Applied Biosystems/MDS SCIEX 4800 MALDI TOF/TOF™ Analyzer

Hardware Guide

Version 3.X Series Software

Part Number 1018535A

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Applied Biosystems | MDS SCIEX

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Preface

How to Use This Guide

Purpose of This Guide

The *4800 MALDI TOF/TOF™* Analyzer Hardware Guide provides reference information related to the 4800 MALDI TOF/TOF™ Analyzer. It describes the system, theory of operation, and how to prepare, maintain, and troubleshoot the system.

Audience

This guide is intended for novice and experienced 4800 MALDI TOF/TOF™ Analyzer users who are using the system for high-throughput proteomics and other research.

Assumptions

This guide assumes that your 4800 MALDI TOF/TOF™ Analyzer has been installed by an Applied Biosystems/MDS SCIEX technical representative and that the vacuum pressures are adequate (that is, below the maximum threshold set in the hardware configuration).

This guide uses conventions and terminology that assume a working knowledge of the Windows® operating system, the Internet, and Internet-based browsers.

Text Conventions

This guide uses the following conventions:

- **Bold** indicates user action. For example:

  Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:

  Before analyzing, *always* prepare fresh matrix.
- A right arrow bracket (>) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File > Open > Spot Set**.

  Right-click the sample row, then select **View Filter > View All Runs**.

User Attention Words

Two user attention words appear in Applied Biosystems/MDS SCIEX user documentation. Each word implies a particular level of observation or action as described below:

Note: Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

Examples of the user attention words appear below:

Note: The size of the column affects the run time.

Note: The Calibrate function is also available in the Control Console.

IMPORTANT! To verify your client connection to the database, you need a valid Oracle® Database user ID and password.

IMPORTANT! You must create a separate Sample Entry Spreadsheet for each 96-well plate.

Safety Alert Words

Safety alert words also appear in user documentation. For more information, see "Safety Alert Words" on page xii.

How to Obtain More Information

Related Documentation

The following related documents are shipped with the system:

- *4800 MALDI TOF/TOF™ Analyzer Getting Started Guide* – Provides brief, step-by-step procedures for preparing and analyzing a sample. It is designed to help you quickly learn to use the 4800 MALDI TOF/TOF™ Analyzer.
- *4800 MALDI TOF/TOF™ Analyzer Quick Reference Cards* – Provide abbreviated but key task-related information.
- *4000 Series Explorer™ Software Online Help* – Provides in-depth information on the software and provides detailed procedures and supporting information for 4800 instrument common tasks. Help is available from the Help menu or by pressing F1.

- *4000 Series Explorer™ Database Tools Online Help* – Provides procedures for performing database archiving, restoring, backing up, and recovering. Help is available from the Help menu or by pressing F1 in the 4000 Series Database Tools.
- *Data Explorer® Software Online Help* – Describes Data Explorer® software, and provides procedures for command tasks. Help is available from the Data Explorer software Help menu or by pressing F1.
- *DeNovo Explorer™ Software Online Help* – Describes the denovo sequencing and database search software included with the 4800 instrument, and provides procedures for common tasks. Help is available from the Help menu or by pressing F1.
- *Peak Explorer™ Software Online Help* – Describes the LC/MALDI visualization and analysis software included with the 4800 instrument, and provides procedures for common tasks. Help is available from the Help menu or by pressing F1.

Portable document format (PDF) versions of this guide and the *4800 MALDI TOF/TOF™ Analyzer Getting Started Guide* are also available on the 4000 Series Explorer™ software installation CD.

Note: For additional documentation, see "How to Obtain Support" on page x.

Database Access and Instrument Control API

For software developers or system administrators who need programmatic database access and instrument control, API (application programming interface) and SDK (software development kits) are available on the instrument software CD that is shipped with your instrument.

The following data access and instrument control tools are available:

- **4000 Series Database Access API** – A set of stored functions, stored procedures, and views that provide a method for locally or remotely retrieving 4000 Series data easily and quickly using SQL queries. Installed on the Instrument.
- **4000 Series Database Access API SDK** – A set of sample programs and utilities (such as DumpT2D) that demonstrate the use of the Data Access API to facilitate customer development of similar custom programs for their needs. The following data transfer tools are available.

- **4000 Series Instrument Control API** – A socket-based interface to the 4000 Series Instruments that provides a programmatic means of remotely controlling and retrieving data from the 4000 Series Instrument. Installed on the instrument.
- **4000 Series Instrument Control API SDK** – A set of libraries for generating the XML fed to the server API and for parsing the XML output from the server API. In addition, sample clients for the Server API and the SDKs are provided. There are SDKs for Java and for C++ and examples for using the Server API in VB, VC++, and Java.

Send Us Your Comments

Applied Biosystems/MDS SCIEX welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@sciex.com

How to Obtain Support

To contact Applied Biosystems/MDS SCIEX Technical Support from North America by telephone, call **1.800.899.5858**.

For the latest services and support information for all locations, go to **http://www.appliedbiosystems.com**, then click the link for **Support**.

At the Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems/MDS SCIEX user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

In addition, the Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems/MDS SCIEX Technical Support and Sales facilities.

Safety and EMC Compliance Information

This section includes the following topics:

Safety Conventions Used in This Document

Safety Alert Words

Four safety alert words appear in Applied Biosystems/MDS SCIEX user documentation at points in the document where a user needs to be aware of relevant hazards. Each alert word–**IMPORTANT**, **CAUTION**, **WARNING**, **DANGER**–implies a particular level of observation or action, as defined below:

Definitions

IMPORTANT! – Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

– Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

– Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

– Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Except for IMPORTANTs, each safety alert word in an Applied Biosystems/MDS SCIEX document appears with an open triangle figure that contains a hazard symbol. *These hazard symbols are identical to the hazard icons that are affixed to Applied Biosystems/* MDS SCIEX instruments (see "Safety Symbols" on page xv).

Examples

The following are some specific examples of the use of safety alert words:

IMPORTANT! You must create a separate a Sample Entry Spreadsheet for each 96-well plate.

The lamp is extremely hot. Do not touch the lamp until it has cooled to room temperature.

CHEMICAL HAZARD. **Formamide**. Exposure causes eye, skin, and respiratory tract irritation. It is a possible developmental and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

ELECTRICAL HAZARD. Failure to ground the instrument properly can lead to an electrical shock. Ground the instrument according to the provided instructions.

Symbols on Instruments

Electrical Symbols

The following table describes the electrical symbols that may be displayed on Applied Biosystems/MDS SCIEX instruments.

Symbol	Description
	Indicates the **On** position of the main power switch.
	Indicates the **Off** position of the main power switch.
	Indicates the **On/Off** position of a push-push main power switch.
	Indicates a standby switch.
	Indicates a terminal that may be connected to the signal ground reference of another instrument. This is not a protected ground terminal.
	Indicates a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.
	Indicates a terminal that can receive or supply alternating current or voltage.
	Indicates a terminal that can receive or supply alternating or direct current or voltage.

Safety Symbols

The following table describes the safety symbols that may be displayed on Applied Biosystems/MDS SCIEX instruments. Each symbol may appear by itself or in combination with text that explains the relevant hazard (see "Safety Labels on Instruments" on page xvi). These safety symbols may also appear next to DANGERS, WARNINGS, and CAUTIONS that occur in the text of this and other product-support documents.

Symbol	Description
	Indicates that you should consult the manual for further information and to proceed with appropriate caution.
	Indicates the presence of an electrical shock hazard and to proceed with appropriate caution.
	Indicates the presence of a hot surface or other high-temperature hazard and to proceed with appropriate caution.
	Indicates the presence of a laser inside the instrument and to proceed with appropriate caution.
	Indicates the presence of moving parts and to proceed with appropriate caution.

Safety Labels on Instruments

The following CAUTION, WARNING, and DANGER statements may be displayed on Applied Biosystems?MDS SCIEX instruments in combination with the safety symbols described in the preceding section.

English	Francais
CAUTION Hazardous chemicals. Read the Material Safety Data Sheets (MSDSs) before handling.	**ATTENTION** Produits chimiques dangeureux. Lire les fiches techniques de sûreté de matériels avant la manipulation des produits.
CAUTION Hazardous waste. Read the waste profile (if any) in the site preparation guide for this instrument before handling or disposal.	**ATTENTION** Déchets dangereux. Lire les renseignements sur les déchets avant de les manipuler ou de les éliminer.
CAUTION Hazardous waste. Refer to MSDS(s) and local regulations for handling and disposal.	**ATTENTION** Déchets dangereux. Lire les fiches techniques de sûreté de matériels et la régulation locale associées à la manipulation et l'élimination des déchets.
WARNING Hot lamp.	**AVERTISSEMENT** Lampe brûlante.
WARNING Hot. Replace lamp with an Applied Biosystems/MDS SCIEX lamp.	**AVERTISSEMENT** Composants brûlants. Remplacer la lampe par une lampe Applied Biosystems/MDS SCIEX.
CAUTION Hot surface.	**ATTENTION** Surface brûlante.
DANGER High voltage.	**DANGER** Haute tension.
WARNING To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to Applied Biosystems /MDS SCIEX qualified service personnel.	**AVERTISSEMENT** Pour éviter les risques d'électrocution, ne pas retirer les capots dont l'ouverture nécessite l'utilisation d'outils. L'instrument ne contient aucune pièce réparable par l'utilisateur. Toute intervention doit être effectuée par le personnel de service qualifié de Applied Biosystems/MDS SCIEX.
DANGER Class 3B visible and/or invisible laser radiation present when open and interlock defeated. Avoid direct exposure to laser beam.	**DANGER** de rayonnement laser de Classe 3B présent évident et/ou invisible en cas d'ouverture et d'une neutralisation des dispositifs de sécurité. Eviter toute exposition directe avec le faisceau.

English	Francais
DANGER Class 3B visible and/or invisible laser radiation when open. Avoid direct exposure to laser beam.	**DANGER** de rayonnement laser de Classe 3B présent évident et/ou invisible en cas d'ouverture. Eviter toute exposition directe avec le faisceau.
DANGER Class 2 (II) lasers can cause damage to eyes. Avoid looking into a Class II laser beam or pointing a Class II laser beam into another person's eyes.	**DANGER** Les lasers de Classe 2 (II) peuvent provoquer de graves dommages aux yeux. Eviter de regarder directement le rayon du laser ou de pointer ce rayon sur l'oeil d'une autre personne.
CAUTION Moving parts.	**ATTENTION** Parties mobiles.

General Instrument Safety

WARNING **PHYSICAL INJURY HAZARD.** Use this product only as specified in this document. Using this instrument in a manner not specified by Applied Biosystems/MDS SCIEX may result in personal injury or damage to the instrument.

Moving and Lifting the Instrument

CAUTION **PHYSICAL INJURY HAZARD.** The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.

Moving and Lifting Stand-Alone Computers and Monitors

WARNING Do not attempt to lift or move the computer or the monitor without the assistance of others. Depending on the weight of the computer and/or the monitor, moving them may require two or more people.

Things to consider before lifting the computer and/or the monitor:

- Make sure that you have a secure, comfortable grip on the computer or the monitor when lifting.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time.
- Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before actually lifting and carrying.
- Instead of lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone slides the contents out of the box.

Operating the Instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Material Safety Data Sheets (MSDSs).

WARNING **PHYSICAL INJURY HAZARD.** Use this instrument as specified by Applied Biosystems/MDS SCIEX. Using this instrument in a manner not specified by Applied Biosystems/MDS SCIEX may result in personal injury or damage to the instrument.

Chemical Safety

Chemical Hazard Warning

CHEMICAL HAZARD. Before handling any chemicals, refer to the Material Safety Data Sheet (MSDS) provided by the manufacturer, and observe all relevant precautions.

CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to *new* customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

You can obtain from Applied Biosystems/MDS SCIEX the MSDS for any chemical supplied by Applied Biosystems/MDS SCIEX. This service is free and available 24 hours a day.

To obtain MSDSs:

1. Go to **https://docs.appliedbiosystems.com/msdssearch.html**
2. In the Search field, type in the chemical name, part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose
4. To have a copy of a document sent by fax or e-mail, select **Fax** or **Email** to the left of the document title in the Search Results page, then click **RETRIEVE DOCUMENTS** at the end of the document list.
5. After you enter the required information, click **View/Deliver Selected Documents Now**.

Chemical Safety Guidelines

To minimize the hazards of chemicals:

- Read and understand the MSDSs provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. See "About MSDSs" on page xix.
- Minimize contact with chemicals. When handling chemicals, wear appropriate personal protective equipment such as safety glasses, gloves, and protective clothing. For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, a fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the cleanup procedures recommended in the MSDS.
- Comply with all local, state/provincial, and/or national laws and regulations related to chemical storage, handling, and disposal.

Chemical Waste Safety

Chemical Waste Hazard

CAUTION HAZARDOUS WASTE. Refer to Material Safety Data Sheets and local regulations for handling and disposal.

WARNING CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems/MDS SCIEX instruments are potentially hazardous and can cause injury, illness, or death.

WARNING CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical Waste Safety Guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After you empty a chemical waste container, seal it with the cap provided.
- Dispose of the contents of a waste container in accordance with good laboratory practices and local, state/provincial, and/or national environmental and health regulations.

Waste Disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Electrical Safety

ELECTRICAL SHOCK HAZARD. Severe electrical shock can result from operating the 4800 MALDI TOF/TOF™ Analyzer without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.

Power

ELECTRICAL HAZARD. Grounding circuit continuity is vital for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.

ELECTRICAL HAZARD. Use properly configured and approved line cords for the voltage supply in your facility.

ELECTRICAL HAZARD. Plug the system into a properly grounded receptacle with adequate current capacity.

Overvoltage Rating

The 4800 MALDI TOF/TOF™ Analyzer has an installation (overvoltage) category of II, and is classified as portable equipment.

Physical Hazard Safety

Compressed Gases

PHYSICAL HAZARD. Nonflammable compressed gas (such as nitrogen and other gases used as collision gas). Contents are under pressure. Receive proper training on the handling of compressed gases before use. Exposure to rapidly expanding gas may cause frostbite. High concentrations of vapors in the immediate area can displace oxygen and cause asphyxiation. Use only in areas with adequate ventilation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

EXPLOSION HAZARD. Pressurized gas cylinders are potentially explosive and can cause severe injury if not handled properly. Attach the gas cylinder firmly to the wall or gas cylinder cart with approved brackets or chains. Always cap the gas cylinder when it is not in use.

Biological Hazard Safety

General Biohazard

BIOHAZARD. Biological samples such as tissues, body fluids, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective eyewear, clothing, and gloves. Read and follow the guidelines in the following publications:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **http://bmbl.od.nih.gov**)
- Occupational Safety and Health Standards, Toxic and Hazardous Substances (29 CFR §1910.1030; **http://www.access.gpo.gov/nara/cfr/waisidx_01/ 29cfr1910a_01.html**).

Additional information about biohazard guidelines is available at: **http://www.cdc.gov**

Laser Safety

Laser Classification

The 4800 MALDI TOF/TOF™ Analyzer uses a diode-pumped, solid-state laser. Under normal operating conditions, the instrument is categorized as a Class 1 laser. When safety interlocks are disabled during certain servicing procedures, the laser can cause permanent eye damage, and, therefore, is classified under those conditions as a Class 3B laser.

The 4800 MALDI TOF/TOF™ Analyzer has been tested to and complies with the "Radiation Control for Health and Safety Act of 1968 Performance Standard CFR 1040.10 and 1040.11"

The 4800 MALDI TOF/TOF™ Analyzer has been tested to and complies with standard EN60825-1, "Radiation Safety of Laser Products, Equipment Classification, Requirements, and User's Guide."

Laser Safety Requirements

To ensure safe laser operation:

- The system must be installed and maintained by an Applied Biosystems/MDS SCIEX Technical Representative.
- All instrument panels must be in place on the instrument while the instrument is operating. When all panels are installed, there is no detectable radiation present. If any panel is removed when the laser is operating (during service with safety interlocks disabled), you may be exposed to laser emissions in excess of the Class 1 rating.
- Do not remove safety labels or disable safety interlocks.

Additional Laser Safety Information

Refer to the user documentation provided with the laser for additional information on government and industry safety regulations.

LASER HAZARD. Lasers can burn the retina causing permanent blind spots. Never look directly into the laser beam. Remove jewelry and other items that can reflect the beam into your eyes. Do not remove the instrument panels. Wear proper eye protection and post a laser warning sign at the entrance to the laboratory if the top or front panels are removed for service.

LASER BURN HAZARD. An overheated laser can cause severe burns if it comes in contact with the skin. DO NOT operate the laser when it cannot be cooled by its cooling fan. Always wear appropriate laser safety goggles.

Bar Code Scanner Laser Safety

Laser Classification

The optional bar code scanner is categorized as a Class 2 (II) laser.

Laser Safety Requirements

Class 2 (II) lasers are low-power, visible-light lasers that can damage the eyes. Never look directly into the laser beam. The scanner is designed to prevent human access to harmful levels of laser light during normal operation, user maintenance, or during prescribed service operations.

LASER HAZARD. Class 2 (II) lasers can cause damage to eyes. Avoid looking into a Class 2 (II) laser beam or pointing a Class 2 (II) laser beam into another person's eyes.

Workstation Safety

Correct ergonomic configuration of your workstation can reduce or prevent effects such as fatigue, pain, and strain. Minimize or eliminate these effects by configuring your workstation to promote neutral or relaxed working positions.

CAUTION **MUSCULOSKELETAL AND REPETITIVE MOTION HAZARD**. These hazards are caused by potential risk factors that include but are not limited to repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.

To minimize musculoskeletal and repetitive motion risks:

- Use equipment that comfortably supports you in neutral working positions and allows adequate accessibility to the keyboard, monitor, and mouse.
- Position the keyboard, mouse, and monitor to promote relaxed body and head postures.

Biohazardous Material Disposal

- **WARNING** BIOHAZARDOUS MATERIAL Do not dispose of system components or subassemblies, including computer parts, in municipal waste. Dispose of replaced components and instruments according to established waste electrical equipment procedures.

Safety and Electromagnetic Compatibility (EMC) Standards

This section provides information on:

- U.S. and Canadian Safety Standards
- Federal Communications Commission Compliance
- European Safety and EMC Standards

U.S. and Canadian Safety Standards

This instrument has been tested to and complies with standard UL 61010A-1, "Safety Requirements for Electrical Equipment for Laboratory Use, Part 1: General Requirements."

This instrument has been tested to and complies with standard CSA 61010.1, "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements."

This instrument has been tested and complies with the FDA CDRH 21 CFR 1040.1 and 1040.11 requirements for laser safety.

Federal Communications Commission Compliance

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 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 instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at their own expense. Changes or modifications not expressly approved by the manufacturer could void your authority to operate the equipment.

European Safety and EMC Standards

CE

Safety

This instrument meets European requirements for safety (Low Voltage Directive 73/23/EEC). This instrument has been tested to and complies with standards EN 61010-1, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements,"and EN 60825-1, "Safety of Laser Products."

EMC

This instrument meets European requirements for emission and immunity (EMC Directive 89/336/EEC). This instrument has been tested to and complies with standard EN 61326 (Group 1, Class A), "Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements."

Introducing the 4800 MALDI TOF/TOF™ Analyzer 1

This chapter covers:

4800 MALDI TOF/TOF™ Analyzer Overview

Introduction

The 4800 MALDI TOF/TOF™ Analyzer is a floor-standing mass spectrometer (Figure 1-1) that delivers high resolution and accurate mass determination for the MS analysis of biomolecules including protein digests, intact proteins, lipids, and carbohydrates. The system also enables the precise selection of individual precursor masses for further fragmentation enabling definitive identification and characterization for these biological molecules by MS/MS.

Figure 1-1 4800 MALDI TOF/TOF™ Analyzer

Features

The 4800 MALDI TOF/TOF™ Analyzer includes the following features:

- Modular vertical design
- Tandem TOF analyzers
- Linear (optional), reflector, or MS/MS acquisition
- Variable ion path length to accommodate:
  - MS Linear mode
  - MS Reflector mode
  - MS/MS mode
- Automated single-plate sample-loading system
- Optional handheld bar code scanner for bar-coded plates
- Video camera for sample viewing within software interface
- Nd:YAG 200-Hz laser
- Positive and negative ion detection
- Two variable two-stage ion acceleration regions
- Ion-focusing lens technology for increased sensitivity
- Automated matrix ion deflection
- High-resolution precursor selection
- High-energy, collision-induced dissociation (CID) cell
- Metastable ion suppression
- High-performance, two-stage reflector
- High-speed 200-Hz digitizer
- Intuitive Microsoft® Windows® XP-based software

For more information, see Appendix A, “Specifications.”

4800 MALDI TOF/TOF™ Analyzer Technology

Overview

The 4800 MALDI TOF/TOF™ Analyzer takes advantage of the following technologies:

- Mass spectrometry
- MALDI
- Time-of-flight ion detection
- Time-of-flight/time-of-flight ion detection
- Time-delay ion extraction

Mass Spectrometry

Mass spectrometry measures the masses of molecules by measuring their mass-to-charge (*m/z*) ratios. Mass is a molecular attribute that can help identify or confirm the identity of a molecule.

Molecular weight measurements by mass spectrometry are based on the production, separation, and detection of molecular ions. A typical mass spectrometer includes:

- **An ion source –** Ionizes sample and generates gas phase ions.
- **A mass analyzer –** Separates ions according to individual mass-to-charge ratios.
- **A detector –** Detects and amplifies ion signals.
- **A data system –** Converts detection of ions into a readable or graphic display.

Matrix-Assisted Laser Desorption Ionization (MALDI)

In matrix-assisted laser desorption ionization (MALDI), sample is embedded in a low-molecular-weight, UV-absorbing matrix that enhances intact desorption and ionization of the sample (Figure 1-2). The matrix is present in vast excess of sample and, therefore, isolates individual sample molecules.

When the laser pulses, sample molecules are ionized by gas phase proton transfer from the matrix. The laser pulse produces:

- Matrix neutrals (M)
- Matrix ions, $(M+H)^+$ and $(M-H)^-$
- Sample neutrals (A)

The matrix ions then collide with the sample neutrals, producing sample ions, $(A+H)^+$ and $(A-H)^-$.

$$(M+H)^+ + A \rightarrow M + (A+H)^+$$

$$(M-H)^- + A \rightarrow (A-H)^- + M$$

Figure 1-2 illustrates the ionization of sample.

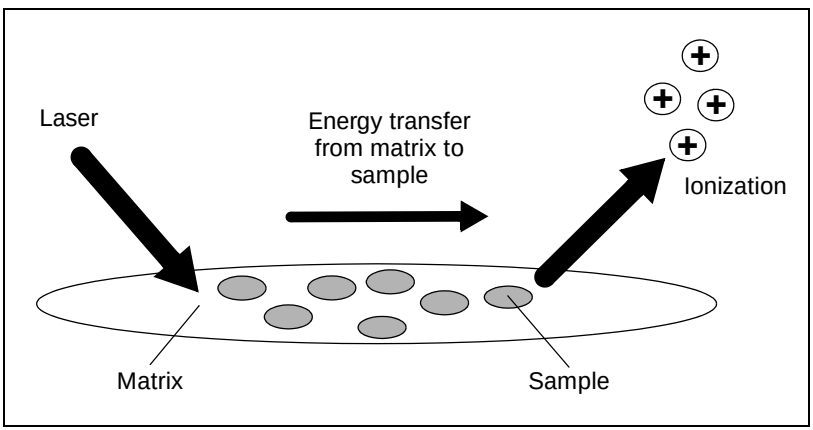


Figure 1-2 Matrix-assisted laser desorption ionization (MALDI)

Time-of-Flight

Time-of-flight (TOF) mass spectrometry works on the principle that when ions are accelerated with the same potential from a fixed point and at a fixed initial time and are allowed to drift, the ions separate according to their mass-to-charge ratios (*m/z*). Lighter ions drift more quickly to the detector. Heavier ions drift more slowly (Figure 1-3).

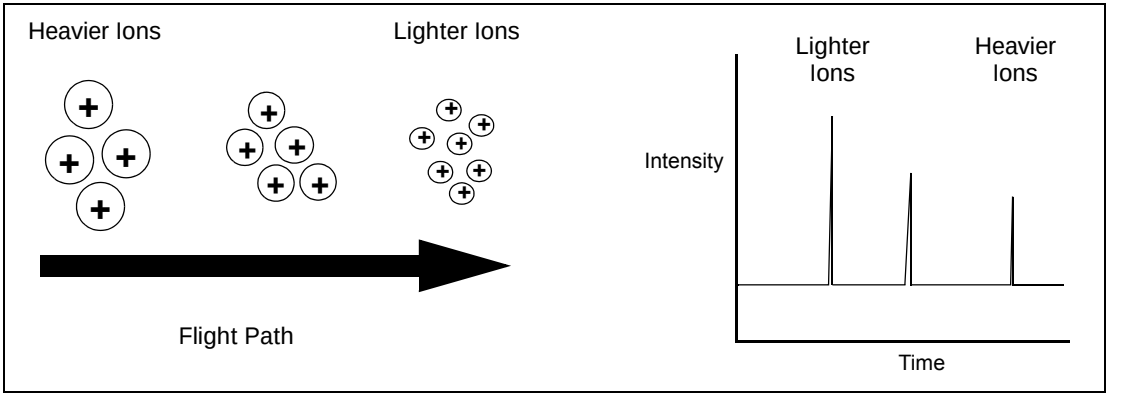


Figure 1-3 Time-of-flight (TOF) analysis

The time required for ions to reach the detector at the opposite end of the flight tube (drift time) is measured. The number of ions reaching the detector at any given time is also measured, and is referred to as ion intensity (abundance) or signal intensity.

Drift time is proportional to the square root of the mass as defined by the following equation:

$$t = s\left(\frac{m}{(2KE)z}\right)^{1/2}$$

where:

t	=	drift time
s	=	drift distance
m	=	mass
KE	=	kinetic energy
z	=	number of charges on ion

Approximate ion mass is determined using the previous equation. However, a calibration procedure using a reference standard of known mass can be used to establish a more accurate relationship between flight time and the mass-to-charge ratio of an ion.

Time-of-Flight/ Time-of-Flight

Time-of-flight/time-of-flight (TOF-TOF), or tandem TOF mass spectrometry (MS), consists of two successive TOF accelerations. In tandem mass spectrometry:

- The first acceleration selects, isolates, and fragments (usually by collision with a neutral gas) a precursor ion of interest.
- The second acceleration reaccelerates the precursor ion and fragments, then measures the masses and intensities of the fragment ions.

When applied to peptides, this technique is more definitive than other MS methods for protein identification and a principal means of protein characterization.

Time-Delay Extraction

Limitations of Continuous Extraction

In traditional MALDI (continuous extraction), ions have a broad kinetic energy distribution that is largely due to the initial velocities imparted to ions during the desorption/ionization process. These initial velocities of desorbed analyte ions are nearly independent of the mass of the analyte, but the initial kinetic energies are proportional to the mass.

In addition, when desorption occurs in a strong electrical field, energy is lost by collisions with the neutral plume, and further mass-dependent energy dispersion results. This leads to peak broadening (poor resolution).

According to the theory of "time-lag energy focusing" as originally developed by Wiley and McLaren[a], the dependence of ion flight time on initial velocity can be corrected, to the first order, by delaying the extraction of ions from the source. If higher-order terms are insignificant, then the mass resolution should be determined by the ratio of the total flight time to the uncertainty in the time measurement. The observed mass resolution should increase in proportion to the effective length of the ion flight path.

a. W. C. Wiley and I. H. McLaren, *Rev. Sci. Instrum*., **1953**, ***26***, 1150–1157, W. C. Wiley, U.S. Patent 2,685,035.

Time-Delay Extraction

Because of the time-delay technology used by the 4800 MALDI TOF/TOF™ Analyzer, ions form in a field-free region. The ions are then extracted or accelerated by applying a high-voltage pulse after a predetermined delay time (Figure 1-4) then a potential gradient is applied by a variable voltage grid. The combination of a time delay with a voltage gradient minimizes the effect of the kinetic energy spread.

