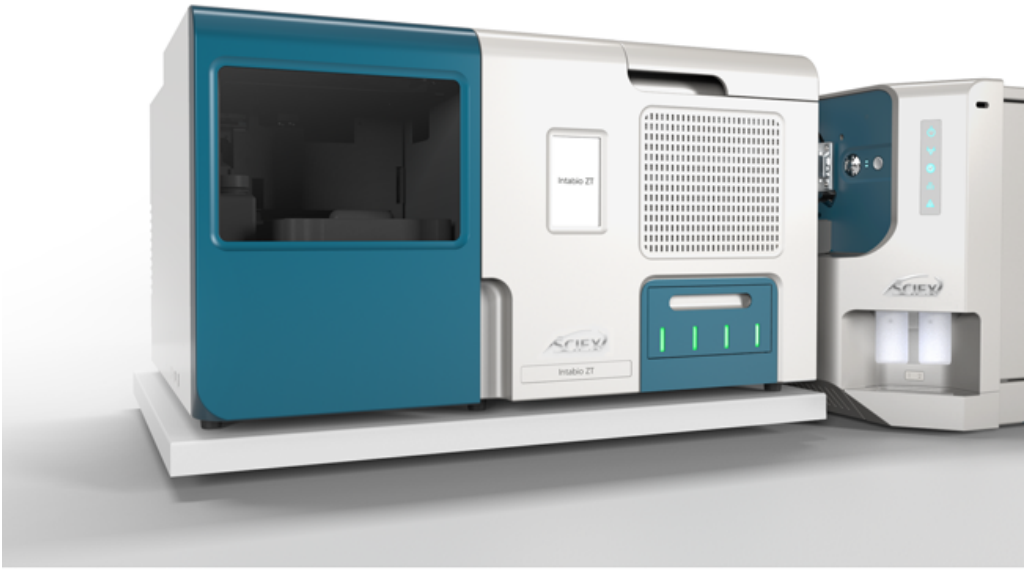
SCIEX

The Power of Precision

Intabio ZT System

User Guide



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Contents

Contents

Operational Precautions and Limitations 1

Note: Before operating the system, carefully read all of the sections of this guide.

This section contains information about general safety and regulatory compliance. This section gives descriptions of possible hazards and the related warnings for the system, and the precautions that should be obeyed to minimize the hazards.

In addition to this section, for information about the symbols that are used in the laboratory environment, on the system, and in this documentation, refer to the section: Glossary of Symbols. For site requirements, refer to the document: *Site Planning Guide*.

General Safety Information

To prevent personal injury or system damage, read, understand, and obey all of the safety precautions and warnings in this document, the manufacturer chemical safety data sheets (SDSs), and product label information. Labels are shown with internationally recognized symbols. Failure to heed these warnings could result in serious injury.

This safety information is intended to supplement federal, state, provincial, and local environmental health and safety (EHS) regulations. It does not cover every safety procedure that should be practiced. Ultimately, the user and the organization are responsible for compliance with federal, state, provincial, and local EHS regulations and for maintaining a safe laboratory environment.

Refer to the correct laboratory reference material and standard operating procedures.

Documentation Symbols and Conventions

The following symbols and conventions are used throughout the guide.

DANGER! Danger identifies an action that can cause severe injury or death.

WARNING! Warning identifies an action that can cause personal injury if precautions are not obeyed.

CAUTION: Caution identifies an operation that can cause damage to the system or corruption or loss of data if precautions are not obeyed.

Note: Notes supply important information in a procedure or description.

Tip! Tips supply information that helps to apply the techniques in a procedure or gives a shortcut, but that is not essential to the completion of a procedure.

Regulatory Compliance

This system complies with the regulations and standards listed in this section. For dated references, refer to the declaration of conformity included with the system and the individual system components. Applicable labels have been affixed to the system.

Australia and New Zealand

- **Electromagnetic Compatibility (EMC):** Radio Communications Act 1992 as implemented in these standards:
  - Electromagnetic Interference—AS/NZS CISPR 11/ EN 55011/ CISPR 11 (Class A). Refer to the section: Electromagnetic Interference.

Canada

- **Electromagnetic Interference (EMI):** CAN/CSA CISPR11. This ISM device complies with Canadian ICES-001. Refer to the section: Electromagnetic Interference.
- **Safety:**
  - CAN/CSA C22.2 No. 61010-1

Europe

- **Electromagnetic Compatibility (EMC):** Electromagnetic Compatibility Directive 2014/30/EU as implemented in these standards:
  - EN 61326-1
  - EN 55011 (Class A)

  Refer to the section: Electromagnetic Compatibility.
- **Safety:**
  - EN 61010-1
- **Waste Electrical and Electronic Equipment (WEEE):** Waste Electrical and Electronic Equipment Directive 2012/19/EU, as implemented in EN 40519. Refer to the section: Waste Electrical and Electronic Equipment.
- **Packaging and Packaging Waste (PPW):** Packaging and Packaging Waste Directive 94/62/EC

- **RoHS Restriction of Hazardous Substances:** RoHS Directive 2011/65/EU and 2015/863/EU

United States

- **Radio Emissions Interference Regulations:** 47 CFR 15, as implemented in FCC Part 15 (Class A)
- **Safety:** Occupational Safety and Health Regulations, 29 CFR 1910, as implemented in these standards:
  - UL 61010-1

International

- **Electromagnetic Compatibility (EMC):**
  - IEC 61326-1
  - IEC CISPR 11 (Class A)

  Refer to the section: Electromagnetic Compatibility.
- **Safety:**
  - IEC 61010-1
  - Machinery Directive 2006/42/EC

Electrical Precautions

WARNING! Electrical Shock Hazard. Do not remove the covers. If the covers are removed, then injury or incorrect system operation can occur. Removal of the covers is not required for routine maintenance, inspection, or adjustment. Contact a SCIEX Field Service Employee (FSE) for repairs that require removal of the covers.

- Obey the required electrical safe work practices.
- Use cable management practices to control electrical cables and decrease the risk of a tripping hazard.

For information about system electrical specifications, refer to the document: *Site Planning Guide*.

Mains Supply

Connect the system to a compatible mains supply as instructed in this guide.

WARNING! Electrical Shock Hazard. Use only qualified personnel for the installation of all of the electrical supplies and fixtures, and make sure that all of the installations adhere to local regulations and safety standards.

WARNING! Electrical Shock Hazard. Make sure that the system can be disconnected from the mains supply outlet in an emergency. Do not block the mains supply outlet.

WARNING! Electrical Shock Hazard. Use only the mains supply cables that are supplied with the system. Do not use mains supply cables that are not correctly rated for the operation of this system.

Protective Earth Conductor

The mains supply must include a correctly installed protective earth conductor. The protective earth conductor must be installed or examined by a qualified electrician before the system is connected.

WARNING! Electrical Shock Hazard. Do not intentionally interrupt the protective earth conductor. Any interruption of the protective earth conductor causes an electrical shock hazard.

UV Radiation Precautions

WARNING! Ultraviolet Radiation Hazard. Prevent exposure to direct or reflected UV radiation. Ultraviolet radiation is harmful to the eyes and skin. Do not operate the UV source without the required system safety interlocks.

Laser Precautions

WARNING! Laser Hazard. Obey all local codes, regulations, and standards, and internal requirements that are applicable to laser safety.

WARNING! Laser Hazard. To prevent exposure to hazardous laser radiation, do not use different equipment and controls or do procedures differently than what is documented in this guide.

WARNING! Personal Injury Hazard. Do not look directly into the anticipated path of the laser beam or at any specular reflections of the laser beam. Invisible ultraviolet radiation from the laser can cause injury to the eyes.

WARNING! Personal Injury Hazard. Do not remove the outer cover of the system. If the cover is not present, then exposure to potentially harmful laser radiation is possible.

The Intabio ZT system contains an embedded Class 3B laser component. The 3B classification means that direct intrabeam viewing of this type of laser is always hazardous to personnel.

The laser assembly contains the laser and several other components in a sealed housing, and has no user-serviceable parts. Service of the laser assembly is restricted to qualified SCIEX Field Service Employees (FSEs). Therefore, the overall laser classification of the system is Class 1, defined as lasers that are safe under reasonably foreseeable conditions of operation.

Chemical Precautions

WARNING! Ionizing Radiation Hazard, Biohazard, or Toxic Chemical Hazard. Before cleaning or maintenance, identify whether decontamination is required. If radioactive materials, biological agents, or toxic chemicals have been used with the system, then the customer must decontaminate the system before cleaning or maintenance.

WARNING! Environmental Hazard. Do not discard system components in municipal waste. To discard components correctly, obey local regulations.

- Before servicing and regular maintenance, identify the chemicals that have been used in the system. For the health and safety precautions that must be obeyed for a chemical, refer to the safety data sheet (SDS). For storage information, refer to the certificate of analysis. To find a SCIEX SDS or certificate of analysis, go to sciex.com/tech-regulatory.
- Always wear assigned personal protective equipment, including powder-free gloves, protective eyewear, and a laboratory coat.

  Note: Nitrile or neoprene gloves are recommended.

- Do work in a well-ventilated area or fume hood.
- When flammable materials such as isopropanol, methanol, and other flammable solvents are in use, do not go near ignition sources.
- Be careful with the use and disposal of any chemicals. If the correct procedures for chemical handling and disposal are not obeyed, then personal injury can occur.
- During cleaning, do not let chemicals touch the skin. Wash hands after use.
- Collect all spent liquids and discard them as hazardous waste.
- Obey all of the local regulations for the storage, handling, and disposal of biohazardous, toxic, and radioactive materials.

System Safe Fluids

CAUTION: Potential System Damage. Do not use any other fluid until confirmation is received from SCIEX that it does not cause a hazard. This is not an exhaustive list.

CAUTION: Potential System Damage. Do not use organic solvents, such as menthol or acetone, to clean the UV window. Organic solvents can leave residue on the UV window that might interfere with detection.

Any substance supplied with the Intabio ZT system, or referenced in the documentation for the system, can safely be used with the system. In addition, the following fluids can also be used with the system. To determine compatibility with other chemicals, contact sciex.com/request-support.

- **Reagents**
  - Fresh 18 MΩ water
  - Acetonitrile
  - Acetic acid, up to 25%
  - Formic acid, up to 1%
  - Isopropanol
  - 1% Diethylamine

Physical Precautions

WARNING! Lifting Hazard. Use a mechanical lifting device to lift and move the system. If the system must be moved manually, then at least four people are required to move the system safely. Follow established safe lifting procedures. We recommend the use of a professional moving service.

Note: Follow established safe lifting procedures. For the weights of system components, refer to the document: *Site Planning Guide*.

Environmental Precautions

Use qualified personnel for the installation of electrical mains, heating, ventilation, and plumbing supplies and fixtures. Make sure that all of the installations comply with local bylaws and biohazard regulations. For information about the required environmental conditions for the system, refer to the document: *Site Planning Guide*.

When the system is set up, make sure that there is sufficient access space around the equipment.

WARNING! Biohazard. For biohazardous material use, always obey local regulations for hazard assessment, control, and handling. Neither this system nor any part is intended to be used as a biological containment.

WARNING! Environmental Hazard. Follow established procedures for disposal of biohazardous, toxic, radioactive, and electronic waste. The customer is responsible for disposal of hazardous substances, including chemicals, waste oils, and electrical components, in accordance with local laws and regulations.

Electromagnetic Environment

Electromagnetic Compatibility

Basic Electromagnetic Environment: Environment existing at locations characterized by being supplied directly at low voltage from the public mains network.

The equipment is intended for use in a basic electromagnetic environment.

Make sure that a compatible electromagnetic environment for the equipment can be maintained so that the device will operate as intended. If the power supply line is subject to high electrical noise, then install a surge protector.

Electromagnetic Interference

Group 1 Equipment: This equipment is classified as industrial, scientific, and medical (ISM) equipment that might use RF energy for internal operation.

Class A Equipment: Equipment which 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. [Derived from CISPR 11:2009, 5.3] Class A equipment shall meet Class A limits.

CAUTION: Potential Radio Interference. This equipment is not intended for use in residential environments and may not supply adequate protection to radio reception in such environments.

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC (Federal Communications Commission) Compliance Rules.

These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the operator's manual, can cause harmful interference to radio communications.

Operation of this equipment in a residential area is likely to cause harmful interference in which case you will be required to correct the interference, at your own expense. Changes or

modifications not expressly approved by the manufacturer could void your authority to operate the equipment.

Decommissioning and Disposal

WARNING! Environmental Hazard. Follow established procedures for disposal of biohazardous, toxic, radioactive, and electronic waste. The customer is responsible for disposal of hazardous substances, including chemicals, waste oils, and electrical components, in accordance with local laws and regulations.

Before decommissioning, obey local regulations to decontaminate the entire system.

When the system is removed from service, obey national and local environmental regulations to divide and recycle different materials.

Note: SCIEX will not accept any system returns without a completed *Decontamination Form*. Contact an FSE to get a copy of the form.

Do not discard system components or subassemblies, including computer parts, as unsorted municipal waste.

Waste Electrical and Electronic Equipment

Obey local municipal waste ordinances for the correct disposal provisions to decrease the environmental impact of waste, electrical, and electronic equipment (WEEE). To discard this equipment safely, contact a local Customer Service office for complimentary equipment pick-up and recycling.

Laboratory Conditions

Safe Environmental Conditions

The system is designed to operate safely in these conditions:

- Indoors
- Altitude: Up to 2,000 m (6,560 ft) above sea level
- Ambient temperature: 15 °C (59 °F) to 30 °C (86 °F)
- Relative humidity: 20% to 80%, noncondensing
- Mains supply voltage fluctuations: ±10% of the nominal voltage
- Transient overvoltages: Up to the levels of Overvoltage Category II
- Temporary overvoltages on the mains supply
- Pollution Degree 2

Equipment Use and Modification

WARNING! Electrical Shock Hazard. Do not remove the covers. If the covers are removed, then injury or incorrect system operation can occur. Removal of the covers is not required for routine maintenance, inspection, or adjustment. Contact a SCIEX Field Service Employee (FSE) for repairs that require removal of the covers.

WARNING! Personal Injury Hazard. Use SCIEX-recommended parts only. The use of parts that are not recommended by SCIEX or the use of parts for any purpose other than their intended purpose can put the user at risk of harm or have a negative effect on system performance.

WARNING! Lifting Hazard. Use a mechanical lifting device to lift and move the system. If the system must be moved manually, then at least four people are required to move the system safely. Follow established safe lifting procedures. We recommend the use of a professional moving service.

Use the system indoors in a laboratory that has the environmental conditions recommended in the document: *Site Planning Guide*, or contact an FSE.

If the system is used in an environment or in a method that is not approved by the manufacturer, then the performance and protection that is supplied by the equipment might be decreased.

