



EASY-ETD, PTCR, and EASY-IC

Ion Sources User Guide

For the Orbitrap Tribrid Series Mass Spectrometer

80000-98088 Revision A • August 2019



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Release history: Rev A, August 2019

Software version: (Thermo) Foundation 3.1 SP7 and later, Xcalibur 4.3 and later, Tune 3.3 and later

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- [Orbitrap Fusion Lumos and Orbitrap Eclipse](#)
- [Orbitrap Fusion and Orbitrap ID-X](#)

Orbitrap Fusion Lumos and Orbitrap Eclipse

Low Voltage Directive 2014/35/EU

This device complies with Low Voltage Directive 2014/35/EU and the harmonized safety standards: EN 61010-1:2010, UL 61010-1: 2012, CAN/CSA No. 22.2 61010-1:2012; EN 61010-2-081:2015, UL 61010-2-081:2015, CAN/CSA No. 22.2 No. 61010-2-081: 2015.

EMC Directive 2014/30/EU and other EMC test standards

This device was tested by TÜV Rheinland of North America and complies with the following EMC standards:

47 CFR 15, Subpart B, Class A: 2017	EN 61000-3-2: 2006 + A1 + A2: 2009	EN 61000-4-5: 2006
CISPR 11: 2009 + A1: 2010	EN 61000-3-3: 2008	EN 61000-4-6: 2009
ICES-003: 2014	EN 61000-4-2: 2009	EN 61000-4-8: 2010
EN 55011: 2009 + A1: 2010	EN 61000-4-3: 2006 + A1 + A2: 2010	EN 61000-4-11: 2004
EN 61326-1: 2013	EN 61000-4-4: 2004 + A1: 2010	

Orbitrap Fusion and Orbitrap ID-X

Low Voltage Directive 2014/35/EU

This device complies with Low Voltage Directive 2014/35/EU and the harmonized safety standards: EN 61010-1:2010, UL 61010-1: 2012, CAN/CSA No. 22.2 61010-1:2012; EN 61010-2-081:2015, UL 61010-2-081:2015, CAN/CSA No. 22.2 No. 61010-2-081: 2015.

EMC Directive 2014/30/EU and other EMC test standards

This device was tested by TÜV Rheinland of North America and complies with the following EMC standards:

47 CFR 15, Subpart B, Class A: 2012	EN 61326-1: 2013	EN 61000-4-4: 2004 + A1
CISPR 11: 2009 + A1	EN 61000-3-2: 2006 + A1 + A2	EN 61000-4-5: 2006
AS/NZS CISPR 22: 2009 + A1	EN 61000-3-3: 2008	EN 61000-4-6: 2009
ICES-003: 2012	EN 61000-4-2: 2009	EN 61000-4-8: 2010
EN 55011: 2009 + A1	EN 61000-4-3: 2006 + A1 + A2	EN 61000-4-11: 2004

FCC Compliance Statement

THIS DEVICE COMPLIES WITH PART 15 OF THE FCC RULES. OPERATION IS SUBJECT TO THE FOLLOWING TWO CONDITIONS: (1) THIS DEVICE MAY NOT CAUSE HARMFUL INTERFERENCE, AND (2) THIS DEVICE MUST ACCEPT ANY INTERFERENCE RECEIVED, INCLUDING INTERFERENCE THAT MAY CAUSE UNDESIRE OPERATION.



CAUTION Read and understand the various precautionary notes, signs, and symbols contained inside this manual pertaining to the safe use and operation of this product before using the device.

Notice on the Proper Use of Thermo Scientific Instruments

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WEEE Directive 2012/19/EU



Thermo Fisher Scientific is registered with B2B Compliance ([B2Bcompliance.org.uk](https://www.b2bcompliance.org.uk)) in the UK and with the European Recycling Platform ([ERP-recycling.org](https://www.eur-recycling.org)) in all other countries of the European Union and in Norway.

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- Appropriate pick-up time
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For additional information about the Restriction on Hazardous Substances (RoHS) Directive for the European Union, search for RoHS on the Thermo Fisher Scientific European language websites.

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Preface

The *EASY-ETD, PTCR, and EASY-IC Ion Sources User Guide* is intended for the following Thermo Scientific Orbitrap Tribrid™ Series mass spectrometers (MSs):

- Orbitrap Eclipse™
- Orbitrap Fusion Lumos™
- Orbitrap Fusion™
- Orbitrap ID-X™

This guide describes the primary components for the optional Thermo Scientific EASY-ETD™, PTCR™, or EASY-IC™ ion source system installed in these MSs. It also provides instructions to replace the reagent ion source. For a list available ion sources per MS, see [Model Differences](#).

Contents

- [Accessing Documentation](#)
- [Providing Documentation Feedback](#)
- [Special Notices, Symbols, and Cautions](#)
- [Safety Precautions](#)
- [Model Differences](#)
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Accessing Documentation

The Orbitrap Tribrid Series MS includes complete documentation.

- [Viewing the Product Manuals](#)
- [Accessing the Help Menu Options](#)
- [Viewing Online User Documentation](#)

For system requirements, refer to the release notes on the software DVD.

Viewing the Product Manuals

The Thermo Fisher Scientific service engineer installs the instrument control applications and the instrument manuals on the data system computer.

❖ To view the product manuals


From the Microsoft™ Windows™ taskbar, choose **Start > All Apps** (Windows 10) or **All Programs** (Windows 7) > **Thermo Instruments > model x.x**, and then open the applicable PDF file.

Accessing the Help Menu Options

Follow this procedure to view the Help systems for the instrument-control applications.

❖ To view the Help

Do the following as applicable:

- Thermo Tune instrument-control application: Click the **Options** icon, , and choose **Tune Help**.
- Thermo Xcalibur™ Method Editor application: Choose an option from the **Help** menu (or press the F1 key).

Viewing Online User Documentation

Visit the Thermo Fisher Scientific website for product manuals and more.

❖ To view user documentation from the Thermo Fisher Scientific website

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3. In the Refine Your Search box, search by the product name.
4. From the results list, click the title to open the document in your web browser, save it, or print it.

To return to the document list, click the browser **Back** button.

Providing Documentation Feedback

❖ To suggest changes to the documentation or to the Help

Complete a brief survey about this document by clicking the button below.
Thank you in advance for your help.



Special Notices, Symbols, and Cautions

Make sure you understand the special notices, symbols, and caution labels in this guide. Most of the special notices and cautions appear in boxes; those pertaining to safety also have corresponding symbols. Some symbols are also marked on the instrument itself and can appear in color or in black and white. For complete definitions, see [Table 1](#).

Table 1. Notices, symbols, labels, and their meanings (Sheet 1 of 2)








Notice, symbol, or label	Meaning
IMPORTANT	Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the product.
Note	Highlights information of general interest.
Tip	Highlights helpful information that can make a task easier.
	Caution: Read the cautionary information associated with this task.
	Chemical hazard: Observe safe laboratory practices and procedures when handling chemicals. Only work with volatile chemicals under a fume or exhaust hood. Wear gloves and other protective equipment, as appropriate, when handling toxic, carcinogenic, mutagenic, corrosive, or irritant chemicals. Use approved containers and proper procedures to dispose of waste oil and when handling wetted parts of the instrument.
	Heavy object: The Orbitrap Tribrid Series MS, excluding its workbench, weighs over 227 kg (500 lb). Never try to detach and move the instrument from its workbench; you can suffer personal injury or damage the instrument.

Table 1. Notices, symbols, labels, and their meanings (Sheet 2 of 2)

Notice, symbol, or label	Meaning
	Hot surface: Allow any heated components to cool before touching them.
	Risk of electric shock: This instrument uses voltages that can cause electric shock and personal injury. Before servicing the instrument, shut it down and disconnect it from line power. While operating the instrument, keep covers on.
	Risk of eye injury: Eye injury can occur from splattered chemicals, airborne particles, or sharp objects. Wear safety glasses when handling chemicals or servicing the instrument.
	Sharp object: Avoid handling the tip of the syringe needle.

Safety Precautions

Read and understand the following cautions that are specific to the shutdown of the MS system or to the removal of parts for cleaning.



CAUTION If you must turn off the MS in an emergency, turn off the main power switch located on the right-side power panel. This switch turns off all power to the MS, including the forepump, without harming components within the system. However, do not use this method as part of the standard shutdown procedure. Instead, see [Shutting Down the Mass Spectrometer Completely](#). To turn off the LC, autosampler, and data system computer in an emergency, use their respective on/off switch or button.



CAUTION To avoid an electrical shock, be sure to follow the instructions in [Shutting Down the Mass Spectrometer Completely](#).



CAUTION Do not turn the instrument on if you suspect that it has incurred any kind of electrical damage. Instead, disconnect the power cord and contact Thermo Fisher Scientific technical support for a product evaluation. Do not attempt to use the instrument until it has been evaluated. (Electrical damage might have occurred if the system shows visible signs of damage, or has been transported under severe stress.)



CAUTION Do not disconnect the power cord at the MS while the other end is still plugged into the electrical outlet.



CAUTION Do not place any objects (for example, the syringe pump or other containers with liquids) on top of the instrument, unless instructed to in the documentation. Leaking liquids might contact the electronic components and cause an electrical short circuit.



CAUTION Hot surface. Allow heated components to cool to room temperature (approximately 20 minutes) before servicing them.




Model Differences

This table lists the required number of forepumps and the available options for the Orbitrap Tribrid Series MSs.

Instrument	Number of forepumps	Available options						
		EASY-IC ion source	HMR ⁿ	EASY-ETD with PTCR ion source	EASY-ETD ion source	Advanced peak determination	1M Orbitrap resolution	UVPD laser module
Orbitrap Eclipse	1 or 2 ^a	✓	✓	✓	✓	✓	✓	✓
Orbitrap Fusion Lumos	1 or 2 ^a	✓	–	–	✓	✓	✓	✓
Orbitrap Fusion	1	✓	–	–	✓	✓	–	–
Orbitrap ID-X	1	✓	–	–	–	–	–	–

^a Forepump model type determines the number of required forepumps. Refer to the *Orbitrap Tribrid Series Getting Connected Guide* for more information.

Contacting Us

Contact	Email	Telephone	QR Code
U.S. Technical Support	us.techsupport.analyze@thermofisher.com	(U.S.) 1 (800) 532-4752	
U.S. Customer Service and Sales	us.customer-support.analyze@thermofisher.com	(U.S.) 1 (800) 532-4752	
Global Support	<ul style="list-style-type: none"> ❖ To find global contact information or customize your request <ol style="list-style-type: none"> 1. Go to thermofisher.com. 2. Click Contact Us, select the country, and then select the type of support you need. 3. At the prompt, type the product name. 4. Use the phone number or complete the online form. ❖ To find product support, knowledge bases, and resources <p>Go to thermofisher.com/us/en/home/technical-resources.</p> ❖ To find product information <p>Go to thermofisher.com/us/en/home/brands/thermo-scientific.</p> 		
<p>Note To provide feedback for this document, go to surveymonkey.com/s/PQM6P62 or send an email message to Technical Publications (techpubs-lcms@thermofisher.com).</p>			

Introduction

The Orbitrap Tribrid Series MS is a member of the Thermo Scientific family of MSs. The optional EASY-ETD, EASY-IC, and PTCR ion source assemblies can come factory-installed with the purchase of specific Tribrid MSs (see [page xv](#)), or a Thermo Fisher Scientific service engineer can install either ion source as an upgrade at a later date.

The information in this guide applies toward both the Internal Calibration (IC) configuration (EASY-IC ion source assembly) and the [electron transfer dissociation \(ETD\)](#) configuration (EASY-ETD or EASY-ETD/PTCR ion source assemblies), unless otherwise noted.

Note

- “ETD source” refers to both the EASY-ETD and EASY-IC ion source assemblies.
- There are three versions of the EASY-ETD and EASY-IC ion sources. This guide specifies differences among the ETD sources where applicable.

For procedures regarding daily operation, maintenance, and system startup and shutdown, refer to the Hardware Manual.

Contents

- [Internal Calibration](#)
- [Electron Transfer Dissociation and Proton Transfer Charge Reduction](#)
- [Reagent Ion Source](#)
- [Calibration Categories and Solutions](#)
- [ETD and PTCR Scan Parameters](#)
- [ETD/PTCR Readback Measurements](#)

Internal Calibration

An Orbitrap Tribrid Series MS with the Internal Calibration configuration (MS/IC system) incorporates a second ion source—the reagent ion source (RIS). The RIS is installed in the ETD/IC source heater interface, which is located in the API source interface just after the RF lens. For additional information about the ETD/IC source heater interface, see [Chapter 2, “Functional Description.”](#)

The RIS for the IC configuration delivers a regulated number of calibrant ions into the much larger population of analyte ions. The location of the RIS allows the quadrupole mass filter to isolate the internal calibrant (reference) ions. The internal calibrant ions are used as a lock mass (m/z) that significantly improves the [mass-to-charge ratio \(\$m/z\$ \)](#) assignment accuracy to less than 1 ppm (up to m/z 1500) in every [Fourier transform \(FT\) mass spectrum](#).

The instrument software uses the precisely known mass-to-charge ratio of the calibration mass peak to provide real-time fine adjustment of the instrument's mass-to-charge ratio calibration, enabling a correction for otherwise uncompensated errors that are due to temperature changes and scan-to-scan variations in the total charge of the population of ions analyzed.

Electron Transfer Dissociation and Proton Transfer Charge Reduction

Note Proton Transfer Charge Reduction (PTCR) is referred to as “PTR” in the GUIs of the Orbitrap Tune and Method Editor software applications.

Similar to the MS/IC system, an Orbitrap Tribrid Series MS with the ETD configuration (MS/ETD system) incorporates the reagent ion source as a second ion source. The RIS is installed in the ETD/IC source heater interface, which is located in the API source interface just after the RF lens. For additional information about the ETD/IC source heater interface, see [Chapter 2, “Functional Description.”](#) See also [Model Differences](#).

The RIS configuration generates the necessary reagent anions for both the ETD and Proton Transfer Charge Reaction (PTCR) to perform the respective ion-ion reaction. The location of the RIS allows the quadrupole mass filter to select the reagent anion for the appropriate reaction, fluoranthene for ETD, or perfluoroperhydrophenanthrene (PFPP) for PTCR. The MS/ETD/PTCR system also includes the additional electronics that supply RF voltage to the front and center transfer lenses (TL1 and TL2) in the linear ion trap (LIT) mass analyzer to allow [charge sign independent trapping](#) in the LIT.

For each ion-ion reaction, the MS mixes a regulated number of reagent anions to react with a population of precursor analyte species, which could be intact precursors for MS^2 experiments or fragment ions for MS^n experiments. These fragments are selected from a controlled variable width window.

For PTCR-configured systems, the PTCR reagent abstracts a proton from the precursor ion population, thus reducing the charge state and expanding the ion spectrum over a larger mass range, reducing peak overlap.

Because the ETD and ETD/PTCR configurations include the RIS, these configurations also include all of the features of the IC configuration.

Reagent Ion Source

The RIS has its own dedicated continuous introduction system that delivers a highly stable flow of fluoranthene—for the ETD or IC options—or perfluoroperhydrophenanthrene (PFPP)—for the PTCR option (Orbitrap Eclipse only). The reagent distribution assembly receives an initial charge of fluoranthene or PFPP upon installation. You can expect this charge to last at least one year under continuous usage.

Note Only a Thermo Fisher Scientific field service engineer can replace the reagent once it is consumed.

The RIS uses a Townsend discharge to produce the thermal electrons used to ionize reagent molecules (by electron capture) to generate the reagent anions (fluorathene and/or PFPP). The RIS does not incorporate a thermionic filament. The addition of the RIS does not compromise performance when compared to the standard Orbitrap Tribrid Series MS (without the RIS).

The RIS produces a very intense current of radical anions and a moderate current of fluoranthene radical cations. When you activate the IC option, it uses the radical anions (negative polarity) and cations (positive polarity) of fluoranthene as the internal calibration lock mass. When you activate the ETD option, it uses the fluoranthene radical anions for the ETD reagent ions. When you activate the PTCR option, it uses PFPP radical anions for the PTCR reagent ions.

Calibration Categories and Solutions

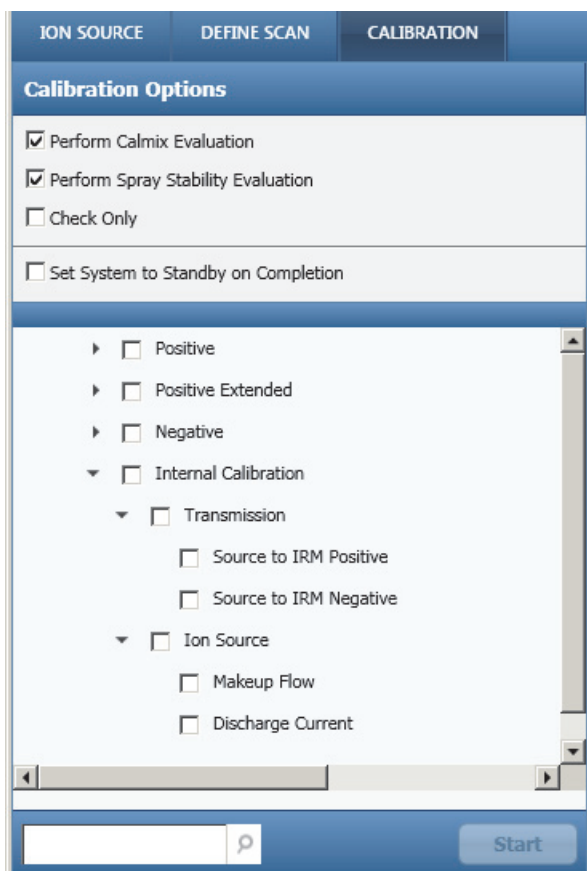
For instructions on how to calibrate the Orbitrap Tribrid Series MS, refer to the Getting Started Guide. After you complete the positive and negative ion polarity calibrations, run the calibration categories under Internal Calibration or ETD, as applicable ([Figure 1](#)). When you set up the MS/IC or MS/ETD system, see [Table 2](#) for the required solution for the IC and ETD categories.