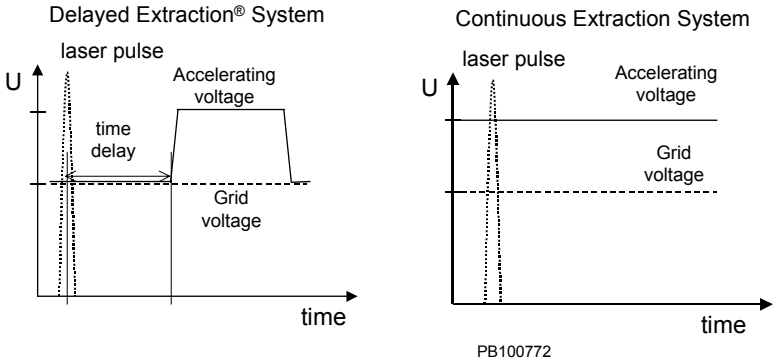


Figure 1-4 Comparison of Delayed Extraction® and continuous extraction systems

Velocity Focusing

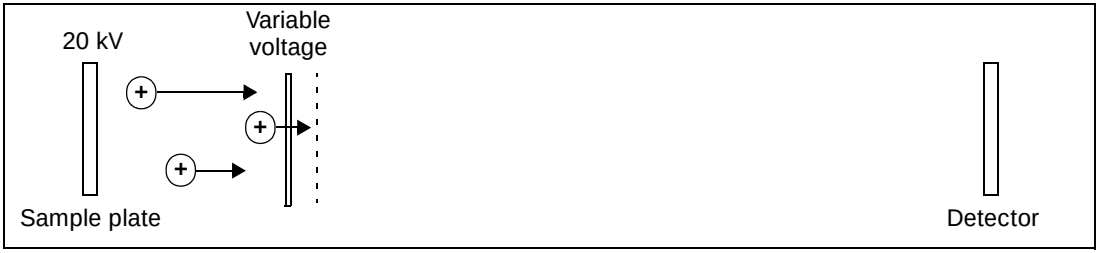
When ions are released from the sample surface, their positions in the ion source correlate with their initial velocity. When the extraction or accelerating voltage pulse is applied after a delay time and a potential gradient is applied, initially slower ions acquire slightly higher energy from the accelerating field than initially faster ions.

By careful tuning of both the delay time and the potential gradient, slow and fast ions of the same mass arrive at the detector at the same time (Figure 1-5 on page 1-9). This is referred to as *velocity focusing*. Velocity focusing enhances both resolution and mass accuracy.

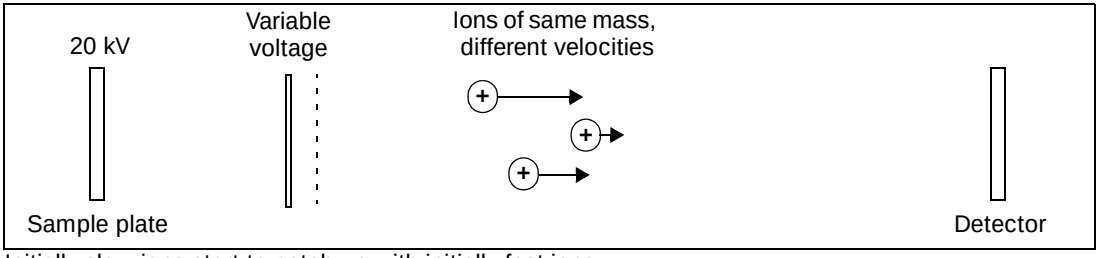
Note: The 4000 Series Explorer™ software automatically determines the optimal delay time and the variable voltage based on the acquisition method parameters and the acquisition type (MS-Linear, MS-Reflector, or MS/MS).

Figure 1-5 shows how Delayed Extraction enables velocity focusing.

No applied electric field. Ions of same mass spread out based on initial velocity.



Accelerating voltage applied after delay time combined with potential gradient (variable voltage). Slow ions accelerated more than fast ions.



Initially slow ions start to catch up with initially fast ions.

Slow ions catch up with fast ions at detector.

Figure 1-5 Time-delay extraction enables velocity focusing

4800 MALDI TOF/TOF™ Analyzer Workflow

Figure 1-6 illustrates the basic steps to acquire, process, and interpret data using the 4800 MALDI TOF/TOF™ Analyzer.

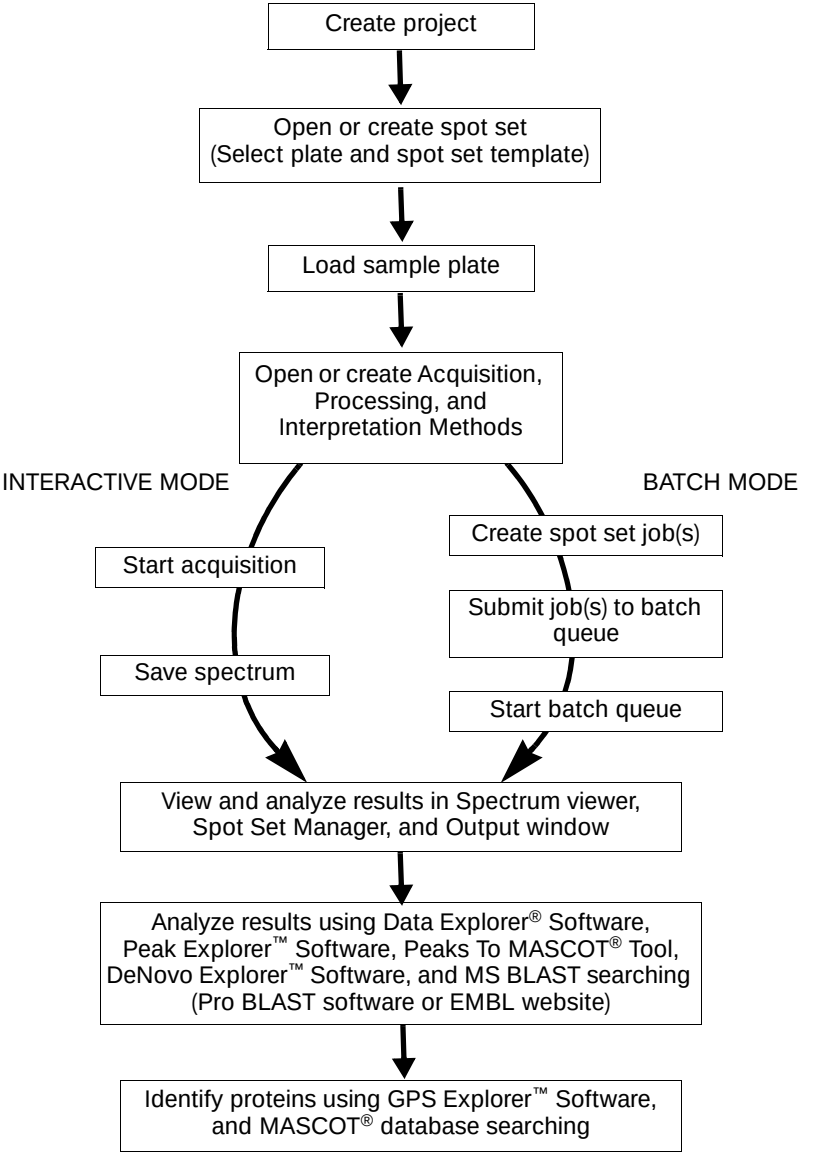


Figure 1-6 4800 MALDI TOF/TOF™ Analyzer work flow

4800 MALDI TOF/TOF™ Analyzer Hardware

This section describes the following components of the 4800 MALDI TOF/TOF™ Analyzer:

- Mass spectrometer
- Vacuum system
- Digitizer
- Computer components
- Optional handheld bar code scanner

Mass Spectrometer

The parts of the 4800 MALDI TOF/TOF™ Analyzer are described below. Figure 1-8 on page 1-13 shows the relative positions of key parts.

Camera

A camera displays, in the software interface, a real-time image of the region of the sample plate in the path of the laser beam, the laser beam position (indicated by a crosshair), and the extraction aperture.

Laser, Attenuator, and Mirrors

An Nd:YAG laser operates at 355 nm to ionize samples. The laser firing rate is 200 Hz.

The laser attenuator varies the intensity of the laser beam reaching the sample. A system of mirrors redirects the laser beam on to the sample plate.

Sample Loading Chamber

The sample loading chamber receives the sample plate from outside the instrument and provides a region of low vacuum before introducing the sample plate into the first source chamber.

The sample loading chamber (Figure 1-7 on page 1-12), has one pad for loading a plate into Source 1 and another for ejecting a plate from Source 1. You can mount a plate on the load pad in the sample loading chamber and add it to the 4000 Series Explorer™ software while another plate is loaded in Source 1. When you finish acquiring from the first plate, the instrument ejects the first plate, then, if applicable, automatically loads the second plate from the load pad in the sample loading chamber.

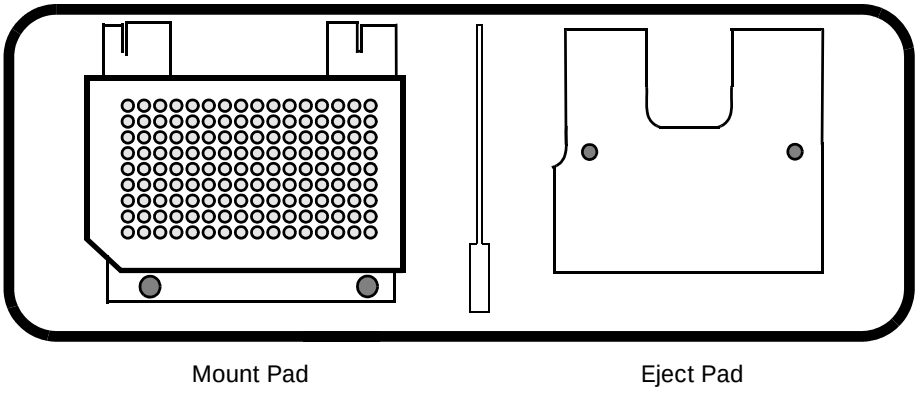


Figure 1-7 Sample loading chamber

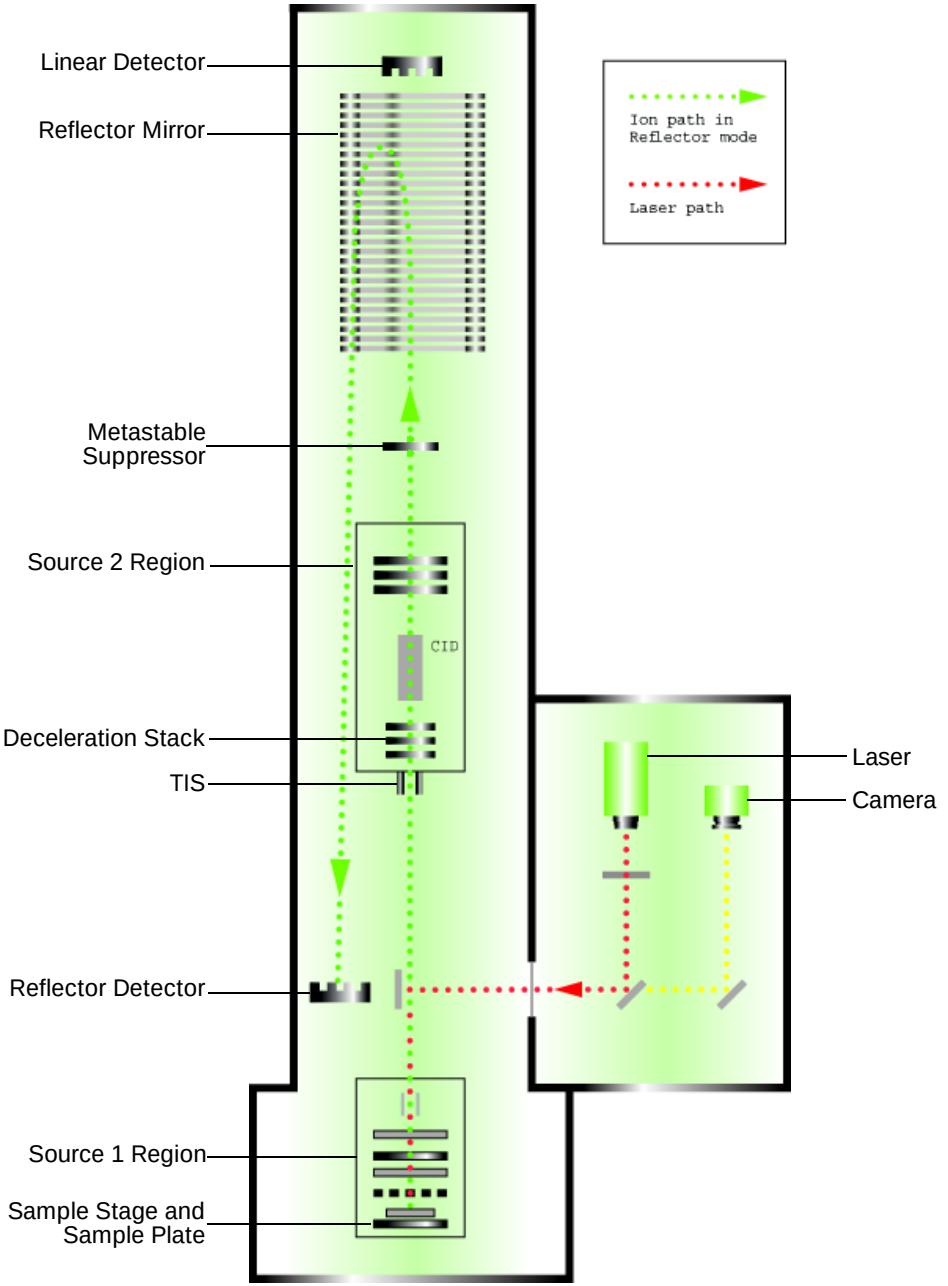


Figure 1-8 4800 MALDI TOF/TOF™ Analyzer mass spectrometer

Source 1 Region

The Source 1 region is a high-voltage region that accelerates ions, then focuses and steers the ion beam.

After the laser strikes the sample, the sample stage and sample plate are supplied with an acceleration voltage (0 to 25,000 V) at a predetermined delay time. This accelerates the ions. A variable voltage and ground are used to form a potential gradient to velocity-focus the ions and subsequently fine-tune the spectrum.

The Source 1 ion optics:

- Focus the ion beam within the flight tube.
- Steer the ion beam by correcting the x and y trajectories of the beam.

The Source 1 region heater maintains the region at approximately 50 °C to reduce the matrix build-up and minimize cleanings.

Flight Tubes

These are field-free regions in which ions drift at a velocity inversely proportional to the square root of their mass-to-charge ratio.

Source 2 Region

The Source 2 region is a high-voltage region that

- Continues to focus and steer the ion beam
- In MS/MS mode, isolates and reaccelerates a precursor ion and its fragments

The Source 2 ion optics:

- Focus the ion beam within the flight tube.
- Steer the ion beam by correcting the x and y trajectories of the beam.
- Isolate the precursor ion of interest (MS/MS mode) through use of a Timed Ion Selector (TIS), which allows only ions of a specific mass to pass through to the detector. (In MS-Linear and MS-Reflector modes, the Timed Ion Selector can be used as a low-mass gate to eliminate saturation effects due to matrix ions.) To optimize the TIS performance, tune the TIS Offset Position. See the *4000 Series Explorer™ Software Online Help*, Automatically Tuning the 4700 or 4800 Instrument.

  Note: You specify the precursor ion of interest by specifying a Precursor Mass in the acquisition method.

- Reduce the energy of the ions before they enter the collision cell using a deceleration stack. This controls the subsequent fragmentation in MS/MS mode.

The Source 2 chamber also includes:

- **Collision cell** – A field-free region that provides collision-induced dissociation (CID) to fragment the precursor ions in MS/MS mode. Precursor ions collide with the gas (typically air) that is introduced into the cell, causing some of them to fragment.

  Note: You can specify the gas type and gas pressure.

- **Acceleration voltage** – In MS/MS mode only, reaccelerates the ions after leaving the collision cell. The fragment ions (formed in the collision cell) are accelerated to a faster velocity than the precursor ions.
- **Variable voltage and ground** – In MS/MS mode only, forms a potential gradient to velocity-focus the ions, and subsequently to fine-tune the spectrum.

Post Source 2 Region

The Post Source 2 region includes the X and Y Mirror deflectors and the metastable suppressor to suppress the precursor ions and deflect the metastable fragment ions formed after the second source (MS/MS mode only). The fragment ions formed in the collision cell and a fraction of the precursor ions reach the detector. (The precursor peaks may be used for calibration of the fragment spectra.)

Mirror Region

The Mirror region includes:

- **Linear detector (optional)** – A dual microchannel plate (MCP) detector that detects ions that travel down the flight tube. The linear detector measures ion abundance over time and sends a signal to the digitizer for conversion. It is used only in MS-Linear mode (not in MS-Reflector or MS/MS modes).
- **Reflector mirror** – Used only in MS-Reflector and MS/MS modes, this two-stage electrostatic mirror (Figure 1-9) consists of a series of plates, which reflect ions back toward the reflector detector. The mirror improves mass accuracy and resolution by:
  - Filtering out neutral molecules
  - Velocity-focusing the ions by correcting the time dispersion due to differences in initial kinetic energy that result in slightly different rates of movement (velocity)

– Increasing separation (resolution) by providing a longer flight path

Ions of the same mass, but with slightly differing initial kinetic energy, enter the mirror at slightly different times. The faster moving ions penetrate deeper into the second stage of the mirror before being reflected back toward the reflector detector. Because these faster moving ions follow a longer flight path, they arrive at the reflector detector at the same time as ions of the same mass, but lower velocity.

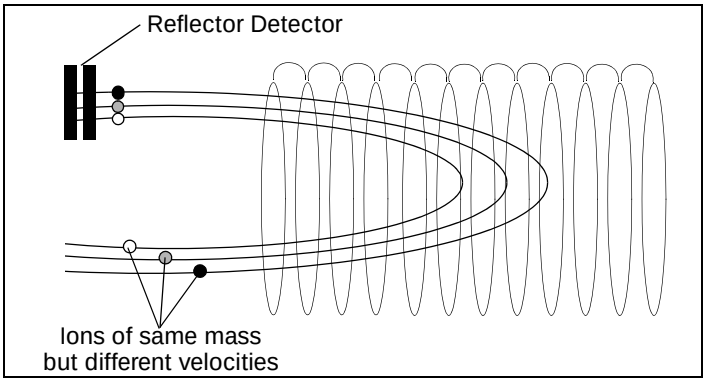


Figure 1-9 Representation of how a two-stage mirror helps velocity-focus ions

- **Reflector detector** – A dual microchannel plate (MCP) detector that detects ions that are reflected by the mirror. The reflector detector measures ion abundance over time and sends a signal to the digitizer for conversion. It is used in MS-Reflector or MS/MS modes only. It is not used in MS-Linear mode.

Vacuum System

The 4800 MALDI TOF/TOF™ Analyzer has a pumping system and sealed enclosures that create and maintain a high-vacuum environment for time-of-flight analysis. This environment:

- Allows unobstructed ion drift
- Provides conditions needed to maintain the high potential differences between system components

Vacuum Regions

The 4800 MALDI TOF/TOF™ Analyzer includes two regions of vacuum:

- Sample loading chamber
- Tower region (includes Source 1, Source 2, and Reflector areas)

Vacuum Pumps

Three vacuum pumps create the vacuum environment:

- **Roughing Pump** – A low-vacuum pump that:
  - Creates a vacuum in the sample loading chamber prior to sample introduction into the first source chamber.
  - Creates a lower-than-atmospheric-pressure condition before the turbo pumps start.
  - Provides backing pressure to the turbo pumps.
  - Provides purging for the collision gas delivery system.
- **CID Turbo Pump** – Enhances a high-vacuum condition in the collision cell.
- **System Turbo Pump** – Creates a high-vacuum condition in the entire system.

During plate loading, vacuum is maintained in the system by the sample loading chamber door. After plate loading, vacuum is maintained in the system by a gate valve between the sample loading chamber and Source 1.

When a CID gas is supplied to the collision cell, a differential pumping baffle helps maintain vacuum in the system.

Vacuum Gauges

The 4800 MALDI TOF/TOF™ Analyzer includes four vacuum gauges:

- **System BA (Bayard-Alpert) Vacuum Gauge** – Monitors pressure in the entire system.
- **CID BA Vacuum Gauge** – Monitors pressure in the collision cell region of the second source chamber.
- **Load TC (Thermocouple) Vacuum Gauge** – Monitors pressure in the sample loading chamber.
- **Foreline TC Vacuum Gauge** – Monitors base roughing pressure, backing pressure of turbo pumps, and CID expansion pressure.

Readings from the vacuum gauges are displayed in the 4000 Series Explorer™ software System Status bar (see Figure 2-1 on page 2-5).

Digitizer

An analog-to-digital converter converts the analog signal from the mass spectrometer to a digital signal that can be transmitted to the computer.

Computer Components

CAUTION Do not enable screen savers on the 4800 MALDI TOF/TOF™ Analyzer computer. Because screen savers use computer memory and processor time, they can decrease system performance or cause other system problems.

The computer that operates the 4000 Series software and controls the mass spectrometer includes the following computer hardware components:

- Minimum computer configuration of a dual Pentium® 4 Xeon™ 2.4-GHz CPU, two 120-GB hard disk drives, and 1-GB RAM (random access memory)
- CD/DVD combination drive (rewritable)
- Integrated Ethernet card (10/100 Base - Tx)
- Additional Ethernet card
- 20-inch color flat-panel monitor
- Dell® keyboard
- Dell optical mouse
- Logitech® Control pad
- Optional computer desk

Software

Software components include:

- Microsoft® Windows® XP
- Applied Biosystems/MDS SCIEX 4000 Series Explorer™ software
- Data Explorer® software (Post-processing software)
- Peak Explorer™ software (Post-processing software)
- Peaks to MASCOT® tool (Database Filtering tool)
- 4000 Series Database tools (Data archiving, restoring, backing up, and retrieving tools)
- DeNovo Explorer™ software (Database searching tool)
- Microsoft Office Professional Edition 2003
- Oracle® Database version 9.2 (Standard Edition)

Optional available software includes:

- 4000 Series Explorer™ Remote Access Client software – Allows acquisition of data by and access to data from a remote computer.
- GPS Explorer™ software and GPS Explorer™ Remote Access Client – In conjunction with 4000 Series Explorer™ software, automates proteomic applications.
- Pro BLAST software – Applied Biosystems/MDS SCIEX proprietary database searching tool.

Remote Access Client Software

If you install the optional 4000 Series Explorer™ Remote Access Client software on a computer that you provide, the computer must meet the following minimum requirements:

- Single processor, 850 Mhz
- 256 MB RAM
- 20 GB of free disk space
- Windows XP Professional with Service Pack 1 or later, or Windows 2000 Professional with Service Pack 3 or later
- Regional Options set to **English (United States)**
- Internet Explorer 6.0 with Service Pack 1 or later
- Microsoft® Office 2000
- 100 Mb/s Ethernet interface
- LAN connection to a 4800 MALDI TOF/TOF™ Analyzer that has 4000 Series Explorer™ software version 3.X series installed

- Either of the following configurations:
  - Oracle® software *not* installed
  - Oracle® software version 9.2 (or later) installed

Note: When simultaneously connecting computers with 4000 Series Explorer™ Software – Remote Access Client to a 4800 MALDI TOF/TOF™ Analyzer, connect no more than four.

Optional Handheld Bar Code Scanner

The optional bar code scanner allows you to scan MALDI plate bar codes in two ways:

- With the scanner mounted in the holder
- By hand

LASER HAZARD. Class 2 (II) lasers can cause damage to eyes. Avoid looking into a Class 2 (II) laser beam or pointing a Class 2 (II) laser beam into another person's eyes.

Note: For optimal operation, the scanner must be approximately 25 cm (10 in.) from the plate. The laser beam must be at a slight angle to and illuminate the entire length of the bar code.

For more information, see the *4800 MALDI TOF/TOF™ Analyzer Getting Started Guide*, the *Handheld Bar Code Scanner Installation Guide* and the *QuickScan® 6000/6000plus AutoSense Stand Instructions,* and "Using the Handheld Bar Code Scanner" on page F-3 of this guide.

Preparing the 4800 MALDI TOF/TOF™ Analyzer 2

This chapter covers:

Starting and Exiting the 4000 Series Explorer™ Software

Starting the Software

To start the 4000 Series Explorer™ software:

1. Log on to the 4800 MALDI TOF/TOF™ Analyzer computer using your User Name and Password. (See your system administrator for your User Name and Password.)
2. Start the software by double-clicking  on the Microsoft® Windows® XP desktop.

   The 4000 Series Explorer™software opens in Interactive mode. You can switch between Interactive mode and Batch mode by clicking  on the toolbar.

   Note: If the system has been shut down, the hardware automatically starts to initialize when you start the software. Initialization may take several minutes to complete. Before using the instrument, allow the vacuum pressures to stabilize.

Exiting the Software

CAUTION Do not exit the 4000 Series Explorer™ software or power off the 4800 MALDI TOF/TOF™ Analyzer unless you need to perform maintenance or move the system (see "Reconnecting the Hardware" on page 5-10).

After you exit the software, you cannot control the analyzer. Exiting the software also powers off high voltages, which can require up to three hours of warm up when you restart the software.

Powering off the system shuts down the vacuum pumps and causes the system to vent.

Accessing Database Software and Tools

The 4000 Series Explorer™ software includes several database software programs and tools. Most of the programs can be accessed from a menu (Table 2-1).

Table 2-1 Database software and tools

Tool	Function	Access
Peak Explorer™ Software	Post-processing of spectral data	Right-click the spot set row(s) of interest.
Data Explorer™ Software 	Post-processing of spectral data	• In the 4000 Series Explorer™ software, click **Tools > Launch Data Explorer**. • Click the icon on the 4000 Series Explorer™ software toolbar. • Click the icon on desktop. • Right-click the spot set row(s) of interest.
Peaks to MASCOT Software 	Peak filtering prior to MASCOT database searching	• In the 4000 Series Explorer™ software, click **Tools > Launch Peaks to Mascot**. • Click the icon on the 4000 Series Explorer™ software toolbar.
DeNovo Explorer™ Software	Spectral database searching	• On the desktop taskbar, click **Start > Applied Biosystems > DeNovo Explorer**. • Right-click the spot set row(s) of interest
Database Tools	• Archive, purge, and restore spot set data • Back up, recover, and migrate the database	On the desktop taskbar, click **Start** > **Applied Biosystems > 4000 Series Database Tools**.

Checking Pressures and High Voltages

Checking Pressures

Before using the mass spectrometer to acquire data, check the pressure readings on the Status bar at the bottom of the 4000 Series Explorer™ software (Figure 2-1 on page 2-5). Check that:

- The vacuum gauge panes (Source 1, Source 2, TCLoad, TCForeline) are green. Green indicates that the pressure readings are adequate (below the maximum set in the hardware configuration).
- The actual pressure readings are adequate for optimal data quality (Table 2-2).

Table 2-2 Chamber pressure for optimal data quality

Chamber	Adequate Pressure (Torr)
Source 1	Less than 5×10^{-7}
Source 2	Less than 5×10^{-7} (if not using CID gas) Less than 5×10^{-6} (if using CID gas)

If pressures are not adequate, allow the system to reach adequate vacuum. Make sure the CID system is off if you are not using it.

CAUTION Do not run the 4800 MALDI TOF/TOF™ Analyzer under inadequate vacuum conditions. Doing so results in inaccurate data.

Checking High Voltages

To acquire the best data, high voltages should be powered on and allowed to stabilize for 30 minutes before using the instrument.

With an acquisition method open, power on high voltages by clicking  in the toolbar. You can verify that high voltages are on by checking the Status bar at the bottom of the 4000 Series Explorer™ software.

Note: An acquisition method must be open to power on high voltages.

Checking System Status

Overview

Three locations display information about the status of the mass spectrometer:

- Status bar
- Hardware Monitor
- CID System viewer

Status Bar

The Status bar (Figure 2-1) is at the bottom of the 4000 Series Explorer™ software window.

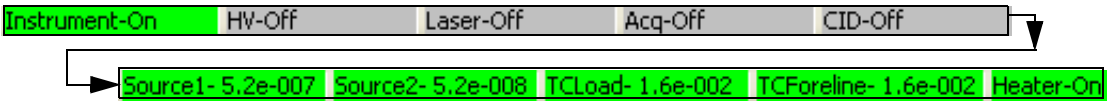


Figure 2-1 Status bar

The status bar displays:

- Instrument status (On, Off, Starting, Busy, Initializing, Deinitializing, Error, or Recovering)
- High-Voltage status (Ramping, On, Off, Interlock, or Error)
- Laser status (Turning On, On, Stabilizing, Turning Off, Off)
- Acquisition status (Starting, On, Stopping, Off, or Error)
- CID System status (Turning On, On, Turning Off, Off, Purging, Purged, or Charging)
- Pressure readings of Source 1, Source 2, and foreline vacuum gauges (TCLoad, TCForeline). See "Checking Pressures" on page 2-4.
- Heater status (Ramping, On, Off, Error)

  Note: Heater status does not affect data quality.

For more information, see *4000 Series Explorer™ Online Help,* Status Bar Parameters.

Each panel in the status bar is color coded (Table 2-3).