Contact an FSE for information about servicing the system. Unauthorized modification or operation of the system might cause personal injury and equipment damage, and might void the warranty. If the system is operated outside the recommended environmental conditions or with unauthorized modifications, then the acquired data might be inaccurate.

Principles of Operation 2

This guide describes the basic operation, troubleshooting, and maintenance of the Intabio ZT system. Read this guide thoroughly before using the product, and operate the product in accordance with the instructions in this guide.

This guide provides safety instructions and precautions to make sure that the user operates the system safely. Obey all Warning and Caution instructions provided in this guide.

System Overview

The system facilitates the robust separation and focusing of compounds in a sample channel before the introduction of the compounds to a mass spectrometer (MS) with gas-assisted electrospray ionization (ESI).

The imaged capillary isoelectric focusing (icIEF) system includes the following components:

- Tools for ESI tip alignment and port coverage:
  - Trigger and interlock cables for contact closure operation
  - An adapter ring for attachment to the mass spectrometer (to be used as an ion source for the mass spectrometer)
  - A cover plate for offline testing
- Solvent vials
- Gas tubing
- A system bench
- Autosampler 12-vial and 48-vial trays, syringe, and bottles
- A laptop with the Intabio software for control and analysis
- Pre-assembled cartridges for separation and electrospray ionization

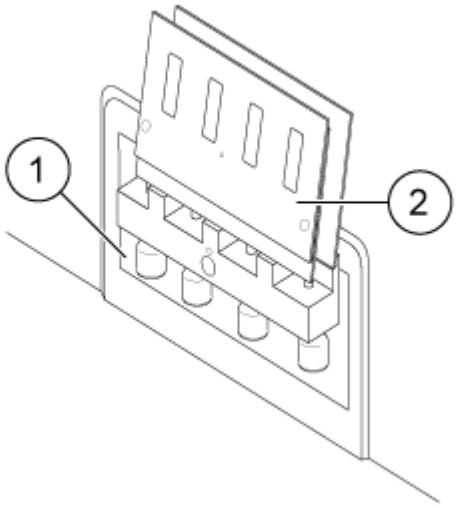
Hardware Overview

CAUTION: Potential System Damage. Take care when moving or operating the system near gas lines or the cables connected to the adapter ring.

The front of the system gives access to the onboard vials and the door to access the system.

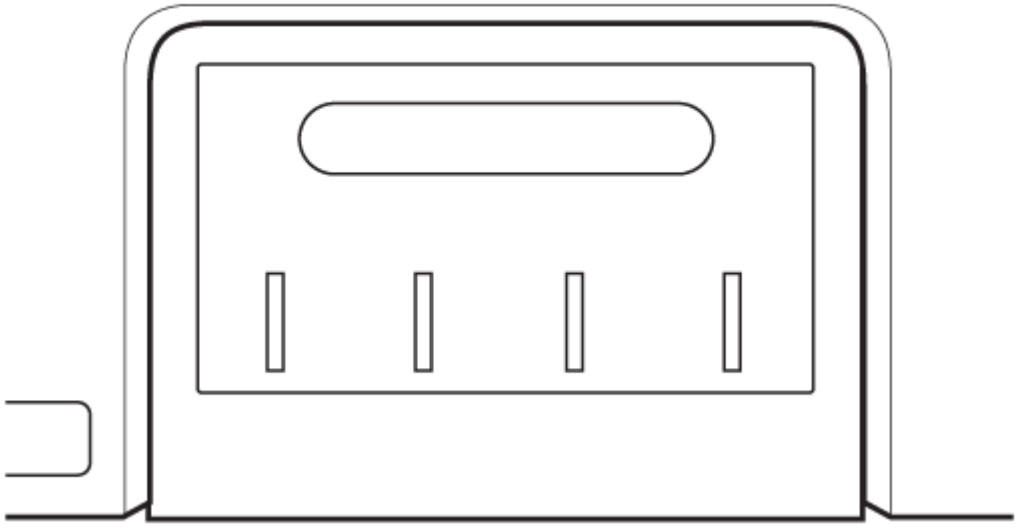
Note: The reagent vial manifold can be pulled out for easy access, but cannot be removed.

Figure 2-1 Reagent Vial Manifold (Open)



Item	Description
1	Reagent vials that supply solution to the sample, anolyte, catholyte and mobilizer channels in the cartridge. **Note:** Dilute the catholyte to 0.25% diethylamine (DEA) with fresh 18 MΩ water by making a 1:3 dilution.
2	Manifold door

Figure 2-2 Reagent Vial Manifold (Closed)

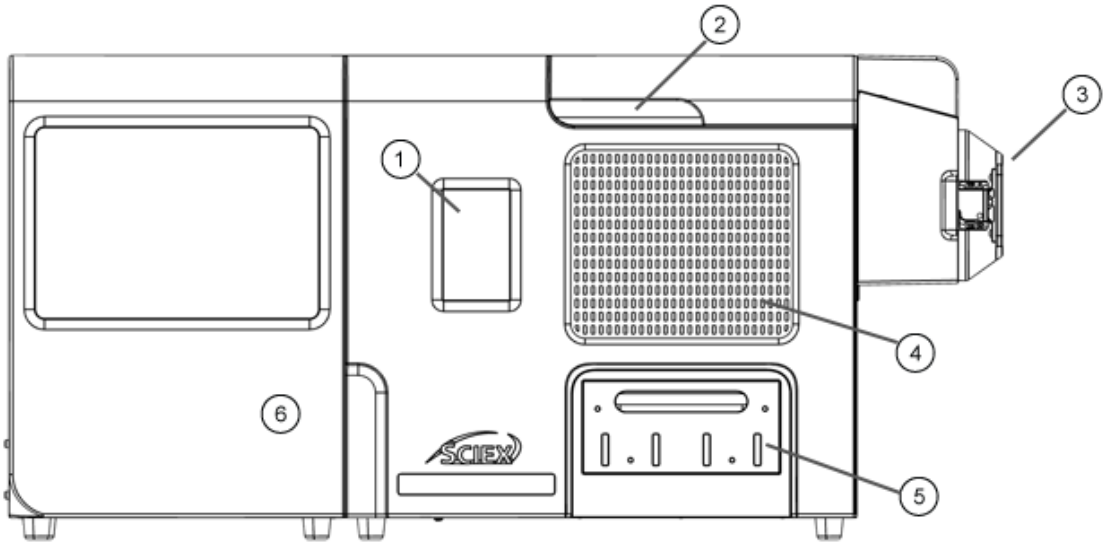


The introduction of the infusion samples, isoelectric focusing solvents, and electrospray ionization solvent occurs through the onboard reagent vial manifold on the front of the Intabio ZT system. Refer to the section: Assess ESI Infusion. The samples to be focused and separated are introduced through the autosampler.

The reagent vials in the manifold are filled manually before use. The reagent vial in the first position is filled with deionized water for washing during standard sample injections, but can also be filled with an infusion sample diluted in the mobilizer reagent before infusion and tuning. For smaller infusion sample volumes, a microcentrifuge tube can be inserted in the first vial position in the reagent vial manifold to reduce the amount of sample required. For a gas-tight seal, make sure to remove the microcentrifuge caps and connectors before insertion of the tube into the infusion sample vial. To prevent air bubbles in the system, make sure that the fluid levels for the reagent vials are above the level of the reagent tubing.

The connector to the mass spectrometer adapter ring is on the side to the right of the front of the system and has a connection for a gas line and a connector for a cable from the adapter ring on the mass spectrometer. The cable for triggering mass spectrometer acquisition is connected to the adapter ring.

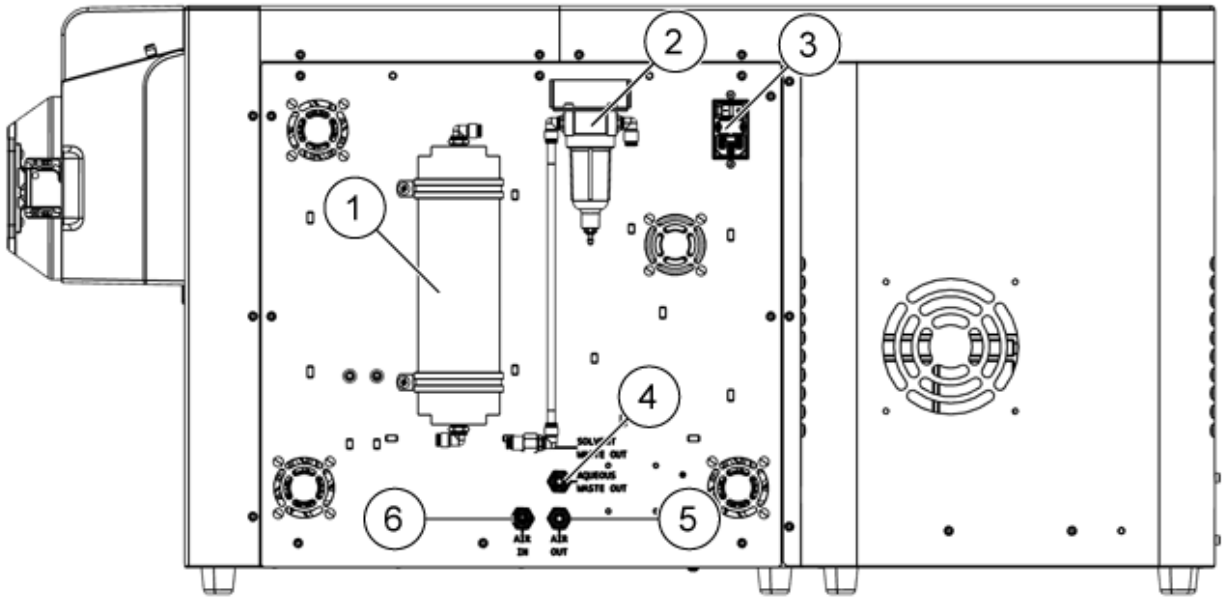
Figure 2-3 Front of the Intabio ZT System



Item	Description
1	Touchscreen
2	Cartridge door
3	Connection for the mass spectrometer adapter ring
4	Vent
5	Reagent vial manifold door
6	Autosampler door

The system fits on the bench. The back of the system has the mains supply connection, power switch, waste line connections, and Ethernet and network communications ports. The system label, which includes the serial number and voltage and fuse requirements, is also on the back panel.

Figure 2-4 Connections on the Back of the Intabio ZT System



Item	Description
1	Ballast
2	Coalescing filter
3	Mains supply input
4	Aqueous waste out
5	Air out
6	Air in

Autosampler

WARNING! Puncture Hazard. Handle the auto-injection system carefully to prevent injuries.

Note: For information about consumables and spare parts, refer to the document: *Parts and Equipment Guide*.

The autosampler is a part of the Intabio ZT system. With regard to the autosampler, references to sample vials refer to analyte sample vials.

Note: Fill the 2 mL analyte sample vials with a maximum of 1.5 mL and a minimum of 0.400 mL of solution. For vials with 300 µL inserts, use a volume of sample between 100 µL and 250 µL.

Do not overfill the vials.

An accessory kit is shipped with the autosampler.

When the door of the autosampler is open, the speeds of the sample tray, syringe, and needle are automatically decreased.

Note: The operating temperature range can vary by ±2 °C.

Specifications for the autosampler include the following:

Operating Ambient Temperature		Sample Tray Compartment Temperature		Relative Humidity (Non-Condensing)	
Minimum	**Maximum**	**Minimum**	**Maximum**	**Minimum**	**Maximum**
15 °C (59 °F)	30 °C (86 °F)	4 °C (39 °F)	Ambient minus 3 °C	20%	80%

Air Needles

The required lengths for the air needles for the autosampler are listed in the following table.

Note: The needle holder lets the needle height be adjusted by 6 mm.

Table 2-1 Available Air Needles

Vial Rack	Needle Type
48 × 1.5 mL to 2.0 mL	62 mm (natural)

Standard Air Needle

The standard air needle is 62 mm long and can be used for a wide range of deep and shallow vial plates.

For non-standard settings, use the corresponding needle types.

Figure 2-5 Standard Air Needle with 2 mL Sample Vials

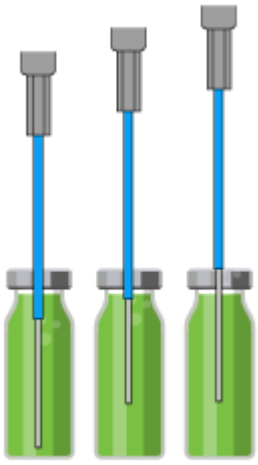
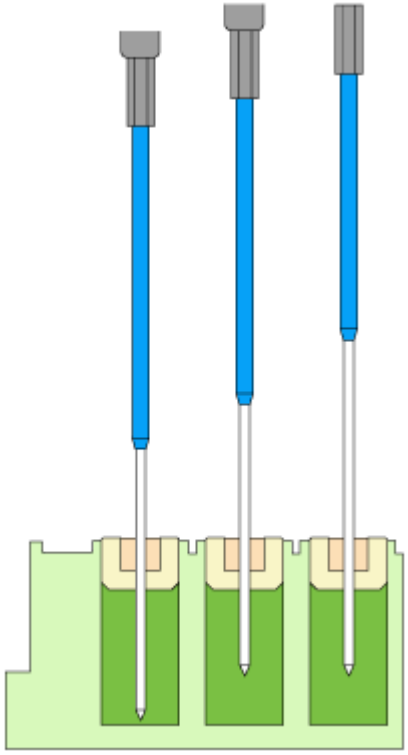


Figure 2-6 Standard Air Needle with Low Microtiter Plates



Sample Vials

When handling the sample vials, follow these guidelines:

Note: Fill the 2 mL analyte sample vials with a maximum of 1.5 mL and a minimum of 0.400 mL of solution. For vials with 300 µL inserts, use a volume of sample between 100 µL and 250 µL.

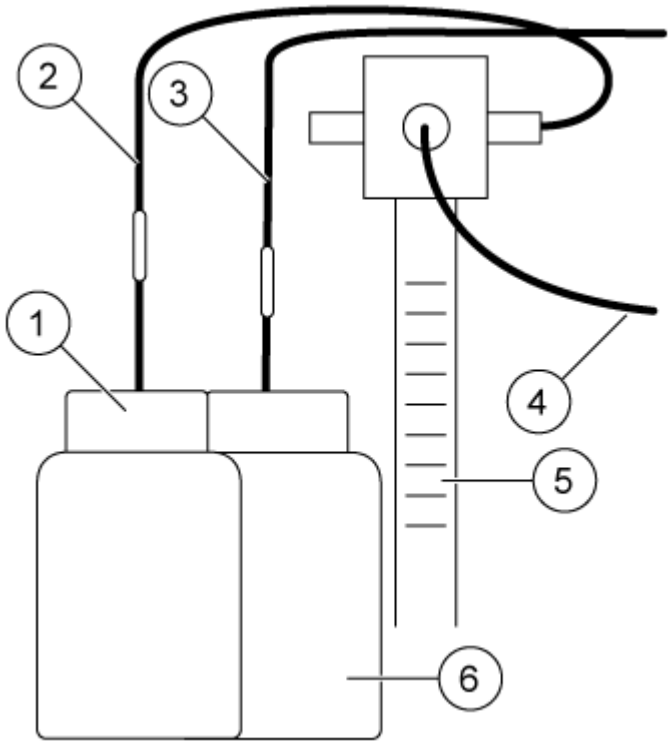
Do not overfill the vials.