1 Introduction

Calibration Categories and Solutions

Figure 1. Calibration pane in the Tune window showing the IC and ETD calibration categories

Internal Calibration categories



ETD calibration categories

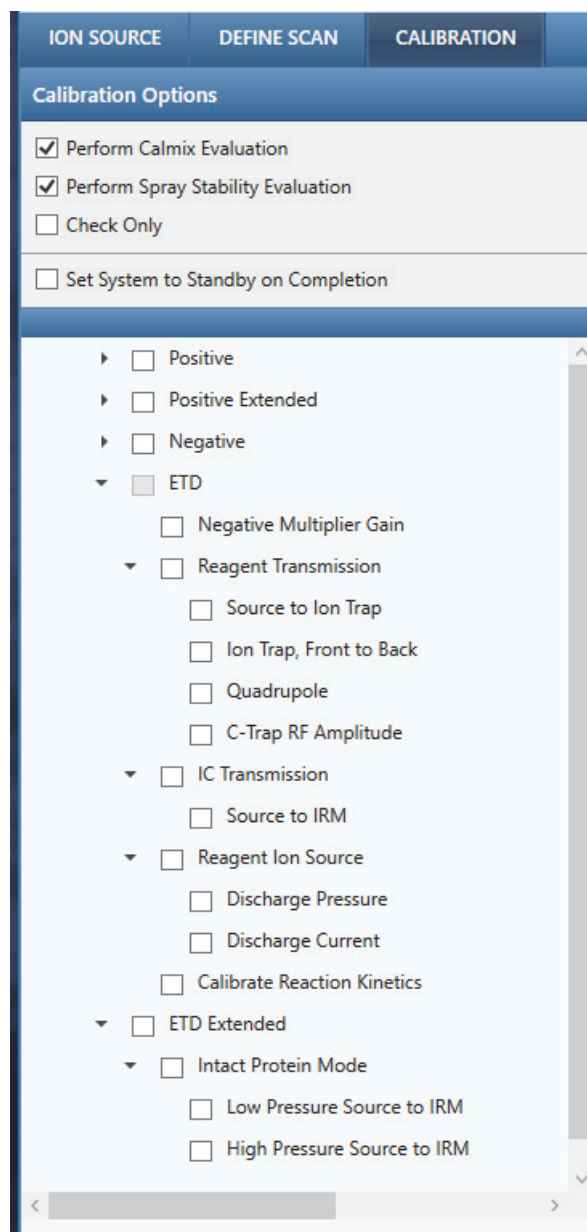


Table 2. Required solutions for the IC and ETD calibration categories (Sheet 1 of 2)

Calibration	Solution
Internal Calibration categories	
Transmission	None. Uses the reagent.
Ion Source	None. Uses the reagent.
ETD calibration categories	
Reagent Transmission	None. Uses the reagent.

Table 2. Required solutions for the IC and ETD calibration categories (Sheet 2 of 2)

Calibration	Solution
IC Transmission	None. Uses the reagent.
Reagent Ion Source	None. Uses the reagent.
Calibrate Reaction Kinetics	Traditional or Pierce™ FlexMix™ (P/N A39239) calibration solution
ETD Extended calibration category	
Intact Protein Mode	None. Uses the reagent.
PTZR Extended calibration category	
Calibration	None. Uses the reagent.

ETD and PTZR Scan Parameters

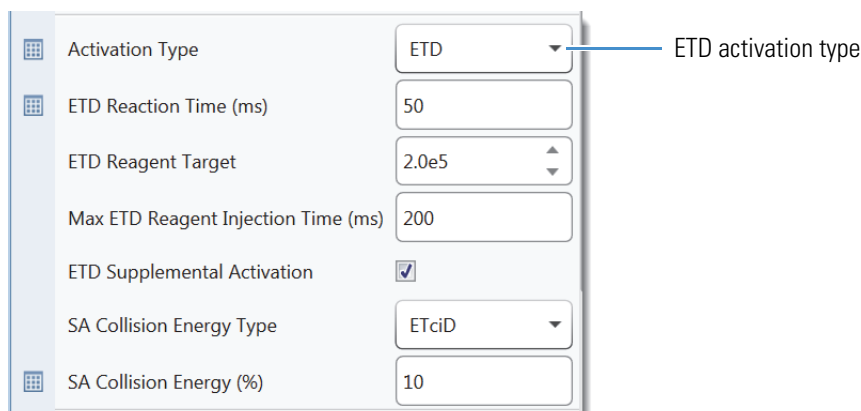
Before you acquire ETD and/or PTZR data with the Tune application, define the scan parameters in the Define Scan pane. The following topics provide ETD and PTZR details.

ETD

Figure 2 shows the default ETD scan parameters that appear after you select the MS² or MSⁿ scan type and the ETD activation type. These parameters are reasonable for a 3+ charge state. See also Table 3.

For instructions on how to acquire data with the Tune application, refer to the Getting Started Guide. For descriptions of the ETD scan parameters, refer to the Define Scan pane topic in the Tune Help.

Figure 2. Define Scan pane (partial) in the Tune window showing the ETD scan parameters



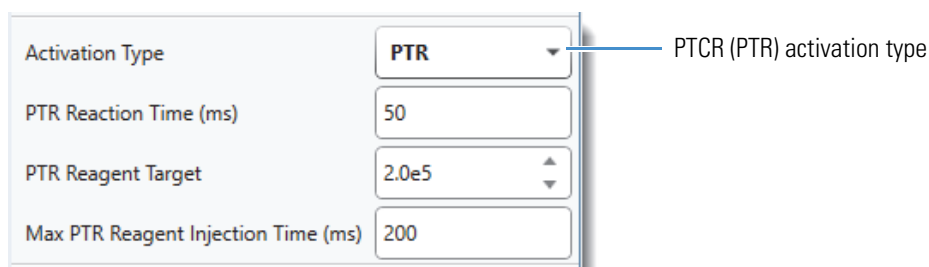
Note The SA Collision Energy Type, ETciD parameter, is off by default.

PTCR

Figure 3 shows the default PTCR scan parameters that appear after you select the MS² or MSⁿ scan type and the PTCR activation type. These parameters are reasonable for a 3+ charge state. See also Table 4.

For instructions on how to acquire data with the Tune application, refer to the Getting Started Guide. For descriptions of the PTCR scan parameters, refer to the Define Scan pane topic in the Tune Help.

Figure 3. Define Scan pane (partial) in the Tune window showing the PTCR scan parameters



ETD/PTCR Readback Measurements

In addition to monitoring the overall system readback status in the Thermo Scientific Tune application, you can monitor individual ETD and PTCR readback values on the By Board and By Function pages in the Status pane. Table 3 and Table 4 list the default values for the ETD and PTCR readback values respectively.

Table 3. Default values for the ETD readbacks

Readback	Default value
Discharge Voltage	approx. -700 V
Oven 1 Temp	75 °C
Split Temp	130 °C
Valve 1	15 SCCM
RIS Heater Current	0.11 A

Figure 4 and Figure 5 show the ETD and PTCR readback measurements, respectively. Normal readback measurements show a green square (■).

Figure 4. Status pane in the Tune window showing the ETD readbacks

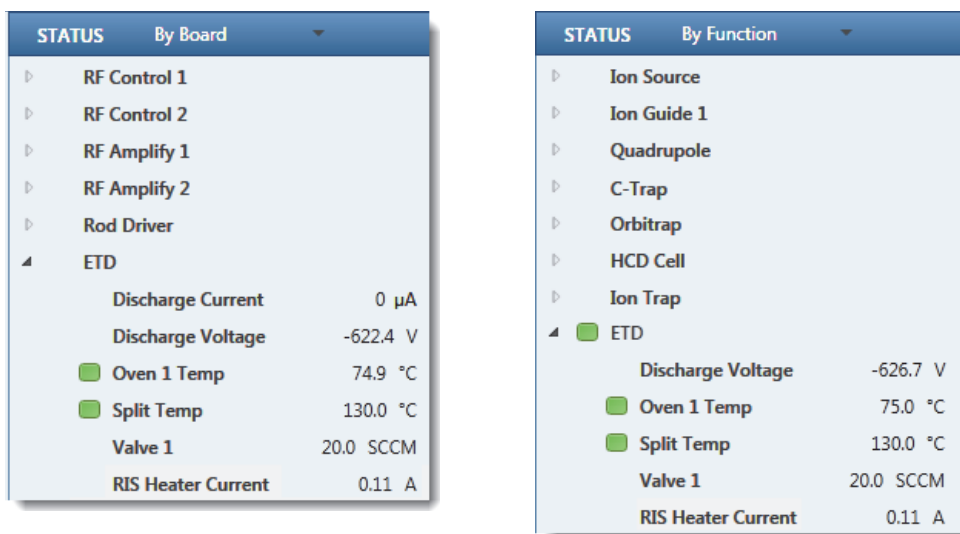
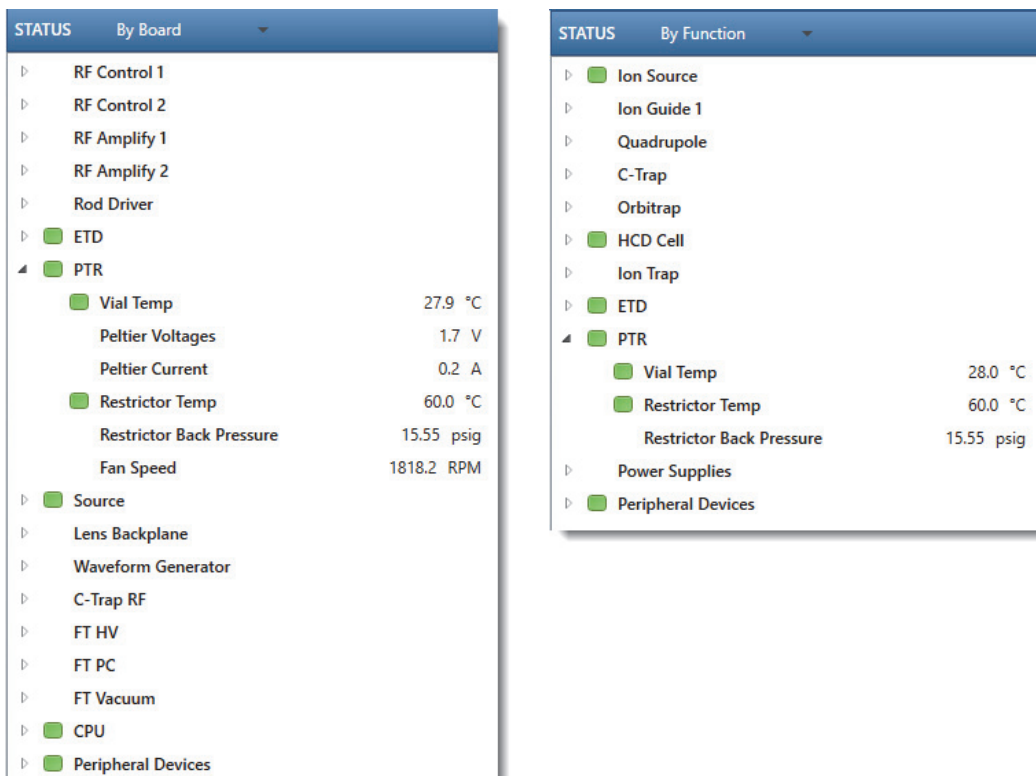


Table 4. Default values for the PTCR readbacks

Readback	Default value
Restrictor temperature	60 °C
Valve 2 (Restrictor Back Pressure)	10 psig
Vial temperature	28 °C

Figure 5. Status pane in the Tune window showing the PTCR readbacks



Functional Description

This chapter describes the principal components and functions of the optional IC, ETD, and PTCR configurations. There are two different designs for the IC and ETD ion source. For a list of which ion source is intended for which MS, see [Model Differences](#).

Contents

- [Electronic Assemblies](#)
- [Reagent Distribution Assembly](#)
- [The IC/ETD/PTCR Source Heater Interface](#)

Electronic Assemblies

Although the Orbitrap Tribrid Series MSs have many electronic assemblies that control their operation, setup for the IC or ETD configuration, as applicable, requires only two or three printed circuit boards (PCBs), respectively.

- ETD Source PCB—Controls the reagent distribution assembly ([Figure 6](#) or [Figure 10](#)) and the ETD/PTCR/IC source heater interface ([Figure 18](#)). It also provides an RF drive signal for the ETD RF PCB for both the Orbitrap Fusion Lumos and Orbitrap Eclipse MSs. The Tune application calibrates the discharge pressure and current. For the Orbitrap Eclipse, it also controls the PTCR reagent oven, restrictor heaters, and Electronic Pressure Regulator (EPR).
- ETD HV and Distribution PCB—Distributes the signals from the ETD Source PCB to various components, including the heaters, the mass flow controller (MFC) for the Orbitrap Fusion and Orbitrap ID-X MSs, or the electronic pressure regulator (EPR) for both the Orbitrap Fusion Lumos and Orbitrap Eclipse MSs. This PCB also powers the high voltage (HV) power supply unit, and contains a pulser circuit that regulates the output current for the HV power supply unit.
- ETD RF PCB—(For instruments with the ETD configuration only) Generates the RF voltage applied to the LIT front and center lenses (TL1 and TL2) during the charge sign independent trapping, which is necessary to enable the ETD reaction.

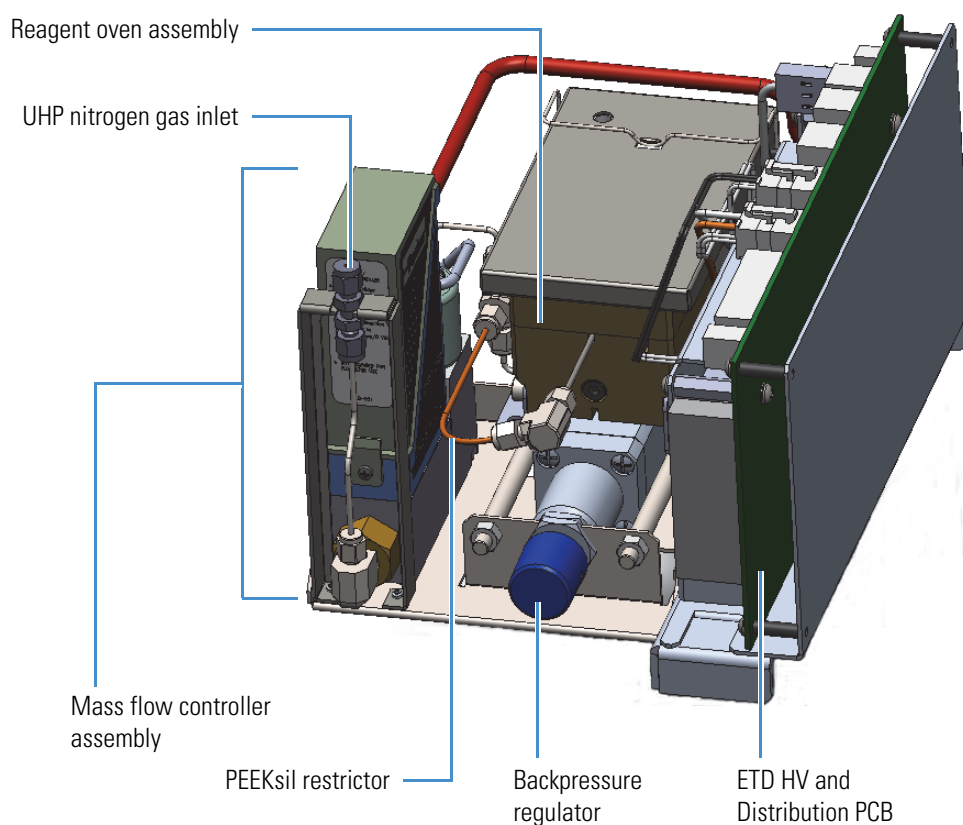
You cannot service the electronic assemblies. For assistance, contact your local Thermo Fisher Scientific field service engineer.

Reagent Distribution Assembly

The IC, ETD, and PTCR configurations include the reagent distribution assembly, of which there are multiple versions (see [Model Differences](#) for a summary). The HV power supply unit in these assemblies supply the discharge current to the RIS.

For the Orbitrap Fusion and Orbitrap ID-X MSs, the reagent distribution assembly ([Figure 6](#)) includes a [gas delivery system](#), three [heaters](#) (in the reagent oven assembly), and an HV power supply unit. [Figure 12](#) shows a block diagram of the gas delivery path to the RIS.

Figure 6. ETD/IC reagent distribution assembly (Orbitrap Fusion and Orbitrap ID-X MSs, back view)



For the Orbitrap Fusion Lumos MS, the reagent distribution assembly (Figure 7) includes two pressure regulators, three restrictors, four heaters (in the reagent oven assembly), and an HV power supply unit.

