipment (Safety) Regulations 2016, The Supply of Machinery (Safety) Regulations 2008, The Restriction of the Use of Certain Hazardous Substances in Electrical and Electronic Equipment Regulations 2012, etc.

Agilent has confirmed that each product complies with the relevant Regulations by testing samples against the designated standards published on GOV.UK.

Proof that a product complies with these regulations is indicated by:

- the UKCA Marking appearing on the rear of the product, and
- the documentation package that accompanies the product containing a copy of the Declaration of Conformity. The Declaration of Conformity is the legal declaration by Agilent that the product complies with the relevant regulations listed above, and shows the designated standards to which the product was tested to demonstrate compliance.

Electromagnetic Compatibility

This product conforms to the following regulations on Electromagnetic Compatibility (EMC) and radio frequency interference (RFI):

- CISPR 11/EN 55011: Group 1, Class A
- EC/EN 61326-1
- AUS/NZ 
- Canada ICES-001 (This Industrial, Scientific and Medical (ISM) device complies with Canadian ICES-001. Cet appareil ISM est conforme à la norme NMB-001 du Canada).

Group 1 ISM equipment Group 1 contains all Industrial, Scientific and Medical (ISM) equipment in which there is intentionally generated and/or used conductively coupled radio- frequency energy which is necessary for the internal functioning of the equipment itself.

Class A equipment This equipment is suitable for use in all establishments other than domestic and those directly connected to a low voltage power supply network which supplies buildings used for domestic purposes.

This device complies with the requirements of CISPR11, Group 1, Class A as radiation professional equipment. Therefore, there may be potential difficulties in ensuring electromagnetic compatibility in other environments, due to conducted as well as radiated disturbances.

If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try one or more of the following measures:

1. Relocate the radio or antenna.
2. Move the device away from the radio or television.
3. Plug the device into a different electrical outlet, so that the device and the radio or television are on separate electrical circuits.
4. Make sure that all peripheral devices are also certified.
5. Make sure that appropriate cables are used to connect the device to peripheral equipment.
6. Consult your equipment dealer, Agilent Technologies, or an experienced technician for assistance.

Changes or modifications not expressly approved by Agilent Technologies could void the user's authority to operate the equipment.

EMC Declaration for South Korea

사용자안내문

This equipment has been evaluated for its suitability for use in a commercial environment. When used in a domestic environment, there is a risk of radio interference.

이 기기는 업무용 환경에서 사용할 목적으로 적합성평가를 받은 기기로서 가정용 환
경에서 사용하는 경우 전파간섭의 우려가 있습니다 .

※ 사용자 안내문은 " 업무용 방송통신기자재 " 에만 적용한다 .

Detachable Power Cord Declaration for Japan

電源コードセットの取扱いについて （日本国内向け）

製品には、同梱された電源コードセットをお使いください。同梱された電源コードセット
は、他の製品では使用できません。

Notice - The power cords for Japanese market

Your product must only use the power cord that was shipped with this product. Do not use this power cord with any other product.

Sound Emission Certification for Federal Republic of Germany

Sound pressure

Sound pressure Lp < 70 dB(A) according to DIN EN ISO 7779.

Schalldruckpegel

Schalldruckpegel LP < 70 dB(A) nach DIN EN ISO 7779.

Waste Electrical and Electronic Equipment (WEEE) Directive

This product complies with the European WEEE Directive marking requirements. The affixed label indicates that you must not discard this electrical/electronic product in domestic household waste.

NOTE

Do not dispose of in domestic household waste.

To return unwanted products, contact your local Agilent office or see **https://www.agilent.com/environment/product/index.shtml** for more information.

6 Appendix

Supplies

Chemicals	Manufacturer	Part No.
Q-TOF LC/MS ESI-X Tuning Mix	Agilent	5191-6449
Melittin chemical standard	Agilent	G1997-85001
Melittin, honey bee	GenScript	RP20415
Ubiquitin from bovine erythrocytes, CAS no. 79586-22-4	MilliporeSigma (Merck)	U6253
Carbonic anhydrase from bovine erythrocytes, CAS no. 9001-03-0	MilliporeSigma (Merck)	C2624

Filament Replacement Supplies	Manufacturer	Part No.
Replacement filament for G1997A ExD cell	Agilent	G1997-67011
Filament Cassette Assembly for G1997A ExD cell	Agilent	G1997-67003 Contact your Agilent Sales representative
Precision Torx Screwdriver T5 X 40 mm	Wiha	SKU: 26617