- Use vial caps with pre-split septa.
- Use a pipette to remove bubbles in the vials.
- To prevent the sample from contaminating the air needle, do not fill the sample vials to the top.
- To prevent air bubbles from forming, and to prevent volatile components from evaporating, only use air-tight closure seals.
- Do not use sample vials without caps.
- Do not use sample vials with hard caps that the sample needle cannot pierce.

Syringe Valve Connections

The autosampler is supplied with a 100 µL syringe. To plumb the 4-port syringe valve, refer to the figure: Figure 2-7.

Figure 2-7 Syringe Valve Connections

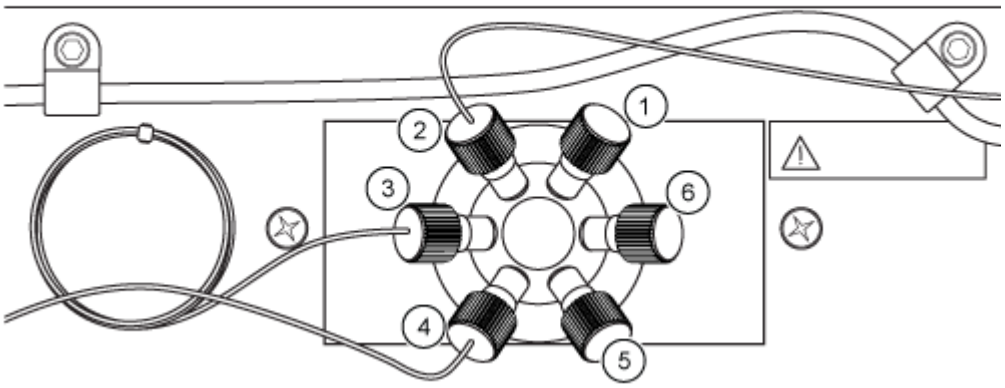


Item	Description
1	Wash bottle in the wash bottle adapter position closest to the autosampler door
2	Wash tubing
3	Waste tubing
4	Buffer tubing
5	Syringe
6	Waste bottle in the bottle adapter position closest to the syringe

Injection Valve Connections

To plumb the 6-port injection valve, refer to the figure: Figure 2-8.

Figure 2-8 Injection Valve Connections



Item	Description
1	PEEK plug, 10-32 coned
2	Sample path to the Intabio ZT system
3	200 µL buffer tubing between syringe valve and injection valve
4	15 µL sample needle
5	PEEK plug, 10-32 coned
6	PEEK plug, 10-32 coned

Connect the Tubing to the Injection Valve

CAUTION: Potential System Damage. Make sure that, after tightening the nut, the tubing is flush with the end of the ferrule to form a tight seal. If the tubing is too long, then the tubing and ferrule will not form a tight seal at the valve. This can cause the connection to leak or the valve internals to be damaged. This might cause carryover, a leak in the connection, or an error with the sample injection.

If it is necessary to install new tubing:
• Make sure that tube ends and ferrule ends are flush.
• To prevent blockage in the flow path, do not overtighten the nuts.

The tubing and ferrule are compressed into the valve when the nut is tightened. To make sure that the connection is leak-resistant, do this:

1. Make sure that the end of the tubing is flush against the end of the ferrule before putting the tubing with the ferrule inside the valve.

   Note: The ferrule on the tubing is compressed in the valve when the nut is tightened. If the ferrule is removed, then it might need to be replaced with a new ferrule to make a tight seal.

2. Make sure that the required nuts, ferrules, and tubings are used.
3. Make sure that the nut and ferrule are over the tubing and that the narrow end of the ferrule is toward the end that will be connected to the valve.
4. Make sure that the tubing in the valve is connected securely before tightening the nut.
5. Make sure that the nut is tight.

   Note: While tightening the nut, use one hand to make sure that the end of the tubing is pressed against the bottom of the valve port.

Autosampler Operation

The autosampler features a two-tray sample compartment that supports adapters for 12 × 10 mL glass vials; 48 × 2 mL glass vials, with or without inserts; and 96-well plates. The sample tray compartment temperature must range from ambient temperature to 4 °C (39 °F) to cool the sample. It is recommended to keep the temperature range between 8 °C (46 °F) and 10 °C (50 °F).

The autosampler has a dual bottle station with one bottle for wash solvent and one bottle for liquid waste. Typically, wash bottle 1 is filled with water for cleaning the fluidic lines of the system between sample injections. Solvents, such as 100% isopropanol (IPA), can be used for stringent

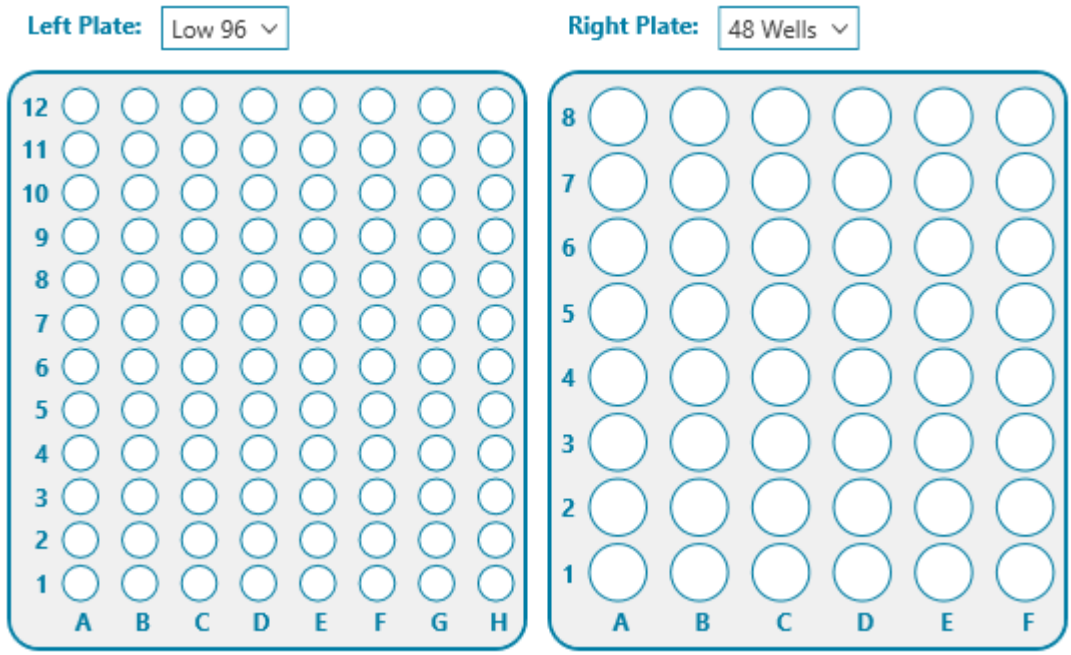
cleaning of the fluidic lines. For more information, refer to the System Helpers in the Intabio software.

Air plugs are aspirated before and after the sample to prevent sample dilution by the autosampler water. Air plugs are not injected, and are instead diverted to the waste stream before and after the sample is injected into the cartridge. After the sample is injected, the Intabio ZT system starts the steps required for separation.

Sample Plates

Samples can be loaded to the sample plates either before or after the injection sequence is configured in the software, but not after a run has begun. The sample plate type used must be selected in the software sequence creation screen before the sample positions are added to the sequence list. Sample well positions in the software match the sample well names on the plates. Make sure that the physical and digital positions of the samples match in the sequence creation screen before proceeding. An incorrect sample position will cause a sample injection to fail.

Figure 2-9 Example of 96-Well Plate and 48-Vial Plate In the Intabio Software



Each sample plate must be positioned with the A1 well in the lower left corner. The autosampler tray shows images of the correct sample plate positioning.

Although the system comes with one 48-vial plate (2 mL vials), one 12-vial plate (10 mL vials), and one 96-well microtiter plate, the system can support up to two plates at a time. The plates can be either the same or different format.

System Bench

CAUTION: Potential System Damage. To prevent damage to the system and mass spectrometer, do not make any bench height adjustments after attaching the system to the mass spectrometer.

The system bench has up and down arrow buttons for height adjustment.

Accessory Components

In addition to the Intabio ZT system and laptop, several other components are essential to successful operation.

The source adapter engages the source interlock and creates a connection between the instrument and mass spectrometer. Push the source adapter against the mass spectrometer interface gently, and then rotate the latches down to lock the source adapter in place.

For more information on installing and configuring the source adapter on the mass spectrometer, refer to the section: Connect to the Mass Spectrometer.

The source adapter identifies the Intabio ZT system on the mass spectrometer.

The nebulizer gas is provided by an external source of zero air to help maintain electrospray ionization. The gas pressure from the external source must be 100 psi (6.9 bar). In the software, users can adjust the nebulizer gas pressure from 0 psi to 90 psi. To remove the nebulizer gas from the gas supply, use the supplied t-piece with the mass spectrometer.

The trigger cable from the source adapter connects to the AUX I/O connector at the left bulkhead of the mass spectrometer.

The cartridge contains:

- A glass chip
- A fluid block with reservoirs for the anolyte, catholyte, and mobilizer solutions, which can be capped
- Ports and electrodes on the bottom for gas, fluidic, and electrical contact with the base of the system

Theory of Operation

About Biotherapeutics and Charge Variants

Biotherapeutics are highly complex, large molecules composed of heterogeneous populations composed of distinct proteoforms that can differ in their physical, chemical, and biological properties, and are measured as differences in their post-translational modifications (PTMs). These differences occur because of cellular processes during bioproduction, chemical modifications during bioprocessing, or storage conditions. The microheterogeneity of protein

therapeutics that results from a population of proteoforms necessitates detailed characterization to ensure Safety, Identity, Strength, Purity, and Quality (SISPQ) of the biotherapeutic. Many PTMs affect charged groups of the biotherapeutic protein, causing changes in the biotherapeutics isoelectric point (pI). These modifications produce charge variants that can be efficiently separated by imaged capillary isoelectric focusing (icIEF).

Imaged Capillary Isoelectric Focusing

Imaged capillary isoelectric focusing (icIEF) is a separation technique that is commonly used in the biopharmaceutical community. icIEF is a popular technique for separating and quantitating the proteoforms of a protein because it is capable of very high-resolution separation at the intact-protein level.

icIEF separates proteoforms by their isoelectric points (pIs) in a gradient of pH generated by ampholyte molecules. A proteoform that is in a pH region below its pI will be positively charged and will migrate toward the negatively charged electrode (cathode). During the migration through a gradient of increasing pH, the charge of the protein decreases until the protein reaches the region in the pH gradient where the pH corresponds to the pI of the proteoform. When it reaches this region, the net charge is zero, at which point an equilibrium between diffusion and electromigration is established. Migration stops and the proteoform is fully focused.

The icIEF process focuses the proteoforms into sharp bands, with each proteoform located at the point in the pH gradient that corresponds to its pI. Focusing of charge variants is monitored and quantitated at the end of the focusing period through UV absorbance imaging. pI markers with well-known pI values are used for qualitative and quantitative analysis. icIEF is a powerful, high-resolution separation approach for separating small differences at the intact protein level. The icIEF focusing process is sensitive enough to resolve proteoforms that differ by only a single charge. Some examples of charge variants include C-terminal lysine variants, proline amidation, glycation, deamidation, succinimide intermediates, and acidic glycans such as sialic acids, among others.

Mass Spectrometry

Mass spectrometry (MS) provides extensive specific information for the identification and characterization of molecules and is also a necessary tool in analytical workflows. The direct coupling of icIEF to MS streamlines decision-making for charge variant peak identification.

Benefits of Using the Intabio ZT System for Peak Identification with icIEF-UV/MS

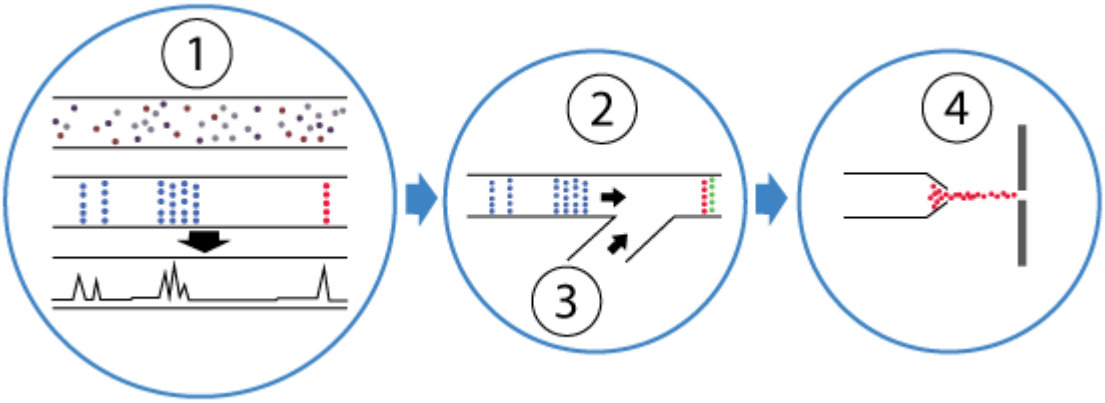
With the Intabio ZT system, scientists can quickly and confidently accelerate decision-making with reduced risk by achieving separation, quantitation, and direct identification of biopharmaceutical charge variants and their proteoforms. The Intabio ZT system, which can be used throughout the development process, provides comprehensive characterization of charge variant-associated quality attributes. It decreases the process time by coupling icIEF separation and high-resolution mass spectrometry into a single workflow.

Theory of the Workflow

Microfluidic chip-based integrated icIEF-UV/MS technology is a powerful analytical approach for increasing the speed of comprehensive characterizations. The Intabio ZT system uses full-channel UV imaging cIEF (icIEF) for sample separation, followed by on-chip chemical mobilization and electrospray ionization (ESI), to analyze the separated proteoforms in the mass spectrometer. On-chip UV detection enables pI determination and relative quantitation of charge isoforms. On-chip electrospray and MS analysis provide direct mass information to identify proteoforms and their post-translational modifications (PTMs). In some cases, these PTMs are the cause of the isoelectric point differences. Continuous imaging during focusing and mobilization enables peak tracking for qualitative and quantitative UV absorbance analysis.