Figure 7. ETC/IC reagent distribution assembly (Orbitrap Fusion Lumos MS, side view)

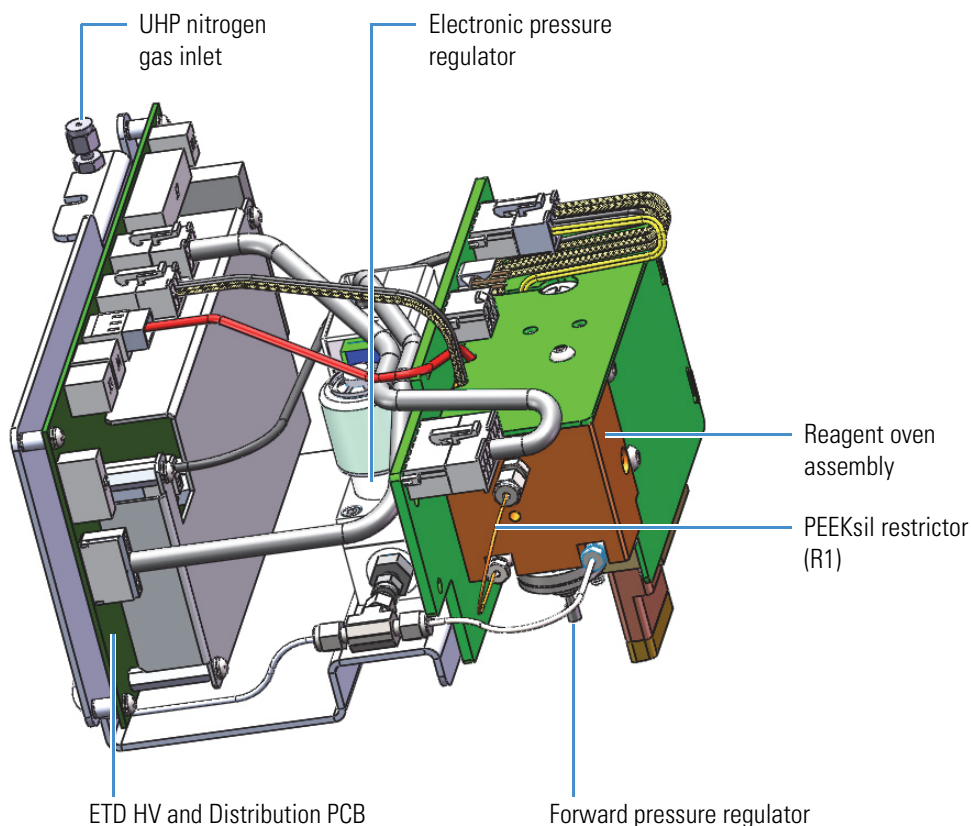
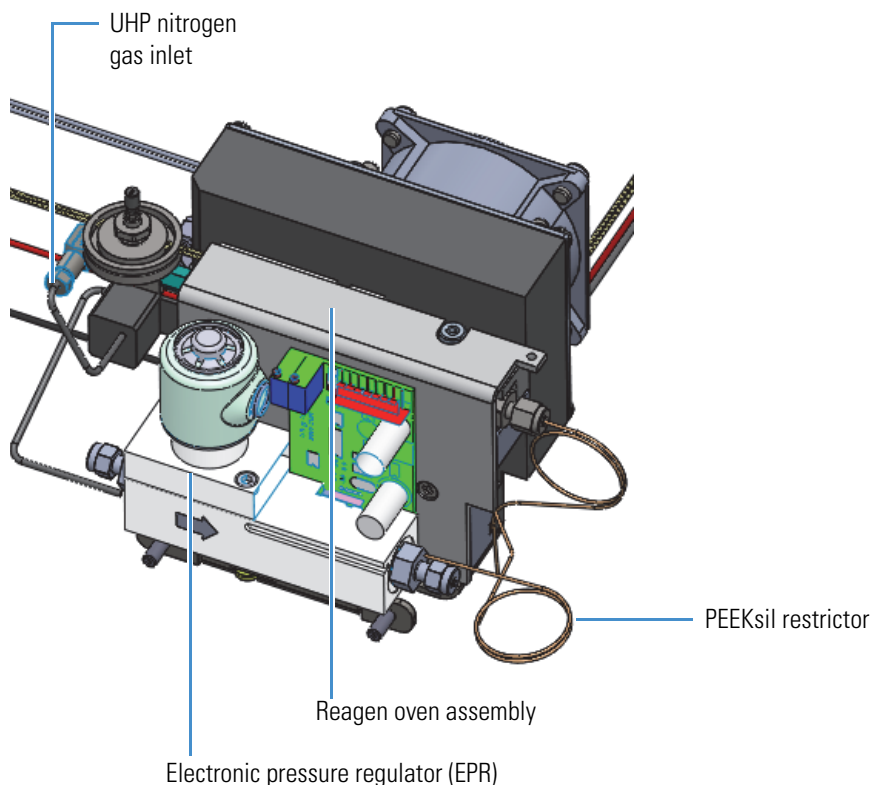


Figure 8 shows the PTCR module assembly. Figure 13 shows a block diagram of the gas delivery path to the RIS.

Figure 8. Reagent distribution assembly (PTCR, Orbitrap Eclipse MS, angled view)



The reagent distribution assembly connects through the vacuum manifold to the terminals of the [The IC/ETD/PTCR Source Heater Interface](#) to deliver the neutral reagent—fluoranthene (ETD) or PFPP (PTCR)—to the RIS for ionization and further IC, ETD, and/or PTCR use. [Figure 10](#) (Orbitrap Fusion and Orbitrap ID-X MSs) and [Figure 11](#) (Orbitrap Fusion Lumos MS) show an expanded view of the reagent's flow path from the reagent oven to the ETD/PTCR/IC source heater interface. It also shows the relative position of the ETD/PTCR/IC source heater interface in the API source interface. See [Figure 9](#) for a view of the ETD and PTCR module assembly for the Orbitrap Eclipse.

Figure 9. ETD and PTCR assembly (Orbitrap Eclipse MS only)

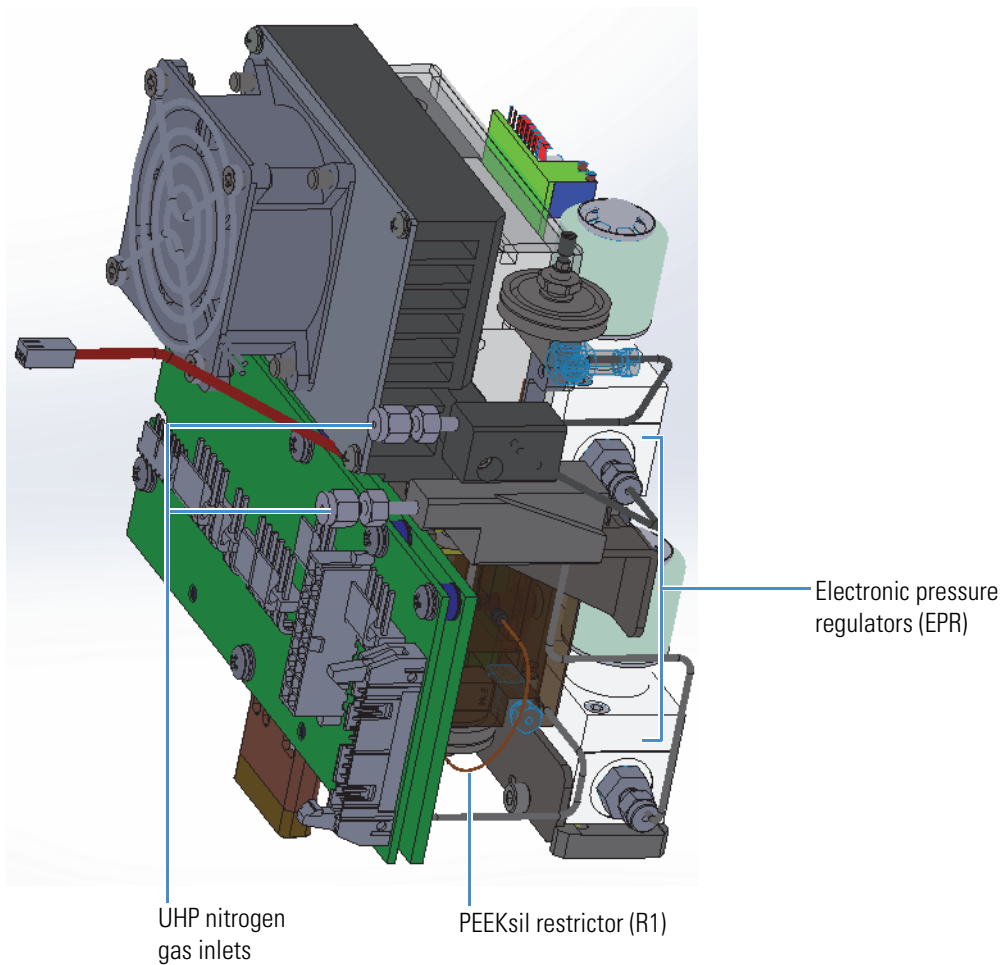


Figure 10. Reagent path from the reagent distribution assembly down into the RIS (expanded view, Orbitrap Fusion and Orbitrap ID-X MSs)

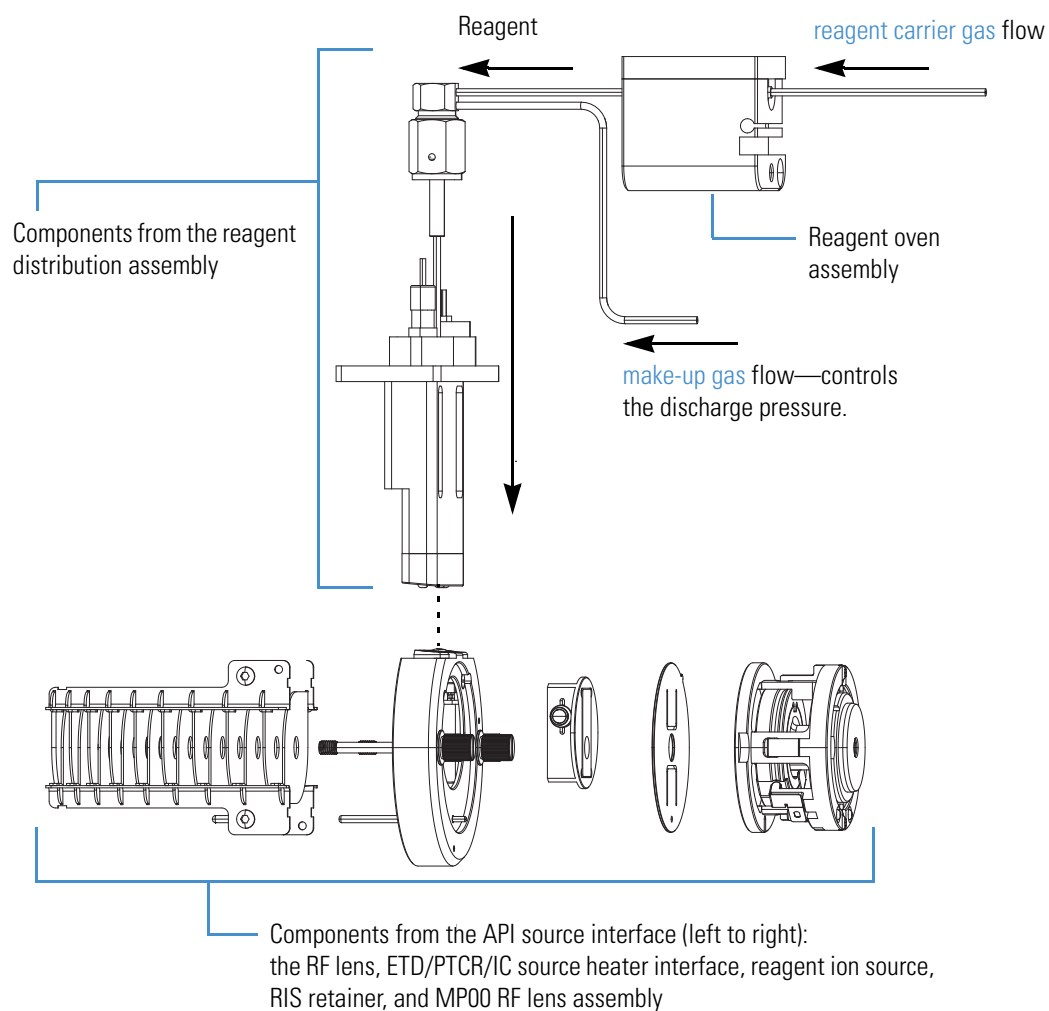
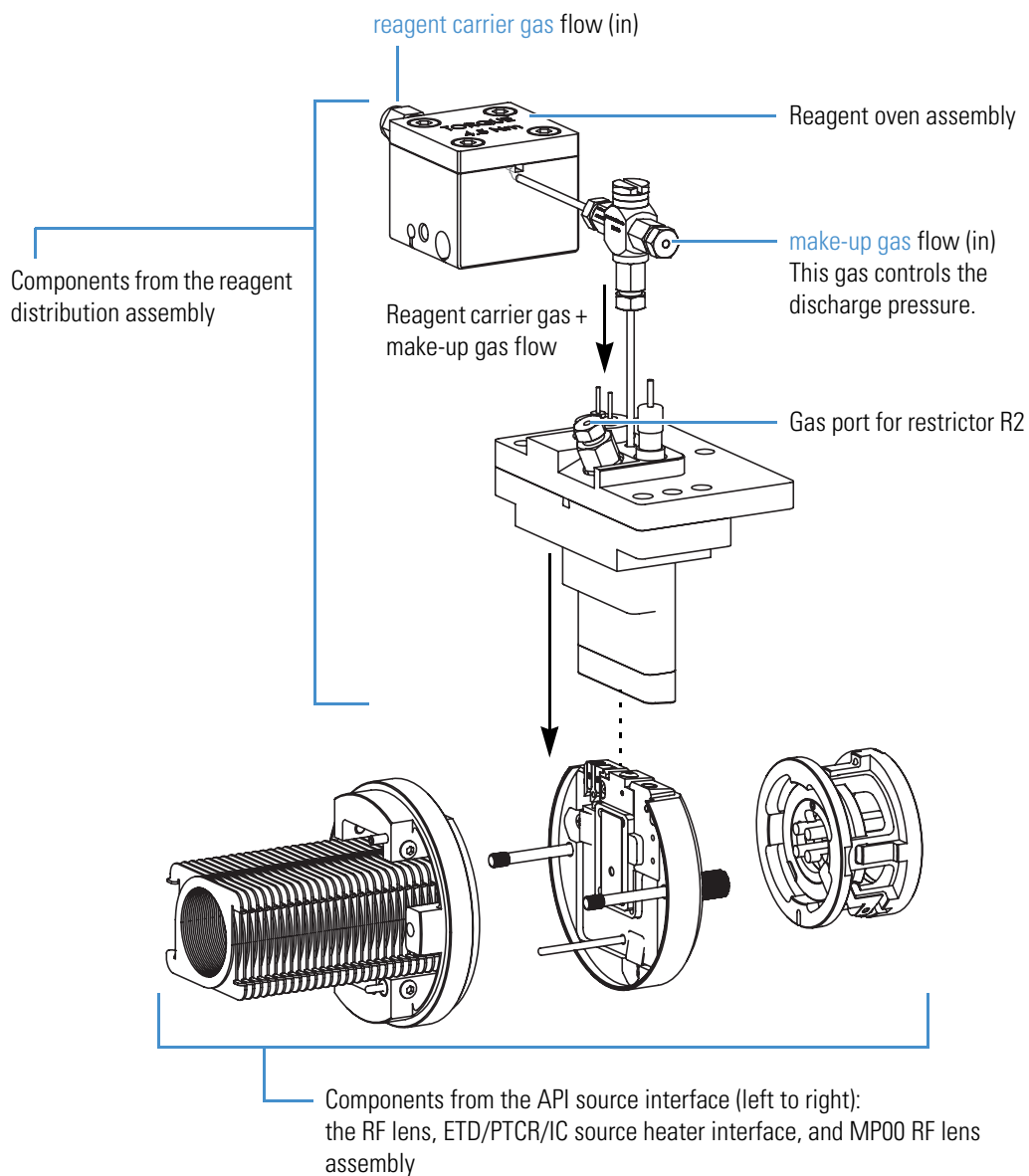


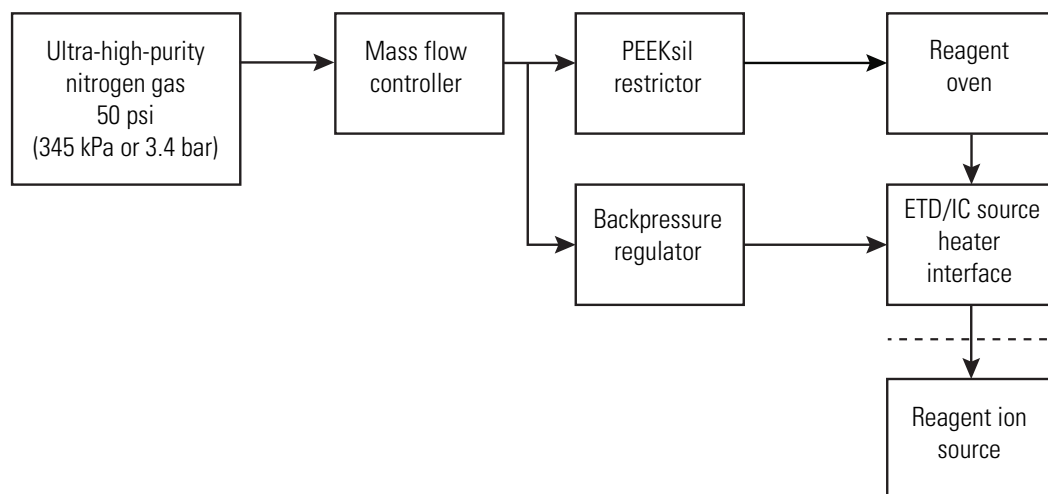
Figure 11. Reagent path from the ETD reagent distribution assembly down into the RIS (expanded view, Orbitrap Fusion Lumos and Orbitrap Eclipse MSs)



Reagent Gas Delivery System—Orbitrap Fusion and Orbitrap ID-X MSs

Figure 12 shows the delivery path for the nitrogen gas to the RIS in the Orbitrap Fusion and Orbitrap ID-X MSs.