Gas Purification Supplies	Manufacturer	Part No.
Big universal trap, Nitrogen, 1/8 inch, 300 psig. Includes brass fittings	Agilent	RMSN-2
Tubing cutter for copper tubing	Agilent	8710-1709
Tubing, copper, 1/8 in OD (Tubing, copper, 1/8 in od x 2.1 mm id, pretested)	Agilent	CP4250
Wrench, open-end, 7/16 inch	–	–

Replacement Parts	Manufacturer	Part No.
Filament Access Door O-Ring	Agilent	G1997-27131

Filament Cassette Cleaning Supplies	Manufacturer	Part No.
Cleaning powder, dielectric capillary, for ion transfer capillary cleaning	Agilent	5190-1401
Cloth, lint-free, 23 x 23 cm, 100% cotton, 15/pk	Agilent	05980-60051
Swabs, cotton, 100/pk	Agilent	5080-5400
Micro fiberglass brush	Micro-Mark	14259

Infusion Supplies	Manufacturer	Part No.
Syringe Pump	Agilent	3162-0178
Syringe Adapter	Agilent	9301-1291
Tubing, red PEEK, 1.6 mm od, 0.13 mm id, 1.5 m	Agilent	0890-1915
Tubing cutter, for plastic/PEEK tubing.	Agilent	8710-1930
Swagelok, 1.6 mm ID, PEEK, finger-tight fitting, 2/pk	Agilent	0100-1516
Manual syringe, 500 µL, fixed needle with LC tip, 22 gauge	Agilent	5190-1522
Zero-dead-volume union, PEEK, with two fittings	Agilent	0100-2441

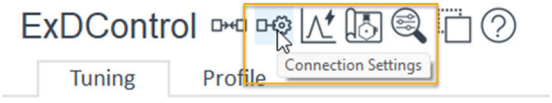
Other Information

Resolve Connection Issues

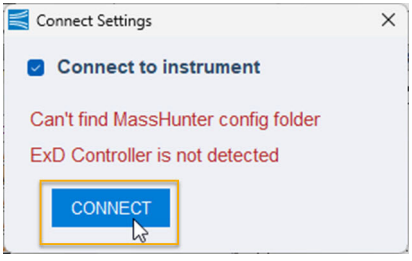
If ExDControl is unable to connect to the instrument, or the following symptoms occur:

- ExDControl status bar indicator reads “Not connected.”
- ExD Controller LCD status indicator reads “Disconnected.”
- Lens voltage and filament current readbacks do not update to match changes to the setpoints.
- When the instrument quadrupole mode changes between Total Ion (MS1) and Isolation (MS2), lens profile voltage readbacks do not update to the values saved in the corresponding MS1 or MS2 *.exd tune files.

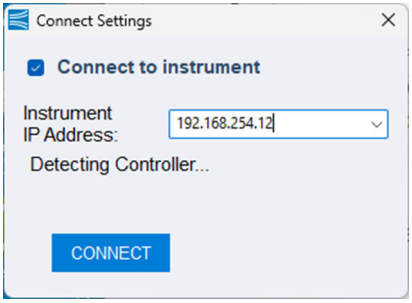
1 Verify that the ExD Controller is on. To turn on, press and hold the ON/OFF power switch on the back panel until the front panel LCD lights
2 Close and reopen ExDControl.
3 Click **Connection Settings**.



4 Press **Shift** + click **Connect** to open the connection settings configuration window.



5 Enter **192.168.254.12** in the IP address field. Press **Enter** after typing in each address.



The statuses update to "Instrument detected" and "Controller is responding,"

6 Click **Connect**.

NOTE

If manually setting the IP address does not resolve connection issues, please contact your Agilent support representative.

Set up Direct Infusion

Equipment

- Syringe pump (3162-0178)
- Syringe (500 µL minimum volume) (5190-1522)
- Tubing, red PEEK, 1.6 mm OD, 0.13 mm ID (0890-1915)
- Syringe adapter (9301-1291)
- Two finger-tight fittings (0100-1516)
- Compatible zero dead volume LC union (0100-2441)

1 Fill a clean syringe with tuning standard and install in the syringe pump.

2 Assemble the infusion line as shown in **Figure 13**.

3 Begin infusion at rate of 10 µL/min. Adjust the infusion rate to achieve a stable signal while avoiding saturation effects.