The Intabio ZT system includes an instrument with the accessories required to operate with a mass spectrometer. A USB-connected laptop with the Intabio software installed is used to control the instrument, develop methods, acquire UV data, and analyze icIEF data. When the system is run as part of a fully-integrated workflow with the mass spectrometer, mass spectrometric data is acquired on the computer connected to the mass spectrometer.

Figure 2-10 View of On-Cartridge Process



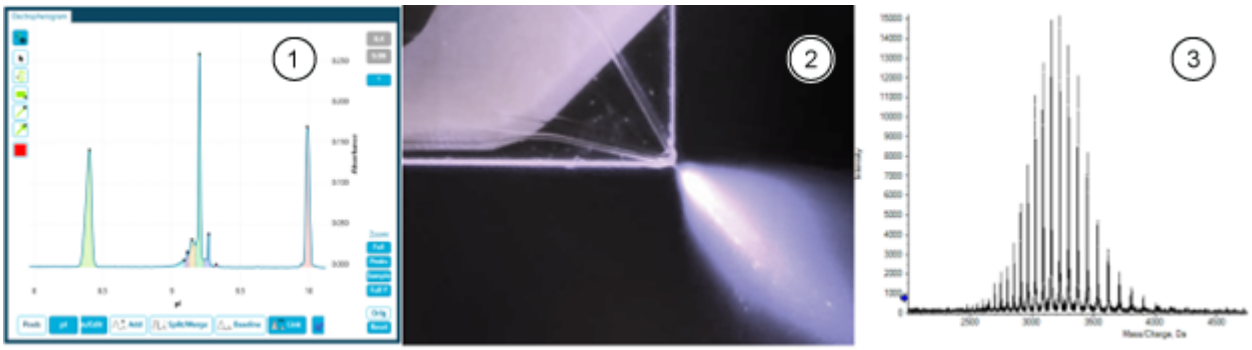
Item	Description
1	Sample focusing and separation
2	Peak mobilization
3	Addition of the acetic acid mobilization solution
4	Electrospray ionization

The electrolytes and mobilizer solutions are formulated to be compatible with mass spectrometry and include acetonitrile, water, diethylamine (DEA), acetic acid, and formic acid. The sample solutions consist of the sample mixed with pI markers, ampholytes, solubilizers, and stacking

reagents. At the beginning of a run, the sample solution is automatically injected onto the separation channel on the chip.

A pH gradient composed of ampholytes is created by applying voltages across the separation channel from the anode to the cathode. The separation process is monitored using 280-nm full-channel UV-based imaging for relative quantitation of the charge-variant peaks. After icIEF focusing of the sample peaks, the peaks are chemically mobilized by adjusting the voltage and driving the sample to the electrospray tip. The separated peaks are then introduced to the adjacent high-resolution mass spectrometer (HRMS) through on-chip electrospray ionization (ESI). Continuous pneumatic nebulization with nitrogen gas improves ESI plume stability and the mass spectrometer signal.

Figure 2-11 icIEF-UV/MS Separation, Focusing, and MS Detection



Item	Description
1	A UV absorbance trace from before mobilization showing focused sample with pI markers
2	Gas assisted electrospray plume
3	Raw mass spectrum

The mass spectrometric analysis benefits both from the pre-separation of proteoforms and the online concentration of the proteoforms with icIEF focusing. The resulting MS data can be analyzed as conventional MS data correlated with the icIEF-UV profile or it can be used to do fully integrated comprehensive analysis of charge heterogeneity, all with the same laptop. The correlation of the UV and MS data gives mass characterization an additional pI dimension to assist in the identification of PTMs.

Operating Instructions 3

Required Materials

The following materials are required for successful integrated icIEF-UV/MS operation:

Note: For information about consumables and spare parts, refer to the document: *Parts and Equipment Guide*.

Equipment
• The Intabio ZT system on an adjustable bench • The source adapter for the mass spectrometer interface • The mass spectrometer with the OptiFlow interface installed **Note:** The mass spectrometer is operating within specifications. • An Intabio ZT cartridge with new caps for fluid block reservoirs • Intabio system—MS Alignment Tool • Intabio system—Cartridge Cap Installation Fixture • Intabio system—System Priming Cartridge • Spin desalting column, 7K MWCO (0.5 mL)

Reagents
• Fresh 18 MΩ water • Acetonitrile • Acetic acid, up to 25% • Formic acid, up to 1% • Diethylamine 1% • Isopropanol (for cleaning)

Customer-Supplied Equipment
• Autopipettes and tips (P20, P200, P1000) • Sample and sample containers: 2 mL glass vials, with or without inserts, with caps, or 96-well plates with plate seals • 1.5 mL microcentrifuge tubes with caps removed for infusion sample • Sample with ampholytes and marker, as required

Intabio ZT System Workflow

Note: To shut down the system between uses, refer to the section: Shut Down the System.

Note: To access the System Helpers in the Intabio software, press **F1**.

To complete the necessary steps to prepare and use the Intabio ZT system, follow this workflow:

Step	To do this...	Refer to...
1	Start the system	Refer to the section: Start the System.
2	Connect to the mass spectrometer	Refer to the section: Connect to the Mass Spectrometer.
3	Prepare the autosampler	Refer to the section: Prepare the Autosampler.
4	Install or replace the cartridge	Refer to the section: Install or Replace the Cartridge or the System Helper: **Install or Replace the Cartridge**.
5	(Optional) Optimize the position for the ESI infusion	Refer to the section: (Optional) Optimize the Position for ESI Infusion or the System Helper: **Optimize the Position for ESI Infusion**.
6	Prepare the reagents	Refer to the document: *Intabio Software User Guide*.
7	Prime the base of the cartridge	Refer to the document: *Intabio Software User Guide*.

Step	To do this...	Refer to...
8	Prepare the cartridge	Refer to the document: *Intabio Software User Guide*.
9	Complete a system performance check	Refer to the section: System Performance Check or the System Helper: **System Performance Check**.
10	Prepare the samples for analysis	Refer to the section: Prepare the Samples for Analysis.
11	Load the cartridge in the system	Refer to the document: *Intabio Software User Guide*.
12	(Optional) Develop a method	Refer to the section: (Optional) Develop a Method.
13	Prepare to run a sequence	Refer to the section: Prepare to Run a Sequence or the System Helper: **Prepare to Run a Sequence**.
14	Analyze the data	Refer to the document: *Intabio Software User Guide*.

Start the System

Note: The Intabio ZT system can stay turned on and idle, and does not need to be shut down when not in use. For short term and long term shutdown procedures, press **F1** for more information in the document: *Help*.

Note: For access to operational controls to see inside of the system, press **F1** for more information in the document: *Help*

1. To turn on the system after a complete shutdown, turn on the power switch on the back panel. Make sure that the laptop has been turned on, and that the Intabio software is open.
2. Make sure that the cartridge clamp is down, and that all of the doors of the system are closed.

   Note: The laser, UV, and HV do not operate if the doors are open, and the stage will not move if the clamp is open.
3. To turn the laser and camera on and off in the Intabio software, click the **Light** and **Laser** toggle buttons.

Tip! When the system is shut down, the motors let the stage move to its unenergized position.

4. To move the stage to the home position, select the **Home** button in the Staging section of the side panel, and then wait a few minutes.

If the system is connected to the mass spectrometer, then use the System Helper: **Home and Align to the MS**. This helper can also be used to align to the mass spectrometer to set the operation positions.

If the system is being operated without being connected to the mass spectrometer, then use the System Helper: **Set the Stage Positions for Offline Operation**.

Connect to the Mass Spectrometer

WARNING! Hot Surface Hazard. Let the ion source interface cool before connecting the mass spectrometer.

CAUTION: Potential Wrong Result. Make sure that the mass spectrometer is calibrated. If the system is not calibrated properly, then a mass might be identified incorrectly or quantitation might be inaccurate.

Note: Do this procedure if the Intabio ZT system is disconnected from the mass spectrometer.

Note: Users must either calibrate with the calibrant delivery system (CDS) in normal tuning mode before connecting the Intabio ZT system, or do a post acquisition calibration based on the infusion instructions that are prompted by the System Helper.

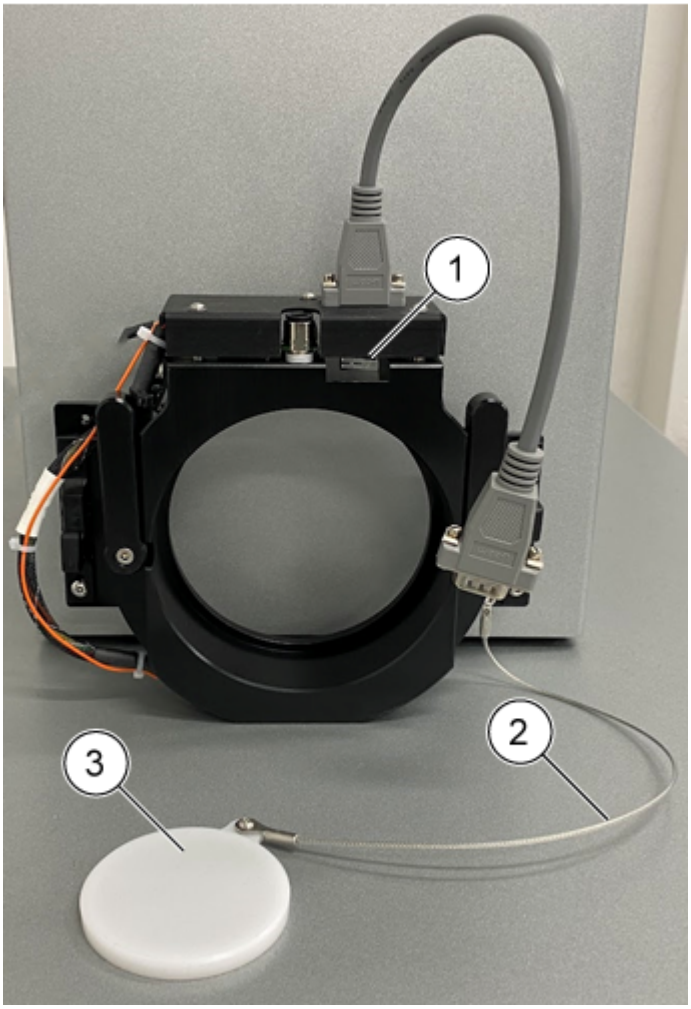
Note: To test basic system integration, use the System Helper: **System Performance Check**.

Prerequisite Procedures
• Make sure that the system bench is not too close to the ion source on the mass spectrometer. **Note:** The bench can be manually moved closer after the ion source is removed.

1. Put the mass spectrometer in Standby state. Refer to the documentation that comes with the mass spectrometer.
2. Remove the ion source. Refer to the documentation that comes with the ion source.
3. Make sure that the OptiFlow interface is installed on the mass spectrometer.
4. Make sure that the flat curtain plate is attached to the mass spectrometer.

5. Align the pins on the mass spectrometer adapter with the corresponding holes, and then turn the side handles to lock the adapter on the interface.
6. Connect the trigger cable to the AUX I/O connector, which is on the left side of the mass spectrometer.
7. Make sure that the exhaust port under the ion source on the mass spectrometer is covered by the adapter ring. The adapter ring is connected to the adapter by a cable.

Figure 3-1 Adapter Ring and Cable



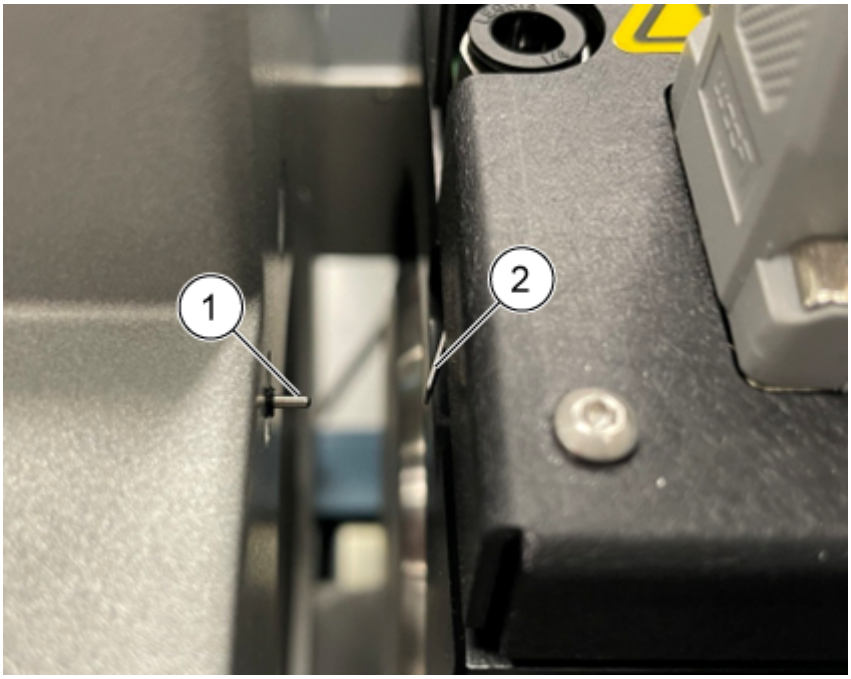
Item	Description
1	Interlock switch
2	Cable

Item	Description
3	Exhaust cap

8. To align the system, slowly move the bench toward the mass spectrometer. Make sure that the hooks on the ion source align with their related holes on the adapter ring.
9. Adjust the height of the bench to align the holes on the adapter with the hooks on the Intabio ZT system.
10. Slowly move the system to the mass spectrometer until the system and mass spectrometer are connected and the mating surface of the Intabio ZT system and the mass spectrometer are parallel to each other.

Note: The interlock plunger on the Intabio ZT system must be in contact with the interlock switch and the interlock switch must be pressed in.

Figure 3-2 System Connections



Item	Description
1	Spring plunger
2	Interlock switch

11. Connect the nebulizer gas line and the source interlock cable.

12. To make sure that interlock is enabled, take the mass spectrometer out of Standby state. The top three LEDs on the mass spectrometer should illuminate.

Note: After the system has been connected to the mass spectrometer, record the height of the system bench.

Prepare the Autosampler

WARNING! Personal Injury Hazard. Do not put hands inside the system while the system is running.

CAUTION: Potential System Damage. Do not put the system in an area subject to excessive dust or shocks.

CAUTION: Potential System Damage. Do not put the system near a source of heat or in direct sunlight. Exposure to heat might influence the internal system temperatures and the cooling capabilities of the autosampler.