Table 2-3 Status bar color code

Color	Description
Green	Displayed during normal operation (for example, when displaying On or normal pressure readings).
Blue	Displayed when a component is changing status (initializing, ramping, starting, stabilizing).
Gray	Displayed when a component is turned off.
Yellow	Displayed when a fault condition occurs (for example, if a pressure reading exceeds the maximum pressure set in the Hardware Configuration - Vacuum Tab)
Pink	Displayed if a pressure reading exceeds the maximum pressure set in the Hardware Configuration - Vacuum Tab for more than 30 seconds. Additionally, high voltages cannot be powered on. This protects the instrument from damage.

Hardware Monitor

Applied Biosystems/MDS SCIEX Technical Support uses the Hardware Monitor when troubleshooting the system. Select **Instrument> Hardware Monitor** to display settings and readings for many hardware components, including the key vacuum system components.

CID System Viewer

To display the CID System viewer (Figure 2-2), select **View > CID System Viewer** or click the CID icon.

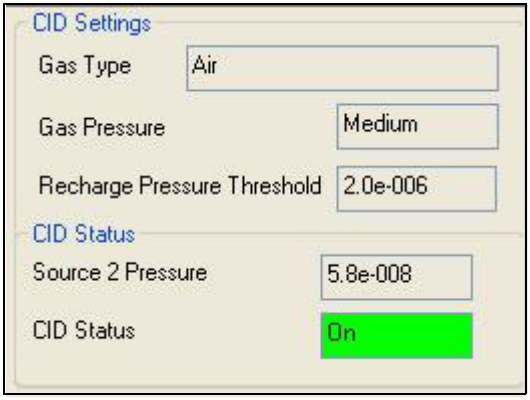


Figure 2-2 CID System viewer

The CID System viewer displays the following settings and statuses:

- **Gas Type** – The type of gas currently used in the CID system (Heavy Weight, Medium Weight, Light Weight, Atmosphere), as set in the spot set job.
- **Gas Pressure** – The currently selected pressure of the CID gas (Medium or High; no CID gas is Low), as set in the spot set job.
- **Recharge Pressure Threshold** – The pressure limit at which the CID system is automatically refilled, as set in the Hardware configuration (Figure 2-6 on page 2-15).
- **Source 2 Pressure** – The current gas pressure in the second source chamber.
- **CID Status** – The current status of the CID System (Off, On, Turning On, Turning Off, Charging, or Purging). To purge or turn off the CID, select the appropriate option in the **Instrument** menu.

Setting Laser Intensity Adjustments

You adjust laser intensity using the fine and coarse laser control buttons in the 4000 Series Explorer™ software Manual Control window and on the Control Pad (Figure F-1 on page F-2).

Note: The Manual Control window has both fine and coarse laser control buttons. The Control Pad has only fine laser control buttons.

The laser control buttons decrease and increase laser intensity when you are in Interactive mode and using Manual control. You cannot manually adjust the laser intensity from the Remote Access Client software or during an automatic control acquisition. For more information, see the *4000 Series Explorer™ Online Help,* Manually Adjusting Laser Intensity.

To set the adjustment increments of the fine and coarse laser control buttons:

1. Select **Tools > Preferences**.
2. In the Preferences dialog box, select the **Laser** tab.
3. Type or select adjustment units in the Fine and Coarse text boxes, then click **Apply**. Default values are 10 and 100, respectively.

   The adjustment units you enter are the units of laser intensity that are increased or decreased each time the laser control buttons are clicked or pressed.

Setting Bar Code Preferences

If you use the handheld bar code scanner with the 4800 MALDI TOF/TOF™ Analyzer, you must configure the software to enable the bar code scanner.

Note: The handheld bar code scanner works only with 4000 Series Explorer™ Software Version 3.0 or higher and with 4700 Explorer™ Software Version 2.0 or later.

To set the bar code scanner preferences:

1. Select **Tools** > **Preferences** .
2. In the Preferences dialog box, select the **Bar Code** tab.
3. Select **Enable Scanner**. The tab expands (Figure 2-3).

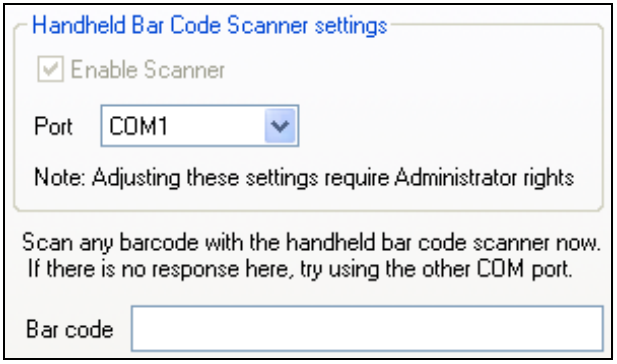


Figure 2-3 Expanded Bar Code tab parameters

4. Select **COM1** in the Port drop-down list.
5. Scan a bar code. The code is displayed in the Bar code field and the scanner is enabled.

   IMPORTANT! If the code is not displayed in the Bar code field, select another port, then rescan.
6. Click **OK**.

Configuring the Hardware

You can configure the:

- Vacuum system
- High Voltage
- Laser
- Light
- CID System
- CID Gases

IMPORTANT! You cannot configure hardware using the 4000 Series Explorer™ Remote Access Client software.

Vacuum Configuration

To check the vacuum configuration:

1. In the 4000 Series Explorer™ software, select **Instrument > Hardware Configuration**.
2. Select the **Vacuum 4800** tab (Figure 2-4).

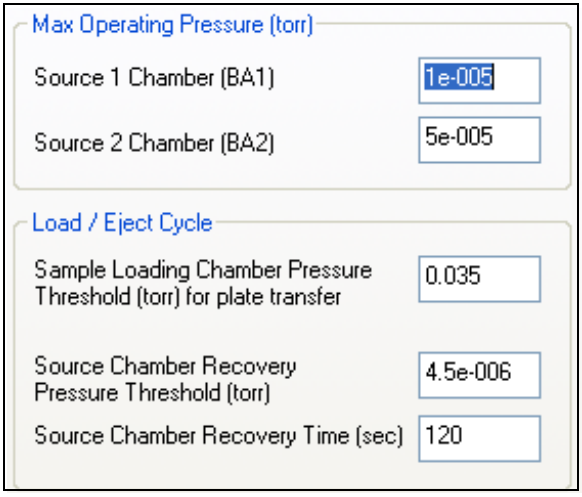


Figure 2-4 Vacuum configuration parameters

3. Specify the values as needed (Table 2-3):

Table 2-4 Vacuum configuration parameters

Parameter	Description
Max Operating Pressure (torr) Pressure above which the high-voltage power supplies are automatically powered off to prevent damage to the instrument. If the chamber pressure is above the Max Operating Pressure, an error message is displayed, and the corresponding pane in the Status Bar turns yellow, then pink if the over-pressure persists for more than 30 seconds. The high voltages cannot be turned on, and the software writes an error in the Windows® 2000 Event log.	
Source 1 Chamber (BA1)	Valid range is 10^{-5} to 10^{-9} torr. Default is 9×10^{-6} torr.
Source 2 Chamber (BA2)	Valid range is 10^{-4} to 10^{-5} torr. Default is 5×10^{-5} torr.
Load/Eject Cycle	
Sample Loading Chamber Pressure Threshold (torr) for plate transfer	Pressure the sample loading chamber must reach before the sample plate moves in or out of the Source Chamber. High values allow faster plate loading. Conversely, a low value is closer to the Source Chamber Recovery Pressure Threshold value (below), decreasing the pump down needed and allowing high voltages to be turned on sooner. Therefore low values allow a smaller Source Chamber Recovery Time value (below).
Source Chamber Recovery Pressure Threshold (torr)	After the sample plate is loaded or ejected, pressure the source chamber must reach before the high voltages can be turned on.
Source Chamber Recovery Time (sec)	After the sample plate is loaded or ejected, the time that the software “waits” for the Source 1 Chamber to reach the Max Operating Pressure. Valid range is 0 to 300 seconds. Default is 120 seconds. If the wait time is exceeded, an error message is displayed that gives you the option of an additional wait time or ejecting the plate.

4. Click **OK** to apply the changes and exit the hardware configuration.

High-Voltage, Laser, and Light Configurations

To check the high-voltage, laser, and light source configurations:

1. In the 4000 Series Explorer™ software, select **Instrument > Hardware Configuration**.
2. Select the **High Voltage/Laser/Light** tab (Figure 2-5).

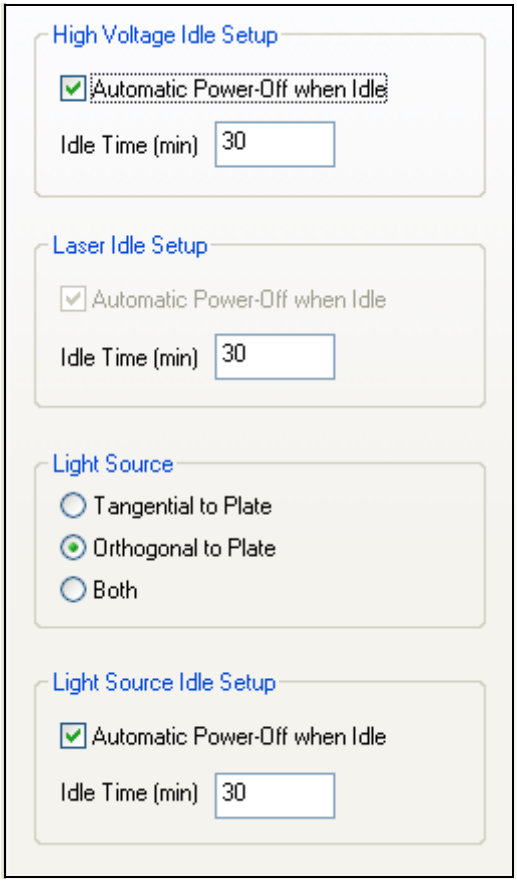


Figure 2-5 High voltage/laser/light tab parameters

3. Specify the High Voltage Idle Set-Up Power-Off and Idle Time (min) parameters.

   IMPORTANT! After restarting the high-voltage power supplies, allow the high-voltage power supplies to warm up for at least 30 minutes before acquiring data.

   - When you select Automatic Power-Off when Idle, specify an Idle Time (the number of minutes of instrument inactivity after which the high-voltage power supplies are automatically powered off). The default is 60 minutes.
   - When you deselect Automatic Power-Off when Idle, the high voltage remains on until overridden by any of the following actions:

     – You select **Instrument > Turn off High Voltage**.

     – Source 1 or Source 2 pressure exceeds the Maximum Operating Pressure.

     – You click **Load** or **Eject**.

     – The interlock is broken.

4. Specify a Laser Idle time (the number of minutes of laser inactivity after which the laser is automatically powered off).The default is 30 minutes.

   Automatic Power-Off when Idle is always enabled. Shutting off the laser extends its lifetime.

5. Select the Light Source:
   - **Tangential to Plate** – The Video Viewer window displays the sample spot as white crystals on a black background. Light illuminating the sample is at an angle to the plate (Figure 1-8 on page 1-13).
   - **Orthogonal to Plate** – The Video Viewer window displays the sample spot as black crystals on a white background. Light illuminating the sample is directly above the plate. Use this option for stainless steel plates (Figure 1-8 on page 1-13).
   - **Both** – The video viewer displays the sample spot as white crystals on a black background. Both lights (tangential and orthogonal) illuminate the sample. Use to achieve a 3 D effect, for example, when viewing 3-HPA matrix.

6. Specify the Light Source Idle Setup and Idle Time (min) parameters.
   - When you select Automatic Power-Off when Idle, you can specify an Idle Time (the number of minutes of inactivity after which the light source power supply automatically turns off). The default is 60 minutes. Powering off the light source extends the lifetime of the tangential light source. The light source powers on again when you perform any one of the following tasks:
     – Start an acquisition
     – Load, eject, mount, unmount, or move a sample plate
     – Turn high voltages on or off
     – Specify any hardware configuration parameter
     – Reinitialize the system
     – Adjust the light source or laser intensity
     – Charge the CID cell or turn CID on or off
   - When you deselect Automatic Power-Off when Idle, the light source remains on.
7. Click **OK** to apply the changes and exit the hardware configuration.

Local Instrument Configuration

To check the local instrument configuration:

1. In the 4000 Series Explorer™ software, select **Instrument > Hardware Configuration**.
2. Select the **Local Instrument** tab.
3. Review the following read-only fields:
   - **Instrument type –** Displays your instrument type.
   - **Serial Number –** Displays the serial number for the connected instrument. The serial number is printed on most printouts from the software.
   - **Laboratory Name –** Displays your laboratory name as entered by the Applied Biosystems/MDS SCIEX representative during installation.
   - **Instrument Name –** Displays your instrument name as entered by the Applied Biosystems/MDS SCIEX representative during installation. The Instrument Name is printed on most printouts from the software.
4. Click **OK** to exit the hardware configuration.

Geometry Configuration

The Geometry tab displays parameters and settings that should be changed only by an Applied Biosystems/MDS SCIEX technical representative. It is password protected. The date of the Last Source Model Calibration, which is done only by an Applied Biosystems/MDS SCIEX technical representative, is also displayed.

Do not change the Geometry Hardware Configuration. Changing these settings can cause your system to malfunction.

CID System Configuration

To check the CID system configuration:

1. In the 4000 Series Explorer™ software, select **Instrument > Hardware Configuration**.
2. Select the **CID System** tab.
3. Specify the Fault Conditions Consecutive Charges Limit. This feature prevents the CID from continually recharging if a leak is present.

   Consecutive Charges Limit is the maximum number of consecutive collision gas charges allowed of the CID system in an effort to exceed a recharge threshold pressure. Valid range is 1 to 20. The default is 20.

   If the limit is exceeded, recharging ceases, the CID valve closes, the system goes into a fault state and will not operate until the problem is resolved.
4. Specify the Gas Inlet 1 Attached Gas Weight. Select:
   - **Heavy** for gases such as xenon
   - **Medium** for gases such as air, nitrogen, and argon
   - **Light** for gases such as helium and neon
5. Click **OK** to apply the changes and exit the hardware configuration.

CID Gases Configuration

To check the CID gases configuration:

1. In the 4000 Series Explorer™ software, select **Instrument** > **Hardware Configuration**.
2. Select the **CID Gases** tab (Figure 2-6).

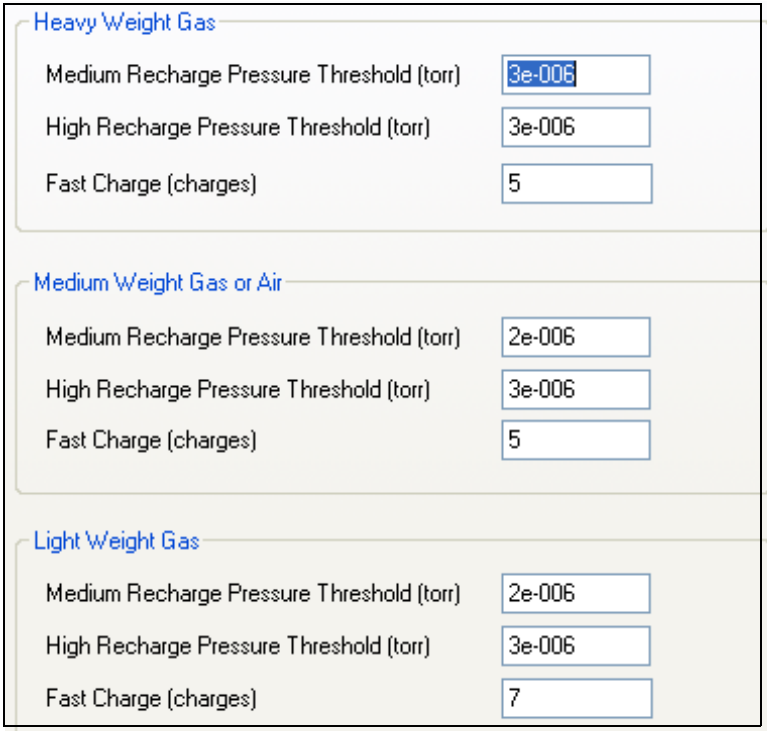


Figure 2-6 CID Gases tab

3. Specify the threshold and fast charge values (Figures 2-6):

   Note: After lowering a recharge threshold value, purge the CID.

   - **Recharge Pressure Threshold** – The Source 2 chamber pressure below which the CID system automatically recharges with the selected gas. The active threshold value is the value corresponding to both the:

     – Selected gas type (Heavy, Medium, Light) in the Hardware Configuration CID System tab

     – Selected gas pressure (Medium, High) in the Spot Set Manager-Job tab.

     The current gas type and pressure are display in the CID System Viewer. Optimize gas pressure for each gas by adjusting the value until you see the desired fragmentation.
   - **Fast Charge** – The number of charges applied the first time you fill the collision cell, for example, when changing from one gas to another.

   Table 2-5 indicates the valid ranges and default values.

Table 2-5 CID Gases parameters

Parameter	Weight Gas		
	Heavy Such as xenon	**Medium or Air** Such as nitrogen or argon	**Light** Such as helium and neon
Medium Recharge Threshold			
Valid range	5×10^{-7} to 1×10^{-5} torr	5×10^{-7} to 1×10^{-5} torr	5×10^{-7} to 1×10^{-5} torr
Default	1.25×10^{-6} torr	1.25×10^{-6} torr	1.25×10^{-6} torr
High Recharge Threshold			
Valid range	5×10^{-7} to 1×10^{-5} torr	5×10^{-7} to 1×10^{-5} torr	5×10^{-7} to 1×10^{-5} torr
Default	2.5×10^{-6} torr	2.5×10^{-6} torr	2.5×10^{-6} torr
Fast Charge			
Valid range	1 to 10	1 to 10	1 to 10
Default	7	7	7

4. Click **OK** to apply the changes and exit the hardware configuration.

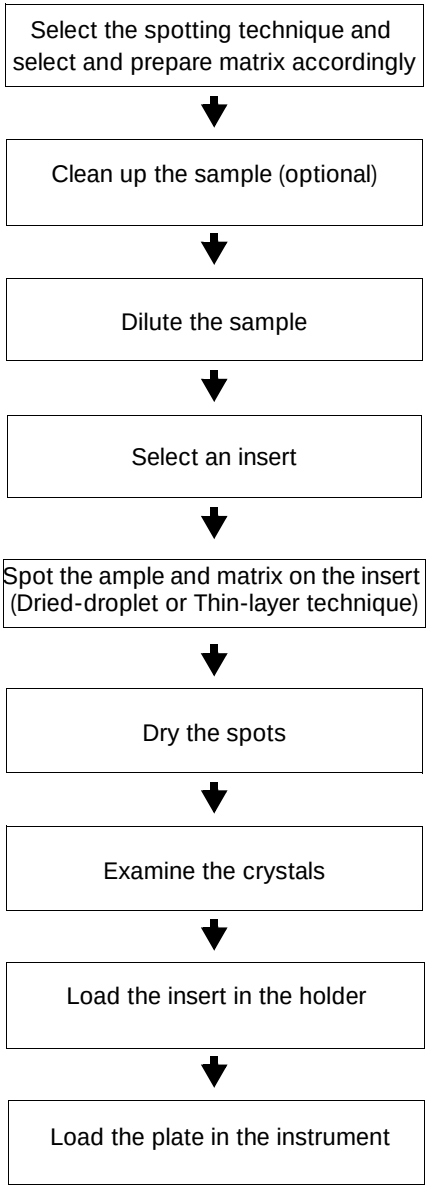
Preparing and Spotting Sample and Matrix

3

This chapter covers:

Overview

In MALDI-TOF applications, the sample preparation technique directly affects the quality of the data you obtain. To prepare samples for analysis:



Selecting a Spotting Technique

The two techniques presented for sample spotting are dried droplet and thin layer. The dried-droplet technique is a simple technique suitable for most applications. The thin-layer technique is used for increased sensitivity, that is, when analyzing peptides with a final concentration <0.1 pmol/mL and CHCA matrix.You can also use the dried-droplet technique to analyze samples with a final concentration <0.1 pmol/mL by decreasing the CHCA matrix concentration and adding ammonium salts (see Zhu, X, and Papayannopoulos, I, 2003).

Dried Droplet

The dried-droplet technique is recommended for most applications. With this technique, you can for mix sample and matrix by one of the methods described in Table 3-1.

IMPORTANT! When using CHCA, follow the corresponding matrix preparation in Table 3-3 on page 3-10.

Table 3-1 Dried-Droplet Technique Mixing Methods

Mixing Method	Description	Conditions for Using
No mixing. Overlaying the sample and matrix solutions	Spot the sample, allow the sample spot to dry, then spot matrix.	• For most applications with CHCA matrix • When you want to preserve the sample
	If the matrix is highly soluble in the sample, spot the matrix first, then the sample.	• When you work with DHB matrix
Mixing on sample plate	Mix the sample solution and matrix solution directly on the sample plate. The sample solution is typically diluted 1:2 with matrix solution.	• When you work with dilute samples and can spot equal volumes of sample solution and matrix solution to create a 1:2 dilution • When you prepare only a few samples • When you are concerned about sample adsorbing to the plastic tubes
Premixing in a tube	Premix the sample solution and matrix solution in a microcentrifuge tube before applying to the sample plate. The sample solution is typically diluted 1:10 with matrix solution.	• When you have concentrated samples with a high salt concentration and need to make dilutions • When you prepare many samples • When you are analyzing nonpolar samples and matrix prepared in high concentrations of organic solvent that evaporates rapidly

Thin-layer

The thin-layer technique requires a polished blank sample plate or insert (no etched positions). The matrix solution and sample solution are spotted and dried separately.

IMPORTANT! When using the thin-layer technique for high-throughput applications, acquire data in interactive mode using Automatic Acquisition Control. In the acquisition method, specify the following:

- In the Spectrum tab, set the Shots/sub-spectrum low (typically less than 20).
- In the Automatic Control tab, Sample Stage Mode, select **Move before every sub-spectrum (pass or fail)**.

Selecting the Matrix

Overview

Below are general guidelines for selecting a matrix for your application. However, when examining a sample mixture, you may need to prepare the mixture more than once, each time with a different matrix.

Proteomics Applications

For proteomics applications, you can use any of the following matrices.

Application	Recommended Matrix
Proteomics applications for peptides and proteins <10,000 Da. Most commonly used matrix because it is easy to prepare and set up and provides good sensitivity.	α-cyano-4-hydroxycinnamic acid (CHCA)
Peptides and proteins >10,000 Da	Sinapinic acid (3,5-dimethoxy-4-hydroxy cinnamic acid)
Peptides and proteins <10,000 Da	2,5-dihydroxybenzoic acid (DHB) (gentisic acid)
• Peptides and proteins >10,000 Da • Glycosylated proteins	2,5-dihydroxybenzoic acid and 5-methoxysalicylic acid (sDHB)

Non-Proteomics Applications

For non-proteomics applications, you can use any of the following matrices.

Application	Recommended Matrix
• Small oligonucleotides (<3,500 Da) • Acidic carbohydrates • Acidic glycopeptides • Acid-sensitive compounds	2,4,6-trihydroxyacetophenone (THAP)
Large oligonucleotides (>3,500 Da)	3-hydroxypicolinic acid (3-HPA) in ammonium citrate
• Neutral or basic carbohydrates • Glycolipids (negative ions), • Polar and non-polar synthetic polymers • Small molecules	2,5-dihydroxybenzoic acid (DHB)

Synthetic Polymers

When analyzing synthetic polymers, choose a matrix according to the type of synthetic polymer sample you are analyzing:

- **Aromatic (for example, polystyrene) –** Use 25 mg/mL dithranol and 1 mg/mL silver trifluoroacetate (Ag TFA) dissolved in tetrahydrofuran (THF)
- **Polar –** Use 10 mg/mL DHB dissolved in deionized water
- **Non-polar –** Use 10 mg/mL indole acrylic acid (IAA) or DHB dissolved in THF, dimethylformamide (DMF), or acetone

Preparing the Matrix

WARNING **CHEMICAL HAZARD.** Refer to the Material Safety Data Sheet (MSDS) provided by the chemical manufacturer before handling solvents or matrices.

Required Materials

- Balance
- 1.5 mL microcentrifuge tubes
- Micropipettor and disposable tips
- Centrifuge
- Vortex mixer
- Matrix
- Solvents (see the requirements for the specific matrix)
- Deionized water

Guidelines

Careful preparation of sample and matrix, and proper spotting, are key to obtaining optimal results. Follow the guidelines below:

- For sample and matrix handling, use high-quality plastic pipette tips, vials, tubes, and trays. Lower-quality consumables are more likely to lead to extraneous peaks caused by plasticizers and other potential contaminants. (Lower-quality consumables also may cause high peptide loss due to adsorption of the peptides to the plastic.) If you detect extraneous peaks, try prewashing the consumables with the same solvent used in your sample or matrix.
- Use a fresh tip each time you pipette a different substance.
- Using a solvent with an organic concentration greater than 50% can cause spreading when spotting sample and matrix on the plate. If sample spreading occurs, decrease the organic concentration in the solvent to less than 50%.
- Use double-deionized water such as Milli-Q® grade 18 mΩ water, which is appropriate for any application. HPLC-grade water can vary in salt concentration and may produce adduct ions in mass spectra. High salt concentrations can interfere with some applications, particularly oligonucleotide analysis.
- Some matrices degrade on exposure to light or humidity. For stability information and storage conditions, refer to the manufacturer's instructions.

- After spotting, example the crystal structure. Appendix C, "Matrices," contains additional information on matrix structure, appearance, and matrix solutions. For additional information on other matrices and their applications, refer to the Bibliography at the end of this guide.
- Matrices other than a-cyano-4-hydroxycinnamic acid (CHCA) may have unevenly distributed crystallization. Acquire data on these spots in Interactive mode using the Video Viewer to manually select the area of the spot you want to acquire. For information, see the *4000 Series Explorer™ Online Help*, Acquiring Data from the Video Viewer.

IMPORTANT! When using the thin-layer technique for high-throughput applications, acquire data in interactive mode using Automatic Acquisition Control. In the acquisition method, specify the following:

- In the Spectrum tab, set the Shots/sub-spectrum low (typically less than 20).
- In the Automatic Control tab, Sample Stage Mode, select **Move before every sub-spectrum (pass or fail)**.

General Preparation Procedure

To prepare a specific matrix, follow the procedure below, using the solvents, concentrations, and modifications indicated in the preparation information for the matrix (refer to the tables listed in Table 3-2).

Table 3-2 Preparation requirements for specific matrices

Matrix	Modifications to the General Preparation Procedures	Recommended Expiration After Preparation[a]
CHCA (a-cyano-4-hydroxycinnamic acid)	Table 3-3 on page 3-10 (dried droplet) Table 3-4 on page 3-13 (thin layer)	One week
Sinapinic acid (3,5-dimethoxy-4-hydroxy cinnamic acid)	Table 3-5 on page 3-14	One day
DHB (2,5-dihydroxybenzoic acid or Gentisic acid)	Table 3-6 on page 3-16 (peptides and proteins <10,000 Da) Table 3-7 on page 3-17 (neutral carbohydrates) Table 3-8 on page 3-18 (small molecules)	One week
sDHB (2,5-dihydroxybenzoic acid and 5-methoxysalicylic acid)	Table 3-9 on page 3-19	One week
THAP (2,4,6-trihydroxyacetophenone)	Table 3-10 on page 3-20	One week
3-HPA (3-hydroxypicolinic acid)	Table 3-11 on page 3-22	One week
Synthetic polymer matrices	Table 3-12 on page 3-23	One week[b]

a. Follow the manufacturer's instructions for storage conditions.

b. Store THF and acetone solutions in tightly sealed vials.

To prepare matrix:

1. Label a 1.5-mL microcentrifuge tube with the name of the matrix, the final concentration, the date prepared, and expiration date. To determine the expiration date, see Table 3-2.
2. Zero the balance with the labeled tube.
3. Weigh out the matrix into the tube.

   Note: You do not need to weigh out the exact amount of matrix. You can record the weight of the matrix and adjust the volume of solvent to achieve the desired final concentration.
4. Add a volume of appropriate solvent to achieve the needed concentration for your matrix. To determine the appropriate solvent and the proper concentration for your matrix, see the specific matrix information. The solvent should also be miscible with the sample.
5. Cap the tube and vortex thoroughly for approximately 1 minute or until dissolved. You can shake the tube by hand if you do not have a vortex mixer.
6. If your matrix requires an additive:
   a. Zero the balance with another microcentrifuge tube and weigh out the additive into the tube. To determine the additive required for your matrix, see the individual matrix information.
   b. Add a volume of appropriate solvent to achieve the needed concentration for the additive. To determine the appropriate solvent and the proper concentration for your additive, see the individual matrix information.
   c. Cap the tube and vortex thoroughly for approximately 1 minute or until dissolved.
   d. Add the additive solution to the matrix solution.
7. Centrifuge the tube for 30 seconds at 2,000 to 5,000 rpm. Alternatively, allow the solution to settle for about 10 minutes. You may see a precipitate at the bottom of the tube.