Figure 12. Schematic of the ETD gas delivery to the RIS



The mass flow controller (MFC) regulates the total gas flow that is delivered to the RIS. You calibrate the MFC setpoint by using the Discharge Pressure calibration routine (Figure 1). The valid range is 0–40 sccm, with 15 sccm as the default.

The gas flow splits at the backpressure regulator to serve two functions:

- (Orbitrap Fusion MS only) Provides a constant vapor pressure of the ETD reagent species to the RIS.
- Provides the correct gas pressure to the discharge region to allow for a stable discharge.

The backpressure regulator applies a constant pressure to the PEEKsil™ restrictor (25 μm \times 10 cm red PEEK tubing) regardless of the MFC setting. This constant pressure establishes a constant flow of approximately 0.1 sccm of nitrogen gas into the reagent oven assembly. In the reagent oven, the nitrogen gas flow equilibrates with the vapor pressure of the ETD reagent molecule (fluoranthene), and is then subsequently delivered to the RIS as the reagent carrier gas flow. The Thermo Fisher Scientific factory sets the backpressure regulator setting. Only Thermo Fisher Scientific service engineers can adjust the backpressure regulator.

For information about the required gases, refer to the Preinstallation Requirements Guide and the Getting Connected Guide.

Reagent Gas Delivery System—Orbitrap Fusion Lumos and Orbitrap Eclipse MSs

Figure 13 shows the delivery path for the nitrogen gas to the RIS in the Orbitrap Fusion Lumos and Orbitrap Eclipse MSs.

Figure 13. Schematic of the gas delivery to the reagent ion source

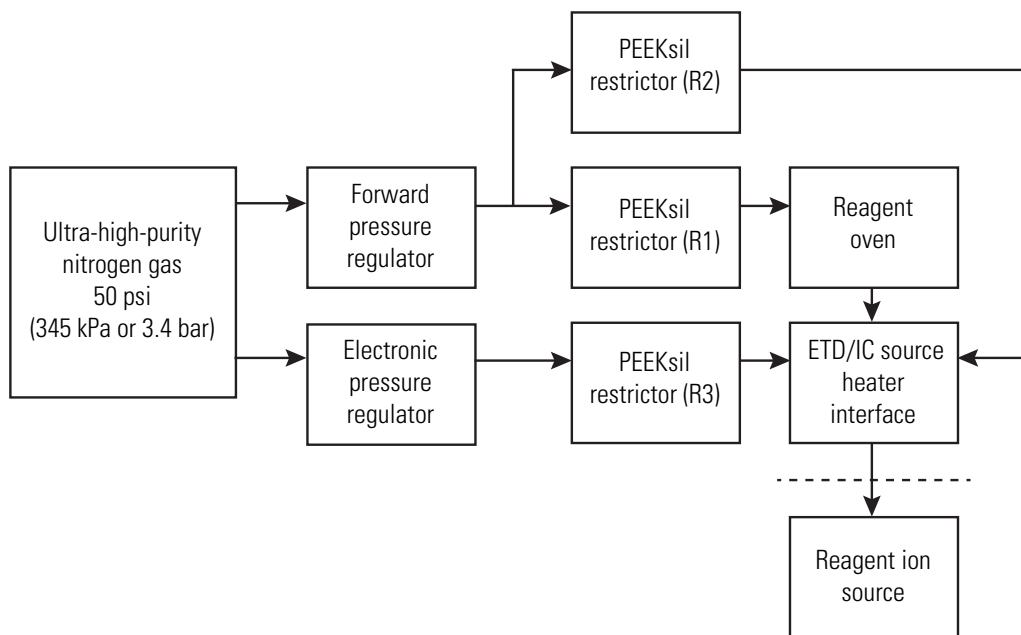
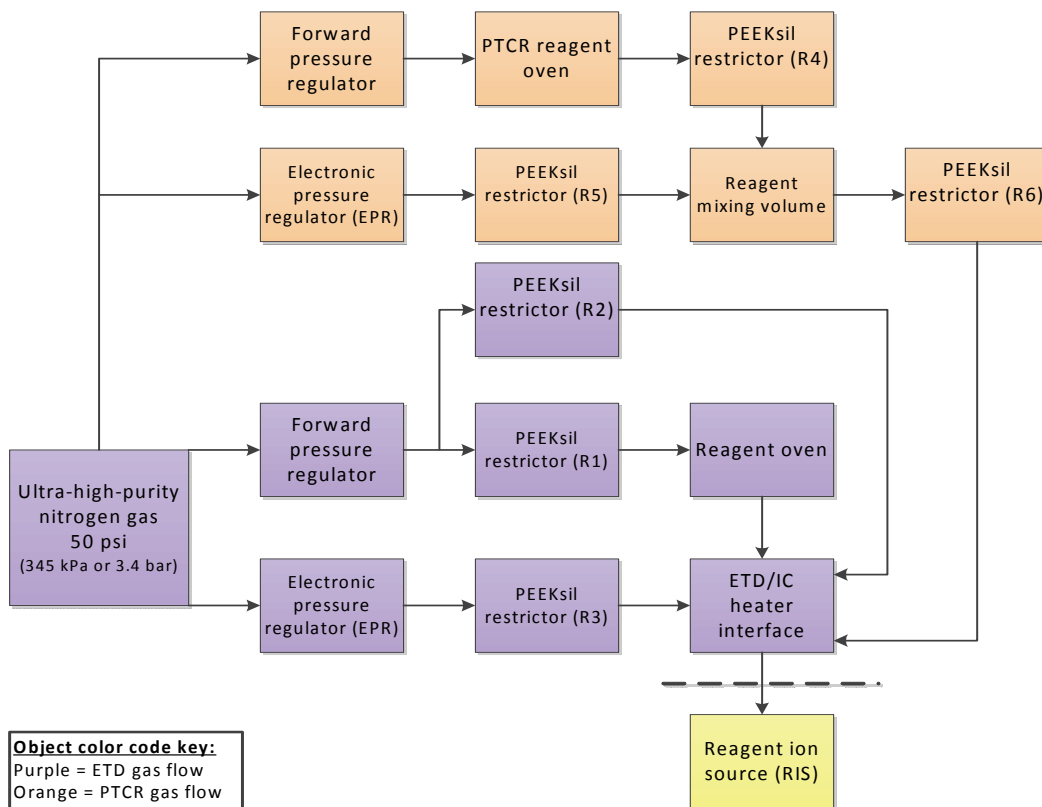


Figure 14 shows the delivery path for the nitrogen gas to the RIS in the and Orbitrap Eclipse MSs including the optional PTCR module.

Figure 14. Schematic of the ETD/PTCR gas delivery to the RIS (Orbitrap Eclipse only)



The use of regulator/restrictor assemblies achieves the following:

- Provides a constant vapor pressure of the ETD reagent species to the RIS.
- Provides the correct gas pressure to the discharge region to allow for a stable discharge.

The forward pressure regulator controls the gas flow through the reagent oven by independently applying a constant pressure into the PEEKsil restrictors (R1 and R2). This constant pressure establishes a constant flow of approximately 0.1 sccm of nitrogen gas into the reagent oven assembly where the nitrogen gas flow equilibrates with the vapor pressure of the ETD/IC reagent molecule (fluoranthene), and is then subsequently delivered to the RIS as the reagent carrier gas flow. The Thermo Fisher Scientific factory sets the forward pressure regulator setting. Only Thermo Fisher Scientific field service engineers can adjust this regulator.

The electronic pressure regulator (EPR) along with restrictor R3 regulates the total gas flow that is delivered to the RIS. You calibrate the EPR by using the Discharge Pressure calibration routine (Figure 1). The valid range is 0–30 psi, with 10 psi as the default.

Tip Thermo Fisher Scientific recommends keeping the ultra-high purity nitrogen on at all times to ensure peak performance of the ETD/PTCR assembly.

For information about the required gases, refer to the *Orbitrap Tribrid Series Preinstallation Requirements Guide* and the *Orbitrap Tribrid Series Getting Connected Guide*.

Ovens, Heaters, and Vial Assemblies

The IC configuration (for all Orbitrap Tribrid Series MSs) or the ETD configuration (for the Orbitrap Fusion, Orbitrap Fusion Lumos, and Orbitrap Eclipse MSs only) contains the heaters listed in Table 5. Together, they heat the reagent-flow regions and control the vapor pressure of the reagent delivered from the ETD/PTCR/IC source heater interface to the RIS.

Table 5. Ovens and heaters (Sheet 1 of 2)

MS	Component	Description	Readback ^a
Orbitrap Eclipse Orbitrap Fusion Orbitrap Fusion Lumos Orbitrap ID-X	Reagent oven assembly (5 W)	Controls the reagent's vapor pressure by maintaining a constant oven temperature. The amount of gas through the oven is determined by a combination of the PEEKsil restrictor and the backpressure regulator (Orbitrap Fusion and Orbitrap ID-X MSs) or the forward pressure regulator (Orbitrap Fusion Lumos MS). The oven temperature setting determines the amount of reagent vapor delivered to the RIS. Default temperature: 75 °C (167 °F)	Oven 1 Temp
Orbitrap Fusion Orbitrap ID-X	Split heater (80 W)	Heats the reagent gas line to prevent condensation of the reagent.	Split Temp
Orbitrap Fusion Lumos Orbitrap Eclipse	Split heaters (two 50 W in parallel; total 100 W)	Default temperature: 130 °C (266 °F)	
Orbitrap Fusion Orbitrap Fusion Lumos Orbitrap ID-X Orbitrap Eclipse	RIS heater (4 W)	Heats the ETD/PTCR/IC source heater interface to prevent internal condensation. Relies on the default RIS heater current (0.11 A), instead of a temperature sensor, to maintain the required temperature.	RIS Current

Table 5. Ovens and heaters (Sheet 2 of 2)

MS	Component	Description	Readback ^a
Orbitrap Eclipse	Vial Peltier	Controls the PTCR reagent's vapor pressure by maintaining a constant vial temperature. The amount of gas through the oven is determined by a combination of the PEEKsil restrictor, the forward pressure regulator and the EPR. The vial temperature setting determines the headspace reagent vapor pressure within the vial Default temperature: 28 °C (82.4 °F)	Vial Temp
	Restrictor heater (10W)	Heats the reagent restrictor to prevent condensation of the reagent. Default temperature: 60 °C (140 °F)	Restrictor Temp

^a Displayed in the Status pane in the Tune application

Figure 15 (Orbitrap Fusion and Orbitrap ID-X MSs) and Figure 16 (Orbitrap Fusion Lumos and Orbitrap Eclipse MSs) show the reagent oven assembly, along with a cross-sectional view of its internal oven.

Figure 15. ETD/IC reagent oven assembly (Orbitrap Fusion and Orbitrap ID-X MSs)

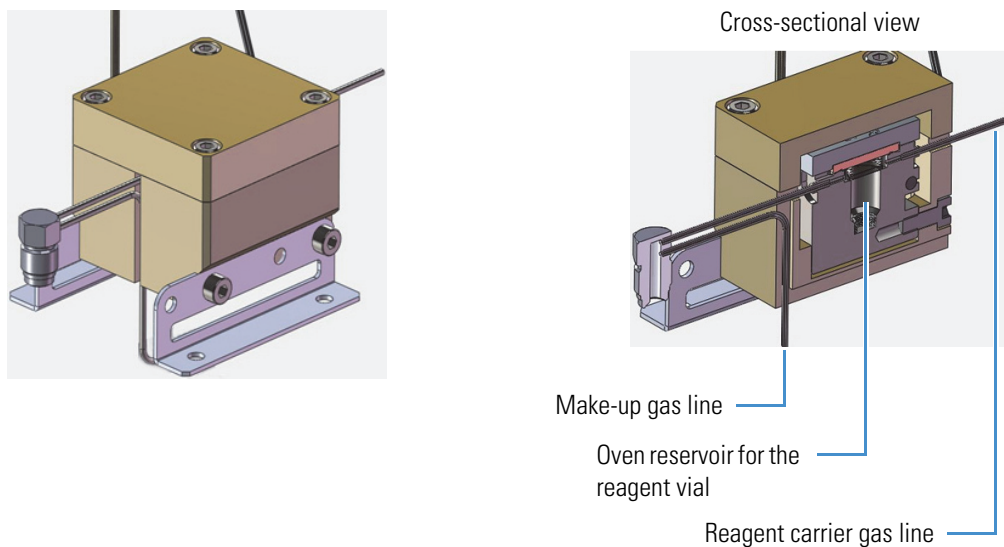


Figure 16. ETD/IC reagent oven assembly (Orbitrap Fusion Lumos and Orbitrap Eclipse MSs)

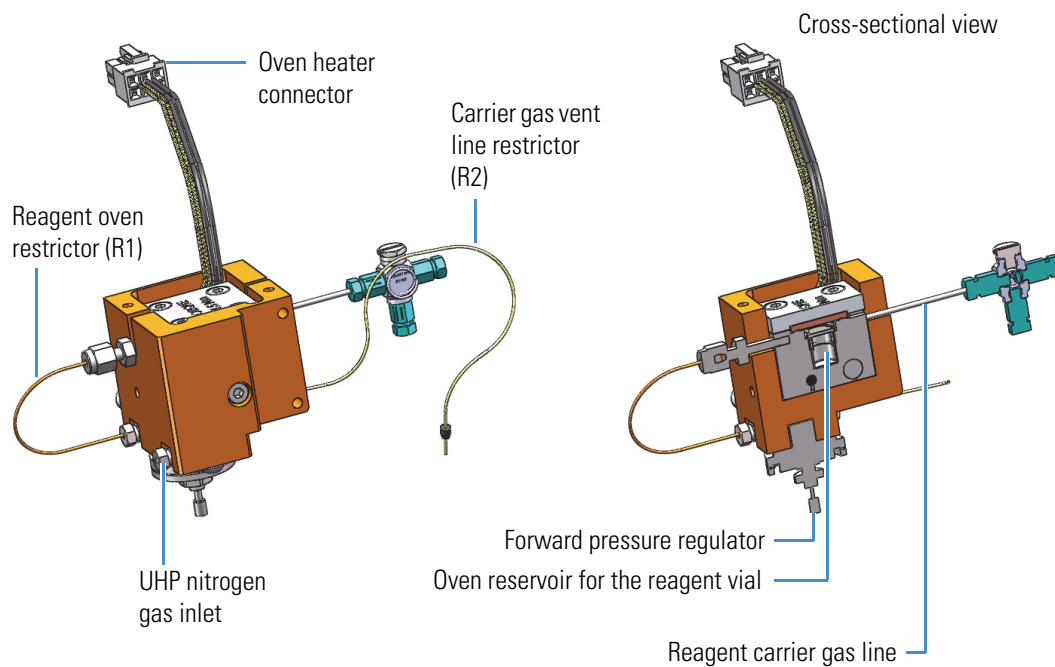
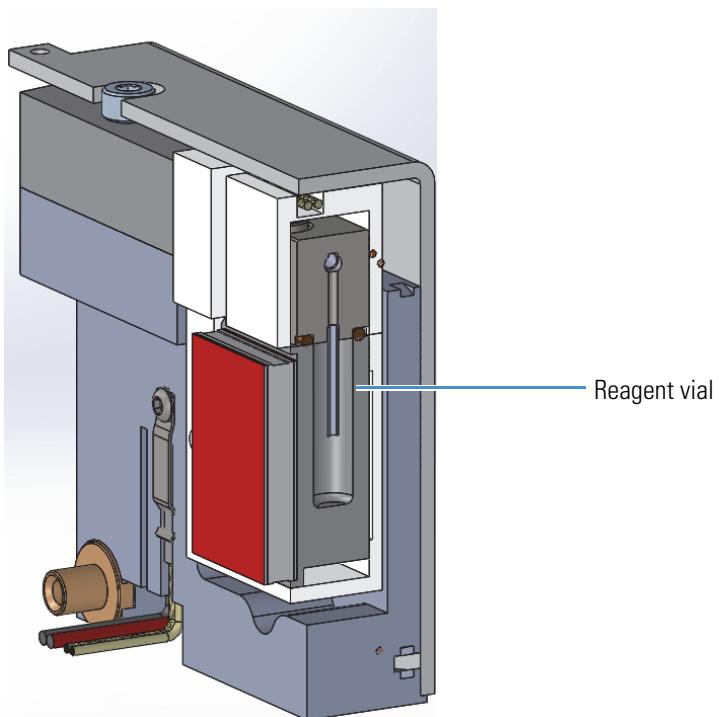
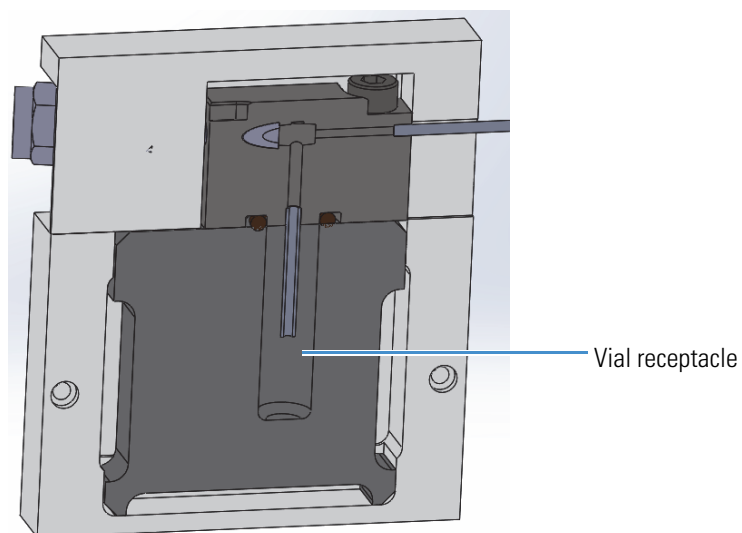


Figure 17 shows the Orbitrap Eclipse PTCR reagent oven and vial assemblies.