Note: Always prime the autosampler at the beginning of each day to prevent air plugs from forming in the fluidic lines.

When selecting the operating site for the Intabio ZT system, make sure that at least 12.7 cm (5 inches) of open space is available on the left side of the system for optimal cooling performance.

Note: Objects on top of the autosampler can have an effect on the cooling capabilities.

Do the following steps at installation:

1. Put the wash bottles in the wash bottle station. Refer to the section: Syringe Valve Connections.
2. Make sure that the 6-port injection valve plumbing is connected. Refer to the section: Injection Valve Connections.
3. Make sure that the syringe valve is connected to the wash bottles. Refer to the section: Connect the Autosampler Tubing.
4. Before the first use, fill the syringe. Refer to the section: Prime the Syringe.

Connect the Autosampler Tubing

WARNING! Flammable Chemical Hazard, Biohazard, Ionizing Radiation Hazard, and Toxic Chemical Hazard. Be sure to use the system in a well-ventilated laboratory environment in compliance with local regulations and with appropriate air exchange for the work performed. Solvents used in high performance liquid chromatography are flammable and toxic. Empty the waste container regularly to prevent it from overflowing. Clean the overflow hole if waste does overflow.

The waste drain system removes all flushing fluids and condensate waste from the autosampler.

Note: If the tubing needs to be replaced, then do this:

1. Make sure that the end of the tubing is flush with the end of the ferrule.
2. Do not overtighten nuts. Nuts that are too tight can cause blockages in the flow path.

1. Connect the tubing to the right side of the syringe valve closest to the autosampler door, and then route the tubing to wash bottle 1. Refer to item 3 in the figure: Figure 2-7.
2. Connect the tubing to the left side of the syringe valve, and then route the tubing to wash bottle 2.
3. Connect the buffer tubing to the center port of the syringe valve and then connect the tubing to port 3 of the injection valve.
4. Connect the drain tubing to the bottom left side of the autosampler.

   Tip! The waste tubing can be installed without removing the front cover of the autosampler.

5. Install the waste bottle under the module.
6. Connect the drain tubing to the autosampler waste bottle. Examine the tubing for kinks that can prevent the liquid from draining and can cause flooding at the autosampler waste drainage site.

   Note: Make sure that the tubing is routed so that there are no parts that prevent liquid from flowing through the waste tube.

Prime the Syringe

CAUTION: Potential System Damage. Do not use salts or buffer solutions. Crystals can block or damage the system.

Note: During installation, the autosampler configuration is created and saved in the ServiceLink software.

Note: Tapping the syringe lightly as the wash solvent is dispensed to the syringe waste might speed up air bubble removal. Air in the syringe affects assay performance because it causes improper volumes to be aspirated or dispensed by the autosampler.

Note: If tubing connections are correctly installed but have leaks, then remove the fitting and ferrule at the leaking connection and replace them with a new fitting and ferrule.

1. Make sure that water has been added to wash bottle 1.
2. Put the end of the wash bottle 1 tubing in wash bottle 1.
3. Open the Intabio software.
4. Click the System Helpers.
5. Open the System Helper: **Prime the Fluidics and Wash the Autosampler**.
6. In the Make Sure that the Autosampler is Ready section, click **Do**.
   The autosampler begins a wash sequence.
7. If there is still air in the syringe, then replace the contents of wash bottle 1 with isopropanol, and then repeat step 6 until the syringe is full of liquid.
8. After the tubing and syringe are filled, replace the wash solvent in wash bottle 1 with LC-MS-grade water.
9. To begin a standard wash routine, repeat step 6.

Install or Replace the Cartridge

WARNING! Puncture Hazard. Keep hands out of covered areas to avoid injury and smudging the optical lens.

CAUTION: Potential System Damage. Do not adjust the mirror or laser while the cartridge is being loaded. Doing so will misalign the optics.

Note: To complete this procedure, use the System Helper: **Install or Replace the Cartridge**.

Note: After the cartridge is secured, make sure that all of the doors on the system are closed.

Note: After removing an old cartridge, put it on a surface with its port side down to minimize the dampness of the membranes in the cartridge fluid block.

Required Materials
• The cartridge with three new caps • Clear syringes or an P200 autopipette with tips that can dispense 165 µL • Intabio system—Cartridge Cap Installation Fixture

(Optional) Optimize the Position for ESI Infusion

Note: To complete this procedure, use the System Helper: **Optimize the Position for ESI Infusion**.

Prerequisite Procedures
• An infusion solution is prepared, consisting of the sample diluted in the mobilizer solution. • The SCIEX OS software is open on the computer that controls the mass spectrometer.

System Performance Check

Note: To complete this procedure, use the System Helper: **System Performance Check**.

Prepare the Samples for Analysis

During preparation of the analyte of interest, record the concentration information for each sample.

Tip! If the sample was desalted for other methods, then the sample must be desalted before analysis with the system.

Note: Fill the 2 mL analyte sample vials with a maximum of 1.5 mL and a minimum of 0.400 mL of solution. For vials with 300 µL inserts, use a volume of sample between 100 µL and 250 µL.

Do not overfill the vials.

1. In a microfuge tube, add 80 µg of the compound of interest to every 200 µL of the icIEF mixture, which includes ampholytes, markers, and stackers.

   Tip! For the platform method of separation, use an equal amount of carrier ampholytes pH 5-8 and pH 8-10.5.

2. Add markers to generate pI information. Make sure that the makers are within linear areas of the gradient with the anticipated sample pI.

   Note: Priming might need to be done more frequently with smaller batches in a centrifuge.

3. If there are solubility issues, then add compounds to the mixture to make sure that the separation is in the window of interest.

   Note: For proteins that aggregate or precipitate during focusing, add formamide to improve solubility.

4. If required, use a pipette to remove any bubbles that are in the sample vial.
5. Add the sample to the tube, and then mix for 3 minutes in a vortex mixer.

(Optional) Develop a Method

CAUTION: Potential System Damage. To prevent system failure, do not use urea with the mass spectrometer.

CAUTION: Potential System Damage. To prevent degradation of the performance of the mass spectrometer, do not use surfactants with the mass spectrometer. If samples contain any detergents or surfactants, then make sure to remove them by using removal spin columns.

CAUTION: Potential System Damage. Do not use concentrations of salt higher than 15 mM. A high concentration of salt and buffer components in the sample can generate high current, greater than 30 µA, which can compress the pH gradient. High concentrations can also cause significant loss in resolution, denature the protein, and decrease the lifespan of the cartridge.

Prerequisite Procedures

- Refer to the document: *Intabio system—Performance Test Mix Product Insert*.
- Make sure that the electrolytes and the formic acid solution are at room temperature. This usually takes 15 minutes to 20 minutes.
- Dilute pI markers 100-fold in the sample solution.
- If the sample requires urea for protein precipitation, then use 10% to 20% formamide to improve solubility.
- Keep the samples on ice until the Intabio ZT system is ready for analysis.
- Make sure that the maximum peak height is less than 0.3 AU.

For troubleshooting, refer to the section: icIEF-UV/MS Method Errors.

Assess ESI Infusion

Note: For desalting methods, refer to the documentation that comes with the spin columns.

The manufacturer recommends a centrifuge speed of 1500 × *g*. Spin 1 minute for storage solution removal and washes and 2 minutes for sample recovery.

Note: For information about consumables and spare parts, refer to the document: *Parts and Equipment Guide*.

1. Remove the bottom closure from the column and then loosen cap. Do not remove cap.
2. Put the column in a collection tube and then spin for 2 minutes to remove the storage solution. Discard the flow-through.
3. Add 300 µL of water on top of the resin and then centrifuge for 2 minutes. Discard the flow-through.

   Repeat this step twice.
4. Blot the bottom of the column to remove any excess liquid and then move the column to a new collection tube.
5. Add 100 µL of the 10 mg/mL NISTmAb standard on top of the resin.
6. Spin the sample in the column for 2 minutes and keep the flow-through that contains the sample. Discard the spin column.
7. Prepare the sample to achieve the final sample concentration of 100 µg/mL. Refer to the tables: Table 3-1 and Table 3-2.

Table 3-1 Infusion Sample Preparation

Master Mix Component	Volume (µL)
10 mg/mL desalted NISTmAb	5
Mobilizer solution (premixed)	495
Total volume	500

Table 3-2 Infusion and Tune File Parameters

Parameter	SCIEX MS
Scan Type	TOF MS
Scan range	2000 to 6000
Curtain gas (psi)	25

Table 3-2 Infusion and Tune File Parameters (continued)

Parameter	SCIEX MS
Nano cell temperature (°C)	1200
Declustering potential (V)	190
Collision energy (V)	55
Accumulation time (s)	0.5
Time bins to sum (s)	150
Polarity	Positive
QJET RF amplitude (V)	270

8. During infusion, optimize the X, Y, and Z coordinates for a maximum signal over 5 mm to 10 mm.

Note: Coordinates can be optimized using 1 mm steps, and then smaller steps, such as 0.1 mm, near the point of optimization.

Note: Make sure that the electrospray is stable.

If the electrospray is highly unstable, if the ESI tip is dripping, or if there is a high total ion chromatogram (TIC) variation, then do this:

1. Make sure that the nitrogen and nebulizer gases are on.
2. Make sure that there are no bubbles in the cartridge. Adjust the pressure of the channel that has bubbles to push the bubbles out of the cartridge through the waste tube.
3. Prime the fluidic lines again.

Assess icIEF-UV/MS System Suitability

For instructions, refer to the section: *System Performance Check* in the Intabio software *Help*.

Finalize the Method

Prerequisite Procedures
• Refer to the sections: Assess ESI Infusion and Assess icIEF-UV/MS System Suitability.

Development of the method starts with a broad pH range method and the optimization of parameters. For molecules with more complex peak profiles or limited solubility, the optimization of a method is done with the troubleshooting guidelines in these steps:

1. Make sure that there is at least 80 µg of the sample in the 200 µL icIEF Master Mix solution.
2. Desalt the sample if it has a high salt concentration or a low protein concentration.
3. Prepare the samples. Refer to the table: Table 3-3

Table 3-3 Sample Preparation

Master Mix Component	Volume (µL)	%
Carrier ampholytes pH 5-8	6	3
Carrier ampholytes pH 8-10.5	6	3
pI Marker 1	2	1
pI Marker 2	2	1
500 mM arginine	5	2.5
10 mg/mL Sample (80 µg)	8	4
Fresh 18 MΩ water	171	85.5
Total volume	200	100

4. Spin the tube in a centrifuge at 1000 × *g* for 3 minutes.
5. Add 200 µL of the sample in an autosampler vial or plate.

Note: Make sure to remove any bubbles at the bottom of the sample vial or well.

Note: For examples of methods, refer to the table: Table 3-4. For all separation and mobilization methods, the scan rate must be 1.

Table 3-4 icIEF Methods

Method	Type	Anode (V)	Cathode Mode	Mobilization Mode	Setpoint	Duration (s)
1	Separation	1500	(V)	Current	0	60
2	Separation	3000	(V)	Current	0	60
3	Separation	4500	(V)	Current	0	240
4	Mobilization	8500	(I)	Maintain	5500	600

Note: The duration might have to be adjusted based on the molecule. To optimize the duration, refer to the table: Table 3-5.

Table 3-5 Duration Range for Separation and Mobilization

Steps	Duration (s)
1	60
2	60
3	240 to 540
4	360 to 600

Prepare to Run a Sequence

Note: To complete this procedure, use the System Helper: **Prepare to Run a Sequence**.

Prerequisite Procedures
• The nebulizer gas is turned on. • The cartridge is installed on the system.

Setup the Mass Spectrometer

Prerequisite Procedures
• The Intabio ZT system has been connected to the mass spectrometer.

1. Make sure that a mass spectrometer method has been made that is 10 minutes long.
2. Make sure that the mass spectrometer is configured to wait for a trigger after contact closure.
3. Make sure that the sample names in SCIEX OS software correspond with the order in the Intabio software.
4. Click **Submit Batch**.
   The mass spectrometer indicates that it is loading to signal that equilibration has begun.

   Note: If the system does not immediately begin equilibrating, then click **Start** in the Queue screen for the appropriate batch.

   Note: If the system does not wait on the loading screen, go to the configuration screen on the mass spectrometer to configure the system to wait for a trigger after contact closure.

Shut Down the System

To complete this procedure, use the System Helper: **System Shutdown Checklist**.

Note: In cases of extended downtime, purge the system fluids with isopropanol, and then leave the cartridge ports covered.

Maintenance 4

WARNING! Fire Hazard or Electrical Shock Hazard. Always turn off the power and then disconnect the system from the mains supply before performing inspection and maintenance. Otherwise, fire, electric shock, or a malfunction might occur.

WARNING! Ionizing Radiation Hazard, Biohazard, or Toxic Chemical Hazard. Before cleaning or maintenance, identify whether decontamination is required. If radioactive materials, biological agents, or toxic chemicals have been used with the system, then the customer must decontaminate the system before cleaning or maintenance.

CAUTION: Potential System Damage. Only use the replacement parts specified in the documentation that comes with the system. Use of any other parts might result in system damage and malfunction.

This section contains information about the maintenance and care of the system. It also provides instructions for maintenance tasks that can be performed by the customer. Regularly clean and maintain the Intabio ZT system for optimal performance. For maintenance procedures that are not included in this guide, contact sciex.com/request-support.

Recommended Maintenance Schedule

A maintenance task for the Intabio ZT system consists of the cleaning or replacement of a system component or component part. Cleaning or replacement of a component or part is required when any one of the following occurs:

- Upon daily inspection, the module, or the area surrounding it, is visibly soiled with spilled fluid or coated with a build-up of dirt or dust.
- The module is determined to be source of a degradation in system performance.
- Usage of the module has been tracked and the number of times that the part has been used reaches or exceeds its maximum usage recommendation.
- The interval for periodic cleaning or replacement of the module has been reached.

Table 4-1 Intabio ZT Maintenance Schedule

Maintenance Task	Frequency	Remarks
Condensation removal	Daily	Condensation can be found in the sample plate or sample tray inserts of the autosampler.
Sample and reagent vial disposal	After each run	To avoid contamination, do not use the same sample and reagent vials in different runs.
Unused reagent disposal and replacement	Daily	Dispose of any unused acetonitrile, acetic acid, and formic acid electrolytes, and then replenish the vials with fresh solutions.
Tuning solution replacement	Before tuning the mass spectrometer	The MS does not have a tuning solution bottle.
Module surface cleaning	Daily	Refer to the section: Clean the Module Surfaces.
Regular system maintenance	Weekly or as needed. Symptoms include: the signal is decreasing or there are changes to voltage or current profiles of the icIEF or UV data.	Refer to the section: Regular Intabio ZT System Maintenance.
Cartridge cleaning	As needed. Symptoms include: visible debris or fibers on the ports on either the bottom of the cartridge, or on the glass chip surface.	Refer to the section: Best Practices for Handling the Intabio ZT cartridge.
Fuse replacement	As needed. Symptoms include: the unit does not start up.	Refer to the section: Replace the Fuse.
General autosampler cleaning	As needed. Symptoms include: dust, debris, or liquid on the autosampler surfaces, including the sample tray and the valve leak bin.	Clean the surface, valve leak bin, sample tray, and drain tubing. Refer to the section: Autosampler Cleaning.