NOTE

For more information on optimizing source and acquisition conditions to set up the instrument for small and large molecule applications, review the following webinar links:

Part 1: Eliminate the Fear of LC/MS: Basics of LC/QTOF Operation

Part 2: Eliminate the Fear of LC/MS: Basics of LC/QTOF Operation

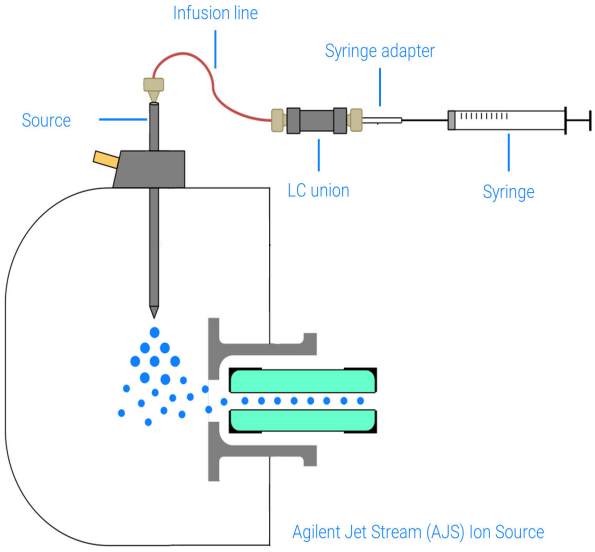


Figure 13. Diagram of direct infusion setup.

Gas Delivery

On ExD-enabled MS systems, the collision cell gas supply line feeds into the ExD cell gas inlet. Gas flows from the ExD cell to the collision cell.

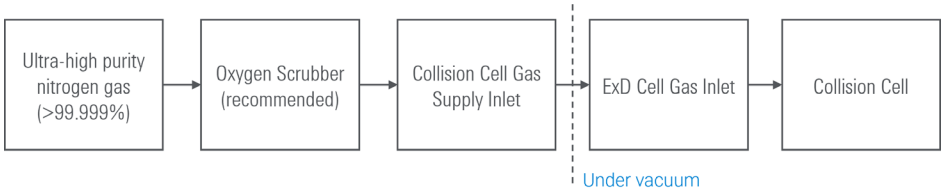


Figure 14. Schematic of gas delivery to the ExD cell and collision cell.

Oxygen Scrubber Set Up

- 1/8” copper tubing (8710-1709)
- 1/8” Swagelock, brass (x2) (8710-1709)
- Wrench
- Trap options:
  - Big universal trap, Nitrogen, 1/8 in, 300 psig (RMSN-2)
  - Optionally add Glass indicating Oxygen trap, stainless steel, 1/8 in, 160 psig (5182-9202), in line after big universal trap.

Be sure to flush lines with nitrogen before connecting tubing to minimize the amount of atmospheric oxygen absorbed by the trap.

NOTE

See Big Universal Trap Installation Sheet for more information: https://www.agilent.com/Library/usermanuals/Public/5184-3513_029108.pdf

Default Tune Parameters

Table 5. Default MassHunter parameters

Parameter	Positive Polarity Value	Negative Polarity Value
Oct1DC	36 V	-36 V
Lens1	34.5 V	-34.5 V
QuadDC	33 V	-33 V
PostFilter DC	32.5 V	-32.5 V
Cell Entrance	32 V	-32 V
HexDC	32 V	-32 V
HexDelta	-5 V	5 V
Hex2DC	14.2 V	-14.2 V
Hex2DV	-1 V	1 V
Collision cell gas pressure	24 psi	24 psi

NOTE

All other parameters can be left as they were. These parameters are fine-tuned with the system tune.