Preparing CHCA Matrix for the Dried-Droplet Spotting Technique

The dried-droplet spotting technique is used for most high-throughput analyses of peptides and proteins <10,000 Da.

Note: When analyzing samples with concentrations of 0.1 pmol/µL or lower, the CHCA matrix sodium and potassium adducts interfere with MS analysis and spectral interpretation. To reduce the interference from these matrix adducts, add ammonium monobasic phosphate or ammonium dibasic citrate when preparing CHCA matrix (see Zhu, X, and Papayannopoulos, I, 2003).

WARNING **CHEMICAL HAZARD.** Read the MSDS before handling any chemical mentioned below, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **Alpha-cyano-4-hydroxycinnamic acid (CHCA)** may cause eye, skin, and respiratory tract irritation. **Acetonitrile (ACN)** is a flammable liquid and vapor. Exposure may cause eye and respiratory tract irritation and blood system damage. **Trifluoroacetic acid (TFA)** causes eye, skin, and respiratory tract burns. It is harmful if inhaled. **Ethanol** is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation and may cause central nervous system depression and liver damage.

Table 3-3 CHCA matrix information for the dried-droplet spotting technique

Attribute/Procedure	Description
Matrix Preparation	
Matrix stability	Prepare weekly.
Matrix solvent	• 0.1% TFA in deionized water • 0.1% TFA in acetonitrile
Matrix concentration	5 mg/mL; 2 mg/mL for samples with a concentration of ≤1 fmol/µL
Matrix additive (samples with concentrations of 0.1 pmol/µL or lower)	ammonium monobasic phosphate or ammonium dibasic citrate (see Zhu, X, and Papayannopoulos, I, 2003)

Table 3-3 CHCA matrix information for the dried-droplet spotting technique *(continued)*

Attribute/Procedure	Description
General preparation modifications[a]	IMPORTANT: If the dry matrix has a mustard-yellow color instead of bright yellow, it may contain impurities. To recrystallize (purify), dissolve the CHCA matrix in warm ethanol. Filter and add about two volumes of deionized water. Let stand overnight in an explosion proof-refrigerator. Filter, then wash the precipitate with cold water. This procedure is not optimized for yield. Follow the procedure in "General Preparation Procedure" on page 3-8. In step 4, prepare the matrix solvent by combining 0.1% TFA in water with 0.1% TFA in acetonitrile. The ratio of water to acetonitrile depends on how you plan to mix the sample and matrix solutions: • **When premixing the sample solution in matrix solution, typically at a 1:10 dilution (sample solution to total volume) –** Use a 1:1 ratio of water to acetonitrile. Combine one part 0.1% TFA in water with one part 0.1% TFA in acetonitrile, and, if using matrix additive, add the matrix additive (skip step 6 of the "General Preparation Procedure" on page 3-8). Add solvent solution to the CHCA matrix. For example, for a final concentration of 5 mg/mL, combine 500 µL 0.1% TFA in water, 500 µL 0.1% TFA in acetonitrile, and matrix additive (if appropriate), then add the mix to 5 mg solid matrix. • **When overlaying or mixing on the plate, typically at a 1:2 dilution (sample solution to total volume) –** Use a 4:6 ratio of water to acetonitrile. Combine four parts 0.1% TFA in water with six parts 0.1% TFA in acetonitrile. Add solvent solution to the CHCA matrix for a final concentration of 5 mg/mL. For example, combine 400 µL 0.1% TFA in water and 600 µL 0.1% TFA in acetonitrile, then add the mix to 5 mg solid matrix. Note: A higher concentration of TFA (up to 3%) may improve sample solubility.
Spotting	
Spotting technique	Dried-droplet
Plate drying method	Air-dry the sample plate after spotting sample and matrix, or use gentle air flow to speed drying.
Matrix crystals	Rounded (see Figure 3-1 on page 3-42)
Final sample concentration	0.1 to 5 pmol/µL

a. Shevchenko, A., M. Wilm, O. Vorm, M. Mann, *Anal. Chem.,* **1996**, *68*, 850–858.

Preparing CHCA Matrix for the Thin-Layer Spotting Technique

The thin-layer spotting technique is used when you need increased sensitivity, for example, when sample concentration is 0.1 pmol/µL or lower. Peptides must be <~3,000 Da.

WARNING **CHEMICAL HAZARD.** Read the MSDS before handling any chemical mentioned below, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **Alpha-cyano-4-hydroxycinnamic acid (CHCA)** may cause eye, skin, and respiratory tract irritation. **Acetone** is a flammable liquid and vapor. Exposure may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry the skin. Exposure may cause central nervous system depression. Keep away from heat, sparks, and flame. **Isopropanol** is a flammable liquid and vapor. Exposure may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin and cause irritation. Exposure may cause central nervous system effects such as drowsiness, dizziness, and headache. **Ethanol** is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation and may cause central nervous system depression and liver damage.

Table 3-4 CHCA matrix information for the thin-layer spotting technique

Attribute/Procedure	Description
Matrix Preparation	
Matrix stability	Prepare weekly.
Matrix solvent	• Pure nitrocellulose (for example, Bio-Rad Laboratories Trans-Blot® 162-0146) • Acetone • Isopropanol
Matrix concentration	20 mg/mL
Matrix additive	None
General preparation modifications[a]	IMPORTANT: If the dry matrix has a mustard-yellow color instead of bright yellow, it may contain impurities. To recrystallize (purify), dissolve the CHCA matrix in warm ethanol. Filter and add about two volumes of deionized water. Let stand in an explosion-proof refrigerator overnight. Filter, then wash the precipitate with cold water. This procedure is not optimized for yield. Follow the procedure in "General Preparation Procedure" on page 3-8. To prepare the solvent solution used in step 4, first prepare 20 mg/mL nitrocellulose in acetone solution. To dissolve nitrocellulose in acetone, vortex for 15 minutes. Next, combine one part nitrocellulose solution with one part isopropanol. Add the solvent solution to the CHCA matrix for a final concentration of 20 mg/mL.
Spotting	
Spotting technique	Thin-layer
Plate drying method	Air-dry the sample plate after spotting matrix solution. After spotting sample solution, dry the sample plate with gentle air flow, such as a heat gun set at low heat and air-flow settings.
Matrix crystals	Rounded (see Figure 3-1 on page 3-42)
Final sample concentration	Up to 0.1 pmol/µL

a. Other special sample preparation techniques are described in Shevchenko, A., M. Wilm, O. Vorm, M. Mann, *Anal. Chem.,* **1996**, *68*, 850–858.

Sinapinic Acid Matrix

Use sinapinic acid matrix for peptides and proteins >10,000 Da. See Table 3-5 on page 3-14.

WARNING **CHEMICAL HAZARD.** Read the MSDS before handling any chemical mentioned below, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **Sinapinic acid** may cause eye, skin, and respiratory tract irritation. **Acetonitrile (ACN)** is a flammable liquid and vapor. It may cause eye, skin, and respiratory tract irritation, central nervous system depression, and heart, liver, and kidney damage. **Trifluoroacetic acid (TFA)** causes eye, skin, and respiratory tract burns. It is harmful if inhaled.

Table 3-5 Sinapinic acid matrix information

Attribute/Procedure	Description
Matrix Preparation	
Matrix stability	Prepare daily.
Matrix solvent	• 0.1% TFA in deionized water • 0.1% TFA in acetonitrile
Matrix concentration	5 mg/mL
Matrix additive	None

Table 3-5 Sinapinic acid matrix information *(continued)*

Attribute/Procedure	Description
General preparation modifications	Follow the procedure in "General Preparation Procedure" on page 3-8. In step 4, combine 0.1% TFA in water with 0.1% TFA in acetonitrile for the matrix solvent. The ratio of water to acetonitrile depends on how you plan to mix the sample solution and matrix solution: • If you plan to premix the sample solution in matrix solution in a 1:10 dilution, combine seven parts 0.1% TFA in water with three parts 0.1% TFA in acetonitrile. Add solvent solution to the sinapinic matrix for a final concentration of 5 mg/mL. For example, add 700 µL 0.1% TFA in water and 300 µL 0.1% TFA in acetonitrile to 5 mg solid matrix. Note: If sample is contaminated with buffer, salt, or detergent, combine 500 µL 0.1% TFA in water and 500 µL 0.1% TFA in acetonitrile. • If you plan to mix the sample solution in matrix solution on the plate in a 1:2 dilution, combine one part 0.1% TFA in water with one part 0.1% TFA in acetonitrile. Add solvent solution to the sinapinic matrix for a final concentration of 5 mg/mL. For example, add 500 µL 0.1% TFA in water and 500 µL 0.1% TFA in acetonitrile to 5 mg solid matrix. Note: A higher concentration of TFA (up to 3%) may improve sample solubility.
Spotting	
Spotting technique	Dried-droplet
Plate drying method	Air-dry sample plate after spotting sample and matrix, or use gentle air flow to speed drying.
Matrix crystals	Uniform rhomboid shape (see Figure 3-1 on page 3-42)
Final sample concentration	0.1 to 5 pmol/µL

DHB Matrix

Use DHB matrix for:

- Peptides and proteins <10,000 Da
- Neutral carbohydrates
- Small molecules

DHB for Peptides and Proteins <10,000 Da

WARNING **CHEMICAL HAZARD.** Read the MSDS before handling any chemical mentioned below, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **Methanol** is a flammable liquid and vapor. Exposure causes eye and skin irritation, and may cause central nervous system depression and nerve damage. **Ethanol** is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation and may cause central nervous system depression and liver damage.

Table 3-6 DHB matrix information for peptides and proteins <10,000 Da

Attribute/Procedure	Description
Matrix Preparation	
Matrix stability	Prepare weekly.
Matrix solvent	10% methanol or ethanol in deionized water
Matrix concentration	5 mg/mL
Matrix additive	None
General preparation modifications	Follow the procedure in "General Preparation Procedure" on page 3-8. No modifications are necessary.
Spotting	
Spotting technique	Dried-droplet
Plate drying method	After spotting sample and matrix, dry down quickly under vacuum for even response. IMPORTANT: If you allow to air-dry, you see an uneven response during analysis, especially during automated acquisitions.
Matrix crystals	Needle-like crystals arranged in a ring if air dried (see Figures 3-3). No obvious crystals if vacuum-dried or if solvent is a fast-drying organic.
Final sample concentration	0.1 to 1 pmol/µL

DHB for Neutral Carbohydrates

WARNING **CHEMICAL HAZARD.** Read the MSDS before handling any chemical mentioned below, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Table 3-7 DHB matrix information for neutral carbohydrates

Attribute/Procedure	Description
Matrix Preparation	
Matrix stability	Prepare weekly.
Matrix solvent	Deionized water
Matrix concentration	10 mg/mL
Matrix additive	None
General preparation modifications	Follow the procedure in "General Preparation Procedure" on page 3-8. No modifications are necessary.
Spotting	
Spotting technique	Dried-droplet
Plate drying method	After spotting sample and matrix, dry down quickly under vacuum for even response. IMPORTANT: If you air-dry, you see an uneven response during analysis, especially during automated acquisitions.
Matrix crystals	Milky amorphous appearance for promoting cationization (see Figure 3-3 on page 3-43) Note: Crystals are difficult to see when the sample plate is vacuum-dried.
Final sample concentration	10 pmol/µL

DHB for Small Molecules

WARNING **CHEMICAL HAZARD.** Read the MSDS before handling any chemical mentioned below, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **Methanol** is a flammable liquid and vapor. Exposure causes eye and skin irritation, and may cause central nervous system depression and nerve damage. **Acetone** is a flammable liquid and vapor. Exposure may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry the skin. Exposure may cause central nervous system depression. Keep away from heat, sparks, and flame.

Table 3-8 DHB matrix information for small molecules

Attribute/Procedure	Description
Matrix Preparation	
Matrix stability	Prepare weekly.
Matrix solvent	Any solvent in which molecules are soluble (deionized water to 100% methanol or acetone).
Matrix concentration	10 mg/mL
Matrix additive	None
General preparation modifications	Follow the procedure in "General Preparation Procedure" on page 3-8. No modifications are necessary.
Spotting	
Spotting technique	Dried-droplet
Plate drying method	After spotting sample and matrix, dry down quickly under vacuum for even response. IMPORTANT: If you air-dry, you see an uneven response during analysis, especially during automated acquisitions.
Matrix crystals	Needle-like crystals arranged in a ring if air-dried (see Figure 3-3 on page 3-43). No obvious crystals if vacuum-dried or if solvent is a fast-drying organic.
Final sample concentration	Highly sample dependent. Ideally a minimum of 0.1 to 10 pmol/µL (15.5 pg/µL to 1.55 ng/µL). With lower concentrations, sample signal may be difficult to distinguish from matrix signal.

sDHB Matrix

Use sDHB matrix for:

- Peptides and proteins >10,000 Da
- Glycosylated proteins

WARNING **CHEMICAL HAZARD.** Read the MSDS before handling any chemical mentioned below, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **Acetonitrile (ACN)** is a flammable liquid and vapor. Exposure may cause eye and respiratory tract irritation and blood system damage. **Trifluoroacetic acid (TFA)** causes eye, skin, and respiratory tract burns. It is harmful if inhaled.

Table 3-9 sDHB matrix information

Attribute/Procedure	Description
Matrix Preparation	
Matrix stability	Prepare weekly.
Matrix solvent	A solution of 80% 0.1% TFA in deionized water and 20% acetonitrile
Matrix concentration	10 mg/mL
Matrix additive	5-methoxysalicylic acid, dissolved in a solution of 50% deionized water and 50% acetonitrile, at a concentration of 10 mg/mL
General preparation modifications	Follow the procedure in "General Preparation Procedure" on page 3-8. In step 6, combine one part additive solution (5-methoxysalicylic acid) with nine parts matrix solution (DHB).
Spotting	
Spotting technique	Dried-droplet
Plate drying method	After spotting sample and matrix, dry down quickly under vacuum for even response. IMPORTANT: If you air-dry, you see an uneven response during analysis, especially during automated acquisitions.
Matrix crystals	Needle-like crystals arranged in a ring if air-dried (see Figure 3-3 on page 3-43). No obvious crystals if vacuum-dried or if solvent is a fast-drying organic.
Final sample concentration	0.1 pmol to 10 pmol/µL

THAP Matrix

Use THAP matrix for:

- Small oligonucleotides <3,500 Da
- Acidic carbohydrates
- Acidic glycopeptides
- Acid sensitive compounds

THAP matrix provides a more even response than 3-HPA matrix. See Table 3-10 on page 3-20.

WARNING **CHEMICAL HAZARD**. Read the MSDS before handling any chemical mentioned below, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Acetonitrile (ACN) is a flammable liquid and vapor. Exposure may cause eye and respiratory tract irritation and blood system damage.

Table 3-10 THAP matrix information

Attribute/Procedure	Description
Matrix Preparation	
Matrix stability	Prepare weekly.
Matrix solvent	50% acetonitrile in deionized water IMPORTANT: Use double-deionized water such as Milli-Q grade 18 mΩ water. HPLC-grade water can vary in salt concentration. A high salt concentration may interfere with oligonucleotide analysis.
Matrix concentration	• **Small oligonucleotides** – 10 mg/mL • **Acidic carbohydrates** – 2 mg/mL
Matrix additive	50 mg/mL diammonium citrate in deionized water
General preparation modifications[a]	Follow the procedure in "General Preparation Procedure" on page 3-8. In step 6, for: • **Small oligonucleotides** – Combine 1 part additive solution (diammonium citrate) with 8 parts matrix solution (THAP). • **Acidic carbohydrates and glycopeptides** – Combine 1 part additive solution (diammonium citrate) with 20 parts matrix solution (THAP).

Table 3-10 THAP matrix information *(continued)*

Attribute/Procedure	Description
Spotting	
Spotting technique	Dried-droplet
Plate drying method	• **Small oligonucleotides** – Air-dry sample plate after spotting sample and matrix, or use gentle air flow to speed drying. • **Acidic carbohydrates and glycopeptides** – Dry sample plate under vacuum after spotting sample and matrix. After drying, allow a few minutes for the sample to absorb ambient humidity (turns faint white). IMPORTANT: If you air-dry, you see an uneven response during analysis, especially during automated acquisitions.
Matrix crystals	Larger than sinapinic acid crystals, overlapping shingles, nonuniform shape (see Figure 3-2 on page 3-43)
Final sample concentration	1 to 10 pmol/µL

a. Other special sample preparation techniques are described in Papac, D.I., A. Wong, A.J.S. Jones, *Anal. Chem.,* **1996**, *68*, 3215–3223.

3-HPA Matrix

Use 3-HPA matrix for large oligonucleotides >3,500 Da.

WARNING **CHEMICAL HAZARD.** Read the MSDS before handling any chemical mentioned below, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **3-Hydroxypicolinic acid (3-HPA)** may cause eye, skin, and respiratory tract irritation. **Acetonitrile (ACN)** is a flammable liquid and vapor. Exposure may cause eye and respiratory tract irritation and blood system damage.

Table 3-11 3-HPA matrix information

Attribute/Procedure	Description
Matrix Preparation	
Matrix stability	Prepare weekly.
Matrix solvent	50% acetonitrile in deionized water Note: HPLC-grade water can vary in salt concentration. Do not use for oligonucleotide analysis.
Matrix concentration	50 mg/mL
Matrix additive	50 mg/mL diammonium citrate in deionized water
General preparation modifications	Follow the procedure in "General Preparation Procedure" on page 3-8. In step 6, combine one part additive solution (diammonium citrate) with eight parts matrix solution (3-HPA).
Spotting	
Spotting technique	Dried-droplet
Plate drying method	Air dry sample plate after spotting sample and matrix, or use gentle air flow to speed drying.
Matrix crystals	Needle-like crystals inside a ring (see Figure 3-2 on page 3-43)
Final sample concentration	1 to 10 pmol/µL

Matrices for Synthetic Polymers

WARNING **CHEMICAL HAZARD.** Read the MSDS before handling any chemical mentioned below, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **Tetrahydrofuran** is a flammable liquid and vapor and may form explosive peroxides. It may be harmful if swallowed. Exposure may cause eye and respiratory tract irritation and central nervous system depression. ***N,N*-Dimethylformamide (DMF)** is harmful if inhaled. It is a flammable liquid and vapor. Exposure may cause eye, skin, and respiratory tract irritation and liver damage. **Acetone** is a flammable liquid and vapor. Exposure may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry the skin. Exposure may cause central nervous system depression. Keep away from heat, sparks, and flame.

Table 3-12 Matrix information for synthetic polymer samples

Attribute/Procedure	Description
Matrix Preparation	
Matrix stability	Prepare weekly. Store THF and acetone solutions in tightly sealed vials.
Matrix solvent	Sample and matrix dependent. Choose solvents in which both the synthetic polymer sample and matrix are soluble.
Matrix concentration	~0.1 M (10^{-1} M)
Matrix additive	None
General preparation modifications	Follow the procedure in "General Preparation Procedure" on page 3-8. No modifications are necessary.
Sample/matrix mixing	Mix the sample solution in matrix solution in a 1:2 dilution. For example, combine 1 µL sample solution and 1 µL matrix solution. For more information, see "Spotting Sample and Matrix Using the Dried-Droplet Spotting Technique" on page 3-39.

Table 3-12 Matrix information for synthetic polymer samples *(continued)*

Attribute/Procedure	Description
Spotting	
Spotting technique	Dried-droplet You can spot polymer samples on sample plates in two ways: • **Thin-layer polymer method –** Yields even response, but provides adequate sample response for only 10 laser shots. Spot 0.3 µL sample/matrix solution in one sample position. • **Thick-layer polymer method –** Yields less even response, but provides adequate sample response for 50 to 100 laser shots. Spot 2 to 3 µL sample/matrix solution in one sample position by placing the pipette tip in the sample position and slowly expelling while keeping the tip in contact with the sample plate. Note: In general, do not touch the pipette tip to the sample plate surface. However, when you use THF as the solvent in the thick-layer method, you must touch the tip to the sample plate to slowly expel the sample onto the sample plate. For more information, see "Spotting Sample and Matrix Using the Dried-Droplet Spotting Technique" on page 3-39. IMPORTANT: Analyze polymer samples within 1 hour of spotting on the sample plate. Many synthetic polymer/matrix mixtures are not stable after they are spotted.
Plate drying method	Apply vacuum drying for nonvolatile solvents (water, DMF). Acetone and THF dry instantaneously.
Matrix crystals	No crystals visible. If sample position looks glassy or shiny, the sample concentration may be too high. Note: Areas in which you see hot and cold spots during acquisition are much smaller with polar compounds than with nonpolar compounds.
Final sample concentration	~0.1 mM (10^{-4} M)

Cleaning the Sample

IMPORTANT! For sample and matrix handling, use high-quality plastic pipette tips, vials, tubes, and trays. Lower-quality consumables are likely to lead to extraneous peaks caused by plasticizers and other potential contaminants. (Lower-quality consumables also may cause high peptide loss due to adsorption of the peptides to the plastic.) If you detect extraneous peaks, try prewashing the consumables with the same solvent used in your sample or matrix.

When to Clean Samples

Sample cleanup is needed if samples:

- Are prepared in phosphate buffers.
- Have ammonium salts or derivatives of organic amines (ammonium bicarbonate, TRIS HCl) in concentrations greater than 50 mM.
- Contain salt, for example, from cation- or anion-exchange purification.
- Are contaminated with detergent.

Clean the sample just before diluting and spotting the sample plate.

Symptoms of Contamination

Cleaning the sample is recommended whenever there is:

- Poor crystallization on the sample plate.
- A large tail on the high-mass side of peaks, which may be unresolved salt or buffer adducts.
- Poor sensitivity with a sample concentration that should yield a strong signal. To detect this condition, mix the sample with a standard that you know yields a strong signal. If the standard does not exhibit the expected signal, a contaminant in the sample is affecting sensitivity.

Selecting a Cleaning Technique

Use the following techniques to clean samples:

- **Washing** – Use this technique when you know the solubility of the contaminant. You can wash a dried sample directly on the sample plate.
- **Drop dialysis (floating membrane)** – Use this technique on polar compounds when you know the contaminants are of low molecular weight. This technique works well for DNA and polar proteins such as glycoproteins.
- **Cation exchange** – Use this technique for nonpolar proteins or DNA when you know the sample contains only a salt contaminant. This technique is faster than dialysis but does not remove contaminants other than salt.
- **ZipTips®** – Use this technique for peptides, proteins, and oligonucleotides when you know the sample contains salt, buffer, and/or glycerol contaminants. This technique is faster, easier, and more effective than dialysis for removing contaminants.

  Millipore ZipTips can be used for a wide range of applications and are compatible with digestion procedures. You can expect 50 to 70% recovery using the following procedure.

  Note: ZipTips can also be used for sample concentration and fractionation of complex mixtures.

Washing

Required Materials

Select a washing solvent in which the contaminant is more soluble than the matrix and the sample. For example:

If the contaminant is …	Then use …
A polar compound, such as a salt or buffer	Cold water (to prevent sample dissolving) with 0.1% TFA
A nonionic detergent such as octyl glucoside	5% isopropanol in water

WARNING **CHEMICAL HAZARD. Isopropanol** is a flammable liquid and vapor. Exposure may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin and cause irritation. Exposure may cause central nervous system effects such as drowsiness, dizziness, and headache. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **Trifluoroacetic acid (TFA)** causes eye, skin, and respiratory tract burns. It is harmful if inhaled.

Procedure

To wash samples:

1. Apply sample and matrix to the sample plate and allow to dry.
2. Add 1 to 3 µL of washing solvent to the dry sample/matrix.
3. Wait 10 seconds, then remove the liquid.

   IMPORTANT! Try not to touch the sample plate with the pipette tip. If you do touch the spot, do not analyze the area you touched.
4. Repeat step 2 and step 3 one more time.
5. Allow the sample plate to dry before analyzing.

   Note: If above washes do not improve results, you can repeat the wash procedure.

Drop Dialysis (Floating Membrane)

Required Materials

- A membrane with a pore size of 0.025 µm or smaller that does not adhere to your sample
- Double-deionized water such as Milli-Q® grade 18 mΩ water
- Forceps
- Acetonitrile

Procedure

To perform drop dialysis:

1. Fill a small container (for example, a pipette tip box) with about an inch of deionized water.
2. Place the container on a stable surface.
3. Use forceps to place the membrane in the water with the appropriate side up (refer to manufacturer's information). If you use your fingers to place the membrane, you can contaminate the membrane with oil and salt.
4. Place about 10 parts of sample (for example, 10 µL) in the center of the membrane.
5. Place 1 part of pure acetonitrile (for example, 1 µL) on top of the sample spot to increase surface area. Do not exceed a 10% concentration of organic. It may dissolve the membrane.

   WARNING **CHEMICAL HAZARD**. **Acetonitrile (ACN)** is a flammable liquid and vapor. Exposure may cause eye and respiratory tract irritation and blood system damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
6. Cover the container to prevent drying and allow it to dialyze for 30 minutes.
7. Note the size of the sample/organic spot, then let it continue to dialyze for 30 minutes.
8. Observe the size of the sample/organic spot again. If the size of the spot is larger than the previous time you checked it, allow the sample to sit for 15 more minutes.

9. Repeat step 8 until the size of the spot remains constant.

   Note: Sample also passes through the membrane during dialysis, particularly low-mass samples. Dialyze small molecules for a shorter time than larger molecules. In general, do not dialyze for more than 2 hours.

10. When the size of the sample spot stabilizes, dialysis is complete. Remove the sample and place it in a microcentrifuge tube.

    Note: The size of the sample drop can increase by a factor of 10 when salt concentration is high.

Cation Exchange

Required Materials

- Cation-exchange beads, 200-mesh. Cation-exchange beads in the ammonium form work best for MALDI applications.
- 1 M ammonium acetate
- Acetone
- Hexane
- Buchner funnel

Preparation of Beads in Ammonium Form

To prepare cation-exchange beads in the ammonium form:

1. Place the beads in twice the bead volume of 1 M ammonium acetate and leave beads in ammonium acetate overnight.

   IMPORTANT! **Ammonium acetate.** Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

2. Remove the beads, then wash with water, acetone, and finally hexane through a Buchner funnel under vacuum.

   WARNING **CHEMICAL HAZARD**. Read the MSDS before handling any chemical mentioned below, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **Acetone** is a flammable liquid and vapor. Exposure may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry the skin. Exposure may cause central nervous system depression. Keep away from heat, sparks, and flame. **Hexane** is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation. Exposure may cause central nervous system depression. It is harmful or fatal if swallowed as it can enter the lungs and cause damage. Keep away from heat, sparks, and flame.

   After washing, you can store the beads indefinitely for reuse as needed.

Procedure

To perform cation exchange:

1. Place about 0.1 mg (a small spatula-tip-full) of cation-exchange beads on a piece of laboratory film.
2. Add 5 to 10 µL of sample for 200-mesh cation-exchange beads. If sample quantity is limited, you can make up the difference with deionized water.
3. Add 5 to 10 µL of matrix on top of the sample, as matrix may also contain salt.
4. Mix by withdrawing and expelling about 20 times with a pipette.

   Note: The volume of sample decreases as the beads absorb water.
5. Allow the beads to settle for 30 seconds.
6. Remove the supernatant with a clean pipette tip, then spot on the sample plate.

ZipTips®

Required Materials

- Micro-adsorptive C_{18} pipette ZipTips
- Acetonitrile (ACN)
- TFA

WARNING **CHEMICAL HAZARD.** Read the MSDS before handling any chemical mentioned below, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **Acetonitrile (ACN)** is a flammable liquid and vapor. Exposure may cause eye and respiratory tract irritation and blood system damage. **Trifluoroacetic acid (TFA)** causes eye, skin, and respiratory tract burns. It is harmful if inhaled.

Procedure

To clean samples with ZipTips:

1. Wash a C_{18} ZipTip in the following order with:
   a. 10 µL of ACN
   b. 10 µL of 50:50 ACN:0.1%TFA
   c. 10 µL of 0.1% TFA
   d. Repeat wash of 10 µL of 0.1% TFA

   To wash, draw a few microliters of a wash solution up into the ZipTip and expel to waste. Repeat with the remaining wash solution.
2. Draw a few microliters of the sample up and down in the ZipTip several times.
3. Discard the liquid. The sample is bound to the C_{18} surface in the ZipTip.
4. Wash the ZipTip again with 10 µL of 0.1% TFA by drawing up into the ZipTip and expelling to waste.
5. Repeat step 4 two times.
6. To elute the sample, draw up into the ZipTip 2 to 3 µL of organic solvent or matrix containing approximately 50% ACN. A 50% organic concentration should give 50 to 70% sample recovery. You can vary the organic concentration to optimize recovery.