Table 4-1 Intabio ZT Maintenance Schedule (continued)

Maintenance Task	Frequency	Remarks
Autosampler rotor seal replacement and cleaning	Annually or as needed. Symptoms include: the signal is decreasing, the pressure is high in the sample line, or liquid is dripping from the valve.	Refer to the section: Replace and Clean the Rotor Seal.
Autosampler sample needle replacement	As needed. Symptoms include: the needle is bent or otherwise damaged, or the signal is decreasing.	Refer to the section: Replace the Sample Needle.
Autosampler air needle replacement	As needed. Symptoms include: the needle is damaged or a different length of needle is required.	Refer to the section: Replace the Air Needle.
Autosampler syringe replacement	As needed. Symptoms include: the syringe contains air bubbles that cannot be removed by flushing, or the signal is decreasing.	Refer to the section: Replace the Syringe.
Autosampler stator cleaning	As needed. Symptoms include: the signal is decreasing, the pressure is high in the sample line, or liquid is dripping from the valve.	Refer to the section: Clean the Stator.
Autosampler buffer tubing replacement	As needed. Symptoms include: the signal is decreasing from blockages or kinks in the tubing.	Refer to the section: Replace the Buffer Tubing.
Reagent and consumable replacement	As needed. Symptoms include: reagents or consumables run out or expire.	Contact sciex.com/request-support.

Regular Intabio ZT System Maintenance

Note: The washing cartridge must be set to 500 mbar.

Note: Dilute the catholyte to 0.25% diethylamine (DEA) with fresh 18 MΩ water by making a 1:3 dilution.

1. Fill all of the reagent vials in the reagent vial manifold with 100% acetonitrile.
2. Do a wash for 30 minutes.
3. Fill all of the vials with fresh 18 MΩ water.
4. To remove any residual acetonitrile solution, do a wash for 30 minutes.
5. Fill the first vial with water, and then fill the anolyte, catholyte, and mobilizer vials with the corresponding reagents.
6. To equilibrate the reagents in the fluidic lines, do a wash for 30 minutes.

The system should be primed daily. To prime the system, do this:

1. Replace the contents of wash bottle 1 and the sample vial on the reagent vial manifold with fresh 18 MΩ water.
2. Replace the contents of the anolyte, catholyte, and mobilizer reagent vials in the reagent vial manifold with the applicable reagents.
3. Use the reagent vial manifold to prime the fluidic paths of the autosampler and the Intabio ZT system.

Clean the Module Surfaces

Required Materials
• Dry, soft rags, or tissue paper • For persistent stains: water

CAUTION: Potential System Damage. Do not let spilled water stay on the instrument surface, and do not use alcohol or thinner-type solvents to clean the surfaces.

1. Wipe the module surfaces with the rag or tissue paper.
2. If the stains persist, then do this:
    a. Dampen a rag in water, and then wring it dry.
    b. Wipe the module surfaces.
    c. Dry the surfaces with a dry rag.

Best Practices for Handling the Intabio ZT cartridge

CAUTION: Potential System Damage. Do not touch the recessed optical windows of the cartridge, the liquid seals, or electrodes on the bottom of the cartridge. Fingerprints on the chip can disrupt proper imaging.

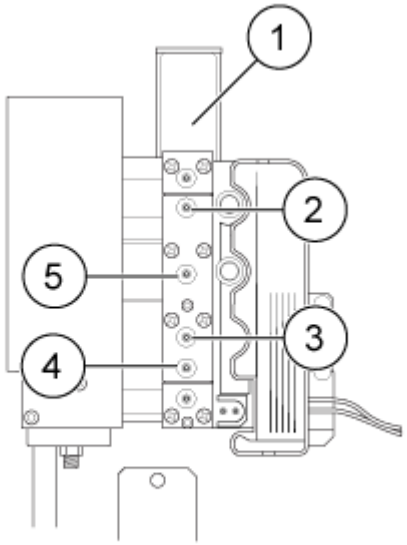
CAUTION: Potential System Damage. Make sure that the cartridge chip tip does not come in contact with any surfaces when the cartridge is handled or removed from its packaging. Damage to the chip tip can cause poor electrospray, which can lead to poor mass spectrometer data acquisition.

CAUTION: Potential System Damage. Make sure to always add anolyte to the reservoir on the left, catholyte to the reservoir in the middle, and mobilizer to the reservoir on the right. If electrolytes are added to the wrong reservoir, then the cartridge might be damaged. These reservoirs are labeled with letters that correspond with the correct solutions. Refer to the table: Table 4-2.

Table 4-2 Cartridge Labels

Letter	Reagent	Contents
A	Anolyte	Formic acid
C	Catholyte	Diethylamine
M	Mobilizer	Acetonitrile and acetic acid

Figure 4-1 Intabio ZT cartridge



Item	Description
1	Drip shield
2	Mobilizer port
3	Anolyte port
4	Sample port
5	Catholyte port

The Intabio ZT cartridge is shipped dry, and is designed to run a batch of injections at once. The cartridge must be replaced after one batch of injections is complete. When using a cartridge, keep this in mind:

- Only handle the cartridges with clean gloves.
- If spikes are visible in the UV data, then clean the outside of the cartridge with canned air.

To clean the Intabio ZT cartridge when spikes are visible, do this:

1. Put the nozzle or the opening of the air source 25.4 cm (10 inches) to 30.5 cm (12 inches) from the cartridge surface. Then, press the aerosol actuator down about halfway for a gentle flow of air.
2. Sweep the air stream across the entire length of the optical window.

3. Turn the cartridge over and then repeat steps 1 and 2.
4. Turn the cartridge over again and then gently clean the top surface of the cartridge with a dry rag before reinstalling the cartridge.

Autosampler Maintenance

Before all maintenance procedures, do this:

1. Open the door of the autosampler.
2. If necessary, remove the insulation cover by pulling it out slowly.

Note: Do not disconnect the autosampler from the power supply. The autosampler must stay on for the System Helpers in the Intabio software to be used.

Autosampler Cleaning

To prime the autosampler, use the System Helper: **Prime the Fluidics and Wash the Autosampler**. If the outside of the autosampler becomes dirty, then wipe it with a rag dampened with isopropanol or water. Other types of general maintenance might include:

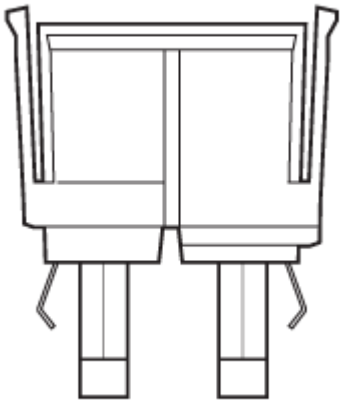
- **Valve leak bin**: A special leak bin is installed below the injection valve. Clean this bin with a rag dampened with isopropanol or water.
- **Sample tray**: If the sample has been spilled on the sample tray, then clean the tray with a rag dampened with isopropanol or water.
- **Drain tubing**: Regularly flush the drain tubing with solvent to prevent blockages and to remove any liquids and condensate.

Replace the Fuse

WARNING! Fire Hazard or Electrical Shock Hazard. Before replacing fuses, turn off the system and disconnect it from the mains supply. Replace a fuse only with a fuse of the correct type and rating. Failure to follow these guidelines might result in fire, electric shock, or instrument malfunction.

Required Materials
• 10 A 250 V fuse, marked T10A250V • Small, flat-bladed screwdriver

Figure 4-2 Fuse



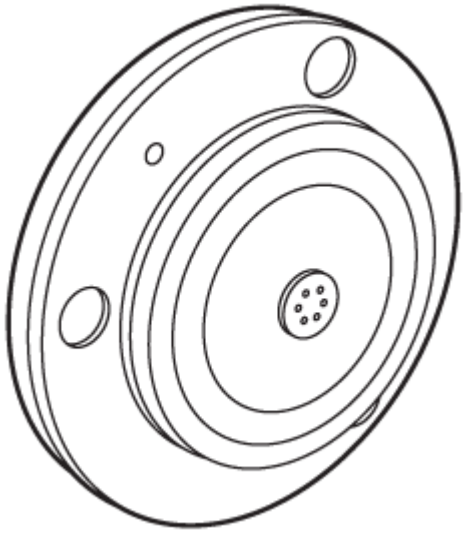
1. Turn off the system.
2. Disconnect the mains supply cable from the mains supply outlet and from the back of the system.
3. Use a small flat-bladed screwdriver to remove the fuse holder located above the connector for the mains supply cable.
4. Remove the fuse from the fuse-carrier assembly.
5. Install the fuse in the fuse-carrier assembly, and then install the assembly in the system.
6. Connect the mains supply cable to the back of the system and the mains supply outlet.
7. Turn on the system.
8. On the desktop of the laptop, open the Intabio software and then log on to the software.
9. If the system does not operate normally, or if the fuse blows again, then contact sciex.com/request-support.

Clean the Stator

Prerequisite Procedures
• Open the door on the autosampler. • Disconnect the tubings from the valve.

Required Materials
• 3 mm hex key

Figure 4-3 Stator



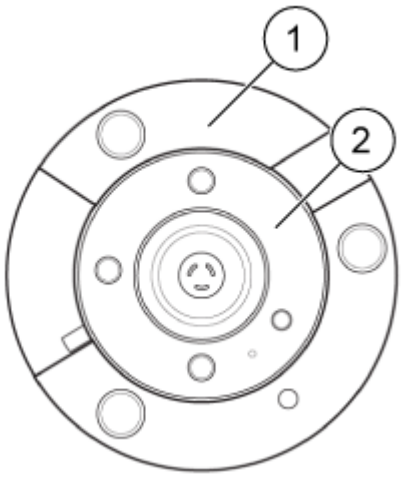
1. Disconnect all of the tubing and fittings from the valve.
2. Remove the three hex screws at the front of the valve. Alternately loosen the screws by half a turn until the tension on the spring assembly is released.
3. Gently remove the stator.
4. Clean the stator by sonicating it in isopropanol for 10 minutes. Refer to the figure: Figure 4-3. Make sure that the side shown in the figure is facing up when sonicating the stator.
5. Install the stator on the valve.
6. Put the three hex screws in the holes, and then tighten them.
7. Connect all of the tubing and fittings to the valve again.
8. To complete an autosampler wash, use the System Helper: **Prime the Fluidics and Wash the Autosampler**.
   The autosampler is now ready for use.

Replace and Clean the Rotor Seal

Prerequisite Procedures
• Open the door on the autosampler. • Disconnect the capillaries from the valve. • Remove the stator. Refer to the section: Clean the Stator.

Required Materials
• 3 mm hex key

Figure 4-4 Valve Components



Item	Description
1	Valve body
2	Rotor seal

1. Gently remove the rotor seal.
2. Clean the rotor seal by sonicating it in isopropanol for 10 minutes.
3. Dry and replace the rotor seal.
4. Put the stator back on the valve.

5. Put the three hex screws in the holes, and then tighten them.
6. Connect all of the tubing and fittings to the valve again.
7. To complete an autosampler wash, use the System Helper: **Prime the Fluidics and Wash the Autosampler**.
   The autosampler is now ready for use.

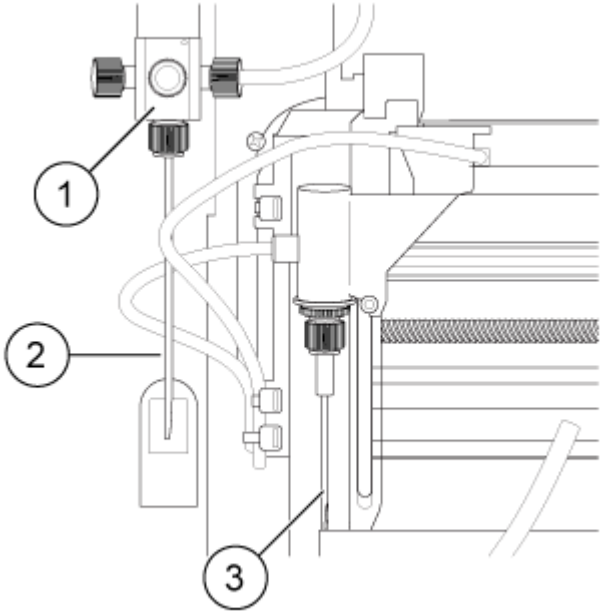
Replace the Buffer Tubing

1. Rotate the tubing fittings counterclockwise to disconnect the buffer tubing from the center port of the syringe valve. Refer to the figure: Figure 2-7.
2. Rotate the tubing fittings counterclockwise to disconnect the buffer tubing from port 3 of the autosampler injection valve.
3. Remove the buffer tubing from the autosampler inside of the Intabio ZT system.
4. Rotate the tubing fittings clockwise to connect the new buffer tubing to the center port of the syringe valve.
5. Rotate the tubing fittings clockwise to connect the new buffer tubing to port 3 of the autosampler injection valve.
6. To remove air from the new buffer tubing, use the System Helpers in the Intabio software to prime the autosampler.

Replace the Sample Needle

Prerequisite Procedures
• Close the Intabio software.

Figure 4-5 Sample Needle



Item	Description
1	Syringe valve
2	Syringe
3	Air needle

1. Open the Maintenance section in the ServiceLink software.
2. Click **Exchange** in the **Needle** section.
   The needle moved into the exchange position.
3. Loosen the air nut.
4. Loosen the nut that connects the tubing to port 4 of the injection valve.
5. Pull the tubing up to remove the needle from the needle assembly.
6. Install a new needle.
7. Make sure that the air nut is tightened to hold the needle assembly firmly.
8. Connect the other end of the needle connection tubing to port 4 of the injection valve.

Note: Do not over-tighten the nut that connects the tubing to the port. Doing so can cause the tubing to become blocked.

9. To move the sample needle to the home position, click **Initialize** in the ServiceLink software.
10. To clean the new needle, complete an autosampler wash.
11. Adjust the needle and tray height settings as required.

Note: If trays with 12-vials or 48-vials are being used, then make sure that the needle height setting is greater than or equal to 2 mm to prevent the needle from touching the bottom of the vials.

Note: If the air needle needs to be replaced, refer to the section: Replace the Air Needle. If the air needle does not need to be replaced, close the ServiceLink software, and then open the Intabio software.