Figure 17. PTCR reagent oven and vial assemblies (Orbitrap Eclipse MS)



Reagent oven with vial (cross-section view)



Reagent vial (cross-section view)

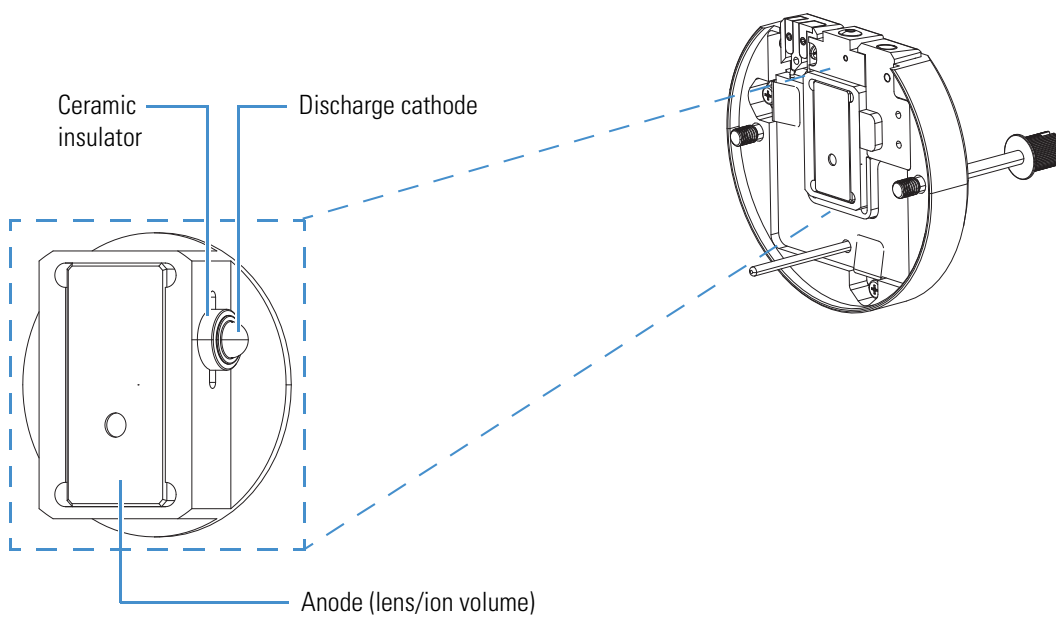
The IC/ETD/PTCR Source Heater Interface

The IC/ETD/PTCR source heater interface is located in the API source interface region between the RF lens and the MP00 RF lens (Figure 10). It consists of a mounting assembly that includes the RIS heater, a gas conduit, an HV contact, and the RIS.

Figure 18 shows the IC/ETD/PTCR source heater interface with a close-up view of the front of the RIS. The RIS contains the internal ion volume where reagent ion species are created, the discharge cathode, the anode, and a ceramic insulator.

The RIS (P/N 70005-20793) is a consumable part. For replacement instructions, see [Removing the RIS](#).

Figure 18. Close-up view of the reagent ion source in the IC/ETD/PTCR source heater interface



2 Functional Description

The IC/ETD/PTCR Source Heater Interface

Calibrating the ETD Reaction Kinetics

You can use the traditional or FlexMix calibration solution to calibrate the ETD reaction kinetics.

❖ To calibrate the ETD reaction kinetics


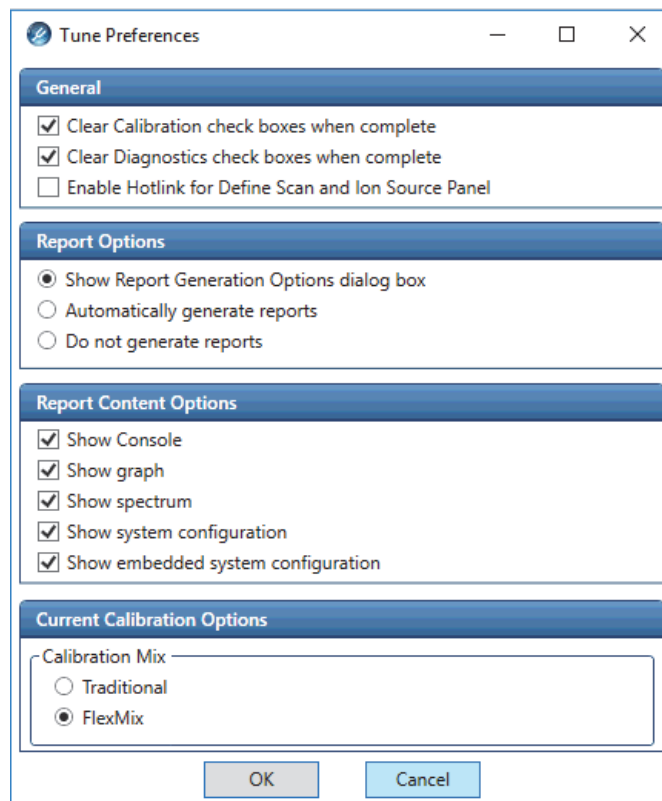
1. Load separate, clean, 500 μ L syringes with 500 μ L of the applicable calibration solution.
2. In the Tune window, specify the calibration solution as follows:
 - a. Click the **Options** icon, , and choose **Preferences**.
 - b. Under Current Calibration Mix (Figure 19), select the **Traditional** or **FlexMix** option, and then click **OK**.

Figure 19. Tune Preferences dialog box

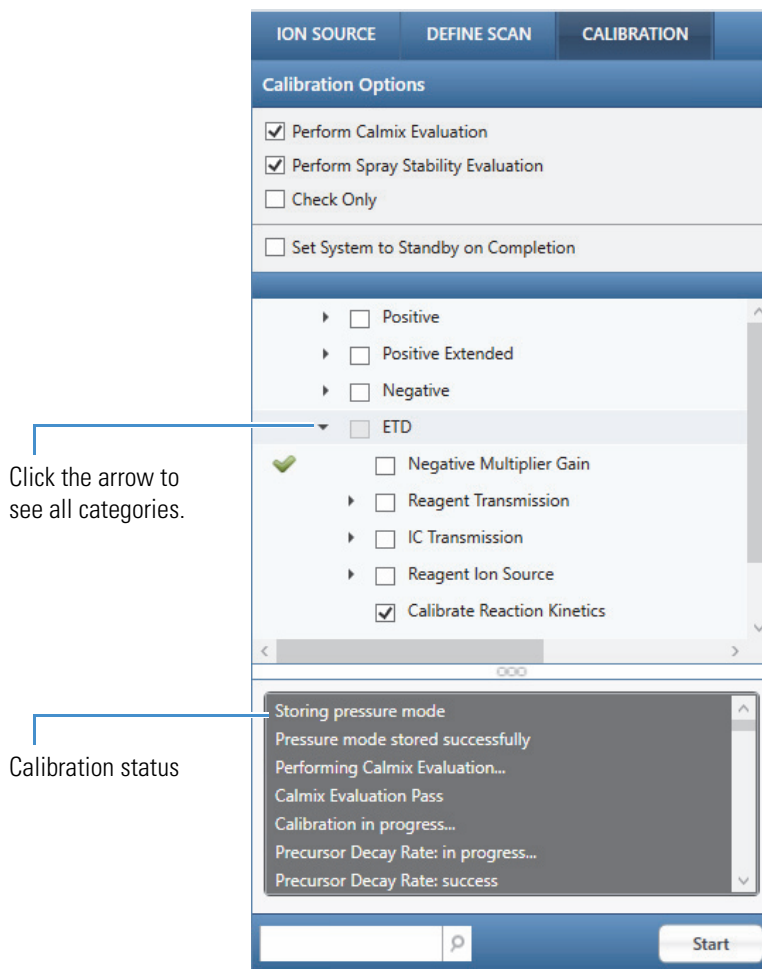


3. In the Calibration pane (Figure 20), do the following:
 - a. Select the **Perform Spray Stability Evaluation** check box.
 - b. (Optional) Select the **Set System to Standby on Completion** check box.
 - c. Click the arrow next to the ETD check box to display the calibration categories.
 - d. Select the **Calibrate Reaction Kinetics** check box, and then click **Start**.

The calibration takes a few minutes to determine the ETD reagent target and reaction time. The plots shown on the following pages appear while the calibration is in progress. When the calibration finishes, you have the option to save the data to a calibration report.

You can now go to the Xcalibur™ data system to set up and run an ETD experiment. For additional information, refer to the Xcalibur Help.

Figure 20. Calibrate Reaction Kinetics, in progress (example)



The Tune application displays multiple plots during this calibration procedure. Figure 21 shows an example of the spray stability results that appear for a few seconds before the calibration starts.

Figure 21. Plots for calibrating the ETD reaction kinetics (1 of 4)

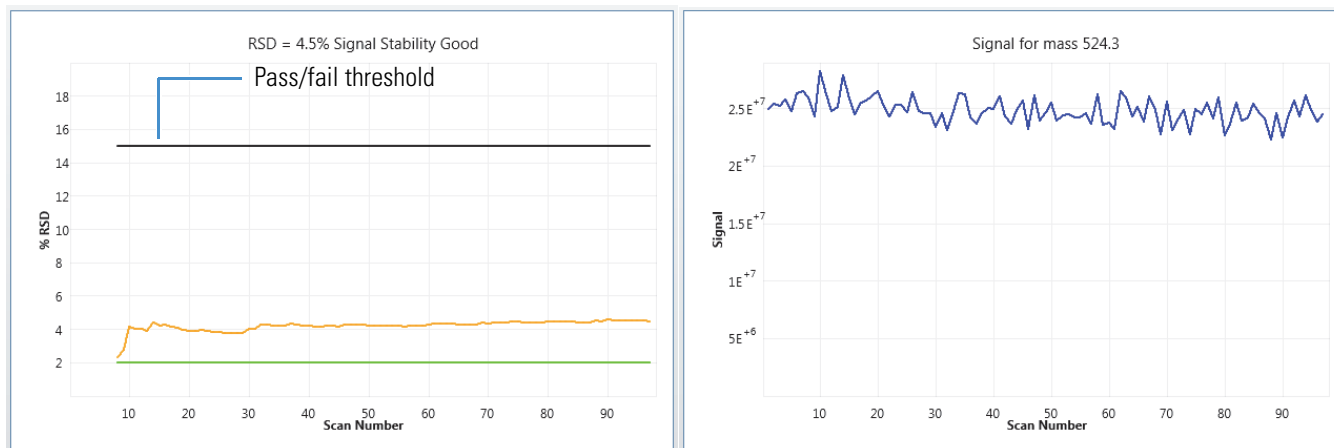
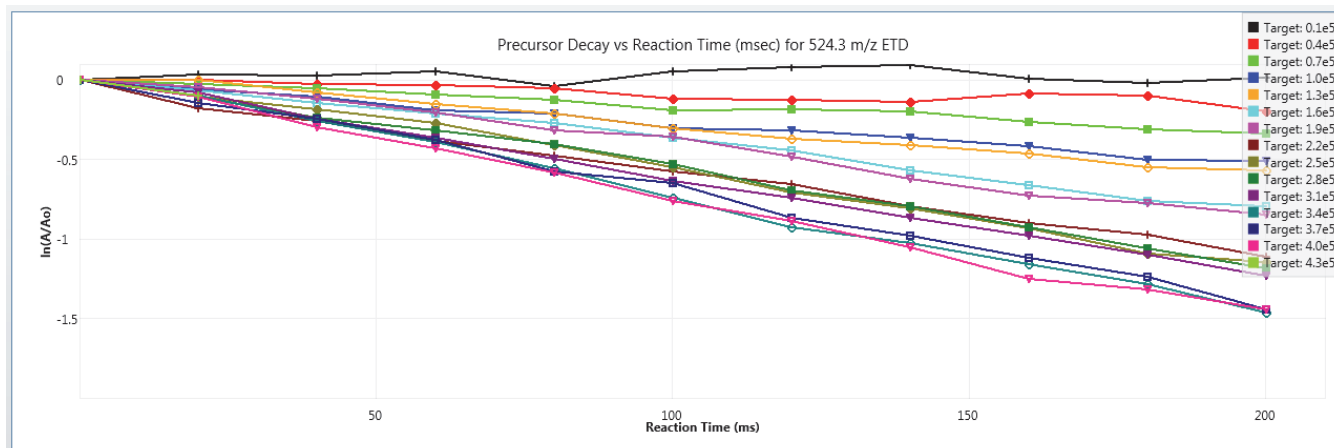


Figure 22 shows several measurements of the MRFA (m/z 524.3) precursor decay versus the preset target reaction times. These plot lines represent the natural logarithm base e of the final reagent population divided by the initial reagent population, written as " $\ln(A/A_0)$," and are used to determine the reaction rate coefficient.

Figure 22. Plots for calibrating the ETD reaction kinetics (2 of 4)



3 Calibrating the ETD Reaction Kinetics

Figure 23 uses the results from the previous plot (Figure 22) to fit the data to a kinetic model, which extracts the optimal reagent target. From these two plot lines, the green vertical line indicates the ideal reagent target at the onset of reagent saturation.

Figure 23. Plots for calibrating the ETD reaction kinetics (3 of 4)

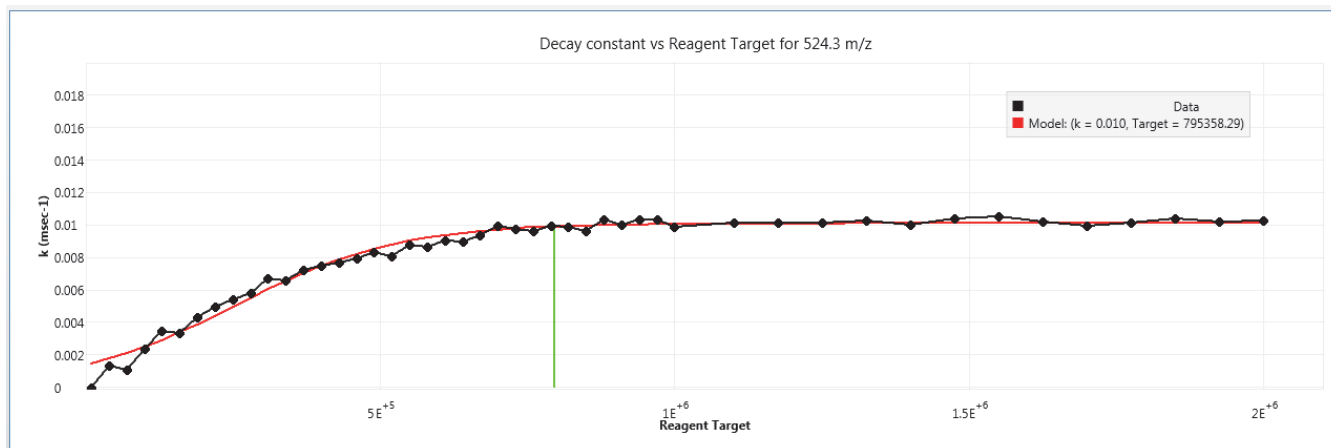
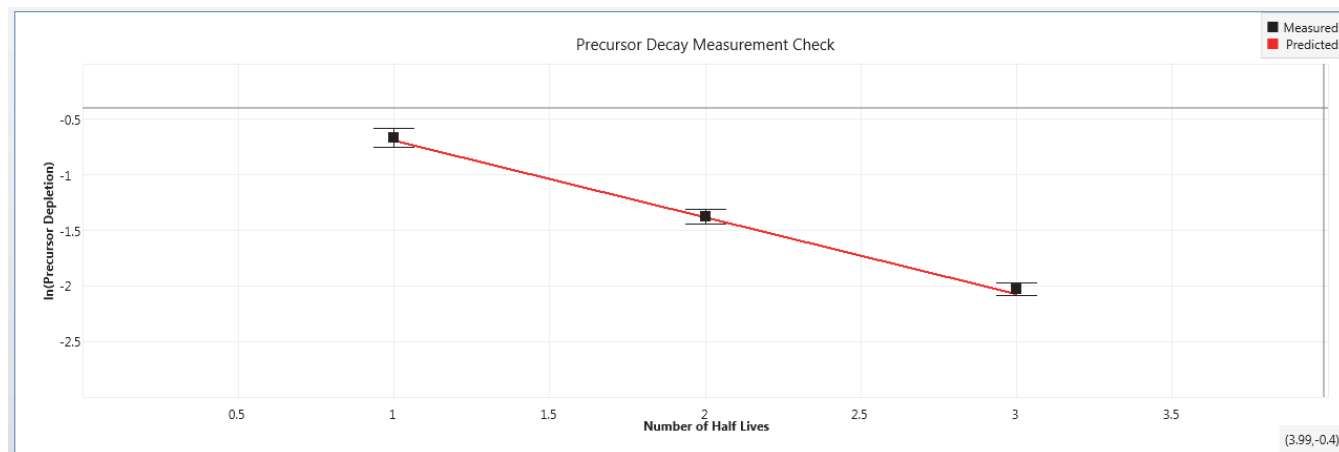


Figure 24 shows the example calibration results.