7. If spotting a plate, expel the eluate (sample) directly onto the plate as described in "Spotting Sample and Matrix Using the Dried-Droplet Spotting Technique" on page 3-39

   If storing the sample or mixing it with matrix, expel the eluate (sample) into a microcentrifuge tube.

Diluting the Sample

Before Diluting Samples

Refer to "Cleaning the Sample" on page 3-25, to determine if your samples should be cleaned before preparing.

IMPORTANT! Prepare sample just before spotting the sample plate.

IMPORTANT! For sample and matrix handling, use high-quality plastic pipette tips, vials, tubes, and trays. Lower-quality consumables are likely to lead to extraneous peaks caused by plasticizers and other potential contaminants. (Lower-quality consumables also may cause high peptide loss due to adsorption of the peptides to the plastic.) If you detect extraneous peaks, try prewashing the consumables with the same solvent used in your sample or matrix.

Diluting Samples for Dried-Droplet Spotting

Prepare a sample solution by diluting the sample in an appropriate liquid to an appropriate *initial* concentration. You will be further diluting to the *final* sample concentration when you mix the sample solution with matrix solution.

If you are unsure of the starting concentration of sample, make a serial dilution to prepare various dilutions of the same sample.

Dilution Liquid

WARNING **CHEMICAL HAZARD.** Read the MSDS before handling any chemical mentioned below, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **Acetonitrile (ACN)** is a flammable liquid and vapor. Exposure may cause eye and respiratory tract irritation and blood system damage. **Trifluoroacetic acid (TFA)** causes eye, skin, and respiratory tract burns. It is harmful if inhaled.

To determine the appropriate dilution liquid, first dilute the sample with deionized water, then add acetonitrile. Add 0.1% TFA to increase solubility, if needed. A higher concentration of TFA may enhance sample ionization and improve sensitivity in samples prepared in buffers.

Many samples adhere strongly to plastic tubes and pipette tips. You can minimize sample loss by preparing samples in 30% acetonitrile with 5 to 10% TFA.

IMPORTANT! Do not dilute sample with phosphate-buffered saline (PBS) or other buffer solutions. A high salt concentration can interfere with sample ionization and may cause increased sodium and potassium adduct peaks.

IMPORTANT! Using a dilution liquid with an organic concentration greater than 50% can cause spreading when spotting sample and matrix on the plate. If sample spreading occurs, decrease the organic concentration in the dilution liquid to less than 50%.

Diluting Samples for Thin-Layer Spotting

Prepare a sample solution by diluting the sample in deionized water to a concentration of <0.1 pmol/µL.

IMPORTANT! Do not dilute a sample in an organic solvent. Organic solvents dissolve the matrix applied to the sample plate.

Preparing Internal Standards

If you require mass accuracy greater than the accuracy provided by external calibration, use an internal standard.

Add two standards of known molecular weight to the sample solution. Use standards that:

- Closely bracket the molecular weight of your unknown
- Can be easily distinguished from the unknown

As a starting point, use an internal standard concentration in the same range as your sample concentration. Because an internal standard can affect the intensity of the sample signal, prepare sample with several different internal standard concentrations.

Selecting an Insert

The 4800 MALDI TOF/TOF™ Analyzer accepts stainless steel 8 × 12 cm (3 × 5 in) holders that are fitted with inserts.

The reusable holder and single-use insert design provides an inexpensive sample plate format that eliminates sample carryover and provides a convenient format for archiving sample plates for additional analysis.

CAUTION Before spotting the insert, always use dry, compressed gas to blow off any fibers that may be on the insert. Take precautions during spotting to reduce the exposure of the insert to fibers. Fibers on the insert can be transferred to the ion optics components during sample acquisition and severely inhibit sensitivity.

Table 3-13 summarizes the available inserts and corresponding plate types and spot set templates to enter in the 4000 Series Explorer™ software. For part numbers, see Appendix B, "Spare Parts."

Table 3-13 Insert types and their corresponding plate types and spot set templates

Insert Name	Description	4000 Series Explorer™ Software	
		Plate Type Name	Spot Set Template Name
Opti-TOF™ 96-well insert, 123 × 81 mm	Etched with 96 sample spots and 13 CAL spots	96 Opti-TOF 123 × 81mm Rev A	96 Opti-TOF 123 × 81 mm Rev A
Opti-TOF™ 384-well insert, 123 × 81 mm	Etched with 384 sample spots and 13 CAL spots	384 Opti-TOF 123 × 81mm Rev A	384 Opti-TOF 123 × 81 mm Rev A
Opti-TOF™ Cal Mix 5 Calibration insert, 123 × 81 mm	Spotted with 384 spots of Cal Mix 5, etched with 8 CAL spots	Blank 3 × 5	Cal Mix 5 Calibration 384 Opti-TOF 123 × 81 mm Rev A

Table 3-13 Insert types and their corresponding plate types and spot set templates

Insert Name	Description	4000 Series Explorer™ Software	
		Plate Type Name	Spot Set Template Name
Opti-TOF™ Mass Standards Calibration insert, 123 × 81 mm	Spotted with 384 spots of five different Cal Mixes, etched with 8 CAL spots	Blank 3 × 5	Mass Standards Calibration 384 Opti-TOF 123 × 81 mm Rev A
Opti-TOF™ LC/MALDI insert, 123 × 81 mm	Blank insert with 8 etched CAL spots for LC-MALDI and for use with the thin-layer spotting technique	Blank 3 × 5	LC 384 PROBOT (use as is with corresponding default Probot template.) LC 384 2 Chromatogram Examples (use as an example to follow when creating your own template)

Adapting 4700 Inserts or Plates

You can use the 4700 Proteomics Analyzer inserts or 2 × 2 in plates with a specially designed holder (see Appendix B). Use Blank 3 × 5 in as the Plate Type name and create your own spot set template (see the *4000 Series Explorer™ Software Online Help*, "Creating Spot Set Templates").

IMPORTANT! Before reusing a 2 × 2 in plate, ensure that the plate was cleaned using the MALDI Plate Cleaning Kit for Stainless Steel or Gold Plates to restore the hydrophobicity. Hydrophobicity minimizes sample spreading and pooling of samples. Follow the instructions provided with the kit.

Spotting Samples and Matrix

Guidelines

Handling Inserts and Sample Plates

To help reduce contamination of your analysis:

- Start with a clean insert. If you use the 4700 2 × 2 in. plate, clean the plate using the MALDI Plate Cleaning Kit.
- Use powder-free gloves.
- Handle the insert and sample plate by the edges.

CAUTION Before and after spotting an insert, always use dry, compressed gas to blow off any fibers that may be on the insert. Take precautions during spotting to reduce the exposure of the insert to fibers. Fibers can be transferred to the ion optics components during sample acquisition and severely inhibit sensitivity.

IMPORTANT! For sample and matrix handling, use high-quality plastic pipette tips, vials, tubes, and trays. Lower-quality consumables are likely to lead to extraneous peaks caused by plasticizers and other potential contaminants. (Lower-quality consumables also may cause high peptide loss due to adsorption of the peptides to the plastic.) If you detect extraneous peaks, try prewashing the consumables with the same solvent used in your sample or matrix.

Spotting Guidelines

- Use finely tapered pipette tips to dispense sample and matrix on the sample plate. Tips with blunt ends do not easily dispense the small volumes used for spotting.
- Spot the insert, allow the spots to dry, then place the insert in the holder.
- For optimum mass accuracy when spotting blanks inserts, do not spot the outer edges of the insert. Be sure the sample spots are no closer to the edge than the etched calibration spots.
- When spotting matrix, use the supernatant, not the precipitate.
- Do not touch the tip of the pipette to the surface of the sample well or the sample spot. Touching can cause uneven crystallization.

  Note: If you spot synthetic polymer samples and use the thick-layer polymer method, you need to touch the pipette tip to the insert. For more information, see "Selecting a Spotting Technique" on page 3-3.

- Try to spot the sample in the center of the sample position. For Automatic data collection, the plate is aligned so that the laser beam strikes the center of the sample position. If the sample is not in the center of the well, some laser shots may not strike the sample, and data quality may be reduced. You can position the sample with respect to the laser shot in Manual control, using the control pad.
- To ensure good crystallization, if you use a welled plate, fill the entire well, if possible, especially if acquiring in Automatic control. Surface tension and sample availability may determine whether or not you can fill the well completely.
- If the sample solution contains organic solvents, try to spot smaller volumes (0.5 µL or less). Some organic solvents such as methanol, acetone, and THF spread very easily on metal surfaces.
- Make sure the sample is evenly applied to the spot.
- To ensure good crystallization, mix sample solution and matrix solution before spotting, if possible.

Spotting Sample and Matrix Using the Dried-Droplet Spotting Technique

1. Using a clean pipette tip for each new sample, mix and/or spot the sample and matrix as described below:

Mixing Method	Procedure
No mixing. Overlaying the sample and matrix solutions.	Note: If your sample is highly soluble in the matrix, spot the matrix first. 1. Spot 0.5 to 1 µL of sample solution on the plate. (Make sure the concentration is appropriate to yield the necessary final sample concentration, as listed in Table 3-3 on page 3-10.) 2. Allow the sample spot to dry. 3. Spot the same volume of matrix solution on top of the dried sample spot.
Mixing on the sample plate IMPORTANT! When mixing directly on the plate, you may need to increase the concentration of organic in the matrix solution. With the 1:2 dilution used in this strategy, the typical concentration of organic may not be high enough to keep the matrix in solution.	1. Spot 0.5 to 1 µL of sample solution on the plate. (Make sure the concentration is appropriate to yield the necessary final sample concentration, as listed in Table 3-3 on page 3-10.) Note: Applying the sample solution before the matrix solution prevents the matrix from drying before it mixes with sample. 2. Spot the same volume of matrix solution. 3. Use the pipette to mix by aspirating and dispensing the solution one to two times. (A homogeneous mixture is critical for good crystallization.)

Mixing Method	Procedure
Premixing in tube	1. In a microcentrifuge tube, dilute the sample solution 1:10 with matrix. For example, for peptides and proteins, mix 1 µL of sample solution (previously diluted to a concentration of 10 fmol/µL to 10 pmol/µL) and 9 µL of matrix solution in a microcentrifuge tube, for a final concentration of 1 fmol/µL to 1 pmol/µL. Mix gently. For other compounds, make sure the initial concentration of sample solution yields the appropriate final sample concentration (see Table 3-3 on page 3-10) when mixed with matrix solution in a 1:10 dilution. Note: If the sample concentration is too high, the sample signal may be suppressed. If the sample concentration is too low, sample signal may be absent. 2. Spot 0.5 to 2 µL of sample/matrix solution on the appropriate position. Volume depends on the well size of the sample plate you are using.
Preparing sample with ZipTips®	Spot 0.5 to 2 µL of sample/matrix solution on the appropriate position.

2. Dry the sample plate. When using CHCA, allow the sample plate to dry for at least 5 minutes. Preparations with high water or salt content may require longer to dry.
3. Visually examine the sample spots to make sure they are dry.

   IMPORTANT! If you load the sample plate into the 4800 MALDI TOF/TOF™ Analyzer before the plate is dry, the pressure in the sample loading chamber rises, and an error message may be displayed.
4. Place a protective cover over the plate until you are ready to load the plate. Do not allow the cover to touch the surface of the plate.

CAUTION Before loading the plate in the instrument, always use dry, compressed gas to blow off any fibers that may be on the plate. Fibers on the plate can be transferred to the ion optics components during sample acquisition and severely inhibits sensitivity.

Spotting Sample and Matrix Using the Thin-Layer Spotting Technique

IMPORTANT! When using the thin-layer spotting technique for high-throughput applications, use one of the following methods to avoid burning through the sample:

- If acquiring using manual control, move the sample plate after every 5 to 10 laser shots.
- If acquiring using automatic control, edit the acquisition method. In the Spectrum tab, set the Shots/sub-spectrum low (typically less than 20). In the Automatic Control tab, select Move before every sub-spectrum (pass or fail) for the Sample Stage Mode.

Spotting Matrix and Sample

To spot matrix solution and sample solution using the thin-layer technique:

1. Dispense 0.5 µL of matrix solution prepared for thin-layer spotting technique on the sample plate to form a thin-layer. It dries in seconds. For information on preparing matrix solution for thin-layer spotting, see "Preparing CHCA Matrix for the Thin-Layer Spotting Technique" on page 3-12.
2. Spot 0.5 to 1 µL of the sample solution in each sample position onto the thin matrix layer applied in step 1.
3. Dry the sample plate with gentle air flow, such as a heat gun set at low heat and air-flow settings.
4. After drying, spot 2 to 3 µL of double-distilled water on top of each dried sample to wash the sample.
5. Wait 5 seconds, and blow off the water droplet with compressed air.
6. Repeat step 4 and step 5 two more times.
7. Place a protective cover over the plate until you are ready to load the plate. Do not allow the cover to touch the surface of the plate.

CAUTION Before loading the plate in the instrument, always use dry, compressed gas to blow off any fibers that may be on the plate. Fibers on the plate can be transferred to the ion optics components during sample acquisition and severely and adversely affect sensitivity.

Examining Crystals on Sample Plates

Overview

Proper crystallization is necessary to acquire high quality data. Ideally, you want small crystals that are evenly distributed in the sample well.

CAUTION Clumped matrix crystals interfere with efficient data acquisition. Clumping can require varying laser intensity across a sample position and decrease mass resolution. In MS/MS mode, clumping may cause inaccurate timing of the Timed Ion Selector and yield poor precursor selection.

You can view crystallization structure of sample and matrix:

- Under a microscope, at a magnification of 30×.
- In the Video Viewer in the 4000 Series Explorer™ software.

CHCA Matrix

Figure 3-1 illustrates good patterns of crystallization for CHCA and sinapinic acid matrices.

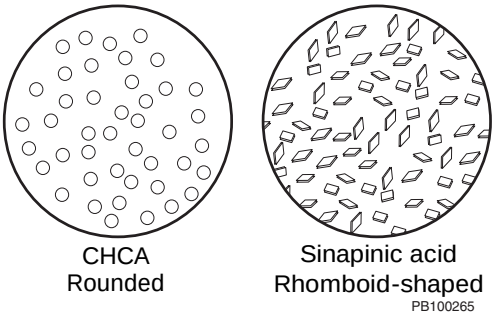


Figure 3-1 Microscopic view of sample spots with CHCA and sinapinic acid matrices

With good crystallization, you see small, equally-sized crystals that are evenly distributed on the plate. Clumping is not desirable. Clumped crystals with CHCA or sinapinic acid may indicate one or more of the following:

- Matrix concentration is too high
- Organic concentration is too high
- Sample plate was dried too quickly
- Sample contains contaminants

IMPORTANT! You can acquire data from a spot that does not have an ideal crystallization pattern. However, when a sample spot contains unevenly distributed crystallization, it may be difficult to acquire quality data. For best results, acquire data in Interactive mode, using the Video Viewer and manually selecting the area of the spot with the best crystallization pattern. For information, see the *4000 Series Explorer™ Online Help*, Acquiring Data from the Video Viewer.

3HPA, THAP, DHB, and sDHB Matrices

Typical appearances of other matrices under magnification are shown in Figure 3-2 and Figure 3-3.

When analyzing 3-HPA crystals, aim the laser at the base of the fan-like crystals for best response.

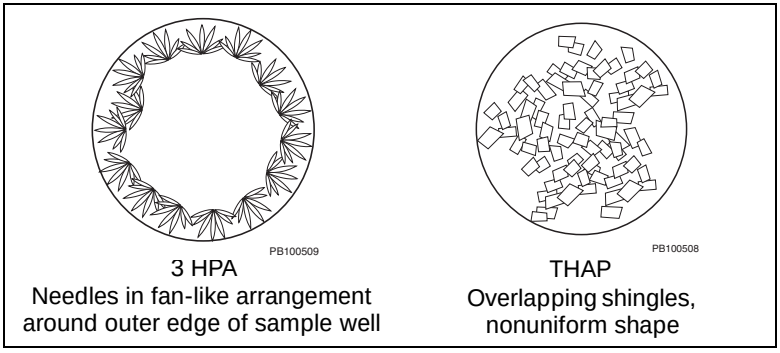


Figure 3-2 Microscopic view of sample spots with 3-HPA and THAP matrices

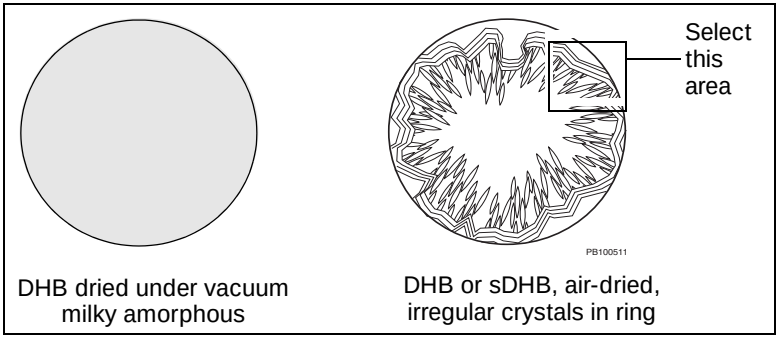


Figure 3-3 Microscopic view of sample spots with DHB or sDHB matrix

Loading Sample Plates

CAUTION Before mounting a plate in the instrument, always use dry, compressed gas to blow off any fibers that may be on the insert or plate. Fibers on the plate can be transferred to the ion optics components during sample acquisition and severely inhibit affect sensitivity.

Note: If you load the sample plate into the 4800 MALDI TOF/TOF™ Analyzer before the spots on the plate are dry, the pressure in the sample loading chamber rises, and an error may be displayed in the 4000 Series Explorer™ software. Wait a few minutes for the chamber to reach vacuum before starting an acquisition.

To load a sample plate:

1. Open the sample loading chamber (Figure 3-4).

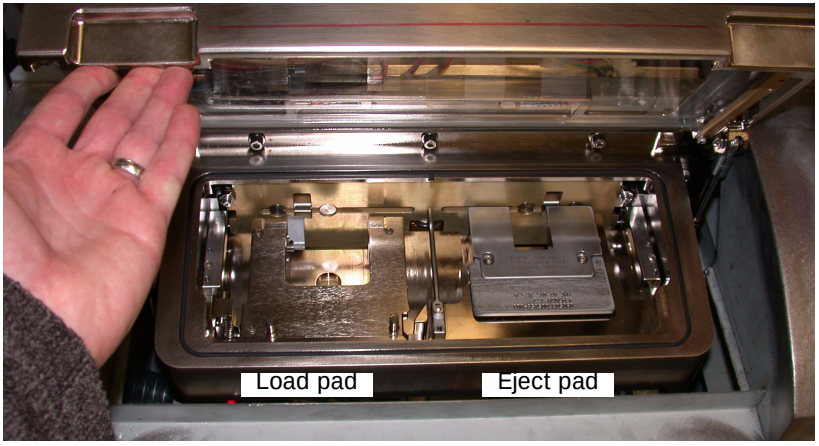


Figure 3-4 Sample loading chamber

2. If a sample plate is on the eject pad (left side), remove it.
3. Place the sample plate on the load pad (right side), with the notched corner of the plate at the front left, and the notched edge of the plate against the pins on the front of the load pad (Figure 3-5).

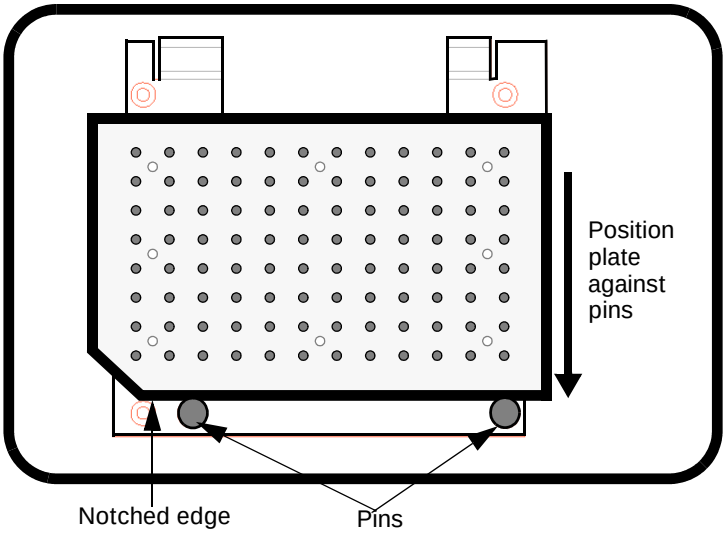


Figure 3-5 Loading a sample plate

4. Close the sample loading chamber.

   WARNING PHYSICAL INJURY HAZARD. Fingers can get caught in the plate loading mechanism. To avoid injury, do not click Load when the sample loading chamber is open.

5. Select the plate you want to load by doing one of the following:
   - Scan the bar code on the bottom of the plate using the optional handheld bar code scanner.
   - Select the spot set corresponding to the plate in the Select Spot Set dialog box, then click **Select**.
   - In the Select Spot Set dialog box, click **Manual Bar Code**. Then, enter the bar code number in the Manual Bar Code dialog box, then click **OK**.

   The Load Sample Plate dialog box opens, displaying the plate and spot set information for the plate you selected.

6. Click **Load** in the Load Sample Plate dialog box. The following occur:
   - A Load/Eject Cycle Status message box displays hardware status during the load sequence.
   - The sample loading chamber is pumped down.
   - High voltage is turned off.
   - If a sample plate is in the main source chamber, it is moved out of the source chamber into the sample loading chamber, and is placed on the eject pad.
   - The new sample plate is loaded from the load pad into the main source chamber.
   - The sample loading chamber is vented.

Note: You can use the Load Inventory function to prepare a second sample plate to be loaded without ejecting the plate in the main source chamber. You can then create and submit spot set jobs for both plates. Once all jobs for the first plate have been completed, the second plate is automatically loaded.

For information on instrument tuning, the instrument process, and using the 4000 Series Explorer™ software, see the *4000 Series Explorer™ Software Online Help* and the *4800 MALDI TOF/TOF™ Analyzer Getting Started Guide*.

Troubleshooting 4

This chapter covers

Overview

This chapter describes potential problems and corrective actions for the 4800 MALDI TOF/TOF™ Analyzer, the bar code scanner, and the database.

If you cannot solve a problem using the information in the following tables, contact Applied Biosystems/MDS SCIEX Technical Support (see "How to Obtain Support" on page x).

4800 MALDI TOF/TOF™ Analyzer Troubleshooting

The following table lists symptoms and their related causes and actions.

Table 4-1 Hardware troubleshooting

Symptom	Possible Cause	Action
System does not power on when the main power switch is on.	The other main power switch is off. For the instrument to power on, both switches must be on.	Turn on the main power switch on the other side of the system.
Cannot start 4000 Series Explorer™ software due to a database connection error	The Computer Name was changed without the Oracle database being reconfigured	Reconfigure the Oracle database: 1. Restart the computer. 2. Select **Start > Run**. 3. In the Run dialog box, click **Browse**, then navigate to C:\WINNT\SYSTEM32\ ChangeWSName.cmd or .vbs. 4. Click **Open**. When a screen flashes briefly on the computer screen, the reconfiguration is complete.
Cannot turn on high voltages	An acquisition method is not open	Open an acquisition method.
	Hardware error occurred	Reinitialize the hardware.

Table 4-1 Hardware troubleshooting *(continued)*

Symptom	Possible Cause	Action
High whining sound when you power on the mass spectrometer	Normal startup operation of the turbo pump	No action. Normal occurrence. Before using the instrument, allow the vacuum pressures to stabilize.
In Automatic control, center of sample position is not aligned with laser spot, as observed in the Video Viewer window	Sample plate not aligned	Align the sample plate. See the *4000 Series Explorer™ Software Online Help*.
The Video Viewer window is black.	The light source is set to Automatic Power-Off when Idle and the idle time has expired.	See "High-Voltage, Laser, and Light Configurations", step 6 on page 2-13.
	Default settings have been lost.	Reset the default settings: • Right click on the Video Viewer • Select **Configure** • Select **Defaults**
Buttons on the Control Pad do not change laser intensity	The system is in Automatic control, and Fixed laser intensity is not selected	Select an acquisition method that specifies **Manual** control or **Automatic** control with **Fixed laser intensity** (Instrument tab) *or* Verify that the acquisition method is the active acquisition method. In the Acquisition Method dialog box, select the Spectrum tab, then select **Manual** control or **Automatic** control with **Fixed laser intensity**.
Cracking sound in mass spectrometer	Arcing caused by faulty electronic components, PCBs, or cables	Shut down the high voltages. Do not use the instrument or restart the computer or software. Contact Applied Biosystems/ MDS SCIEX Technical Support.

Table 4-1 Hardware troubleshooting *(continued)*

Symptom	Possible Cause	Action
TC foreline vacuum pressure remains high after start up.	Roughing pump is off.	If necessary, turn on the roughing pump.
TC foreline vacuum pressure fluctuates.	Roughing pump is malfunctioning.	Check the pump oil level (see Figure 5-2 on page 5-6). If necessary, contact Applied Biosystems /MDS SCIEX Technical Support.
Instrument-ERROR is displayed- in the Status bar at the bottom of the 4000 Series Explorer™ software	Hardware error occurred	Reinitialize the hardware. See "Reinitializing Hardware" on page 5-3.
Vacuum over pressure	Vacuum leak	Contact Applied Biosystems/ MDS SCIEX Technical Support.
	Gauge fault	Reinitialize the hardware. See "Reinitializing Hardware" on page 5-3.
	Turbo pump pressure is out of range	If necessary, turn the roughing pump on. Wait for turbo pressure to come within range. If the turbo pressure remains out of range, contact Applied Biosystems/ MDS SCIEX Technical Support.
	BA gauge is off	
System-wide problem with timed events	Time on the computer clock changed from International English to European format	Reset Regional Settings to English (United States). If necessary, reset the Time format to h:mm:ss tt. Exit and restart the 4000 Series Explorer™ software.
	Time on the computer clock was updated while the software was running IMPORTANT! When updating the computer Date/Time properties, be sure to exit the 4000 Series Explorer™ software first.	Exit and restart the 4000 Series Explorer™ software.

Table 4-1 Hardware troubleshooting *(continued)*

Symptom	Possible Cause	Action
Light source stays on	Automatic Power-Off is disabled	When Automatic Power-Off is disabled, you can not shut off the light source (see "High-Voltage, Laser, and Light Configurations" on page 2-11).

Handheld Bar Code Scanner Troubleshooting

LASER HAZARD. Class 2 (II) lasers can cause damage to eyes. Avoid looking into a Class 2 (II) laser beam or pointing a Class 2 (II) laser beam into another person's eyes.

Table 4-2 Bar Code Scanner troubleshooting

Symptom	Possible Cause	Action
Scanner does not work.	Poor connection.	Check the connections between the bar code scanner and the computer.
	Com port not set	Set the com port. Refer to "Setting Bar Code Preferences" on page 2-8.
Scanning is inconsistent.	Bar code label on the MALDI plate is dirty, scratched or damaged.	Check that barcoding is working properly by scanning the bar code on a source plate. If scanning is working properly, use a different MALDI plate, or type in the bar code instead of scanning. If scanning is not working properly, contact Applied Biosystems/MDS SCIEX Technical Support.
	The scanner holder height may need adjusting.	Adjust the height of the bar code scanner holder to between 20 and 25 cm (8 and 10 in.) For information, see the *Handheld Bar Code Scanner Installation Guide*.
Scanner does not work when mounted in the holder.	Scanner is incorrectly inserted in the holder (trigger is not depressed).	Reinsert the scanner in the holder.
	AutoSense® Stand Mode is disabled.	Reenable AutoSense Stand Mode. Refer to the *QuickScan® 6000/6000plus AutoSense® Stand Instructions.*

Database Computer Troubleshooting

Table 4-3 Database computer troubleshooting

Symptom	Possible Cause	Action
Noticeably slower 4800 instrument performance.	Database is too full.	Purge unneeded spot sets. If performance remains slow after purging, contact Applied Biosystems/ MDS SCIEX Technical Support.
	Computer files are fragmented.	Have your system administrator defragment the hard drive.
When restoring an archived spot set that was created on one (source) 4800 instrument to another (target) 4800 instrument, an error message appears similar to: "Error step 4.5: check Method - Failed - See rest_0000000300.log for details".	The database on the target 4800 instrument does not contain the identical operating mode name that the archived spot set used when it was created on the source 4800 instrument. For example, the source 4800 instrument used an earlier 4000 Series Explorer™ software version that included discontinued operating modes, or a custom operating mode was used on the source 4800 instrument.	1. From the 4000 Series Explorer™ software on the *source* 4800 instrument, select **File** > **Open** > **Operating Mode**. 2. Record all operating modes listed in the Open Operating Mode dialog box. 3. Repeat steps 1 and 2 at the *target* 4800 instrument, noting any operating modes that are not listed in the Open Operating Mode dialog box on the target 4800 instrument. 4. From the 4000 Series Explorer™ software on the *source* 4800 instrument, export only those operating modes that do not appear on the target 4800 instrument. See the *4000 Series Explorer™ Software Online Help*, "Exporting XML Files".