Replace the Air Needle

Prerequisite Procedures
• Remove the sample needle. Refer to the section: Replace the Sample Needle.

1. Remove the air needle from the height adjustment nut.
2. Install the new air needle.
3. Install the sample needle again.
4. To rinse the needle, complete an autosampler wash.
   The needle is ready for use.
5. Close the ServiceLink software, and then open the Intabio software.

Replace the Syringe

1. Fill wash bottle 1 with 150 mL of isopropanol.
2. Close the Intabio software, and then open the ServiceLink software.
3. In the Maintenance section of the ServiceLink software, click **Exchange** in the Syringe section.
4. Disconnect the syringe from the syringe valve.
5. Disconnect the plunger from the syringe drive.
6. Fill the new syringe with wash solvent, preferably isopropanol.
   Make sure that any air bubbles are removed from the syringe.
7. Connect the plunger of the filled syringe to the syringe drive.
8. Install the syringe in the syringe valve, making sure it is tight.

9. In the Maintenance section of the ServiceLink software, click **Home** in the Syringe section. The syringe moves to the home position and then its content is dispensed to the internal syringe waste.
10. If there is air in the syringe, then click **End** in the ServiceLink software to fill the syringe with isopropanol.
11. Repeat steps 7 and 8 until there is no more air in the syringe.
12. After all of the air has been removed from the syringe, replace the contents of wash bottle 1 with LC-MS-grade water, if necessary.
13. To complete two autosampler washes, use the System Helper: **Prime the Fluidics and Wash the Autosampler**.
14. Close the ServiceLink software, and then open the Intabio software.
    The syringe is now ready for use.

Troubleshooting 5

If an issue cannot be resolved by the corrective actions in this section, or if a symptom is not included in the tables in this section, then contact a SCIEX representative.

To avoid some fault conditions, if required, change the duration for each applicable module in the method.

icIEF-UV/MS Method Errors

Issue	Cause	Corrective Action
The signal of the largest peak is greater than 0.3 AU.	The sample concentration too high or there is too much camera saturation.	Reduce the sample concentration or exposure time to avoid camera saturation.
The current is greater than 30 µA.	The ionic strength of the sample is too high.	Desalt or dilute the sample of interest. Prime all of the fluidic lines again, and then examine the cartridge for any air bubbles, precipitation, or blockages.
An unreproducible peak profile is observed.	The sample has too much protein.	Add 20% formamide to the sample solution to increase protein solubility. If the resulting peak profile is reproducible, then no other action is needed. However, if the resulting peak profile is not reproducible, then increase the percentage of formamide to 40%. Optimize focusing time and sample concentration, if required.
The peak resolution is too low.	There is not sufficient carrier ampholytes in the pI region of the sample peaks.	If the pI values of sample peaks are below 8, then add more pH 5-8 narrow range carrier ampholytes. If the pI values of the sample peaks are above 8, then add more pH 8-10.5 narrow range carrier ampholytes.

Issue	Cause	Corrective Action
The intact mass in the deconvoluted MS spectra does not match the theoretical mass.	The MS is not accurate.	Assess the adducts, the mass accuracy of the MS, and the time segments of deconvoluted data. Make sure that the MS ionization conditions are not harsh or causing fermentation.
The ion signal is being suppressed.	The Master Mix is too concentrated.	Dilute the Master Mix of the sample 1:1 with fresh 18 MΩ water.
The deconvoluted MS spectrum and the time segments assigned do not correlate with the icIEF data.	The MS peak resolution is poor.	Examine the mobilization electropherogram to make sure that the peak resolution is kept.

Autosampler

Table 5-1 Autosampler: Analytical Errors

Possible Cause	Corrective Action
Analytical Errors	• Make sure that the application has run previously without errors and that no changes have been made to the analytical system since the last successful run. • Determine whether the fault is caused by the autosampler or other modules in the system.
Errors in the injection and method configuration have resulted in wear.	• Examine the autosampler for wear, especially wear to the rotor seal and syringe.
The volumes of the buffer tubing, sample needle, and syringe are incompatible.	• Make sure that the software settings for the volumes of the syringe, buffer tubing, and sample needle match the volumes of the physically installed parts.
Environmental conditions do not meet the requirements.	• Make sure that the laboratory conditions meets the requirements in the document: *Site Planning Guide*.
Light levels are too high for light-sensitive samples.	• Make sure that light exposure levels are appropriate. • Make sure that the insulation cover is installed over the sample tray area.

Table 5-2 Autosampler: Poor Reproducibility

Possible Cause	Corrective Action
There is air in the aspiration path.	• Initialize the autosampler. Examine the syringe for air bubbles, and then remove them, if applicable.
The syringe is leaking.	• If the syringe is leaking at the top, then make sure that it has been installed correctly, including the PTFE seal. • If the syringe is leaking at the bottom, then replace it.
The syringe valve is leaking.	• Replace the syringe valve. • Examine the valve and then contact sciex.com/request-support.
The rotor seal is worn.	• Replace the rotor seal, and then examine the stator of the valve.
Tubing connections contain dead volume.	• Install new fittings on the tubing connections.
The sample signal has been reduced due to air bubbles in the syringe.	• Remove the air bubbles from the syringe using isopropanol in the wash bottle 1. • Replace the contents of wash bottle 1 with water, and then prime the autosampler.

Table 5-3 Autosampler: Excessively Large Peak for a Blank Sample

Possible Cause	Corrective Action
Fluidic components contain residual sample.	• Flush the sample needle, inside and outside. • Replace the rotor seal. • Replace the tubing and fittings between the autosampler and the fluidic path connections.
A blank sample is contaminated.	• Use a new blank sample.
The cause is unknown.	• Try to resolve the issue by using different solvents and liquids.

Table 5-4 Autosampler: No Injection

Possible Cause	Corrective Action
The flow path is blocked.	• Disconnect the fitting of the needle from the injection valve. • Start flushing the system. • If solvent flows out at the free port (port 4), then examine the needle. • If no solvent flows out at the free port (port 4), then disconnect the buffer tubing from the injection valve (port 3). • Start flushing the system. • If solvent flows out at the open end of the buffer tubing, then examine the rotor seal and stator. • If no solvent flows out of the open end of the buffer tubing, then disconnect it from the syringe valve. • Start flushing the system. • If solvent flows out of syringe valve, then examine the buffer tubing and replace it if required. • If no solvent flows out of the syringe valve, then examine the connections of the flow path to determine whether they are too tight and examine the syringe valve.

Autosampler Messages

If the module shows error messages other than those listed in the following sections, then restart the module once. If error messages are shown repeatedly, then contact sciex.com/request-support.

After resolving the error, press **ENTER** to continue.

Table 5-5 Autosampler Error Messages

Error Message	Description
Autosampler is in run mode.	• Close the software, and then open it again. Turn the module off, and then on.

Table 5-5 Autosampler Error Messages (continued)

Error Message	Description
Autosampler is not responding. Please check communication settings and ensure the device is online.	• Turn the module off, and then on. Make sure that the network configuration is correct. If the message is shown again, then contact sciex.com/request-support.
Cannot run autosampler.	• Turn the module off, and then on. Make sure that the network configuration is correct. If the message is shown again, then contact sciex.com/request-support.
Cannot stop autosampler.	• Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.
Communication port for autosampler was not initialized. Please check the configuration settings.	• Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.
Configuration settings do not match with the device. Run cannot start.	• Correct the parameters in the ServiceLink software.
Deviation of more than ±2 mm towards home.	• Remove any blockages that prevent the vial plate from moving. Make sure that the belt for the vial plate has the correct tension.
Dispenser error.	• Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.
Electronics error.	• Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.
Error occurred during initialization, the Autosampler cannot start.	• Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.
Error resetting output.	• Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.

Table 5-5 Autosampler Error Messages (continued)

Error Message	Description
Error running user defined program.	• Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.
Error setting the tray configuration.	• Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.
Error setting the tray temperature.	• Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.
Home sensor activated when not expected.	• Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.
Home sensor not de-activated.	• Remove any blockages that prevent the vial plate from moving. Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.
Home sensor not reached.	• Remove any blockages that prevent the vial plate from moving. Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.
Horizontal: home sensor activated when not expected.	• Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.
Horizontal: home sensor not de-activated.	• Remove any blockages that prevent the needle unit from moving. Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.
Horizontal: home sensor not reached.	• Remove any blockages that prevent the needle unit from moving. Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.

Table 5-5 Autosampler Error Messages (continued)

Error Message	Description
Horizontal: needle position is unknown.	• Initialize the needle unit using the ServiceLink software.
Incorrect amount of steps executed to reach the home position	• Remove any blockages that prevent horizontal movement or cause too high torque in the movement.
Injection needle unit error.	• Remove any blockages that prevent the needle unit from moving. If the message is shown again, then contact sciex.com/request-support.
Injection valve or ISS unit error.	• Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.
Invalid configuration. ISS option not installed on autosampler. Please switch off this option in configuration dialog.	• Correct the parameters in the ServiceLink software. If the message is shown again, then contact sciex.com/request-support.
Invalid configuration. SSV option not installed on autosampler. Please switch off this option in configuration dialog.	• Correct the parameters in the ServiceLink software. If the message is shown again, then contact sciex.com/request-support.
Invalid instrument is detected.	• Correct the parameters in the ServiceLink software. If the message is shown again, then contact sciex.com/request-support.
Invalid tray temperature (number) °C. The temperature should be between 4 °C and 22 °C.	• Correct the parameters in the Intabio software.
Invalid wait time. The time should be between 0 and 9 h 50 min 59 sec.	• Correct the parameters in the Intabio software.
ISS valve error.	• Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.
Needle movement error.	• Make sure that the position of the needle unit is correct. Turn the module off, and then on.

Table 5-5 Autosampler Error Messages (continued)

Error Message	Description
Missing vial.	• Make sure that the position of the needle unit is correct. Turn the module off, and then on.
No user defined or mix program is running.	• Correct the parameters in the Intabio software.
Setting mix program error.	• Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.
Setting service mode failed.	• Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.
Syringe dispenser unit error.	• Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.
Syringe home sensor not de-activated.	• Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.
Syringe home sensor not reached.	• Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.
Syringe position is unknown.	• Initialize the syringe unit using the ServiceLink software.
Syringe rotation error.	• Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support
Syringe valve did not find destination position.	• Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.
Temperature above 48 °C at cooling ON.	• Turn off cooling and make sure that the ambient temperature sensor is functioning properly. If the message is shown again, then contact sciex.com/request-support.

Table 5-5 Autosampler Error Messages (continued)

Error Message	Description
ISS option not installed on autosampler. Please switch off ISS-B option in configuration dialog.	• Correct the parameters in the ServiceLink software.
The autosampler is not ready. Please try later.	• Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.
Tray error.	• Correct the parameters in the Intabio software.
Tray position is unknown.	• Turn the module off, and then on.
Valve error.	• Correct the parameters in the Intabio software.
Vertical: home sensor not de-activated.	• Remove any blockages that prevent the needle unit from moving. Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.
Vertical: home sensor not reached.	• Remove any blockages that prevent the needle unit from moving. Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.
Vertical: needle position is unknown.	• Initialize the instrument in the Intabio software.
Vertical: stripper did not detect plate (or wash/ waste). Missing vial.	• Make sure that the sample vial and plate are installed correctly. Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.
Vertical: stripper stuck.	• Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.
Vertical: The sample needle arm is at an invalid position.	• Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.
Wear-out limit reached.	• Turn the module off, and then on. If the message is shown again, then contact sciex.com/request-support.

Table 5-5 Autosampler Error Messages (continued)

Error Message	Description
Wrong tubing volume. The largest tubing volume for standard injections is 200 µL.	• Correct the parameters in the Intabio software.

Syringe Dispenser Unit Error Messages

Table 5-6 Syringe Dispenser Unit Error Messages

Error Message	Description
Syringe valve didn't find wanted position.	• Make sure that the syringe valve pulley is not damaged.
Syringe home sensor not reached.	• Examine the spindle and transport block. • Do a wash using Direct Control in the ServiceLink software to make sure that the flow is not restricted.
Syringe home sensor not de-activated.	• Examine the spindle and transport block. • Do a wash using Direct Control in the ServiceLink software to make sure that the flow is not restricted.
Syringe position is unknown.	• Initialize the module using Direct Control in the ServiceLink software.
Syringe rotation error.	• Do a wash using Direct Control in the ServiceLink software to make sure that the flow is not restricted.

Needle Unit Error Messages

Table 5-7 Needle Unit Error Messages

Error Message	Description
Horizontal: needle position is unknown.	• Initialize the module using Direct Control in the ServiceLink software.
Horizontal: home sensor not reached.	• Make sure the needle movement is not blocked.
Horizontal: home sensor not de-activated.	• Make sure the needle movement is not blocked.

Table 5-7 Needle Unit Error Messages (continued)

Error Message	Description
Incorrect amount of steps executed to reach the home position	• Make sure the horizontal needle movement is not blocked.
Vertical: needle position is unknown.	• Initialize the module using Direct Control in the ServiceLink software.
Vertical: home sensor not reached.	• Make sure the needle movement is not blocked.
Vertical: home sensor not deactivated.	• Make sure the needle movement is not blocked.
Vertical: home sensor activated when not expected.	• Contact sciex.com/request-support
Vertical: stripper did not detect plate (or wash/waste).	• Make sure that plates or vials are installed.
Vertical: stripper stuck.	• Make sure that the vial stripper is fully in the down position. • Examine the spring mechanism for the stripper. • Examine the vial stripper for any obstructions or dirt.
Vertical: The sample needle arm is at an invalid position.	• Contact sciex.com/request-support

Tray Unit Error Messages

Table 5-8 Tray Unit Error Messages

Error Message	Description
Home sensor not reached.	• Make sure that there are no obstructions to the tray movement. Move the tray forward and backward.
Deviation of more than +/-2mm towards home.	• Make sure there are no visible obstructions in the tray area.
Home sensor not de- activated.	• Make sure that the transport foam is removed from the tray compartment. • Make sure that there are no obstructions to the tray movement. Move the tray forward and backward.

Table 5-8 Tray Unit Error Messages (continued)

Error Message	Description
Tray position is unknown.	• Initialize the module using Direct Control in the ServiceLink software.

Electronics Error Messages

Table 5-9 Electronics Error Messages

Error Message	Description
Error occurred during initialization, Autosampler cannot start.	An error occurred during start-up. • Start the module again and verify the error code.

Cooling Unit Error Messages

Table 5-10 Cooling Unit Error Messages

Error Message	Description
Temperature above 48 °C at cooling ON.	• Turn cooling off, wait for 30 minutes, and then inspect the temperature sensor to make sure that it shows the ambient temperature. If it does not, then replace the sensor.