Figure 24. Plots for calibrating the ETD reaction kinetics (4 of 4)



Conducting an ETD Infusion Experiment

This chapter describes how to set up the Orbitrap Tribrid Series/ETD system for an ETD infusion experiment using the angiotensin I sample solution.

Contents

- [Setting Up the Syringe Pump for Direct Infusion](#)
- [Setting Up the MS/ETD System for an ETD or ETD/PTCR Experiment](#)

Setting Up the Syringe Pump for Direct Infusion

Use the syringe pump to infuse the angiotensin I sample solution directly into the API source.

IMPORTANT To minimize the possibility of cross-contamination, use a different syringe and length of PEEK tubing for each type of solution.

❖ To set up the syringe pump for direct infusion of the sample solution

1. Load a clean, 500 μ L syringe with the angiotensin I sample solution.

For instructions on how to prepare the sample solution, see [Appendix B, “Preparing the Angiotensin I Solutions.”](#)

2. Plumb the inlet for direct infusion as described in the Getting Started Guide.
3. Turn on the syringe pump’s power switch (on the back of the device).
4. In the Tune window, place the MS in **Standby** mode.



CAUTION To prevent electric shock, verify that the grounding union is made of stainless steel. A grounding union made of a nonconductive material, such as PEEK, creates an electric shock hazard.

Setting Up the MS/ETD System for an ETD or ETD/PTCR Experiment

Before you run the ETD or ETD/PTCR experiment, set up the operational parameters and verify the infusion of the angiotensin I sample solution.

See these topics:

- [Preparing the MS for an ETD or ETD/PTCR Experiment](#)
- [Setting the MS's Optimal Pressure](#)
- [Defining the Scan Parameters for the Angiotensin I Sample Solution \(Orbitrap Fusion\)](#)
- [Verifying the Reagent Anion Intensity](#)



CAUTION Before beginning normal operation of the MS each day, verify that there is sufficient nitrogen for the API source. If you run out of nitrogen, the MS automatically turns off to prevent atmospheric oxygen from damaging the source. The presence of oxygen in the source when the MS is on can be unsafe. In addition, if the MS turns off during an analytical run, you might lose data.

Preparing the MS for an ETD or ETD/PTCR Experiment

❖ To prepare the MS for an ETD or ETD/PTCR experiment


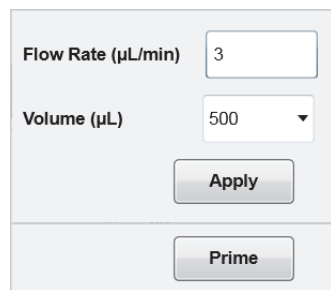
1. In the Tune window, place the MS in **On** mode, .
2. Click **Positive (Negative)** to select the positive ion polarity mode.
3. On the Ion Source – Ion Source page, enter **3** in the Current LC Flow ($\mu\text{L}/\text{min}$) box.
4. Set the syringe pump parameters as follows:
 - a. Click **Syringe On (Off)** to turn on the syringe pump.
The button name changes to Syringe On.
 - b. Click the arrow next to the Syringe On/Off button to open the syringe pump settings box (Figure 25), and then enter the following:
Flow Rate ($\mu\text{L}/\text{min}$): **3**
Volume (μL): **500**

Figure 25. Syringe parameters box



The image shows a software interface for syringe parameters. It contains two input fields: 'Flow Rate (µL/min)' with the value '3' and 'Volume (µL)' with the value '500'. Below these fields are two buttons: 'Apply' and 'Prime'.

- c. Click **Apply**.
5. Verify that the inlet plumbing connections do not leak.
6. Open the syringe pump settings box again, and press and hold **Prime** to prime the syringe.

The preset priming flow rate is 100 µL/min.



7. Verify that the system readback is normal.

Setting the Instrument's Optimal Pressure

For the Orbitrap Eclipse, Orbitrap Fusion Lumos, and Orbitrap Fusion MSs, use the Peptide Application Mode, which uses the Standard Pressure mode for the reserpine example. (For Orbitrap ID-X MS, the Small Molecules application mode also uses the standard pressure mode.)

❖ To set the instrument's optimal pressure

1. From the Application Mode menu, choose either Intact Protein, Small Molecules, or Peptide, based on your application requirements.
 - Small Molecules and Peptide modes will set the IRM pressure at 0.008 (Torr)
 - For Intact Protein mode, select either the low pressure mode at 0.002 (Torr), or the standard pressure mode at 0.008 (Torr), or the high pressure mode at 0.02 (Torr) according to your application requirements.

For intact protein or native protein experiments, do one of the following:

- For intact protein experiments and top-down experiments with large fragment ions, select **Low Pressure (Intact Protein)** mode.
- For native protein experiments, select **High Pressure** mode.

Defining the Scan Parameters for the Angiotensin I Sample Solution (Orbitrap Fusion)

❖ To define the scan parameters for the angiotensin I sample solution (Orbitrap Fusion)

1. In the Tune window, click **Define Scan** to open the Define Scan pane.
2. Set the full-scan parameters as listed in [Table 6](#).

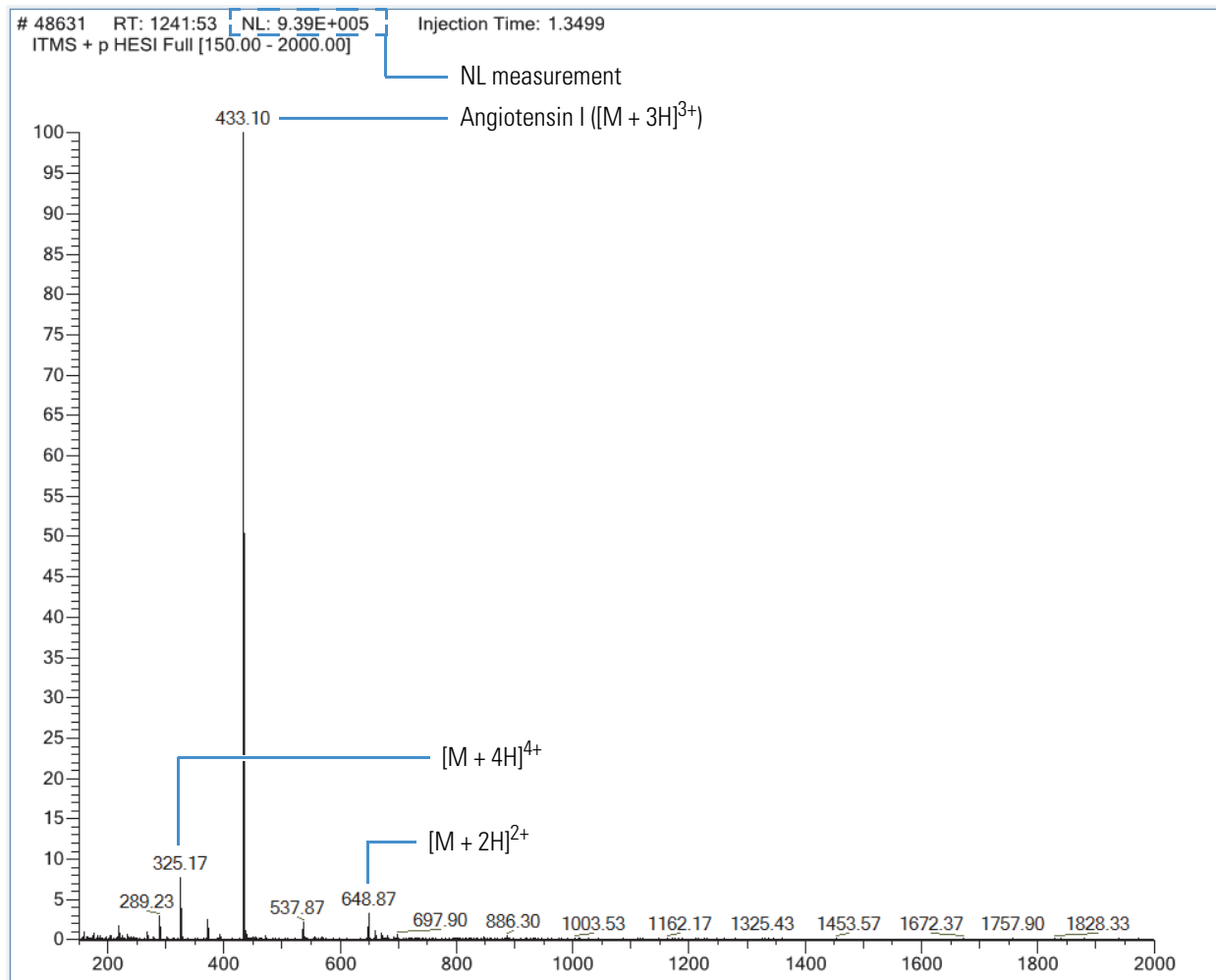
Table 6. Full-scan parameters (angiotensin example)

Parameter	Value
Scan Type	MS Scan
Detector Type	Ion Trap
Ion Trap Scan Rate	Normal
Mass Range	Normal
Scan Range (m/z)	150–2000
RF Lens (%)	<ul style="list-style-type: none"> • Orbitrap Fusion: 60 (default) • Orbitrap Fusion Lumos and Orbitrap Eclipse: 30
AGC Target	100%

3. Click **Apply**.
4. In the profile mode spectrum, verify that the normalization level (NL) for the triply charged ions (m/z 433) is between 1×10^5 and 5×10^7 ([Figure 26](#)). If the NL value is outside this range, recalibrate the MS.

For calibration instructions, refer to the Getting Started Guide.

Figure 26. Full-scan spectrum of the angiotensin I sample solution (profile mode)



Verifying the Reagent Anion Intensity

❖ To verify that the reagent anion intensity is sufficient

1. In the Define Scan pane, select **View EASY-ETD Reagent** in the Scan Type list.
2. Click **Apply**.
3. In the profile mode spectrum, verify that the NL value for the reagent anions is between 1×10^6 and 2×10^7 (Figure 27). If the profile mode NL is below this value, run the ETD reagent related calibrations.

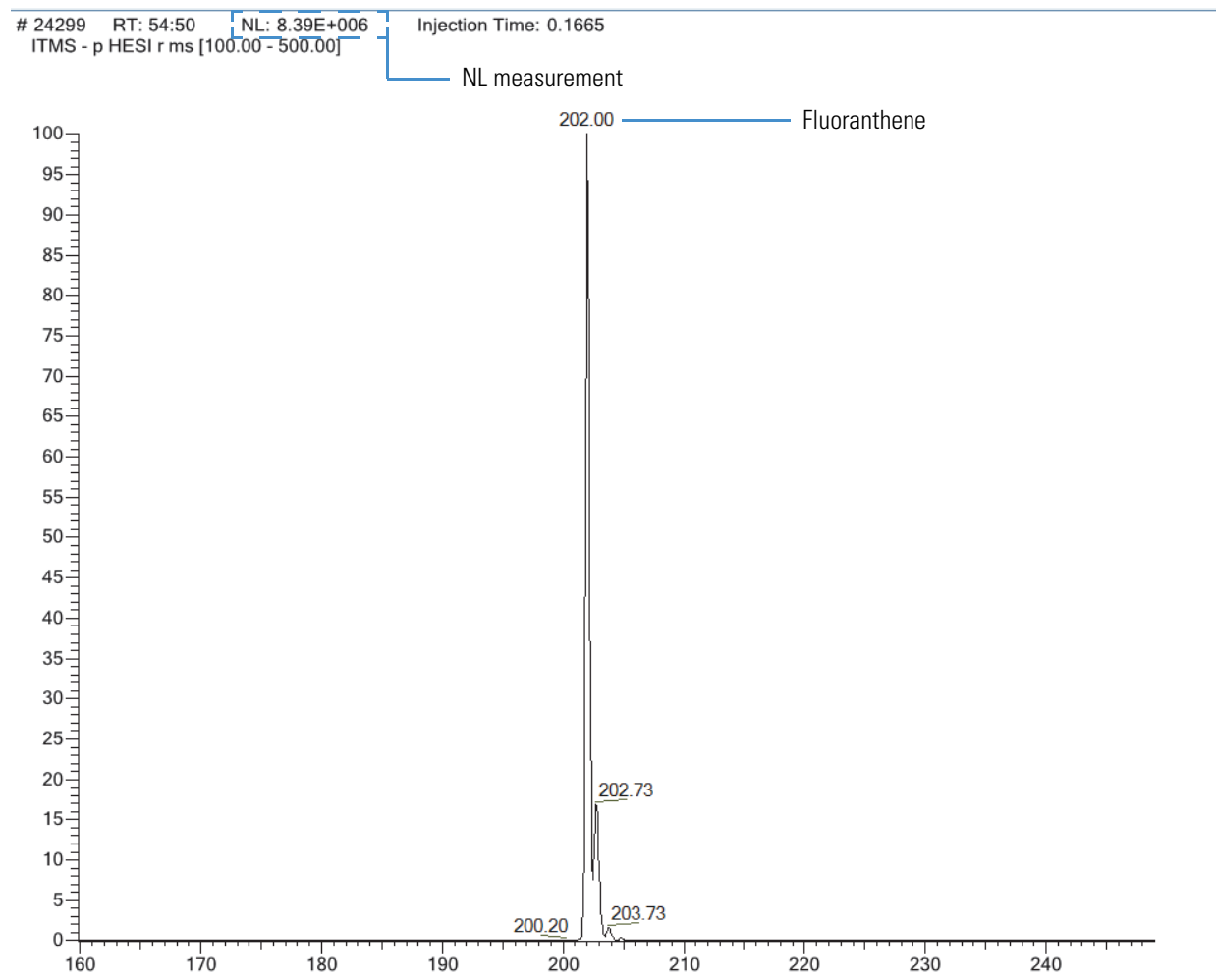
For a new RIS, the NL abundances for m/z 202 is above 5×10^6 (profile mode). The attainable reagent ion abundance decays slowly with the source usage due to the gradual contamination of the RIS. When execution of the appropriate calibrations no longer restores the reagent ion abundance to at least 1×10^6 in profile mode, you might need to replace the RIS (see [Maintaining the Reagent Ion Source](#)).

4 Conducting an ETD Infusion Experiment

Setting Up the MS/ETD System for an ETD or ETD/PTCR Experiment

For calibration instructions, refer to the Getting Started Guide.

Figure 27. Spectrum of the ETD reagent anion (m/z 202, profile mode)



Maintaining the Reagent Ion Source

Over time, the ion signal from the RIS decreases as the internal components become dirty, need to be recalibrated, or both. The expected lifetime of the RIS is six months to one year.

Note You can service (replace) the reagent ion source, but you cannot service the reagent vial. For information about replacing the reagent, see [Appendix A, “Reagent Species.”](#)

Replace the RIS under these conditions:

- For instruments with the IC configuration—When the MS’s Orbitrap™ mass analyzer cannot lock to the internal calibrant mass and the injection time for the internal calibrant exceeds 10 msec for a target of 200 internal calibration ions. (Assumes that the instrument has had a full calibration in positive, negative, and IC modes, and that the Orbitrap mass accuracy is within specification.)
- For instruments with the ETD configuration—When a full instrument calibration (positive, negative, and ETD modes) does not result in a reagent anion signal above the 1×10^6 profile intensity in the View Reagent mode (see [Verifying the Reagent Anion Intensity](#)).



CAUTION Heavy object. The Orbitrap Tribid Series MS, excluding its workbench, weighs over 227 kg (500 lb). Never try to detach and move the instrument from its workbench; you can suffer personal injury or damage the instrument. For additional information, contact your local Thermo Fisher Scientific field service engineer.

Contents

- [Guidelines](#)
- [Supplies](#)
- [Removing the RIS](#)
- [Cleaning the RIS](#)
- [Installing the New RIS](#)
- [Reassembling the API Source Interface](#)

Guidelines

For optimal results, follow these guidelines when performing the procedures in this chapter:

- Always wear a new pair of lint- and powder-free gloves when handling internal components. Never reuse gloves after you remove them because the surface contaminants on them recontaminate clean parts.
- Always place the components on a clean, lint-free work surface.
- Have nearby the necessary supplies and replacement parts.
- Always wear protective eye wear when you handle the internal parts.
- Proceed methodically.

IMPORTANT

- Put on a new pair of lint- and powder-free gloves before starting each removal and reinstallation procedure.
- Make sure that you do not introduce any scratches or surface abrasions while handling the internal components. Even small scratches can affect performance if they are close to the ion transmission path. Avoid using tools, such as metal pliers, that might scratch these components.

Supplies

The Orbitrap Tribrid Series MS requires very few tools to perform routine maintenance procedures. [Table 7](#) lists the supplies for replacing the RIS.