Table 4-3 Database computer troubleshooting *(continued)*

Symptom	Possible Cause	Action
When restoring an archived spot set that was created on one (source) 4800 instrument to another (target) 4800 instrument, an error message appears similar to: "Error step 4.5: check Method - Failed - See rest_0000000300.log for details".*(continued)*		5. From the 4000 Series Explorer™ software on the *target* 4800 instrument, import the missing operating modes. See the *4000 Series Explorer™ Online Help*, "Importing XML Files". 6. Repeat the steps to restore the archived spot set.

Maintenance 5

This chapter covers:

Shutting Down and Starting Up

CAUTION Do not exit the 4000 Series Explorer™ software or power off the 4800 MALDI TOF/TOF™ Analyzer unless you need to perform maintenance or move the system.
After you exit the software, you cannot control the analyzer. Exiting the software also powers off high voltages, which can require up to three hours of warm up when you restart the software.
Powering off shuts down the vacuum pumps and causes the system to vent. If you need to exit the software or power off the 4800 MALDI TOF/TOF™ Analyzer for maintenance or moving, see "Starting and Exiting the 4000 Series Explorer™ Software" on page 2-2.

Before You Begin

During the start up procedure, you are prompted for a User Name and Password. See your system administrator for your User Name and Password.

Shutting Down

To shut down the 4800 MALDI TOF/TOF™ Analyzer:

1. Make sure that high voltages are off, the Batch Queue is stopped, and the CID system is off.
2. Exit the 4000 Series Explorer™ software by selecting **File > Exit**. The 4000 Series Explorer™ software closes.
3. On the Windows® XP desktop, click **Start > Shut Down**.
4. In the Shut Down windows dialog box, click **Shut down**, then click **OK**.
5. After a message is displayed, power off the monitor and printer. Power off the main power switch, on the left side panel of the instrument, to the right of the power cord.

Starting Up

To start up the 4800 MALDI TOF/TOF™ Analyzer:

1. Power on the main power switch, on the left side panel of the instrument, to the right of the power cord.

   IMPORTANT! Both main power switches (one on the left side panel and one on the right side panel) must be in the ON position for the instrument to power on. You can power off the instrument using either switch.

   The hardware starts to initialize. The roughing pump starts. After approximately 10 minutes, the turbo pumps start. You hear a subtle whine as the vacuum pumps spin up to speed.

   After venting, it can take up to 24 hours for the system to reach adequate vacuum.

2. Power on the computer monitor and printer. Log on using your User Name and Password.
3. Start the software by double-clicking  on the Windows® XP desktop.

   The software initializes and prepares the hardware for acquisition.

Reinitializing Hardware

The 4800 MALDI TOF/TOF™ Analyzer hardware automatically starts to initialize when you start the software.

You need to reinitialize the hardware if a hardware error occurs, indicated in the software status bar ("Status Bar" on page 2-5) by Instrument-ERROR.

To reinitialize the hardware, select Instrument > Reinitialize.

Note: You cannot reinitialize hardware from the Remote Access Client.

If reinitializing does not work, restart the computer. If restarting the computer does not work, contact Applied Biosystems/MDS SCIEX Technical Support. See "How to Obtain Services and Support" on page xii.

Maintaining the System

Planned Maintenance

Regular planned maintenance helps to keep your 4800 MALDI TOF/TOF™ Analyzer functioning properly. Perform the following procedures as indicated:

Task	When to Perform	Procedure (page)
Drain oil from the roughing pump mist filter	Every month	5-5
Check roughing pump oil level. If the oil level is low, contact Applied Biosystems/ MDS SCIEX Technical Support (see "How to Obtain Support" on page x).	Every month	5-5
Clean the large and small fan filters	Every 3 months	5-2
Hardware maintenance performed by an Applied Biosystems/MDS SCIEX service representative.[a]	Every 6 months	NA
Archive and/or purge spot sets	As needed to manage your data storage space. To ensure good system performance, archive and purge spot sets when less than 20% of the database space is free.	5-16
Back up database	On a regular basis. If a backup has not been performed in a week, the 4000 Series Explorer™ software displays a reminder.	5-20

a. Contact Applied Biosystems/MDS SCIEX for information on service contracts for planned maintenance.

Maintenance Log

The maintenance log (Appendix G) includes a log sheet for you to copy and use to track maintenance on your 4800 MALDI TOF/TOF™ Analyzer.

Hardware Maintenance Procedures

Roughing Pump

The roughing pump (see "Vacuum Pumps" on page 1-17) is equipped with an oil mist filter to capture oil residues in the pump exhaust. The collected oil must be drained periodically.

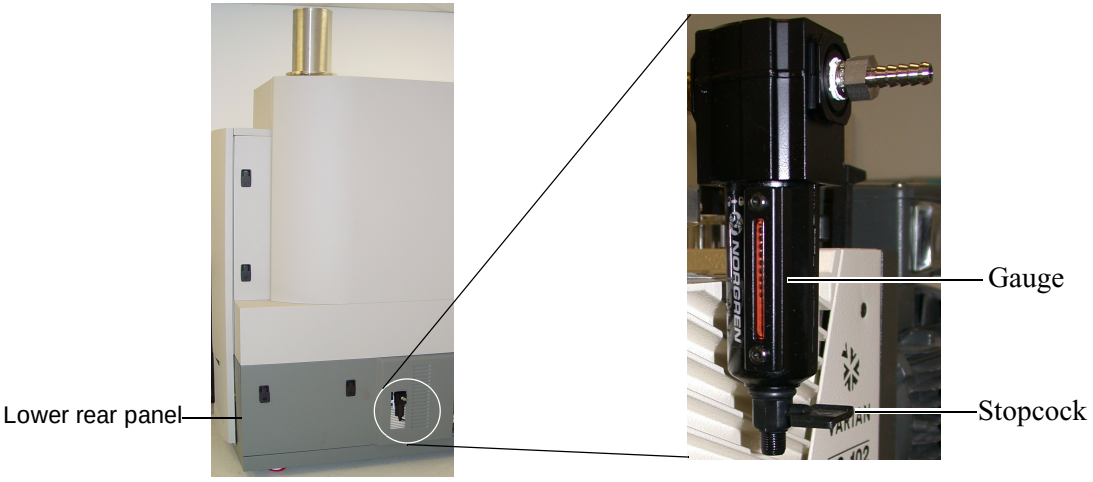


Figure 5-1 Oil mist filter on the 4800 instrument rear panel

To drain the oil mist filter and check the pump oil level:

1. Shut down the system as described in "Shutting Down and Starting Up" on page 5-2.
2. If necessary, move the system so you have access to the rear panel (Figure 5-1).
3. Check the oil mist filter gauge (Figure 5-2).
4. If the gauge indicates the presence of oil, place a waste container under the stopcock. Open the stopcock to drain the accumulated oil. Close the stopcock.
5. Carefully clean up any oil residues. Discard the cleaning materials and oil in accordance with federal, state, and local laws.
6. Remove the rear lower panel.

7. Check the roughing pump oil gauge (Figure 5-2). If the oil level is low, contact Applied Biosystems/MDS SCIEX Technical Support (see "How to Obtain Support" on page x). The oil level should be between the visible arrows that indicate the maximum and minimum acceptable levels.
8. Reinstall the rear lower panel.
9. Start up the instrument as described in "Shutting Down and Starting Up" on page 5-2.

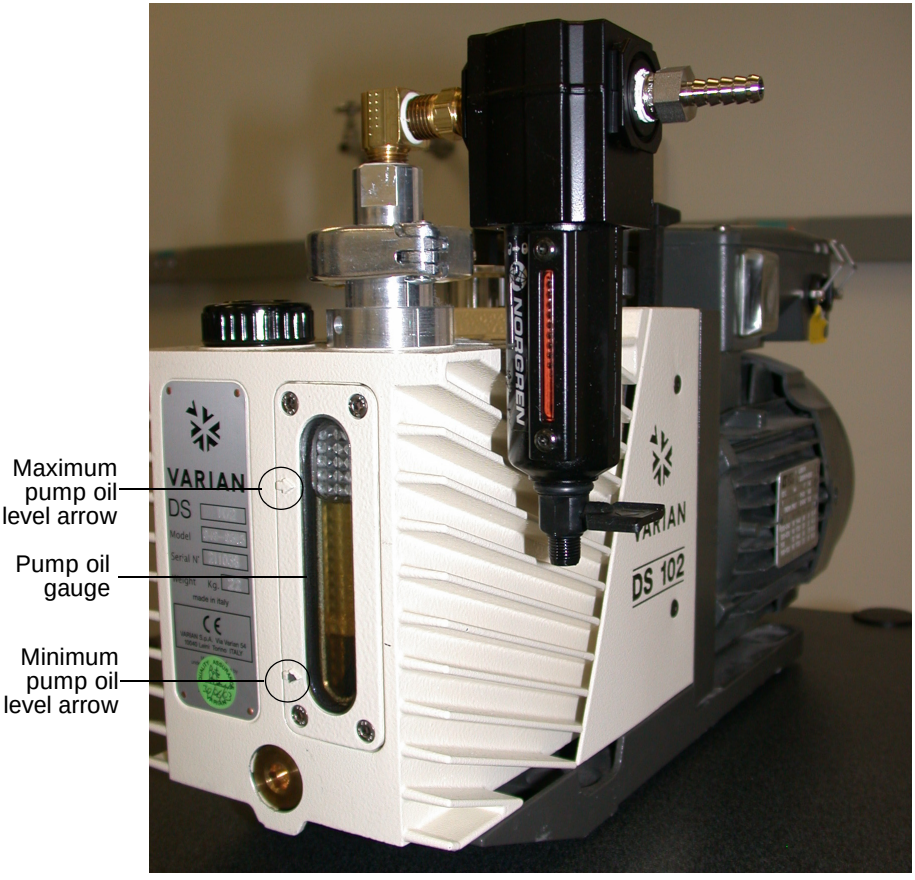


Figure 5-2 Roughing pump oil gauges

Fan Filters

Clean the fan filters every 3 months. Dirty fan filters may inhibit adequately cooling, leading to temperature increases which can cause electronic failures.

To clean the fan filter:

1. Shut down the system as described in "Shutting Down and Starting Up" on page 5-2.
2. If necessary, move the system so you have access to the rear and right panels.

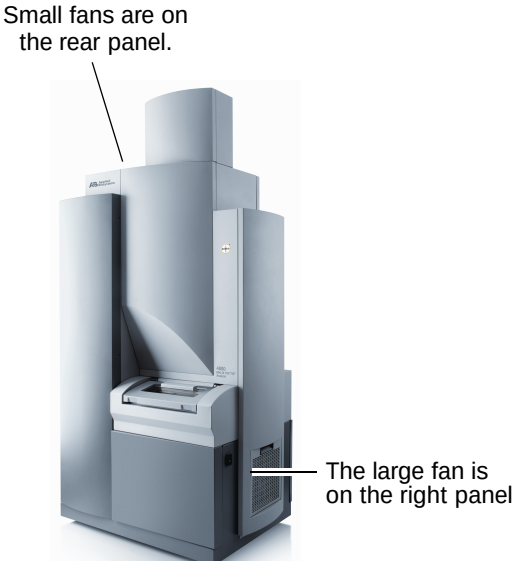


Figure 5-3 Fan locations

3. For the one large fan on the right panel, remove the large fan filter cover, then remove the filter (see Figure 5-4).

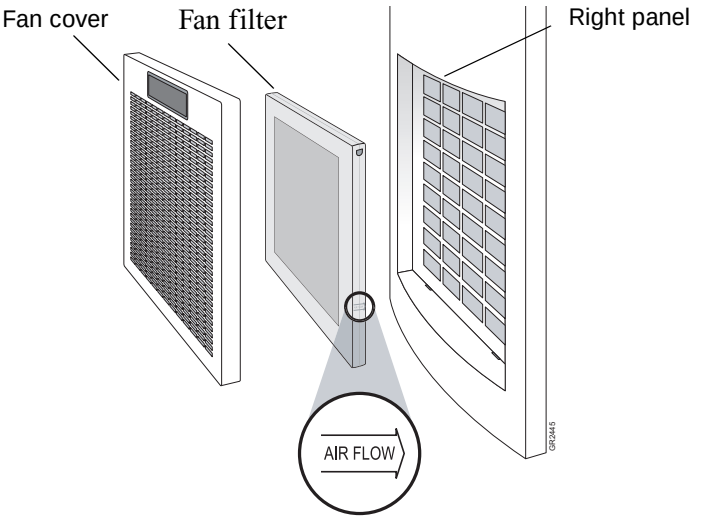


Figure 5-4 Removing the large fan filter cover and filter

1. For the two small fans on the upper rear panel, remove the small fan covers (hard plastic piece) and filters (Figure 5-5).

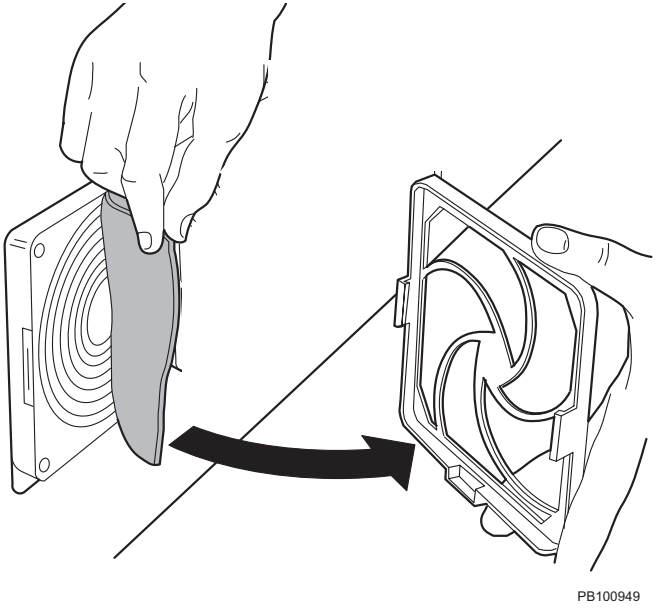


Figure 5-5 Removing the small fan filter cover and filter

2. Clean the filters under running water and allow to dry.

   Note: If you prefer to replace a filter with a new filter, see Appendix B, "Spare Parts," for the part number to order.

3. Reinstall the filters and filter covers. Position the large filter such that the air flow arrow points toward the inside of the instrument (see Figure 5-4 on page 5-8).

4. Start up the system as described in "Shutting Down and Starting Up" on page 5-2.

Reconnecting the Hardware

Overview

The 4800 MALDI TOF/TOF™ Analyzer is installed by an Applied Biosystems/MDS SCIEX service engineer. If you need to move the 4800 MALDI TOF/TOF™ Analyzer, the following sections describe how to reconnect the hardware.

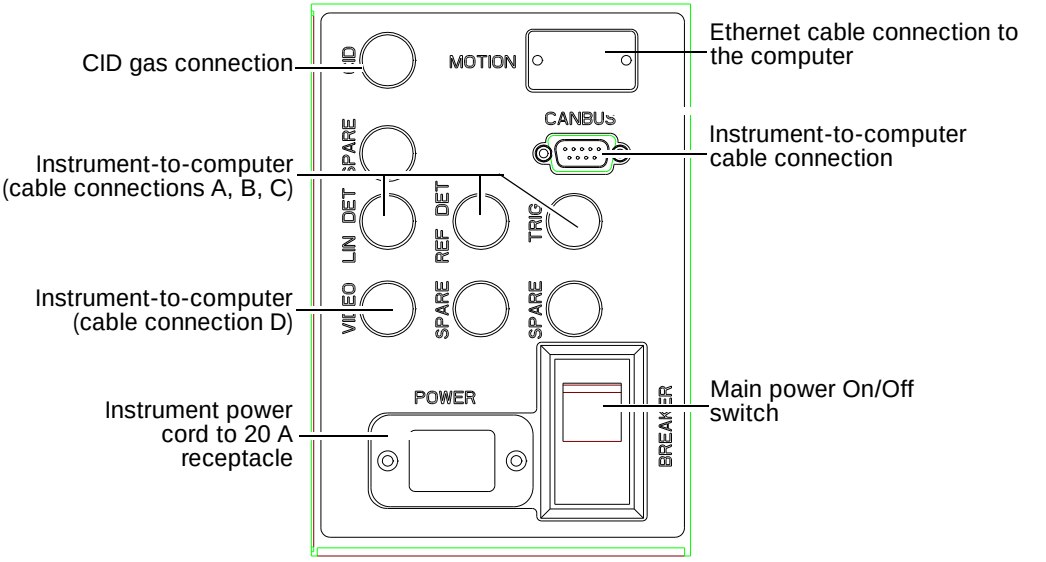


Figure 5-6 Connections on the left side of the instrument

CAUTION Before disconnecting and moving the instrument, shut down the instrument as described in "Shutting Down and Starting Up" on page 5-2.

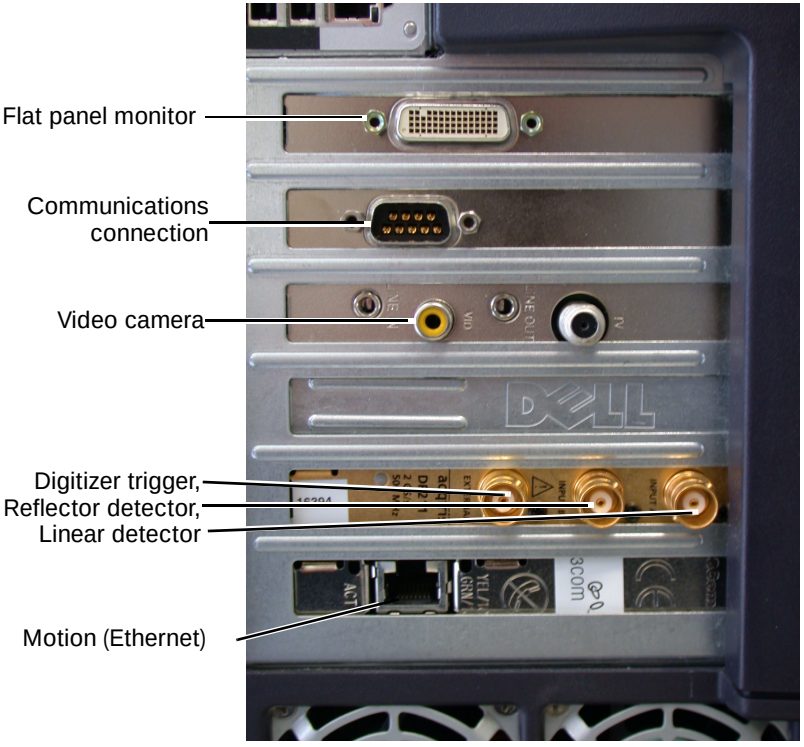


Figure 5-7 Instrument connections on the computer

Instrument Power Connection

Plug the 4800 MALDI TOF/TOF™ Analyzer power cord (on the left of the instrument) into a 20 A receptacle. This provides power for both the mass spectrometer and the internal computer.

Roughing Pump Power Connection

Plug the roughing pump power cord (on the rear of the instrument) into a 15 A receptacle.

Connecting Collision Gas

Connect the collision-induced dissociation (CID) gas to the GAS connectors on the left side of the mass spectrometer using the provided Teflon® tubing (1/8-inch O.D., 1/16-inch I.D.). Adjust the pressure gauge on the gas cylinder up to 5 PSIA.

WARNING **PHYSICAL HAZARD. Nonflammable compressed gas (such as nitrogen and other gases used as collision gas).** Contents are under pressure. Receive proper training on the handling of compressed gases before use. Exposure to rapidly expanding gas may cause frostbite. High concentrations of vapors in the immediate area can displace oxygen and cause asphyxiation. Use only in areas with adequate ventilation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

CAUTION Do not set the gas pressure above 5 PSIA. Doing so can damage the CID system.

Connecting Computer Components

Monitor – Plug the monitor into a standard wall receptacle. The monitor cable connects to the VGA connector on the rear of the computer (Figures 5-9).

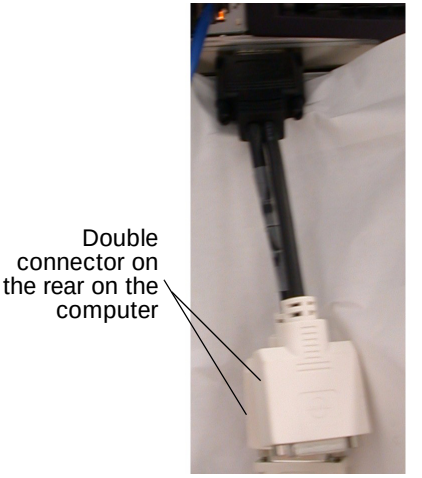


Figure 5-8 Monitor connector

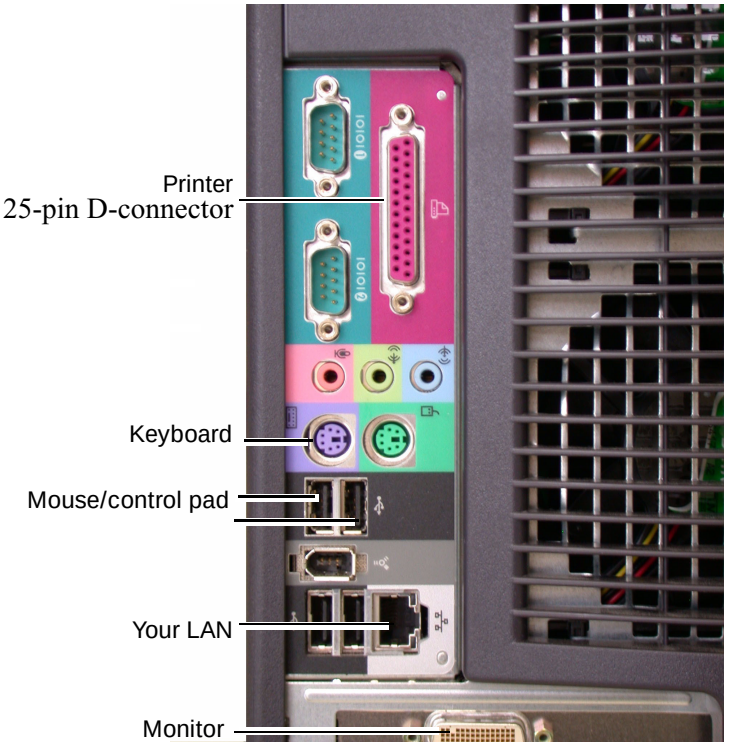


Figure 5-9 Computer component connections

Keyboard – Connect to the keyboard connector on the rear of the computer.

Remote Mouse – Connect the mouse receiver to one of the two USB connectors on the rear of the computer.

Control Pad – Connect the Control Pad (Figure F-1 on page F-2) cable to one of the two USB connectors on the rear of the computer.

Connecting a Printer

Connect an optional printer to the PRINTER connector (25-pin D-connector) or a USB connector on the computer.

Connecting to a Network

Connect one end of the provided RJ-45 cable to the NETWORK connector on the rear panel of the computer. Connect the other end to your LAN connection. Avoid connecting to the motion connector (see Figure 5-7 on page 5-11).

Connecting Exhaust to Hood

If you operate the 4800 MALDI TOF/TOF™ Analyzer in a clean room environment, you can vent the exhaust to a hood using PVC tubing (1/4-inch I.D., 3/8-inch O.D.). Connect the tubing to the exhaust connector on the oil mist filter on the rear panel of the 4800 MALDI TOF/TOF™ Analyzer (Figure 5-10).

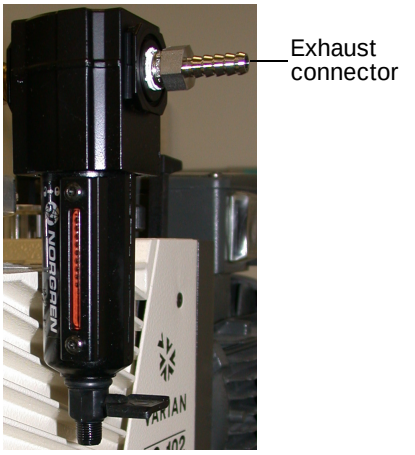


Figure 5-10 Exhaust connector at rear of the 4800 MALDI TOF/TOF™ Analyzer

Connecting the Handheld Bar Code Scanner

For information on connecting the optional handheld bar code scanner, see the *Handheld Bar Code Scanner Installation Guide*.

4000 Series Explorer™ Database Maintenance

Data archive, restore, back up, and recovery tools let you:

- Archive and/or purge selected spot sets, including associated job and user information, sample information, raw spectral data, associated methods, results, and peak lists. To ensure good system performance, archive and purge spot sets when less than 20% of the database space is free.
- Restore selected spot sets, including associated job and user information, sample information, raw spectral data, associated methods, results, and peak lists if you need to access previously archived spot set data or transfer the data to another system.
- Back up the entire database on a routine basis. The importance of your data, how often you use your system, and how much data is stored or processed determine how frequently you should back up your system. At a minimum, consider backing up once per week.
- Recover the entire database in the event of a catastrophic failure. Perform a recovery (from your latest backup) only if you experience an unrecoverable failure (typically, a disk failure). Contact Applied Biosystems/MDS SCIEX Technical Support for recovery procedures.
- Migrate the database. Migration updates the database to make it compatible with the latest instrument software. Perform a migration only after the installation of a new version of 4000 Series Explorer™ software.

Note: The 4000 Series Explorer™ Database Tools are not available on computers running 4000 Series Explorer™Software – Remote Access Client.

Archiving/Purging Spot Sets

For optimal system performance, archive and/or purge spot sets from the database to an archive destination folder. Spot sets include associated job and user information, sample information, raw spectral data, associated methods, results, and peak lists.

IMPORTANT! Archive and purge spot sets when less than 20% of the database space is free. To view the current percentage of database utilization, select **Help** > **About 4000 Series Explorer** from the 4000 Series Explorer™ software.

Before You Begin

- If necessary, see your system administer for the purge password.
- Check the file size of the spot set(s) and estimate the time needed, generally 20 minutes to 40 minutes per gigabyte of file size.
- Next, make sure there are no remote users connected. You cannot use the 4000 Series Database Restore tool if the 4000 Series Explorer™ software is running. After all remote users disconnect, save any unsaved data, then exit the 4000 Series Explorer™ software.

Archiving/purging spot sets

To archive and/or purge spot sets:

1. On the desktop taskbar, click **Start**> **All Programs** > **AB 4000 Series Explorer > 4000 Series Database Tools**.
2. In the 4000 Series Database Tools dialog box, click **Archive**.
3. In the 4000 Series Database Archive Tool dialog box, click **Browse** (to the right of Folder, Project, or Spot Set) to open the 4000 Series Database Project Tree dialog box.
4. Expand the Project tree as required to access the folder, project, or spot set that you want to archive. (You can archive a folder that contains projects and associated spot sets, a project that contains spot sets, or an individual spot set.)
5. Click to select the desired folder, project, or spot set to be archived, then click **OK**. The selected item appears in the Folder/Project/Spot Set Name field.

6. Unless lack of disk space requires that you archive to a different location, accept the default archive location (C:\Program Files\Applied Biosystems\4000 Series Explorer\archive) displayed in the Destination Root Archive Folder box.

   To select a different archive location:

   - Click **Browse** (to the right of Destination Root Archive Folder).
   - In the Browse For Folder dialog box, navigate to and select the folder to use as a destination for the archive.
   - Click **OK**. The selected folder name appears in the Destination Root Archive Folder field.

   Note: If you are archiving to the default location (or any other location on the 4800 computer), it is good practice to periodically check the amount of free hard disk space. If free disk space is 10% or less, contact Applied Biosystems/MDS SCIEX Technical Support for suggestions to make more disk space available.

7. Set options for the archive by selecting check boxes in the Options section. Depending on your selections, you can archive and purge, archive without purging, or purge without archiving.

   - **Archive –** Copies the selected spot set data from the database to the archive destination folder.
   - **Overwrite Files in File System –** Overwrites files that exist in the archive destination folder with identically named files. This check box is available only if the Archive check box is selected.
   - **Purge –** Deletes unneeded selected spot set data (see step 4) from the database. If the Archive check box is also selected, spot set data are also archived to the archive destination folder. If the Archive check box is not selected, spot set data are *deleted and not archived*.