Injection Valve Unit Error Messages

Table 5-11 Injection Valve Unit Error Messages

Error Message	Description
Indicated position not reached.	• Contact sciex.com/request-support
Wear-out limit reached.	• Examine the injection valve for leaks and wear. Contact sciex.com/request-support

Intabio ZT cartridge A

The Intabio ZT cartridge is used for microfluidic chip-based isoelectric focusing separation with integrated on-chip electrospray ionization for MS detection using the ZenoTOF 7600 system.

The Intabio ZT cartridge includes the following components:

- A microfluidic chip that combines isoelectric focusing separation with integrated on-chip electrospray ionization for MS detection
- Sealed reservoirs for electrolytes and mobilizer solutions
- Ports and electrodes on the bottom for the gas, fluidic, and electrical contact with the base of the Intabio ZT system.

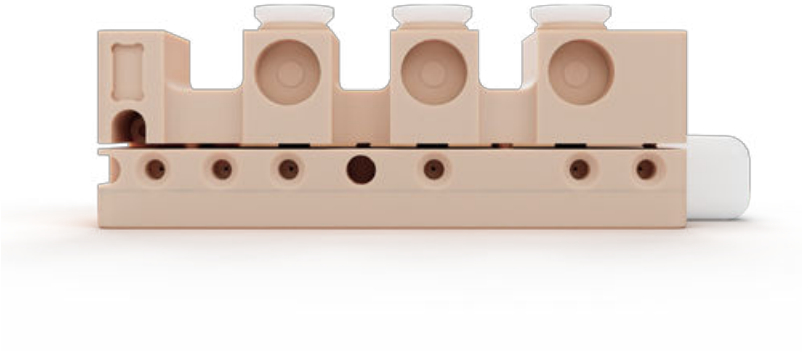
The notch on the left side of the cartridge as well as the hole between the anolyte and the catholyte port assist with the mounting alignment on the base of the system.

Table A-1 Kit Contents

Kit Contents	
Intabio ZT cartridge, including three reservoir caps and chip cover	One cartridge per pack
Capacity	15 injections per cartridge **Note:** Do the 15 injections in a 16-hour period. During this period, do not remove the cartridge or let the cartridge become dry. The cycle time for each injection is about 30 minutes.
Storage conditions before use	Room temperature

Figure A-1 Top View of the Intabio ZT cartridge

Figure A-2 Bottom View of the Intabio ZT cartridge with Sealed Reservoirs



For a list of reagents and consumables, refer to the document: *Parts and Equipment Guide*.

Care and Handling

WARNING! Electrical Shock Hazard. Do not spill any liquid on the cartridge. Wipe away any liquid before installing the cartridge or use canned air to gently dry the cartridge.

CAUTION: Potential System Damage. Do not touch the recessed optical window or the electrodes of the cartridge to avoid damaging the equipment.

CAUTION: Potential System Damage. Make sure that the electrospray ionization (ESI) tip does not touch any surfaces when removing the cartridge from the packaging or handling the cartridge to avoid damaging the cartridge.

CAUTION: Potential System Damage. Make sure to always wear clean gloves when handling the cartridge to avoid leaving any contaminants, including skin oils, on the surface of the cartridge. Contaminants can create high voltage paths that will impede proper operation of the cartridge and the instrument.

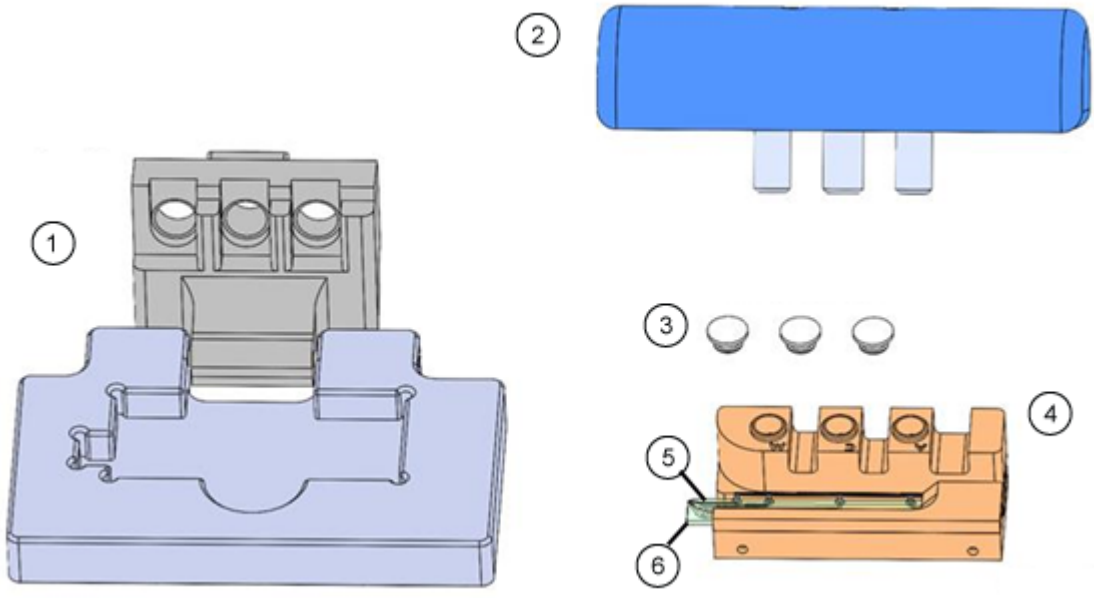
CAUTION: Potential System Damage. Make sure to always add an anolyte solution to the reservoir on the left, catholyte solution to the middle reservoir, and the mobilizer solution to the reservoir on the right. Adding the electrolytes and mobilizer solutions to the incorrect reservoirs can damage the cartridge.

For more information about preparing the cartridge for analysis, use the System Helpers in the Intabio software.

Refer to the following figure for a description of the parts of the cartridge and Intabio system—Cartridge Cap Installation Fixture. To make sure that the cartridge operates correctly, use this tool to apply equal pressure to the caps to seal the filled reservoirs.

Note: To make sure that the system performs optimally, the cartridge caps must be installed using the Intabio system—Cartridge Cap Installation Fixture and the Cap Sealing Tool.

Figure A-3 Components of the Intabio system—Cartridge Cap Installation Fixture and the Intabio ZT cartridge



Item	Description
1	Base and Aligner. Shipped with the system.

Item	Description
2	Cap Sealing Tool. Shipped with the system.
3	Caps. Shipped with the cartridge.
4	Intabio ZT cartridge
5	ESI tip
6	Optical window

Intabio system—Performance Test Mix Kit

B

Intabio system—Performance Test Mix kit is designed for use with the NISTmAb to evaluate the overall performance of the Intabio ZT system. For use, 480 µL of the performance text mix is added to 20 µL of the NISTmAb (stock 10 mg/mL). The 500 µL solution can be used for about 9 injections. For more instructions, refer to the section: *System Performance Check* in the Intabio software *Help*.

Equipment and Materials Required

Note: For information about consumables and spare parts, refer to the document: *Parts and Equipment Guide*.

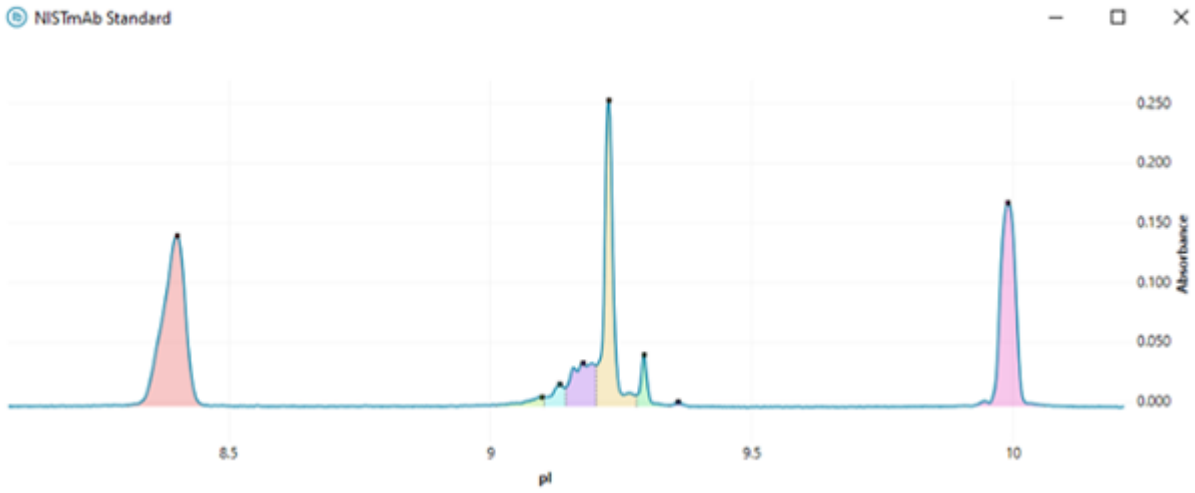
Table B-1 Intabio system—Performance Test Mix Kit

Component	Composition	Quantity	Storage
Intabio system—Performance Test Mix	Solution with: • 1% carrier ampholytes pH 3-10 • 3% carrier ampholytes pH 8-10.5 • 2.5% 500 mM arginine • 1.25% 200 mM iminodiacetic acid • 1% pI 8.40 marker • 1% pI 9.99 marker	4 × 500 µL	Upon receipt, store the Intabio system—Performance Test Mix vials between +2 °C and +8 °C.

Customer Supplied Solutions and Equipment

- NISTmAb (Makes 8 × 100 µL aliquots)
- Sample vials with or without integrated inserts and pre-slit caps
- Spin desalting column, 7K MWCO (0.5 mL) (1 per aliquot)
- Microcentrifuge tubes: 2 for desalting each aliquot, with caps cut off
- Table-top centrifuge, autopipettes, and tips

Figure B-1 Example of a NISTmAb Standard

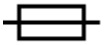


Glossary of Symbols C

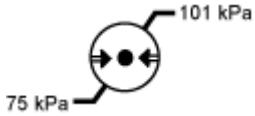
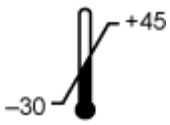
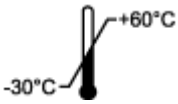
Note: Not all of the symbols in the following table are applicable to every instrument.

Symbol	Description
	Australian Regulatory Compliance Mark. Indicates that the product complies with Australian Communications Media Authority (ACMA) EMC and Electrical Safety Requirements.
	Alternating current
A	Amperes (current)
	Asphyxiation Hazard
EC REP	Authorized representative in the European community
	Biohazard
CE	CE Marking of Conformity
REF	Catalog number
	Caution. Consult the instructions for information about a possible hazard. **Note:** In SCIEX documentation, this symbol identifies a personal injury hazard.

Symbol	Description
	China RoHS Caution Label. The electronic information product contains certain toxic or hazardous substances. The center number is the Environmentally Friendly Use Period (EFUP) date, and indicates the number of calendar years the product can be in operation. Upon the expiration of the EFUP, the product must be immediately recycled. The circling arrows show the product is recyclable. The date code on the label or product indicates the date of manufacture.
	China RoHS logo. The device does not contain toxic and hazardous substances or elements above the maximum concentration values and the device is an environmentally-friendly product that can be recycled and reused.
	Consult instructions for use.
	Crushing Hazard
	cTUVus mark for TUV Rheinland of North America
	Data Matrix symbol that can be scanned by a barcode reader to obtain a unique device identifier (UDI)
	Environmental Hazard
	Ethernet connection
	Explosion Hazard

Symbol	Description
	Eye Injury Hazard
	Fire Hazard
	Flammable Chemical Hazard
	Fragile
	Fuse
Hz	Hertz
	International safety symbol "Caution, risk of electric shock" (ISO 3864), also known as High Voltage symbol If the main cover must be removed, then contact a SCIEX representative to prevent electric shock.
	Hot Surface Hazard
	In Vitro Diagnostic Device
	Ionizing Radiation Hazard
	Keep dry. Do not expose to rain. Relative humidity must not exceed 99%.

Symbol	Description
	Keep upright.
	Lacerate/Sever Hazard
	Laser Radiation Hazard
	Lifting Hazard
	Magnetic Hazard
	Manufacturer
	Moving Parts Hazard
	Pacemaker Hazard. No access to people with pacemakers.
	Pinching Hazard
	Pressurized Gas Hazard
	Protective Earth (ground)

Symbol	Description
	Puncture Hazard
	Reactive Chemical Hazard
SN	Serial number
	Toxic Chemical Hazard
	Transport and store the system within 66 kPa to 103 kPa.
	Transport and store the system within 75 kPa to 101 kPa.
	Transport and store the system within the specified minimum (**min**) and maximum (**max**) levels of relative humidity, noncondensing.
	Transport and store the system within –30 °C to +45 °C.
	Transport and store the system within –30 °C to +60 °C.
	Ultraviolet Radiation Hazard
UK CA	United Kingdom Conformity Assessment Mark
UKRP	United Kingdom Responsible Person

Symbol	Description
VA	Volt Ampere (apparent power)
V	Volts (voltage)
	WEEE. Do not dispose of equipment as unsorted municipal waste. Environmental Hazard
W	Watts (power)
	*yyyy-mm-dd* Date of manufacture

Glossary of Warnings D

Note: If any of the labels used to identify a component become detached, then contact a SCIEX field service employee (FSE).

Label	Translation (if applicable)
FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.	FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Contact Us

Customer Training

- In North America: NA.CustomerTraining@sciex.com
- In Europe: Europe.CustomerTraining@sciex.com
- Outside the EU and North America, visit sciex.com/education for contact information.

Online Learning Center

- SCIEX Now Learning Hub

Purchase Supplies and Reagents

Reorder SCIEX supplies and reagents online at store.sciex.com. To set up an order, use the account number, found on the quote, order confirmation, or shipping documents. Currently, customers in the United States, Canada, United Kingdom, Belgium, Netherlands, France, Germany, and Switzerland have access to the online store, but access will be extended to other countries in the future. For customers in other countries, contact a local SCIEX representative.

SCIEX Support

SCIEX and its representatives maintain a staff of fully-trained service and technical specialists located throughout the world. They can answer questions about the system or any technical issues that might arise. For more information, visit the SCIEX website at sciex.com or contact us in one of the following ways:

- sciex.com/contact-us
- sciex.com/request-support

CyberSecurity

For the latest guidance on cybersecurity for SCIEX products, visit sciex.com/productsecurity.

Documentation

This version of the document supercedes all previous versions of this document.

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Contact Us

To find software product documentation, refer to the release notes or software installation guide that comes with the software.

To find hardware product documentation, refer to the documentation that comes with the system or component.

The latest versions of the documentation are available on the SCIEX website, at sciex.com/customer-documents.

Note: To request a free, printed version of this document, contact sciex.com/contact-us.