CAUTION Avoid exposure to potentially harmful materials.

By law, producers and suppliers of chemical compounds are required to provide their customers with the most current health and safety information in the form of Material Safety Data Sheets (MSDSs) or Safety Data Sheet (SDS). The MSDSs and SDSs must be freely available to lab personnel to examine at any time. These data sheets describe the chemicals and summarize information on the hazard and toxicity of specific chemical compounds. They also provide information on the proper handling of compounds, first aid for accidental exposure, and procedures to remedy spills or leaks.

Read the MSDS or SDS for each chemical you use. Store and handle all chemicals in accordance with standard safety procedures. Always wear protective gloves and safety glasses when you use solvents or corrosives. Also, contain waste streams, use proper ventilation, and dispose of all laboratory reagents according to the directions in the MSDS or SDS.

Table 7. Supplies for replacing the RIS

Description	Part number
Aluminum foil, heavy gauge	Fisher Scientific™ 01-213-104
Foam-tipped swabs	Fisher Scientific 14-960-3J
Gloves, lint-free and powder-free	Fisher Scientific 19-120-2947 ^a
	Unity Lab Services:
	<ul style="list-style-type: none"> • 23827-0008 (medium size) • 23827-0009 (large size)
Industrial tissues, lint-free	—
Methanol, LC/MS-grade	Fisher Scientific A456-1
Nitrogen gas, clean and dry	—
Protective eye wear	—
Reagent ion source	70005-20793

^a Multiple sizes are available.

Removing the RIS

The RIS is a consumable part—do not attempt to disassemble and clean it. To remove the RIS, follow these topics in order:

1. [Shutting Down the Mass Spectrometer Completely](#)
2. [Removing the API Source Interface](#)
3. [Removing the IC/ETD/PTCR Source Heater Interface](#)
4. [Removing the RIS from the IC/ETD/PTCR Source Heater Interface](#)

Note

- Before you continue, read [Special Notices, Symbols, and Cautions](#) and [Safety Precautions](#).
- The following components are slightly different between the Orbitrap Fusion MS, and both the Orbitrap Fusion Lumos and Orbitrap Eclipse MSs: sweep cone, ion transfer tube, API source interface, RF lens, MP00 RF lens, and lens L0.
- Unless otherwise noted, use the Orbitrap Fusion MS procedures.


IMPORTANT

- Prepare a clean work surface by covering the area with lint-free paper or a large sheet of clean aluminum foil.
- Put on a new pair of lint- and powder-free gloves before starting the removal and reinstallation procedures.

Shutting Down the Mass Spectrometer Completely

Shut down the Orbitrap Tribrid Series MS completely only when you are not using it for an extended period of time or when you must shut it down for maintenance or service.

❖ To shut down the MS completely

1. Place the MS in **Standby** mode, .
2. Place the electronics service switch in the Service Mode (down) position.

This turns off all LEDs on the front panel and the power to the nonvacuum system electronics.

3. Turn off the Main Power switch.

The following occurs:

- All power to the MS, including the turbomolecular pumps and the forepump or forepumps, turn off. All LEDs on the front panel are off.
 - After approximately 5 seconds, power to the vent valve solenoid shuts off, the vent valve opens, and the vacuum manifold vents with dry nitrogen. You can hear a hissing sound.
 - After about 2 minutes, the vacuum manifold is at atmospheric pressure.
4. Unplug the MS's power cord from the electrical outlet.



CAUTION Do not disconnect the power cord at the MS while the other end is still plugged into the electrical outlet.

Removing the API Source Interface

❖ To remove the API source interface

1. Follow the procedure [Shutting Down the Mass Spectrometer Completely](#), vent the system, and let it cool to room temperature.

Venting the mass spectrometer can take several minutes.



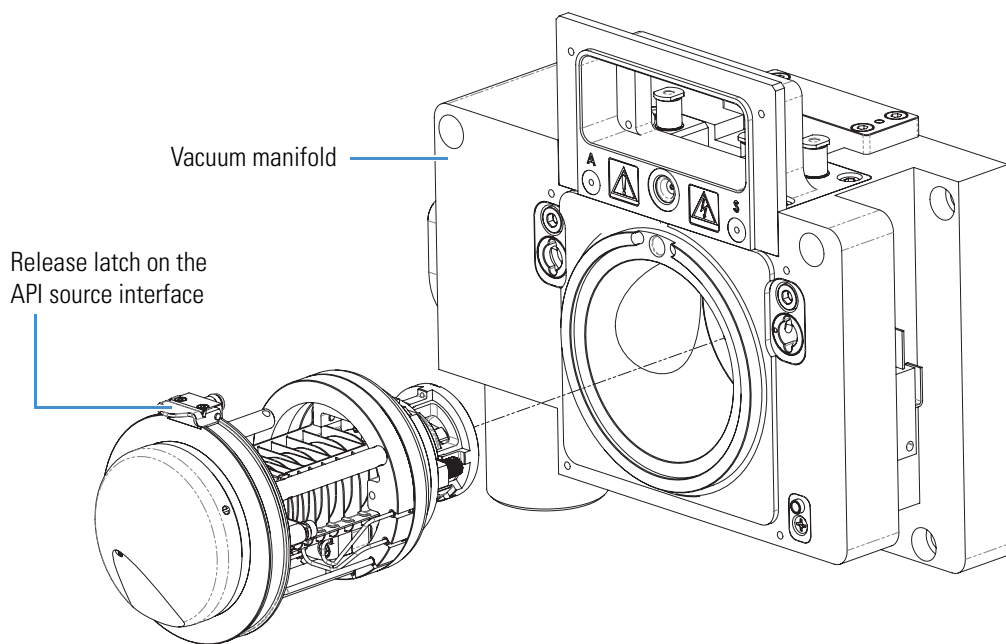
CAUTION Hot surface. Allow heated components to cool to room temperature (approximately 20 minutes) before you touch or service them.

2. Remove the API source interface housing from the MS.

For instructions, refer to the Getting Started Guide.

3. Lift up the release latch ([Figure 28](#)), grasp the API source interface with your fingers, and then carefully pull it out of the vacuum manifold.

Figure 28. API source interface removed from the vacuum manifold (Orbitrap Fusion and Orbitrap ID-X MSs)

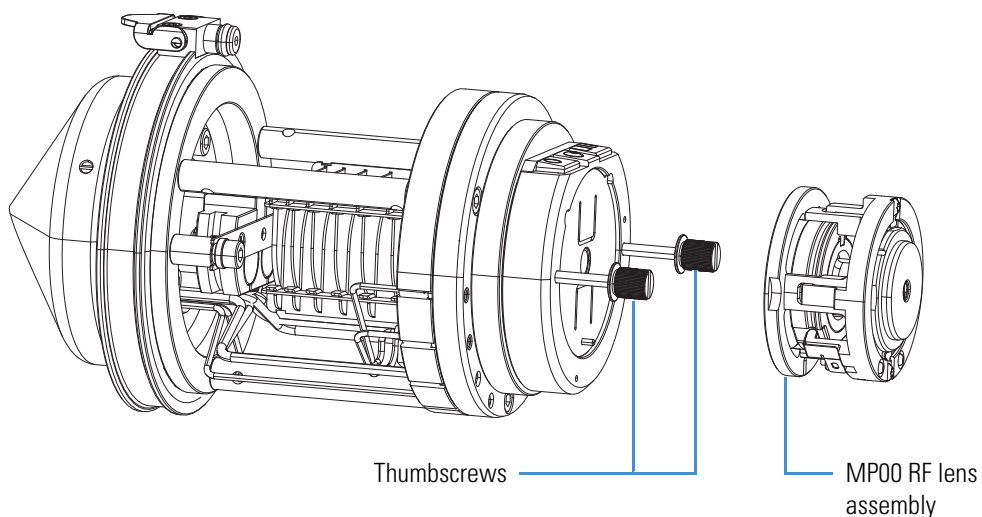


Removing the IC/ETD/PTCR Source Heater Interface

❖ **To remove the IC/ETD/PTCR source heater interface**

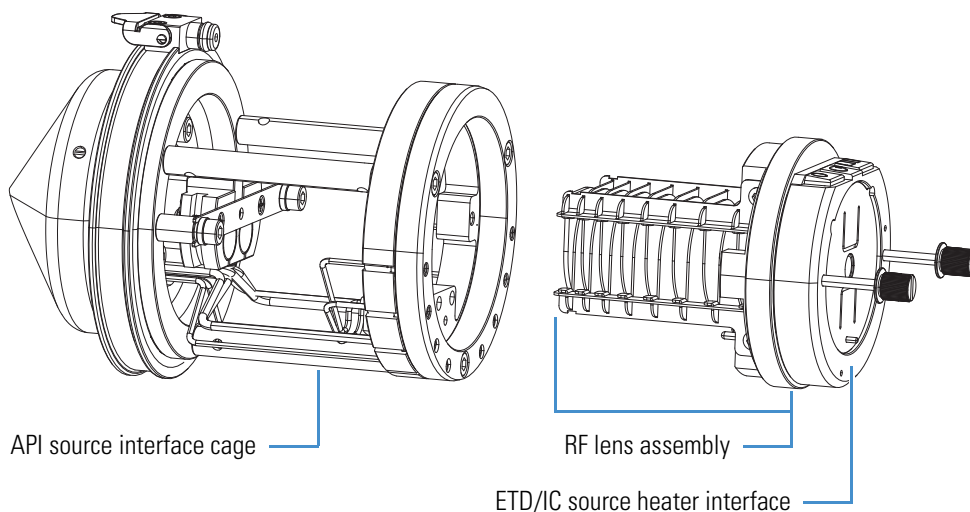
1. Follow the procedure in [Removing the API Source Interface](#).
2. Loosen and extend the two thumbscrews on the back of the API source interface, and then remove the MP00 RF lens assembly ([Figure 29](#)).

Figure 29. MP00 RF lens assembly removed from the API source interface (Orbitrap Fusion and Orbitrap ID-X MSs)



3. Continue to loosen the two thumbscrews and use them to carefully pull out the RF lens assembly from the API source interface cage ([Figure 30](#)).

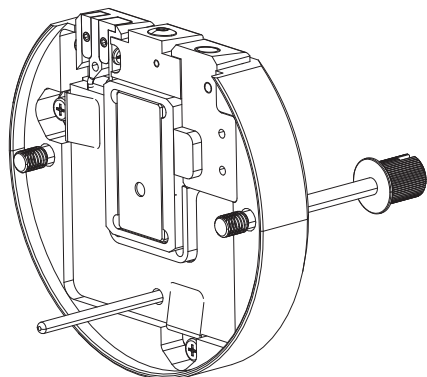
Figure 30. RF lens removed from the API source interface cage (Orbitrap Fusion and Orbitrap ID-X MSs)



4. Loosen the two thumbscrews even further and use them to pull out the IC/ETD/PTCR source heater interface (Figure 31).

Hold the heater interface with the thumbscrews facing upward to prevent the RIS from falling out of the IC/ETD/PTCR source heater interface.

Figure 31. IC/ETD/PTCR source heater interface (front view)



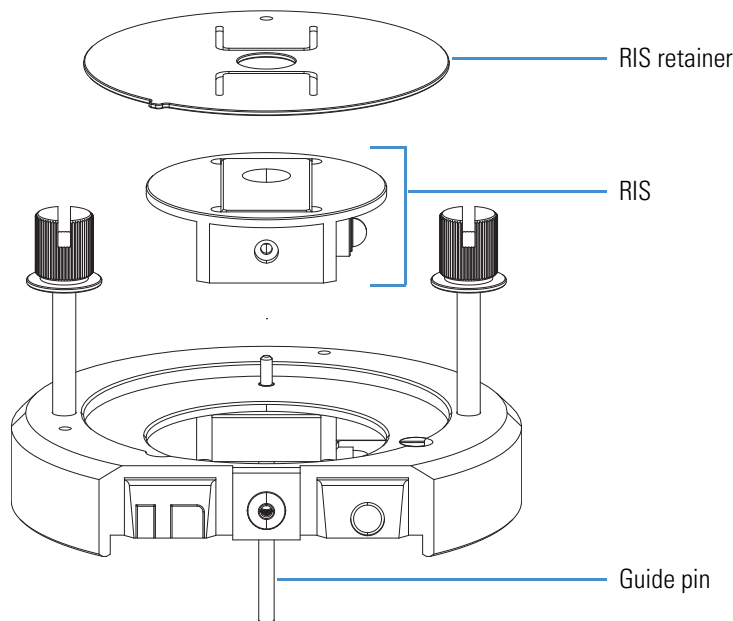
Removing the RIS from the IC/ETD/PTCR Source Heater Interface

❖ To remove the RIS

1. Hold the IC/ETD/PTCR source heater interface with the thumbscrews facing up.
2. From the bottom, press upward with your fingers to remove the RIS retainer and the RIS (Figure 32).

To dispose of the old RIS, see [WEEE Directive 2012/19/EU](#).

Figure 32. RIS removed from the IC/ETD/PTCR source heater interface



Cleaning the RIS

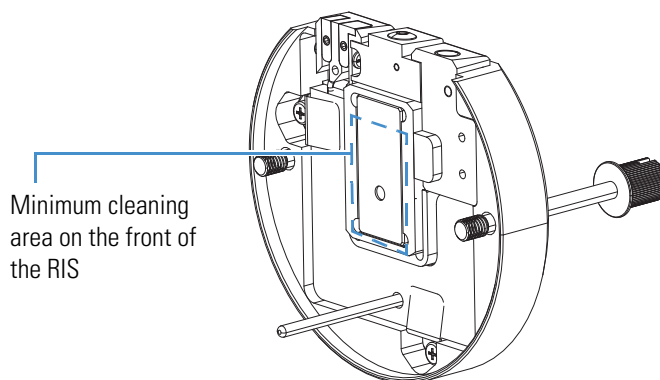
When you remove the API source interface from the instrument to clean the various internal components, Thermo Fisher Scientific recommends that you also clean the RIS areas as noted in this section—do not disassemble the RIS for additional cleaning.

Tip You do not need to remove the RIS retainer or the RIS from the IC/ETD/PTCR source heater interface to clean the designated areas.

❖ To clean the RIS

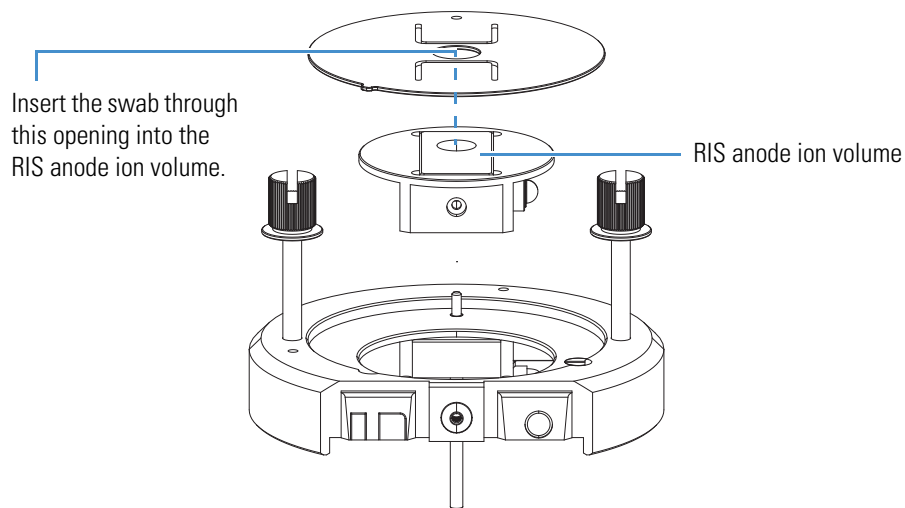
1. Follow the procedure [Removing the IC/ETD/PTCR Source Heater Interface](#).
2. Soak foam-tipped swabs in a 50:50 solution of methanol/water.
3. Clean the front of the IC/ETD/PTCR source heater interface with the swabs, as shown in [Figure 33](#).

Figure 33. Front of the IC/ETD/PTCR source heater interface



4. On the back of the IC/ETD/PTCR source heater interface, insert a swab through the RIS retainer into the opening of the RIS anode ion volume (Figure 34), and then clean inside the opening.

Figure 34. Back of the IC/ETD/PTCR source heater interface (exploded view)



5. Dry the component with nitrogen gas to make sure that all the solvent evaporates.
6. Using a magnification device, inspect the opening for any residual lint or particulates.

Note Inspect the inside surface and edges to confirm that no lint or particulates are present. Use plastic tweezers or a similar tool to remove the lint or particulate.

7. Follow these procedures:
 - a. [Reinstalling the RF lens and the IC/ETD/PTCR Source Heater Interface.](#)
 - b. [Reinstalling the MP00 RF Lens Assembly.](#)
 - c. [Reinstalling the API Source Interface.](#)

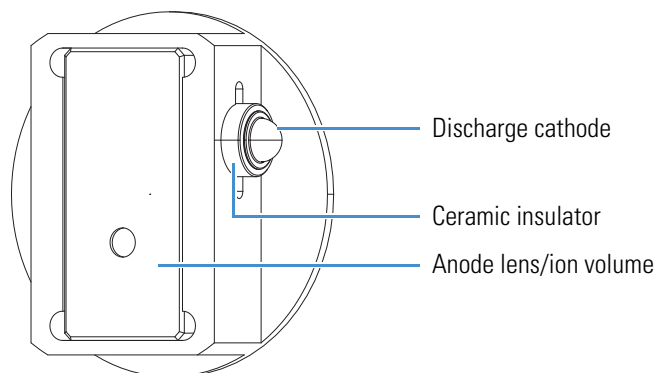
Installing the New RIS

If you determine that a new RIS is needed, follow this procedure.