8. Click **Start**. If you selected the Purge check box, type the purge password, then click **OK**. The selected spot set data are archived to the archive destination folder and/or purged. The Status and Progress fields show the state of the ongoing archive.

9. A message appears when the archive is finished. Click **Yes** if you want to read the log file that lists the results of the archive.

   The log file is automatically created and saved to the following location:

   C:\Program Files\Applied Biosystems\4000 Series Explorer\Archive\Log.

   The destination folder structure matches the folder structure in the 4000 Series database. Each spot set and its associated data are saved in a folder with the same name as the spot set. The spot set information is in a .dmp file.

Restoring Spot Sets

Restore selected spot sets from an existing archive folder to the database on any 4000 Series computer with the same or later version of the 4000 Series Explorer™ software. Spot sets include associated job and user information, sample information, raw spectral data, associated methods, results, and peak lists.

Before You Begin

- You cannot restore a spot set if an identically named spot set already exists in the 4800 system (the restore for that spot set fails). If both spot sets must exist in the system, rename the existing spot set before performing the restore.
- Check the file size of the spot set(s) and estimate the time needed, generally 20 minutes to 40 minutes per gigabyte of file size.
- Make sure there are no remote users connected. You cannot use the 4000 Series Database Restore tool if the 4000 Series Explorer™ software is running. After all remote users disconnect, save any unsaved data, then exit the 4000 Series Explorer™ software.

Restoring Spot Sets

1. On the desktop taskbar, click **Start** > **All Programs** > **AB 4000 Series Explorer** > **4000 Series Database Tools**.
2. In the 4000 Series Database Tools dialog box, click **Restore**.
3. Click **Browse** to open the 4000 Series Database Archive Files dialog box.
4. Navigate to the folder that contains your previously archived data.
5. Perform one of the following:
   - To restore all spot sets in a project or folder, select the desired project or folder in the file selection box, then click **Open Project/Folder**. The project or folder name appears in the Folder/Project/Spot Set Name field.
   - To restore a spot set, navigate to, then select the desired .dmp file (containing all spot set data) in the file selection box. The file name appears in the File Name field. Click **Open**. The spot set name and path to the project/folder appears in the Folder/Project/Spot Set Name field.

6. Click **Start**. The selected spot sets and associated data are restored to the database. The Status and Progress fields show the state of the ongoing restore operation. A DOS window may appear briefly during the restore operation.
7. A message appears when the restore is finished. Click **Yes** if you want to read the log file that lists the results of the restore.

   The log file (for example: `rest_000010004.log`) is automatically generated by the restore operation and is saved to:

   C:\Program Files\Applied Biosystems\4000 Series Explorer\Archive\Log.

   If necessary, see "Database Computer Troubleshooting" on page 4-7 for more information.

Backing Up the Database

Regularly back up the 4800 database to protect data and ensure that you can recover from any catastrophic system failure. Your backup schedule depends on how you use the system. For more information, see Backup Guidelines below.

You can back up the database:

- Automatically, by setting an automated backup schedule (recommended, see page 5-22)
- Manually, by running a backup any time one is required (see page 5-25)

Backup Guidelines

Developing and following an effective backup schedule for your system ensures that you have a recent copy of your data in the event of a hardware failure or user error. Your backup schedule depends on how you use the system. High-volume or high-throughput workflows with daily generation of new data require frequent backups. A good question to ask when determining your backup schedule is "how much data can I afford to lose?" Whatever your backup schedule, keep in mind that:

- For good laboratory practices, develop a standard protocol for backing up, and enforce it consistently. It is good practice to schedule automatic backups.

- If you do not back up daily, take into account the importance of your data and system usage (how much data is stored or processed) to determine how frequently you should back up your system. At a minimum, consider backing up once per week.
- Back up to a network drive or reusable media. Depending on the size of your database, the designated location for the backup must have 20 to 100 GB of free space.

  IMPORTANT! If you back up to reusable media, rotate through three sets of backup media, overwriting the oldest set with the next backup. With three sets of backups, you always have at least one good copy if you should lose data.

- To view the percentage of 4000 Series database utilization, select **Help** > **About 4000 Series Explorer** (from the 4000 Series Explorer™ software).
- If the 4800 MALDI TOF/TOF™ Analyzer computer displays warnings that database utilization is over 80%, perform an immediate backup, then archive and purge spot sets.
- The 4000 Series Explorer™ Backup Tool temporarily backs up the database files to a folder on your hard drive, then copies the files to a network device. The free space required on both the local hard drive and the network device must be adequate for the size of your backup files. Depending on the size of your database and the amount of generated spectra and peak data, the free space requirement can range from 10 to 40 GB. See "Estimating Free Space" below to estimate the required free space (on the temporary location on the local hard drive and on the network device) for a backup.

Estimating Free Space

Option 1

If you have performed daily backups, a recent backup is likely to reflect the size of your next backup (assuming similar 4800 instrument run conditions). Navigate to the remote backup destination and observe the size of the TSQBackup.dmp and TSQBackup.log files. The total size of these files should provide a close estimate for the space required for your next backup. For more information on backup files, see "Files in the Backup Destination" on page 5-28.

Option 2

a. Start the 4000 Series Explorer™ software.

b. Select **Help** > **About 4000 Series Explorer**.

c. On the line that lists Percent Database Utilization, locate the value (in MB) for **Used:**

d. Multiply the Used: value by 5. The result provides a safe estimate of the space required for your backup.

Backing Up the Database Automatically

To back up the database automatically:

1. On the desktop taskbar, click **Start** > **All Programs** > **AB 4000 Series Explorer** >**4000 Series Database Tools**.
2. In the 4000 Series Database Tools dialog box, click **Backup**.
3. In the Local Backup Directory Used for Temporary Storage field, select the folder to be used for temporary storage of the backup.

   The software automatically (and temporarily) backs up the database to your selected folder, copies the backup to the remote backup drive (see step 4), then purges the data in the selected folder. This process provides the fastest backup possible.

   Note: Unless a lack of disk space prevents you from doing so, select the default **D:\Backup** folder.
4. In the Remote Backup Directory Used for Archived Backups field, select the destination drive for the backups. The drive:
   - Must be a writable network or removable drive with a 20 to 100 GB capacity.
   - Cannot be a local hard drive, a CD-ROM drive, a RAM drive, or an unmounted drive.
5. In the Maximum Number of Archived Backups field, enter the number of backups that you want to archive. For example, if you select the default (4), your fourth backup overwrites your first (oldest) backup in the folder. For more information on the backup files, see "Files in the Backup Destination" on page 5-28.
6. Click **Schedule Auto Backup**. The Scheduled Tasks dialog box opens.
7. Double-click **Add Scheduled Task** then use the Scheduled Task wizard to schedule an automatic backup.

Table 5-1 Scheduled Task wizard

Wizard Page	Action
First	Click **Next** to continue.
Second	a. Click **Browse** to select the program you want to run. The Select Program to Schedule dialog box opens. b. Navigate to the following program: C:\Program Files\Applied Biosystems\4000 Series Explorer\Bin\TSQBackup.exe. c. Make sure TSQBackup.exe is selected, then click **Open**.
Third	Select how often (weekly, for example) or at which event (when you log on, for example) you want to schedule the automatic backup, then click **Next**.
Fourth	If you selected Daily, Weekly, Monthly, or One Time Only in the previous step, select a Start Time and any other scheduling details, then click **Next**.
Fifth	a. In the User name field, type the name of the person who is to perform backups on the database. (This person must have network permissions on the Remote Backup Directory). Note: If the 4800 MALDI TOF/TOF™ Analyzer computer is part of a domain, you must also enter the domain name in the User name field. Use the following format: Domain\User b. Enter the Windows logon password for the user, confirm the password, then click **Next.**
Sixth	Select **Open advanced properties for this task when I click Finish**, then click **Finish**.

8. When the Task tab opens, click to position the cursor at the end of the last quotation mark in the Run field. Type **-R** in the Run field. Figure 5-11 on page 5-24 shows how the updated Run field should appear.

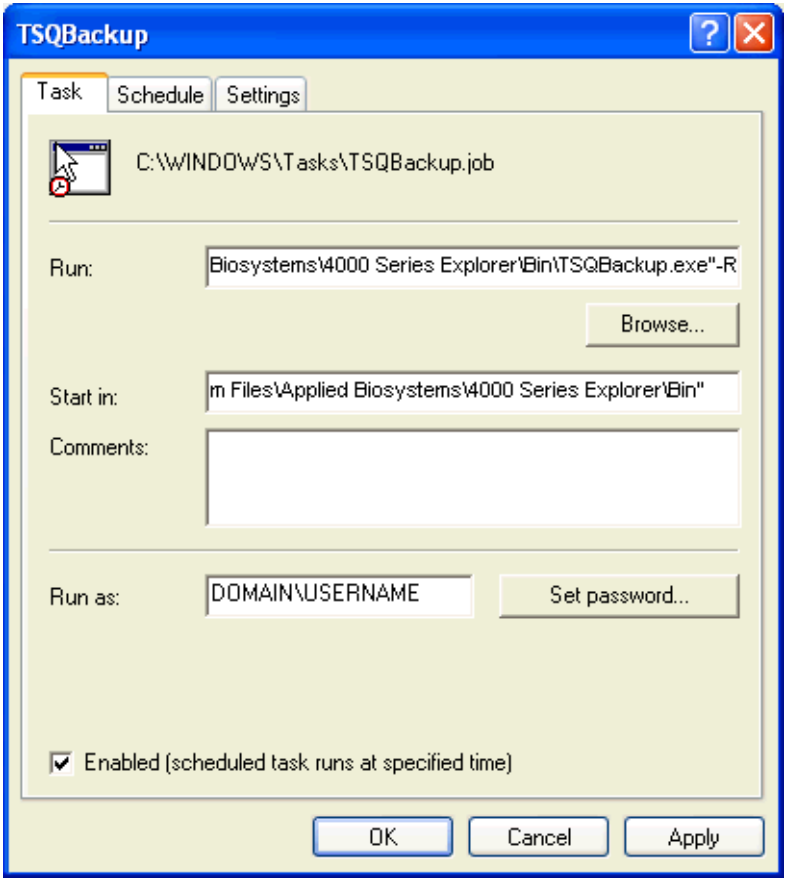


Figure 5-11 Run field

9. Click **OK**.
10. In the Set Account Information dialog box, re-enter, then confirm the password you entered above, then click **OK**. The TSQBackup task appears in the Scheduled Task dialog box.
11. Close the Scheduled Task dialog box. Your backup is scheduled to run automatically.

Backing Up the Database Manually

To back up the database manually:

1. On the desktop taskbar, click **Start** > **All Programs** > **AB 4000 Series Explorer** >**4000 Series Database Tools**.
2. In the **4000 Series** Database Tools dialog box, click **Backup**.
3. In the Local Backup Directory Used for Temporary Storage field, select the folder to be used for temporary storage of the backup.

   The software automatically (and temporarily) backs up the database to your selected folder, copies the backup to the remote backup drive (see step 4), then purges the data in the selected folder. This process provides the fastest backup possible.

   Note: Unless a lack of disk space prevents you from doing so, select the default **D:\Backup** folder.
4. In the Remote Backup Directory Used for Archived Backups field, select the destination drive for the backup. The drive:
   - Must be a writable network or removable drive with a minimum capacity of 20 GB.
   - Cannot be a local hard drive, a CD-ROM drive, a RAM drive, or an unmounted drive.
5. In the Maximum Number of Archived Backups field, enter the number of backups that you want to archive. For example, if you select the default (4), your fourth backup overwriting your first (oldest) backup in the folder. For more information on the backup files, see "Files in the Backup Destination" on page 5-28.
6. Click **Start Backup Now**. The database is first (temporarily) backed up to the Local Backup Directory on drive D, then copied to your specified backup drive.

After a successful backup, the data in the Local Backup Directory is automatically purged. If you want to view the results of the backup, navigate to the TSQBackup.log file in My Computer, then double-click the file.

Recovering the Database

If you experience a catastrophic database or disk failure, recover the database using the 4000 Series Explorer™ Database Recover Tool and your latest backup. If you have any questions about performing a database recovery, contact Applied Biosystems/MDS SCIEX Technical Support before starting the recovery.

Before You Begin

IMPORTANT! Recovering the database destroys all data currently on your 4800 MALDI TOF/TOF™ Analyzer database and replaces it with the data from your last backup.

- **Identify the last backup** – Make sure that you can clearly identify the last backup before starting the recovery. For more information, see "Files in the Backup Destination" on page 5-28.
- **Estimate the time needed** – Check the file size of the spot set(s) and estimate the time needed, generally 20 minutes to 40 minutes per gigabyte of file size. Restoring an archived spot set that was created on one (source) 4800 instrument to another (target) 4800 instrument can take twice as long as restoring to the source instrument.
- **Make sure there are no remote users connected** – You cannot use the 4000 Series Database Recover tool if the 4000 Series Explorer™ software is running. After all remote users disconnect, save any unsaved data, then exit the 4000 Series Explorer™ software.
- **If necessary, import/export operating mode** – When restoring an archived spot set that was created on one (source) 4800 instrument to another (target) 4800 instrument, the database on the target 4800 instrument must contain the identical operating mode name that the archived spot set used when it was created on the source 4800 instrument. To import the operating mode, if necessary, see Table 4-3 on page 4-7, second item, action steps 1 through 6.

Recovering the Database

To recover the database:

1. On the desktop taskbar, click **Start**> **All Programs** > **AB 4000 Series Explorer** > **4000 Series Database Tools**.
2. In the 4000 Series Database Tools dialog box, click **Recover**. A warning dialog box opens.

3. Read the statement in the warning dialog box, then click **Yes** to continue. The 4000 Series Database Recover dialog box opens.
4. Click **Browse**, then navigate to the remote folder where your backup is located.
5. Select your most recent backup, then click **OK**.

   Note: Unless you have changed the default backup file name, your most recent backup is named **TSQBackup_01.dmp**.
6. In the Password field, enter the recover password. (If necessary, see your system administrator for the password)
7. Click **Start**. The recovery starts and a DOS window opens for the duration of the recovery.

   IMPORTANT! Do not manually close, click on, or interact with the DOS window.

   When the recovery finishes, the DOS window closes and the Status field displays a successful recovery message.
8. Click **Close** to close the 4000 Series Explorer™ Database Recover dialog box.
9. Start the 4000 Series Explorer™ software and verify that your system is operational and your backup data is recovered.

Migrating the Database

Migration updates the database to make it compatible with the latest instrument software. Install the new version of 4000 Series Explorer™ software, then perform a migration.

1. On the desktop taskbar, click **Start**> **All Programs** > **AB 4000 Series Explorer** > **4000 Series Database Tools**.
2. In the 4000 Series Database Tools dialog box, click **Migrate**.
3. Click **Start Migration**. Messages in the dialog box display the migration status.
4. When a prompt displays that the data migration is complete, click **OK**.
5. Click **Close** to exit the Database Migration dialog box.

Files in the Backup Destination

The following example presumes that you have selected "3" for Maximum Number of Archived Backups (in the 4000 Series Explorer™ Software Database Backup Tool dialog box).

After the First Backup

When your *first* backup finishes, the following files exist in your specified (remote) backup destination:

- **TSQBackup_01.dmp** – The current backup.
- **TSQBackup_01.log** – A log file that shows the results of the backup.

After the Second Backup

When your *second* backup finishes, the following files exist in your specified (remote) backup destination:

- **TSQBackup_01.dmp** – The current backup.
- **TSQBackup_02.dmp** – Copy of the first backup.
- **TSQBackup_01.log** – A log file that shows the results of the current backup.
- **TSQBackup_02.log** – A log file that shows the results of the first backup.

After the Third Backup

When your *third and subsequent* backups finish, the following files exist in your specified (remote) backup destination:

- **TSQBackup_01.dmp** – The current backup.
- **TSQBackup_02.dmp** – Copy of the second-most current backup.
- **TSQBackup_03.dmp –** Copy of the third-most current backup.
- **TSQBackup_01.log** – A log file that shows the results of the current backup.
- **TSQBackup_02.log** – A log file that shows the results of the second-most current backup.
- **TSQBackup_03.log** – A log file that shows the results of the third-most current backup.

Specifications

A

This chapter covers:

Mass Spectrometer Specifications

Table A-1 Mass spectrometer specifications

Condition	Specification
Throughput	>400 samples per hour, depending on application
Ion detection	Positive, negative
Sample plate loading	Automated single-plate sample-loading system
Sample acquisition	• Manual control using control pad, mouse, or keyboard • Automatic control using Interactive or Batch mode
Ion acceleration	Two acceleration regions. The first region has three stages; the second has two-stages.
Ion path length	• MS Linear mode – 150 cm (59 in.) • MS Reflector mode – 300 cm (118 in.) • MS/MS mode – 240 cm (94 in.)
Reflector mirror	Two-stage
Laser	Nd:YAG, 355 nm wavelength, 3 to 7 ns pulse width, 200 Hz firing rate
Digitizer	Acqiris® DP211 • Samples/second – 2 gigasample/second • Analog bandwidth – 500 MHz • Available bin sizes (ns) – 0.5, 1, 2, 4, 10, 20 • Input bandwidth – 25 or 500 MHz • Recording rate – 200 Hz
Vacuum system	• Triple differential turbomolecular pumping for high vacuum • Multi-vacuum gauge capability for independent monitoring of source and reflector chambers
Dimensions	• Depth – 81 cm (32 in. • Width – 109 cm (43 in.) • Height – 239 cm (94 in.)
Weight	657 kg (1,448 pounds) including the roughing pump

Miscellaneous Specifications

Table A-2 Miscellaneous specifications

Condition	Specification
Environmental	• Operating temperature: 20 to 25 °C (68 to 77 °F) • Stable within 2 °C over 24 hours • Relative humidity: 30 to 80%, noncondensing • Altitude: ≤2,000 m (6,500 feet) above sea level • For indoor use only • Installation category (overvoltage category) of II; classified as portable equipment • Pollution degree rating of 2; can be installed in an environment that has only nonconductive pollutants
Computer	Minimum supplied configuration: • Dual Pentium® 4 Xeon™, 2.4-GHz CPU, two 120-GB hard disk drives, and 1-GB RAM (random access memory) • 20-inch color flat-panel monitor

Spare Parts

B

This appendix contains the following:

The following items are available from Applied Biosystems/MDS SCIEX.

Item Name	Description	Part Number
MALDI Plate Cleaning Kit for Stainless Steel or Gold Plates	For cleaning the 51 × 51 mm (2 × 2 in.) plates	4342532
Plates and Plate Holders		
Opti-TOF™ 96-well insert, 123 × 81 mm	Etched with 96 sample spots and 13 CAL spots, stainless steel, 5-pack	4352801
Opti-TOF™ 384-well insert, 123 × 81 mm	Etched with 384 sample spots and 13 CAL spots, stainless steel, 5-pack	4352802
Opti-TOF™ Cal Mix 5 Calibration insert, 123 × 81 mm	Spotted with 384 spots of Cal Mix 5, etched with 13 CAL spots, stainless steel, 1 per pack	4358091
Opti-TOF™ Mass Standards Calibration insert, 123 × 81 mm	Spotted with 384 spots of five different Cal Mixes, etched with 13 CAL spots, stainless steel, 1 per pack	4358092
Applied Biosystems/MDS SCIEX 4000 MALDI TOF/TOF Analyzer Calibration Inserts Kit	• Opti-TOF™ Cal Mix 5 Calibration Insert 123 x 81 mm (1 ea.) • Opti-TOF™ Mass Standards Calibration Insert 123 x 81 mm (1 ea.)	4358098
Opti-TOF™ LC/MALDI insert, 123 × 81 mm	Blank 123 × 81 mm (3 × 5 in.) insert with 8 etched CAL spots, stainless steel, 5-pack	4352797
Holder, Opti-TOF™, 4800 System 123 × 81 mm	Stainless steel plate holder that accepts the 123 × 81 mm (3 × 5 in.) insert, 1 per pack	4350840
Holder, 4800 System, 2 × 2 in	Stainless steel plate holder that accepts the 51 mm × 51 mm (2 × 2 in.) plate, 1 per pack	4352605
Holder, 4800 System, 44 × 44 mm	Stainless steel plate holder that accepts the 44 mm × 44 mm (1.7 × 1.7 in.) insert, 1 per pack	4351641
Holder, MALDI Spot, 4800 System	Stainless steel plate holder that accepts the MALDI Spot insert, 1 per pack	To be added

Item Name	Description	Part Number
Hardware		
Filter, Plastic, Elite	Small fan filter, 2-pack	V330112
Filter, Intake Right	Large fan filter	4350576
Pump, Roughing DS-102	Roughing pump	4346972
Gamepad, Logitech Dual Action	Control pad	4352556

Matrices

C

This chapter covers:

Overview

This appendix provides information for commonly used matrices. The information includes:

- Matrix mass spectra
- Chemical structures
- Applications
- Descriptions of physical appearance
- Suggested solution concentrations
- Characteristic matrix ions

Refer to Figure C-1 through Figure C-9 for characteristic peak patterns and masses.

Refer to Table C-1 on page C-7 for other matrix information.

For additional matrix information, refer to the Bibliography.

WARNING **CHEMICAL HAZARD.** Read the MSDS before handling any chemical mentioned below, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **Acetonitrile (ACN)** is a flammable liquid and vapor. It may cause eye, skin, and respiratory tract irritation, central nervous system depression, and heart, liver, and kidney damage. **Trifluoroacetic acid (TFA)** causes severe burns to the eyes, skin, and respiratory tract. **Methanol** is a flammable liquid and vapor. Exposure causes eye and skin irritation, and may cause central nervous system depression and nerve damage. **Ethanol** is a flammable liquid and vapor. It may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin. Exposure may cause central nervous system depression and liver damage.

Matrix Mass Spectra

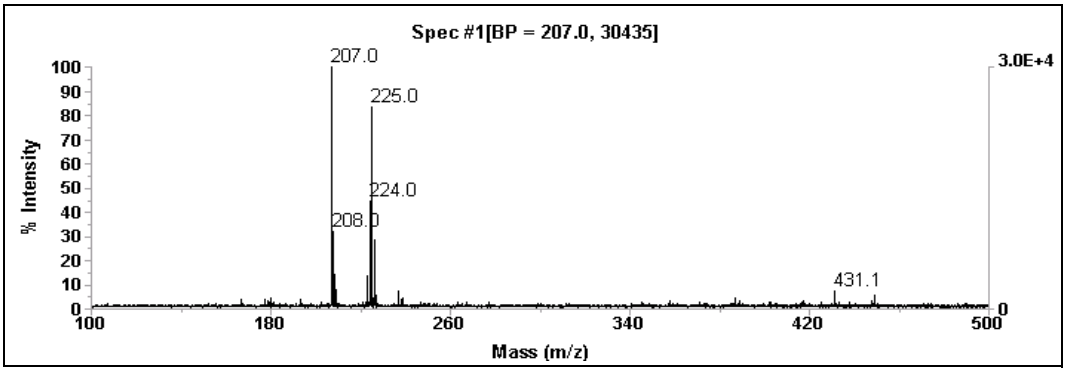


Figure C-1 Sinapinic acid matrix spectrum

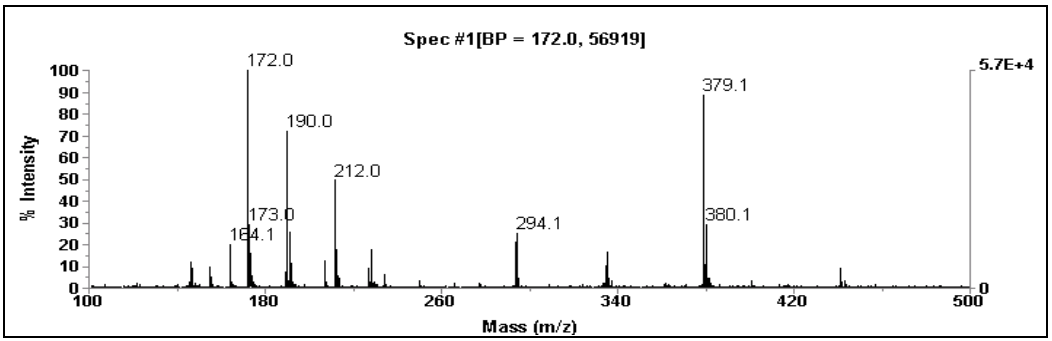


Figure C-2 α-Cyano-4-hydroxycinnamic acid (CHCA) matrix spectrum

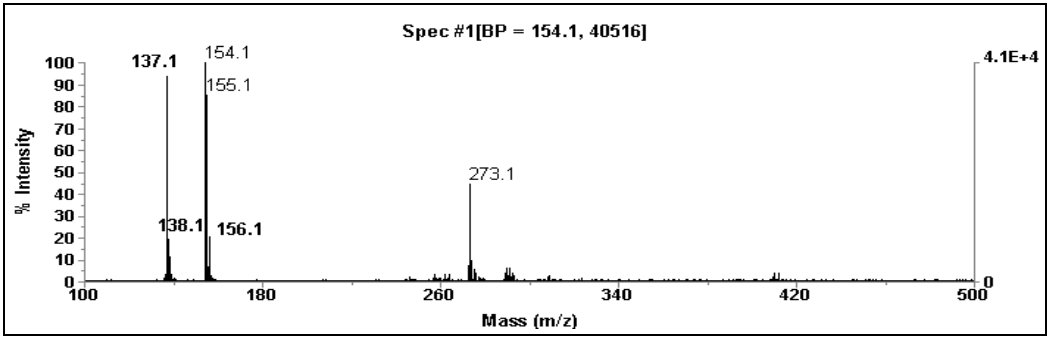


Figure C-3 2,5-Dihydroxybenzoic acid (2,5-DHB) matrix spectrum

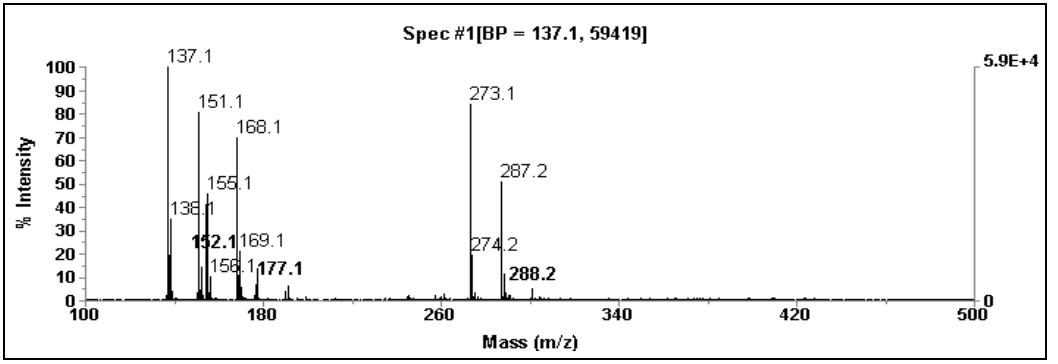


Figure C-4 Mixture of 2,5-dihydroxybenzoic acid and 5-methoxysalicylic acid (sDHB) matrix spectrum