Table 6. ExD cell Lens Profiles in ExDControl

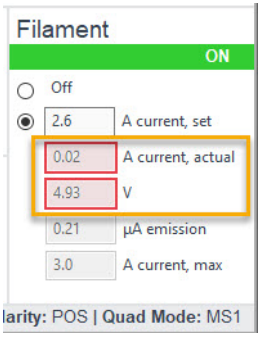
Profile Type	Filament State	L1	L2	LM3	L4	FB	LM5	L6	L7
Trans. Positive	ON	10.0	26.0	26.0	25.0	16.6	20.0	12.0	15.5
ECD	ON	10.0	26.0	26.0	25.0	16.6	20.0	18.0	15.5
Trans. Positive	OFF	30.0	30.0	28.0	24.0	22.0	18.0	14.0	15.5
Trans. Negative	OFF	-26.5	-26.0	-21.0	-16.6	-15.0	-15.0	-12.0	-13.0

Troubleshooting

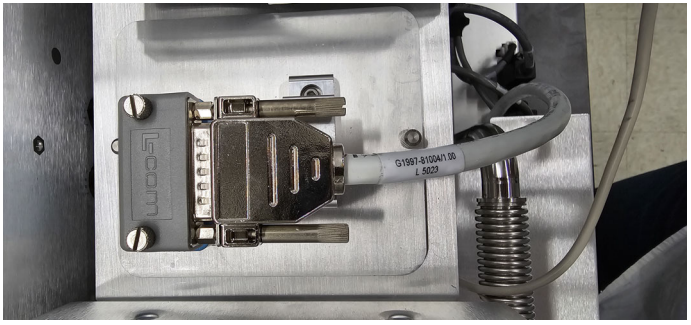
Diagnosing Filament Failure

1 Check for the following symptoms of filament failure:

- No ECD.
- Low sensitivity.
- When the filament is on and the current setpoint is > 0 A, the filament current actual approaches 0 A while the filament voltage drops to 5 V maximum.



2 Verify that the D-sub cable is connected to the ExD Controller and the electrical feedthrough in the modified manifold cover.



NOTE

D-sub cable damage or disconnection mimics symptoms of filament failure.

CAUTION

If the filament has failed after being exposed to the atmosphere while on, contact an Agilent service representative. Rapid filament failure due to oxidation can contaminate nearby parts with conductive debris, causing shorting.

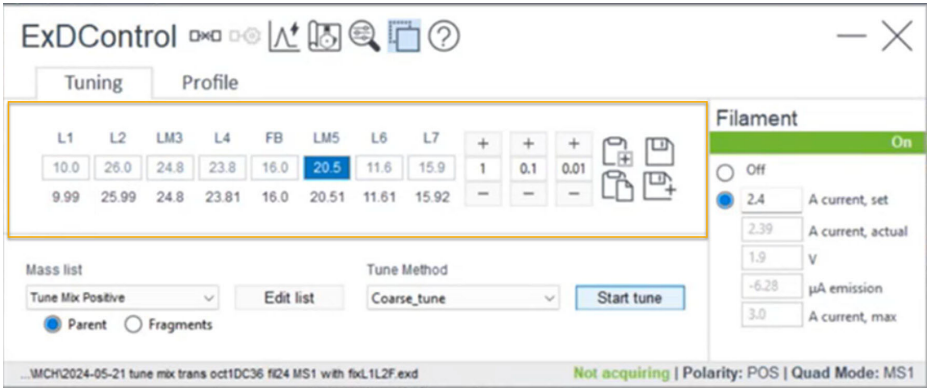
NOTE

When the filament is On, low-abundance products of ECCR and ECD can be present in the spectrum. To adjust the intensity of electron activated products, see **"Tuning the ExD cell for specific experiment types"** on page 46.

Manual Tune

Manual tune when using custom analytes or to troubleshoot autotune results. Manually adjust the ExD cell lens profile in the ExDControl Tuning tab.

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



CAUTION

When the filament is on, L1 should always be set to 10 V. When the filament is OFF, L1 may be increased to as high as 30 V. Any two profiles selected for use in the ExDControl Profiles tab must have the same L1 voltage setting.

To manually tune the lens profile for transmission or ECD on an infused tuning standard or analyte:

1 Check that the source parameters are optimized for the infused tuning standard or analyte and signal is stable.

2 Press **Shift** and click to select L2, LM3, L4, FB, LM5, L6, and L7 together. Adjust these lenses in unison to maximize signal intensity.

   Oct1DC voltage defines ion energy. With the filament OFF, the ExD cell lens profile does not exceed Oct1DC. With the filament on, the lens profile does not exceed Oct1DC + ~10 V. Set voltages higher with the filament on to compensate for negative electron charge.

3 Adjust FB and L4. For transmission profiles, L4 is usually greater than FB by 0.5-5 V. For ECD profiles, L4 is usually greater than FB by 5-10 V.