❖ To install the new RIS

1. Make sure that the discharge cathode is fully inserted into the ceramic insulator ([Figure 35](#)).

Figure 35. RIS (front view)



2. Align the RIS and the RIS retainer as shown in [Figure 32](#), and then reinstall them into the IC/ETD/PTCR source heater interface.

Reassembling the API Source Interface

After replacing the RIS, follow these topics in order:

1. [Reinstalling the RF lens and the IC/ETD/PTCR Source Heater Interface](#)
2. [Reinstalling the MP00 RF Lens Assembly](#)
3. [Reinstalling the API Source Interface](#)

Reinstalling the RF lens and the IC/ETD/PTCR Source Heater Interface

❖ To reinstall the RF lens and the IC/ETD/PTCR source heater interface

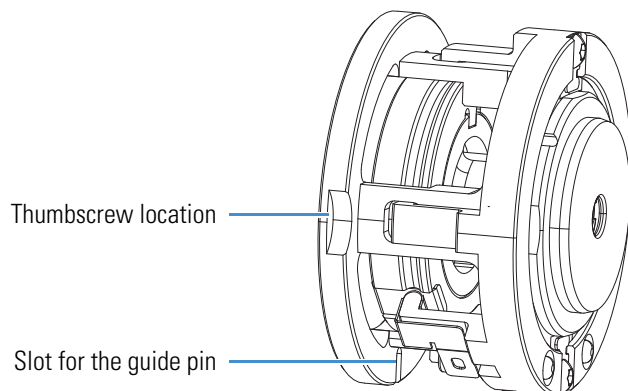
1. Align the guide pin on the IC/ETD/PTCR source heater interface ([Figure 32](#)) with the guide pin socket on the RF lens, and then firmly press the heater interface until it snaps into place.
2. Tighten the two thumbscrews a few turns into the RF lens.
3. Orient the RF lens as shown in [Figure 30](#), and then carefully slide it into the API source interface cage.
4. Tighten the two thumbscrews a few turns into the API source interface cage.

Reinstalling the MP00 RF Lens Assembly

❖ To reinstall the MP00 RF lens assembly

1. Align the bottom slot on the MP00 RF lens assembly (Figure 36) with the guide pin on the ETD/IC source heater interface (Figure 31), and then carefully push the MP00 RF lens onto the heater interface.

Figure 36. Alignment slot and thumbscrew locations on the MP00 RF lens assembly (Orbitrap Fusion and Orbitrap ID-X MSs)



2. Tighten the two thumbscrews so that they are fingertight against the MP00 RF lens assembly.

Reinstalling the API Source Interface

❖ To reinstall the API source interface

1. Orient the API source interface with the release latch at the top (Figure 28).
2. Carefully insert the API source interface into the vacuum manifold.
3. Reinstall the API source interface housing.
4. Start up the system as described in “Starting the System after a Complete Shutdown” in the Hardware Manual.

5 Maintaining the Reagent Ion Source

Reassembling the API Source Interface

Reagent Species

The Orbitrap Tribrid Series uses multiple reagent species depending on the Orbitrap model. The following topics provide details for IC, ETD, and PTCR sources.

Contents

- [Fluoranthene](#)
- [Perfluoroperhydrophenanthrene](#)

Fluoranthene

The Orbitrap Tribrid Series/IC and Orbitrap Tribrid Series/ETD MS systems use fluoranthene as the reagent species. The Thermo Fisher Scientific factory installs the 0.15 g of fluoranthene, which is contained within a stainless steel vial, in the reagent oven assembly ([Figure 15](#)).

With continuous operation of the MS, the reagent is estimated to last for at least one year. Contact your local Thermo Fisher Scientific field service engineer when it is time to replace the reagent vial. Only a Thermo Fisher Scientific field service engineer can order the ETD Reagent Fluoranthene Kit ((P/N 70005-62033) and replace the reagent vial.

Fluoranthene is potentially hazardous. Use it in accordance with its MSDS or SDS.



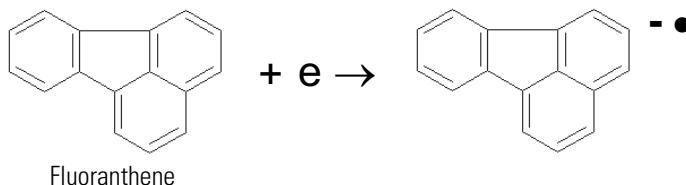
CAUTION Avoid exposure to potentially harmful materials.

By law, producers and suppliers of chemical compounds are required to provide their customers with the most current health and safety information in the form of Material Safety Data Sheets (MSDSs) or Safety Data Sheet (SDS). The MSDSs and SDSs must be freely available to lab personnel to examine at any time. These data sheets describe the chemicals and summarize information on the hazard and toxicity of specific chemical compounds. They also provide information on the proper handling of compounds, first aid for accidental exposure, and procedures to remedy spills or leaks.

Read the MSDS or SDS for each chemical you use. Store and handle all chemicals in accordance with standard safety procedures. Always wear protective gloves and safety glasses when you use solvents or corrosives. Also, contain waste streams, use proper ventilation, and dispose of all laboratory reagents according to the directions in the MSDS or SDS.

The fluoranthene radical anion is generated according to the reaction shown in [Figure 37](#).

Figure 37. ETD reagent (fluoranthene radical anion) generation from fluoranthene



Perfluoroperhydrophenanthrene

The Orbitrap Tribrid Series/ETD/PTCR MS systems use perfluoroperhydrophenanthrene (PFPP) as the reagent species. The Thermo Fisher Scientific factory installs the 0.15 g of PFPP, which is contained within a stainless steel vial, in the reagent oven assembly ([Figure 16](#)).

With continuous operation of the MS, the reagent is estimated to last for at least one year. Contact your local Thermo Fisher Scientific field service engineer when it is time to replace the reagent vial. Only a Thermo Fisher Scientific field service engineer can order the PTCR Reagent Perfluoroperhydrophenanthrene Kit (P/N HAZMAT-01-00145) and replace the reagent vial.

Perfluoroperhydrophenanthrene is potentially hazardous. Use it in accordance with its MSDS or SDS.



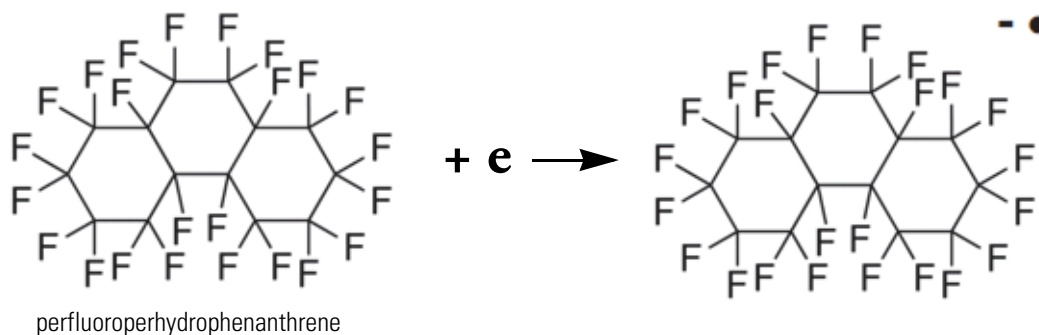
CAUTION Avoid exposure to potentially harmful materials.

By law, producers and suppliers of chemical compounds are required to provide their customers with the most current health and safety information in the form of Material Safety Data Sheets (MSDSs) or Safety Data Sheet (SDS). The MSDSs and SDSs must be freely available to lab personnel to examine at any time. These data sheets describe the chemicals and summarize information on the hazard and toxicity of specific chemical compounds. They also provide information on the proper handling of compounds, first aid for accidental exposure, and procedures to remedy spills or leaks.

Read the MSDS or SDS for each chemical you use. Store and handle all chemicals in accordance with standard safety procedures. Always wear protective gloves and safety glasses when you use solvents or corrosives. Also, contain waste streams, use proper ventilation, and dispose of all laboratory reagents according to the directions in the MSDS or SDS.

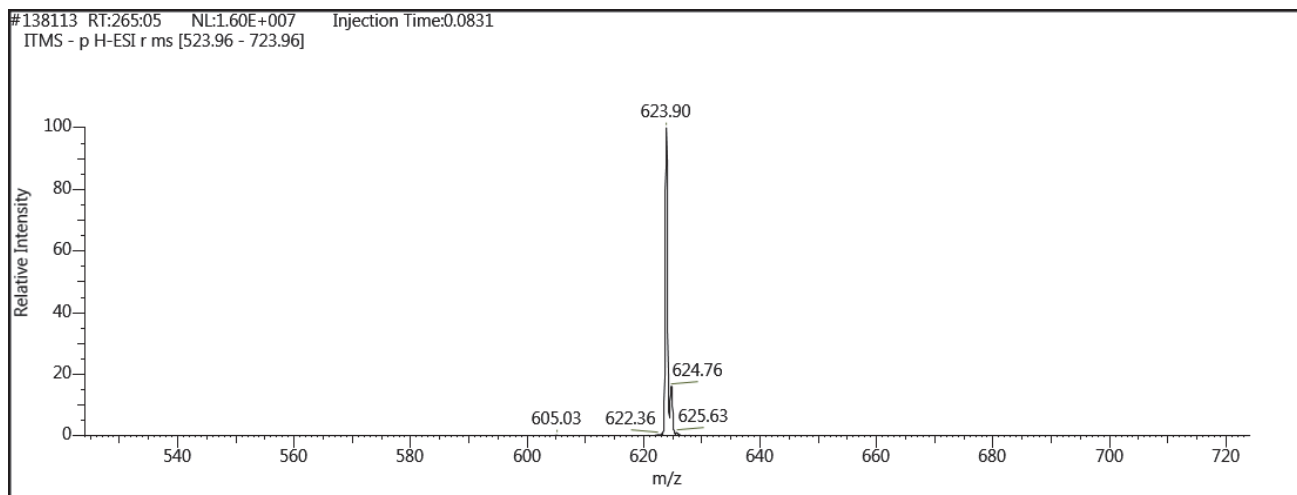
The PFPP radical anion is generated according to the reaction shown in [Figure 38](#).

Figure 38. PTCR reagent (PFFP radical anion) generation from PFPP



[Figure 39](#) shows the example the PTCR calibration results of PFPP (Orbitrap Eclipse).

Figure 39. Plot of the PFPP spectrum (Orbitrap Eclipse only)



A Reagent Species

Perfluoroperhydrophenanthrene

Preparing the Angiotensin I Solutions

This appendix describes how to prepare solutions containing angiotensin I (human acetate hydrate). Dilute a stock solution to make a sample solution (1 pmol/ μ L), which you use to demonstrate the application of the Orbitrap Tribrid Series/ETD MS system and to optimize the reagent ion reaction time.

The procedures in this section include use of other potentially hazardous chemicals, including glacial acetic acid and methanol. Handle these chemicals according to their MSDS or SDS guidelines.

Contents

- [Angiotensin I](#)
- [Preparing the Angiotensin I Stock Solution](#)
- [Preparing the Angiotensin I Sample Solution](#)

Angiotensin I

Angiotensin I is potentially hazardous. Use it in accordance with its MSDS or SDS.



CAUTION Avoid exposure to potentially harmful materials.

By law, producers and suppliers of chemical compounds are required to provide their customers with the most current health and safety information in the form of Material Safety Data Sheets (MSDSs) or Safety Data Sheet (SDS). The MSDSs and SDSs must be freely available to lab personnel to examine at any time. These data sheets describe the chemicals and summarize information on the hazard and toxicity of specific chemical compounds. They also provide information on the proper handling of compounds, first aid for accidental exposure, and procedures to remedy spills or leaks.

Read the MSDS or SDS for each chemical you use. Store and handle all chemicals in accordance with standard safety procedures. Always wear protective gloves and safety glasses when you use solvents or corrosives. Also, contain waste streams, use proper ventilation, and dispose of all laboratory reagents according to the directions in the MSDS or SDS.

Preparing the Angiotensin I Stock Solution

The ETD Chemical Kit (P/N 80000-62047) contains the 1 mg vial of angiotensin I (P/N 00301-15517), which you can also purchase from Sigma-Aldrich™ (#A9650).

❖ To prepare the angiotensin I stock solution

1. Remove the 1 mg vial of angiotensin I from the ETD Chemical Kit.
2. Add the following to the angiotensin I vial (1 mg):
 - 382 μ L of water
 - 382 μ L of methanol
 - 8 μ L of glacial acetic acid
3. Mix the solution thoroughly.

Preparing the Angiotensin I Sample Solution

Before you begin, prepare the angiotensin I stock solution.

❖ To prepare the angiotensin I sample solution

1. Pipet 100 μ L of the stock solution (1 nmol/ μ L) of angiotensin I into a clean, polypropylene microcentrifuge tube.
2. Add 900 μ L of 50:50 methanol/water (0.1% acetic acid) to the tube.
3. Mix the solution (100 pmol/ μ L) thoroughly.
4. Pipet 19.8 mL of 0.1% acetic acid 50:50 methanol/water into a clean, 20 mL glass scintillation vial.
5. Add 200 μ L of the 100 pmol/ μ L solution into the scintillation vial to bring the final volume to 20 mL.
6. Mix the solution (1 pmol/ μ L) thoroughly.
7. Store this sample solution (1 pmol/ μ L) in a refrigerator until it is needed.

Glossary

A B C D E F G H I J K L M N O P Q R S T U V W X Y Z

A

API source The sample interface between the liquid chromatograph (LC) and the mass spectrometer (MS).

atmospheric pressure chemical ionization (APCI) A soft ionization technique operating at atmospheric pressure. Electrons from a corona discharge initiate the process by ionizing the mobile phase vapor molecules, forming a reagent gas. Charged species are generated in the gas phase.

atmospheric pressure ionization (API) Ionization performed at atmospheric pressure by using atmospheric pressure chemical ionization (APCI), heated-electrospray (H-ESI), or nanospray ionization (NSI).

auxiliary gas The outer-coaxial gas (nitrogen) that assists in evaporating sample solution as it exits the ESI, APCI (optional), or APPI (optional) spray insert. The mass spectrometer heats this gas to the user-specified vaporizer temperature.

C

charge sign independent trapping Simultaneous, mutual confinement of positive and negative ions such as the multiply charged analyte precursor cations and the ETD reagent anions in the high-pressure cell of the linear ion trap mass analyzer during ETD.

chemical ionization gas See [make-up gas](#).

collision gas A neutral gas used in the collision cell to undergo collisions with ions.

collision-induced dissociation (CID) A method of fragmentation where ions are accelerated to high-kinetic energy and then allowed to collide with neutral gas molecules such as helium. The collisions break the bonds and fragment the ions into smaller charged product ions and neutral fragments.

E

electron transfer dissociation (ETD) A method of fragmenting peptides and proteins. In ETD, singly charged reagent anions transfer an electron to multiply protonated peptides within the ion trap mass analyzer. This leads to a rich ladder of sequence ions derived from cleavage at the amide groups along the peptide backbone. Amino acid side chains and important modifications such as phosphorylation are left intact.

electrospray (ESI) A soft ionization technique operating at atmospheric pressure. A solution of the analyte passes through a small capillary such that the fluid sprays through an electric field, generating very fine droplets. The droplets evaporate until all ions are in the gas phase.

F

Fourier transform (FT) The mathematical operation that converts the image current signal detected in an ICR trap or Orbitrap mass spectrometer to a set of m/z values. The Fourier components correspond to ion mass and the Fourier coefficients correspond to ion abundance.

Fourier Transform - Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS [or FTMS]) A technique that determines the mass-to-charge ratio of an ion by measuring its cyclotron frequency in a strong magnetic field.

H

heated-electrospray (H-ESI) Converts ions in solution into ions in the gas phase by using electrospray (ESI) in combination with heated auxiliary gas.

I

ion optics Focuses and transmits ions from the API source to the mass analyzer.

L

lens A metal disk with a circular hole in the center that allows the ion beam to pass.

M

make-up gas The gas associated with the optional EASY-IC or EASY-ETD ion source assembly that flows from the mass flow controller to the reagent distribution assembly.

mass spectrum A graphical representation (plot) of measured ion abundance versus mass-to-charge ratio. The mass spectrum is a characteristic pattern for the identification of a molecule and is helpful in determining the chemical composition of a sample.

mass-to-charge ratio (m/z) An abbreviation used to denote the quantity formed by dividing the mass of an ion (in Da) by the number of charges carried by the ion. For example, for the ion $C_7H_7^{2+}$, $m/z = 45.5$.

R

reagent carrier gas Ultra-high-purity nitrogen gas used to transfer the reagent to the reagent ion source that is regulated by the backpressure regulator.

RF voltage (linear ion trap) An AC voltage of constant frequency and variable amplitude that is applied to the quadrupole rods of a linear ion trap mass analyzer or to the rods of a multipole. Because the frequency of this AC voltage is in the radio frequency (RF) range, it is referred to as RF voltage.

S

sheath gas The inner coaxial gas (nitrogen), which is used in the API source to help nebulize the sample solution into a fine mist as the sample solution exits the ESI or APCI nozzle.

source See [API source](#).

sweep gas Nitrogen gas that flows out from the gap between the sweep cone and the ion transfer tube into the API source. Sweep gas aids in solvent declustering and adduct reduction.



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