4 Adjust LM3 and LM5 separately and then in unison. LM3 and LM5 are usually greater than FB.

5 Adjust L2 and L6 separately. For profiles tuned in MS2, lowering L2 tends to improve transmission of the isolated precursor.

6 Adjust L1 and L7 separately in large steps.

7 If tuning for ECD, do the following to maximize ECD fragment ion intensity:

   a Adjust LM3, L4, FB, and LM5 in unison in small steps.

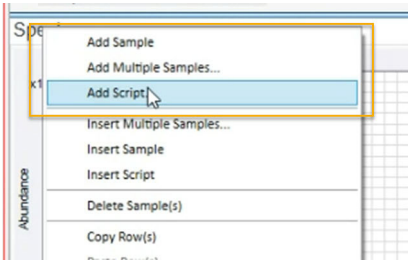
   b Optimize the difference between L4 and FB. Increasing the Δ V roughly corresponds to increasing electron energy.

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

Using ExDControl scripts

Include ExDControl scripts in Worklists to change lens profiles or turn the filament on or off.

1 In MassHunter Acquisition software, right click on the left column of the worklist and select **Insert script** or Add script.



The Select Script window appears.

2 Click the File drop-down and select an option:
   - For ExDControl scripts: **Exd_Acq_Scripts.exe**
   - For MassHunter Acquisition scripts: **MH_Acq_Scripts.exe**

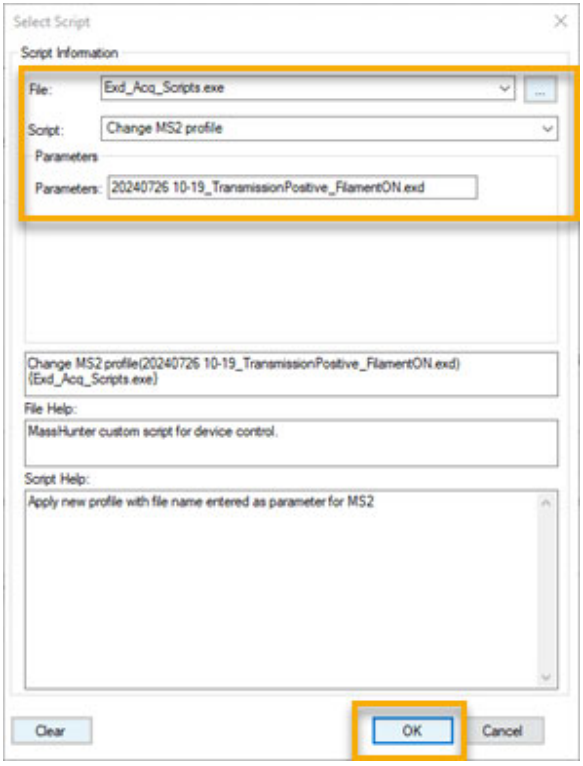
3 Click the **Script** drop down and select an option:
   - **Change MS1 Profile**
   - **Change MS2 Profile**
   - **Filament ON**
   - **Filament OFF**

4 With the options **Change MS1 Profile** or **Change MS2 Profile**, enter the name of a profile .exd file in the Parameters field. For other ExDControl scripts, the Parameters field, leave the field blank.

NOTE

It is not necessary to enter a path to the file if it is in the default Profiles directory (C:\Users\<username>\E-MSION-36\ExDControl\profiles).

5 Click **OK** to enter the script into the worklist.



Resolve Tuning Mix Peak Imbalance

MassHunter Acquisition software tune and calibration procedures cannot be completed if the melittin $[MH]^{3+}$ m/z 949 precursor intensity is greater than tuning mix m/z 922 intensity.

To resolve the peak imbalance, try the following:

1 Verify that the filament is turned on.

NOTE

If working with filament off, use tuning mix ESI-X 10-Fold dilution when tuning in MassHunter Acquisition software.

2 Tune the ExD cell lens profile. See **"Daily Operation"** on page 29 for more information.

3 Raise the filament current in the ExDControl Filament Panel by 0.05 A and tune the ExD cell lens profile.

4 Prepare a fresh batch of tuning mix with melittin. See **"Preparing Tuning Mix with Melittin, ESI-X, 2-Fold Dilution"** on page 58 for guidance.

As a workaround, use Tuning Mix, ESI-X, 10-Fold Dilution for MassHunter Acquisition software tuning and calibration.

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