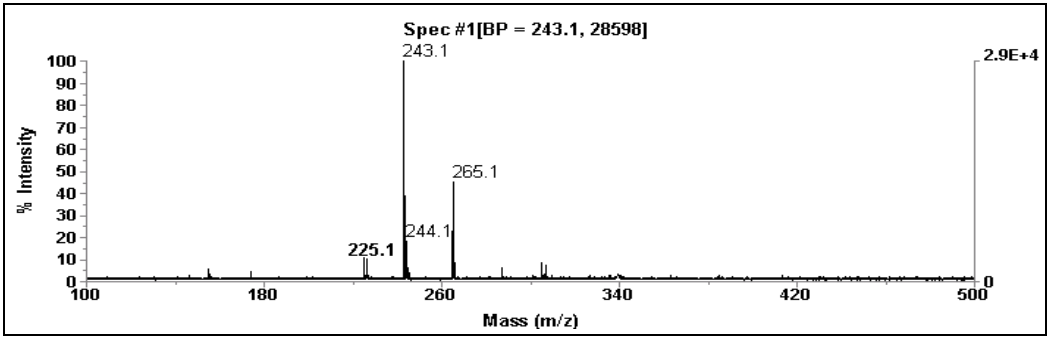


Figure C-5 2-(4-Hydroxy-phenylazo)-benzoic acid (HABA) matrix spectrum

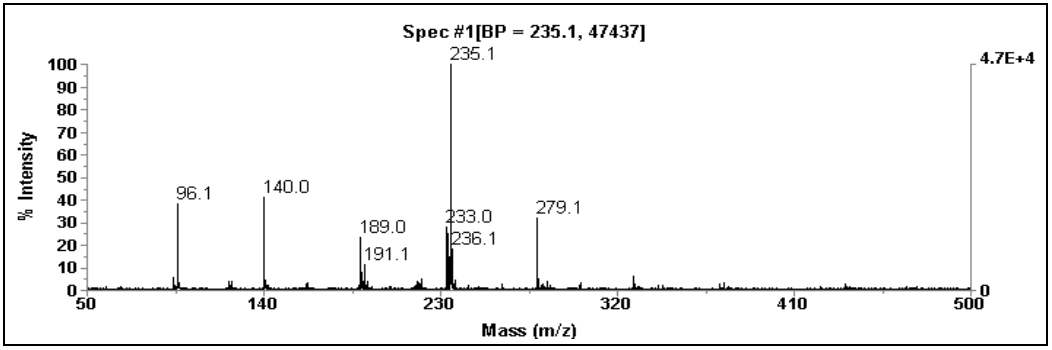


Figure C-6 3-Hydroxypicolinic acid (3-HPA) matrix spectrum

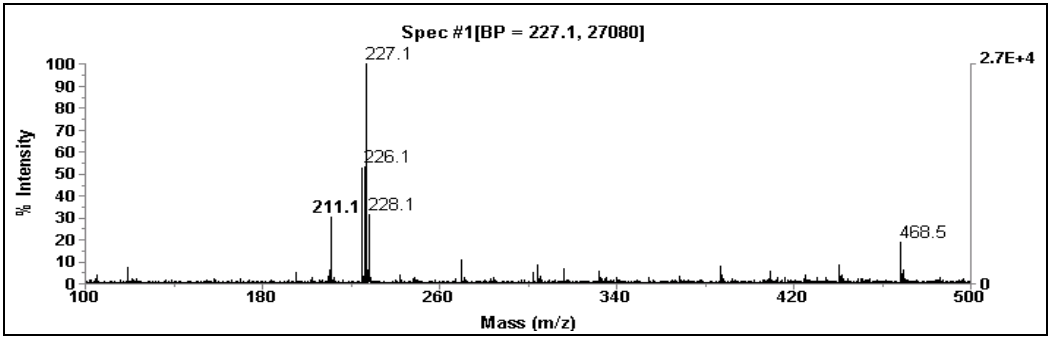


Figure C-7 Dithranol matrix spectrum

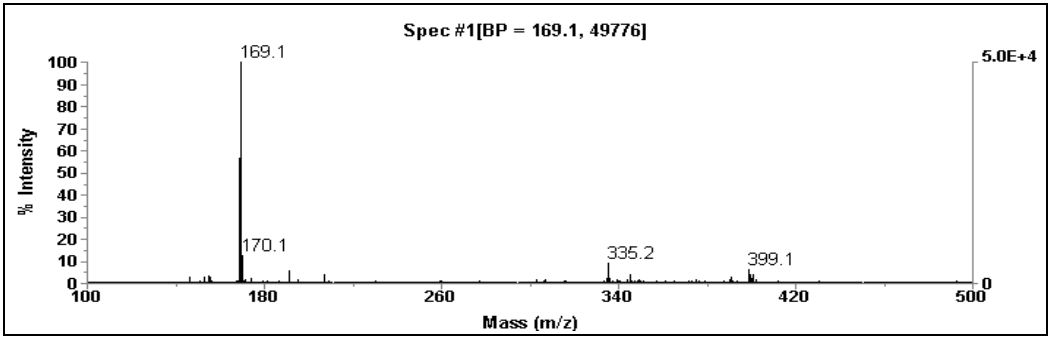


Figure C-8 2,4,6 Trihydroxyacetophenone (THAP) matrix spectrum

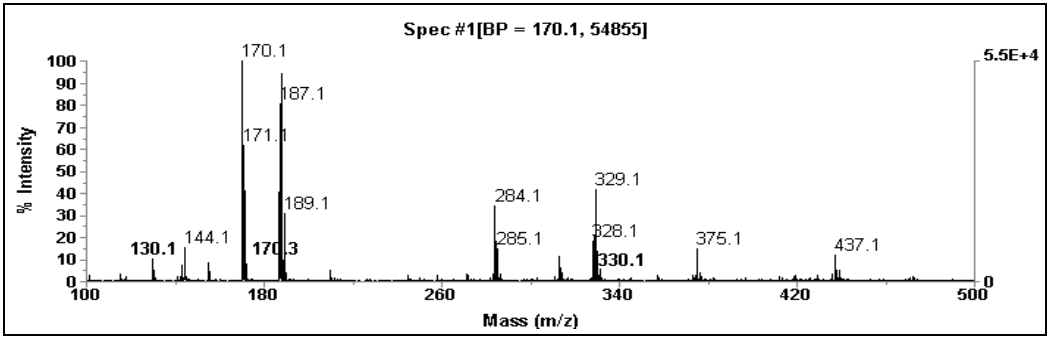


Figure C-9 Trans-3-indoleacrylic acid (IAA) matrix spectrum

Other Matrix Information

Table C-1 Matrix information

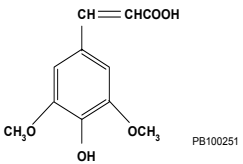
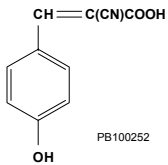
Matrix	Applications/Color	Matrix Solution Concentration	Characteristic Matrix Ions (monoisotopic)
Sinapinic acid (see mass spectrum on page -3) MW 224.07 Da 	**Applications**: • Peptides • Proteins Color of crystals/ solution: White Note: Matrix powder may also contain orange crystals. Do not use crystals when preparing solutions.	• 10 mg/mL in 70:30 water:acetonitrile (0.1% TFA final conc.) • 10 mg/mL in 50:50 water:acetonitrile (0.1% TFA final conc.) if sample contaminated	• 225.076 • 224.068 • 207.066 • 431.134
Alpha-cyano- 4-hydroxycinnamic acid (αCHCA) (see mass spectrum on page C-3) MW 189.04 Da 	Applications: • Peptides • Proteins Color of crystals/ solution: Yellow	5 mg/mL in 50:50 water:acetonitrile (0.1% TFA final conc.)	• 164.047 • 195.050 • 172.040 • 379.093 • 212.032 • 294.076

Table C-1 Matrix information *(continued)*

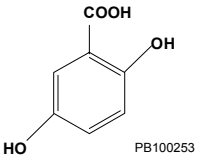
Matrix	Applications/Color	Matrix Solution Concentration	Characteristic Matrix Ions (monoisotopic)
2,5-dihydroxybenzoic acid (2,5-DHB) (see mass spectrum on page -4) MW 154.03 Da  	Applications: • Peptides (mixtures) • Proteins • Carbohydrates • Glycolipids (negative ion mode) • Polar synthetic polymers Color of crystals/ solution: White	Peptides/proteins: 5 mg/mL in 90% deionized water, 10% methanol or ethanol Other compounds: 10 mg/mL in water	• 155.034 • 154.027 • 137.024 • 273.040
	Applications: Small molecules Color of crystals/ solution: White	10 mg/mL in solvent in which sample and matrix are soluble	

Table C-1 Matrix information *(continued)*

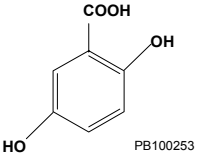
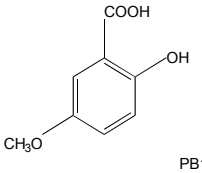
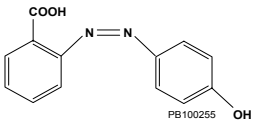
Matrix	Applications/Color	Matrix Solution Concentration	Characteristic Matrix Ions (monoisotopic)
Mixture of 2,5-dihydroxybenzoic acid and 5-methoxysalicylic acid (sDHB) (see mass spectrum on page -4) MW 154.03 Da + MW 168 Da Mixture MW = 322.03 Da   	Applications: Large proteins Color of crystals/ solution: White	10 mg/mL in solvent in which sample and matrix are soluble	• 155.034 • 154.027 • 137.024 • 273.040 • 151.040 • 168.042 • 169.050
2-(4-hydroxy-phenyl azo)-benzoic acid (HABA) (see mass spectrum on page C-4) MW 242.07 Da  	Applications: • Proteins • Lipopolysaccharides • Polar and nonpolar synthetic polymers Color of crystals/ solution: Orange	• ~1.3 mg/mL in 50:50 water: acetonitrile or in 40:40:20 water: acetonitrile: methanol • 10 mg/mL in ethanol or methanol	• 243.077 • 265.059

Table C-1 Matrix information *(continued)*

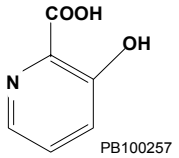
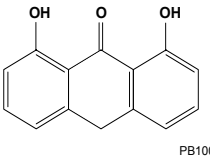
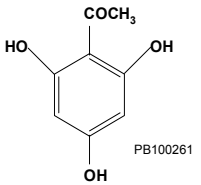
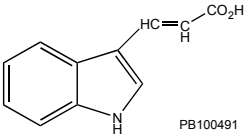
Matrix	Applications/Color	Matrix Solution Concentration	Characteristic Matrix Ions (monoisotopic)
3-hydroxypicolinic acid (3-HPA) (see mass spectrum on page -5) MW 139.03 Da 	Applications: Oligonucleotides Color of crystals/ solution: Light brown	Make 9:1 solution of matrix:diammonium citrate Matrix – 50 mg/mL in 50:50 water:acetonitrile Diammonium citrate – 50 mg/mL in water	• 96.045 • 140.035 • 279.062 • 235.072 • 234.064 • 233.056 • 191.082 • 189.066
Dithranol (see mass spectrum on page -5) MW 226.06 Da 	Applications: Nonpolar synthetic polymers Color of crystals/ solution: Yellow	10 mg/mL in tetrahydrofuran + silver trifluoroacetate (to minimize Na^+ and K^+ adduct formation)	• 225.055 • 226.063 • 227.071 • 211.076
2,4,6 trihydroxy acetophenone (THAP) (see mass spectrum on page -5) MW 168.04 Da 	Applications: Small Oligonucleotides Color of crystals/ solution: White	Make 9:1 solution of matrix:diammonium citrate **Matrix –** 10 mg/mL in 50:50 water:acetonitrile **Diammonium citrate –** 50 mg/mL in water	169.050

Table C-1 Matrix information *(continued)*

Matrix	Applications/Color	Matrix Solution Concentration	Characteristic Matrix Ions (monoisotopic)
trans-3-indoleacrylic acid (IAA) (see mass spectrum on page -6) MW 187.2  	Applications: Non-polar polymers Color of crystals/ solution: White	10^{-1} M in solvent appropriate for sample	• 187.063 • 188.071 • 170.061 • 144.081 • 130.066 • 375.134 • 329.120 • 284.131
Picolinic acid	Tang, K., N.I. Taranenko, S.L. Allman, C.H. Chen, L.Y. Chang, and K.B. Jacobson, “Picolinic Acid as a Matrix for Laser Mass Spectrometry of Nucleic Acids and Proteins,” *Rapid Commun. Mass Spectrom.*, **1994**, *8*, 673–677.		
Nicotinic acid	Ehring, H.M., M. Karas, F. Hillenkamp, *Org. Mass Spectrom.,* **1992**, *27,* 472–480.		

Calibration Standard Information

D

This appendix contains the following:

Calibration Standards

Common Calibration Standards

Table D-1 Common calibration standards

Compound	Molecular Weight		Charge State	Protonated Molecular Ion $[M+H]^+$	
	Monoisotopic	Average		Monoisotopic	Average
Leucine Enkephalin	555.2693	555.63	+1	556.2771	556.64
des-Arg[1] Bradykinin	903.4603	904.04	+1	904.4681	905.05
Bradykinin	1059.5614	1060.23	+1	1060.5692	1061.24
			+2	—	531.1
Angiotensin I	1295.6775	1296.50	+1	1296.6853	1297.51
Substance P-amide	1346.7281	1347.65	+1	1347.7360	1348.66
Glu[1] -Fibrino-peptide B	1569.6696	1570.60	+1	1570.6774	1571.61
Neurotensin	1671.9097	1672.95	+1	1672.9175	1673.96
Adrenocorticotropic hormone (ACTH), clip 1–17	2092.0789	2093.45	+1	2093.0867	2094.46
Bovine Trypsin	2162.0492	2163.34	+1	2163.0574	2164.34
Porcine Trypsin	2211.0968	2211.43	+1	2211.1046	2212.43
Adrenocorticotropic hormone (ACTH), clip 18–39	2464.1910	2465.71	+1	2465.1989	2466.72
Insulin B chain, oxidized	3493.6435	3495.95	+1	3494.6513	3496.96
Adrenocorticotropic hormone (ACTH), clip 7–38	3656.9216	3659.18	+1	3657.9294	3660.19

Table D-1 Common calibration standards *(continued)*

Compound	Molecular Weight		Charge State	Protonated Molecular Ion $[M+H]^+$	
	Monoisotopic	Average		Monoisotopic	Average
Insulin, bovine	—	5733.58	+1	5730.6087	5734.59
			+2	2865.8083	2867.80
Thioredoxin (*E. coli*, oxidized)	—	11673.47	+1	—	11674.48
			+2	—	5837.74
Cytochrome *C* (horse heart)	—	12360.5	+1	—	12361.5
			+2	—	6181.25
Myoglobin (horse heart)	—	16951.55	+1	—	16952.56
			+2	—	8476.78
Trypsinogen	—	23980	+1	—	23981
Carbonic anhydrase	—	29023	+1	—	29024
Enolase (Baker's yeast)	—	46671	+1	—	46672
			+2	—	23336
Bovine serum albumin (BSA)	—	66430	+1	—	66431
			+2	—	33216

Reference Mass Lists

To view reference mass lists for common calibration standards in the 4000 Series Explorer™ software:

1. Select **Tools > Edit Calibration Reference Masses**.
2. In the Edit Calibration Reference Masses dialog box, click … to open the Select Calibration Reference Mass List dialog box.
3. Select **All Objects** for the Project.
4. Click the **Master Reference Mass List** to open it.
5. If desired, create and save your own reference mass lists.

Theoretical Cleavages for Angiotensin I

Table D-2 and Table D-3 list monoisotopic masses (Da) for the theoretical cleavages of angiotensin I as calculated for the positive ion mode. Monoisotopic mass $(M+H)^+$ for the sequence DRVYIHPFHL is 1296.685 Da.l

Table D-2 N-terminal ions of angiotensin

Monoisotopic Mass (Da) for Ion Type				Fragment
a	b	**d_a**	**d_b**	
88.040	116.035	—	—	D
244.141	272.136	—	—	DR
343.209	371.204	329.194	—	DRV
506.273	534.268	—	—	DRVY
619.357	647.352	591.325	605.341	DRVYI
756.416[a]	784.411[a]	—	—	DRVYIH
853.468	881.463	—	—	DRVYIHP
1000.537	1028.532	—	—	DRVYIHPF
1137.596	1165.591	—	—	DRVYIHPFH

a. These peaks are typically the most intense in the spectrum.

Table D-3 C-terminal ions of angiotensin I

Monoisotopic Mass (Da) for Ion Type	**Fragment**
y	
132.103	L
269.161	HL
416.230	FHL
513.283[a]	PFHL
650.341	HPFHL
763.426	IHPFHL
926.489	YIHPFHL
1025.557	VYIHPFHL
1181.658[a]	RVYIHPFHL

a.These peaks are typically the most intense in the spectrum.

Theoretical Cleavages for Glu[1]-Fibrino-Peptide B

Table D-4 and Table D-5 list monoisotopic masses (Da) for the theoretical cleavages of Glu[1]-Fibrino-peptide B as calculated for the positive ion mode. Monoisotopic mass $(M+H)^+$ for the sequence EGVNDNEEGFFSAR is 1570.677 Da.

Table D-4 N-terminal ions of glu[1]-fibrino-Peptide B

Monoisotopic Mass (Da) for Ion Type		Fragment
a	b	
159.077	187.072	E
258.145	286.140	EG
372.188	400.183	EGV
487.215	515.210	EGVN
601.258	629.253	EGVND
730.301	758.296	EGVNDN
859.343	887.338	EGVNDNE
916.365	944.360	EGVNDNEE
1063.433	1091.428	EGVNDNEEG
1210.502	1238.497	EGVNDNEEGF
1297.534	1325.529	EGVNDNEEGFF
1368.571	1396.566	EGVNDNEEGFFS
—	—	EGVNDNEEGFFSA

Table D-5 C-terminal ions of glu[1]-fibrino-Peptide B

Monoisotopic Mass (Da) for Ion Type			Fragment
y	**w_a**	**v**	
175.120[a]	—	—	R
246.157	—	230.125	RA
333.189	298.152	301.162	RAS
480.257	—	388.194	RASF
627.325	—	535.263	RASFF
684.347[a]	—	—	RASFFG
813.390	736.342	739.353	RASFFGE
942.432	865.384	868.395	RASFFGEE
1056.475[a]	994.427	997.438	RASFFGEEN
1171.502	1108.470	1111.481	RASFFGEEND
1285.545	1223.497	1226.508	RASFFGEENDN
1384.613	1351.555	1340.551	RASFFGEENDNV
1441.635[a]	—	—	RASFFGEENDNVG

a.These peaks are typically the most intense in the spectrum.

Theoretical Cleavages for ACTH 2 Clip 18–39

Table D-6 and Table D-7 list monoisotopic masses (Da) for the theoretical cleavages of adrenocorticotropic hormone (ACTH) 2 clip 18–39 as calculated for the positive ion mode. Monoisotopic mass $(M+H)^+$ for the sequence RPVKVYPNGAEDESAEAFPLEF is 2465.199 Da.

Table D-6 N-terminal ions of ACTH 2 Clip 18–39

Monoisotopic Mass (Da) for Ion Type			Fragment
a	b	d	
226.167	254.162	—	R
325.235	353.230	—	RP
453.330	481.325	311.220	RPV
552.399	580.393	396.272	RPVK
715.462	743.457	538.383	RPVKV
812.515	840.510	—	RPVKVY
926.558	954.553	—	RPVKVYP
983.579	1011.574	883.552	RPVKVYPN
1054.616	1082.611	—	RPVKVYPNG
1183.659	1211.654	—	RPVKVYPNGA
1298.686	1326.681	1125.653	RPVKVYPNGAE
1427.728	1455.723	1254.696	RPVKVYPNGAED
1514.760	1542.755	1369.723	RPVKVYPNGAEDE
1585.797	1613.792	1498.765	RPVKVYPNGAEDES
1714.840	1742.835	—	RPVKVYPNGAEDESA

Table D-6 N-terminal ions of ACTH 2 Clip 18–39 *(continued)*

Monoisotopic Mass (Da) for Ion Type			Fragment
a	**b**	**d**	
1785.877	1813.872	1656.835	RPVKVYPNGAEDESAE
1932.946	1960.940	—	RPVKVYPNGAEDESAEA
2029.998	2057.993	—	RPVKVYPNGAEDESAEAF
2143.082	2171.077	—	RPVKVYPNGAEDESAEAFP
2272.125	2300.120	2101.035	RPVKVYPNGAEDESAEAFPL
—	—	2214.120	RPVKVYPNGAEDESAEAFPLE

Table D-7 C-terminal ions of ACTH 2 Clip 18–39

Monoisotopic Mass (Da) for Ion Type			Fragment
y	**w_a**	**v**	
166.087	—	—	F
295.129	—	—	FE
408.213	—	—	FEL
505.266	—	—	FELP
652.335	—	—	FELPF
723.372	—	—	FELPFA
852.414	—	—	FELPFAE

Table D-7 C-terminal ions of ACTH 2 Clip 18–39 *(continued)*

Monoisotopic Mass (Da) for Ion Type			Fragment
y	w_a	v	
923.451	—	—	FELPFAEA
1010.483	—	—	FELPFAEAS
1139.526	—	—	FELPFAEASE
1254.553	—	—	FELPFAEASED
1383.596	—	—	FELPFAEASEDE
1454.633	—	—	FELPFAEASEDEA
1511.654	—	—	FELPFAEASEDEAG
1625.697	—	—	FELPFAEASEDEAGN
1722.750	—	—	FELPFAEASEDEAGNP
1885.813	—	—	FELPFAEASEDEAGNPY
1984.882	—	—	FELPFAEASEDEAGNPYV
2112.977	—	—	FELPFAEASEDEAGNPYVK
2212.045	2178.987	2167.982	FELPFAEASEDEAGNPYVKV
2309.098	2264.040	—	FELPFAEASEDEAGNPYVKVP

Products of Beta-Galactosidase Digest

Table D-8 lists monoisotopic masses (Da) for the digest products of Beta-galactosidase as calculated for the positive ion mode.

Table D-8 Beta-galactosidase peptide mass assignments

Fragment Number	Peptide Fragment	Residues (Start–End)	$(M+H)^+$ Monoisotopic (Calculated)
T10	WVGYGQDSR	159–167	1067.4910
T77	GDFQFNISR	954–962	1083.5223
T64	IDPNAWVER	802–810	1099.5536
T76	ELNYGPHQWR	944–953	1299.6234
T11	LPSEFDLSAFLR	168–179	1394.7320
T3	DWENPGVTQLNR	16–27	1428.6872
T52	LSGQTIEVTSEYLFR	632–646	1742.8965
T72	VNWLGLGPQENYPDR	896–910	1757.8611
T14	WSDGSYLEDQDMWR	192–205	1787.7335
T48	YDENGNPWSAYGGDFGDTPNDR	579–600	2446.9812
T70	IDGSGQMAITVDVEVASDTPHPAR	859–882	2466.1935
T74	WDLPLSDMYTPYVFPSENGLR	919–939	2516.1808
T20	VTVSLWQGETQVASGTAPFGGEIIDER	257–283	2847.4165

Sample Log Sheets

E

This appendix contains blank sample log sheets you can copy and use to track samples spotted on sample plates.

Applied Biosystems/MDS SCIEX 4800 MALDI TOF/TOF™ Analyzer Sample Log												
Date		Bar Code			Plate Name				Spot Set			
	1	3	5	7	9	11	13	15	17	19	21	23
A (1–23)												
A (2–24)												
B (1–23)												
B (2–24)												
C (1–23)												
C (2–24)												
D (1–23)												
D (2–24)												
E (1–23)												
E (2–24)												
F (1–23)												
F (2–24)												
G (1–23)												
G (2–24)												
H (1–23)												
H (2–24)												
	2	4	6	8	10	12	14	16	18	20	22	24

Calibration	CAL 1	CAL 2	CAL 3	CAL 4	CAL 5	CAL 6

Applied Biosystems/MDS SCIEX 4800 MALDI TOF/TOF™ Analyzer Sample Log												
I (1–23)												
I (2–24)												
J (1–23)												
J (2–24)												
K (1–23)												
K (2–24)												
L (1–23)												
L (2–24)												
M (1–23)												
M (2–24)												
N (1–23)												
N (2–24)												
O (1–23)												
O (2–24)												
P (1–23)												
P (2–24)												
	2	4	6	8	10	12	14	16	18	20	22	24

Calibration	CAL 1	CAL 2	CAL 3	CAL 4	CAL 5	CAL 6

Applied Biosystems/MDS SCIEX 4800 MALDI TOF/TOF™ Analyzer Calibration Log Date _____ Bar Code _____ Plate Name _____ Spot Set _____		
CAL #	Calibrant	Matrix
1		
2		
3		
4		
5		
6		
7		
8		

Applied Biosystems 4800 MALDI TOF/TOF™ Analyzer Sample Log

Date _____ Bar Code _____ Plate Name _____ Spot Set _____

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A																								
B																								
C																								
D																								
E																								
F																								
G																								
H																								
I																								
J																								
K																								
L																								
M																								
N																								
O																								
P																								

Applied Biosystems/MDS SCEIX 4800 MALDI TOF/TOF™ Analyzer Sample Log
Date _____ Bar Code _____ Plate Name _____ Spot Set _____

Sample #		Sample	Matrix
1	A_1		
2	A_2		
3	A_3		
4	A_4		
5	A_5		
6	A_6		
7	A_7		
8	A_8		
9	A_9		
10	A_10		
11	A_11		
12	A_12		
13	A_13		
14	A_14		
15	A_15		
16	A_16		
17	A_17		
18	A_18		
19	A_19		
20	A_20		
21	A_21		
22	A_22		
23	A_23		
24	A_24		

Applied Biosystems/MDS SCIEX 4800 MALDI TOF/TOF™ Analyzer Sample Log

Date _____ Bar Code _____ Plate Name _____ Spot Set _____

Sample #		Sample	Matrix
25	B_1		
26	B_2		
27	B_3		
28	B_4		
29	B_5		
30	B_6		
31	B_7		
32	B_8		
33	B_9		
34	B_10		
35	B_11		
36	B_12		
37	B_13		
38	B_14		
39	B_15		
40	B_16		
41	B_17		
42	B_18		
43	B_19		
44	B_20		
45	B_21		
46	B_22		
47	B_23		
48	B_24		

Applied Biosystems/MDS SCIEX 4800 MALDI TOF/TOF™ Analyzer Sample Log Date _____ Bar Code _____ Plate Name _____ Spot Set _____			
Sample #		Sample	Matrix
49	C_1		
50	C_2		
51	C_3		
52	C_4		
53	C_5		
54	C_6		
55	C_7		
56	C_8		
57	C_9		
58	C_10		
59	C_11		
60	C_12		
61	C_13		
62	C_14		
63	C_15		
64	C_16		
65	C_17		
66	C_18		
67	C_19		
68	C_20		
69	C_21		
70	C_22		
71	C_23		
72	C_24		

Applied Biosystems/MDS SCIEX 4800 MALDI TOF/TOF™ Analyzer Sample Log Date _____ Bar Code _____ Plate Name _____ Spot Set _____			
Sample #		Sample	Matrix
73	D_1		
74	D_2		
75	D_3		
76	D_4		
77	D_5		
78	D_6		
79	D_7		
80	D_8		
81	D_9		
82	D_10		
83	D_11		
84	D_12		
85	D_13		
86	D_14		
87	D_15		
88	D_16		
89	D_17		
90	D_18		
91	D_19		
92	D_20		
93	D_21		
94	D_22		
95	D_23		
96	D_24		

Applied Biosystems/MDS SCIEX 4800 MALDI TOF/TOF™ Analyzer Sample Log
Date _____ Bar Code _____ Plate Name _____ Spot Set _____

Sample #		Sample	Matrix
97	E_1		
98	E_2		
99	E_3		
100	E_4		
101	E_5		
102	E_6		
103	E_7		
104	E_8		
105	E_9		
106	E_10		
107	E_11		
108	E_12		
109	E_13		
110	E_14		
111	E_15		
112	E_16		
113	E_17		
114	E_18		
115	E_19		
116	E_20		
117	E_21		
118	E_22		
119	E_23		
120	E_24		

Applied Biosystems/MDS SCIEX 4800 MALDI TOF/TOF™ Analyzer Sample Log Date _____ Bar Code _____ Plate Name _____ Spot Set _____			
Sample #		Sample	Matrix
121	F_1		
122	F_2		
123	F_3		
124	F_4		
125	F_5		
126	F_6		
127	F_7		
128	F_8		
129	F_9		
130	F_10		
131	F_11		
132	F_12		
133	F_13		
134	F_14		
135	F_15		
136	F_16		
137	F_17		
138	F_18		
139	F_19		
140	F_20		
141	F_21		
142	F_22		
143	F_23		
144	F_24		

Applied Biosystems/MDS SCIEX 4800 MALDI TOF/TOF™ Analyzer Sample Log Date _____ Bar Code _____ Plate Name _____ Spot Set _____			
Sample #		Sample	Matrix
145	G1_1		
146	G_2		
147	G_3		
148	G_4		
149	G_5		
150	G_6		
151	G_7		
152	G_8		
153	G_9		
154	G_10		
155	G_11		
156	G_12		
157	G_13		
158	G_14		
159	G_15		
160	G_16		
161	G_17		
162	G_18		
163	G_19		
164	G_20		
165	G_21		
166	G_22		
167	G_23		
168	G_24		

Applied Biosystems/MSD SCIEX 4800 MALDI TOF/TOF™ Analyzer Sample Log Date _____ Bar Code _____ Plate Name _____ Spot Set _____			
Sample #		Sample	Matrix
169	H_1		
170	H_2		
171	H_3		
172	H_4		
173	H_5		
174	H_6		
175	H_7		
176	H_8		
177	H_9		
178	H_10		
179	H_11		
180	H_12		
181	H_13		
182	H_14		
183	H_15		
184	H_16		
185	H_17		
186	H_18		
187	H_19		
188	H_20		
189	H_21		
190	H_22		
191	H_23		
192	H_24		

Applied Biosystems/MDS SCEIX 4800 MALDI TOF/TOF™ Analyzer Sample Log Date _____ Bar Code _____ Plate Name _____ Spot Set _____			
Sample #		Sample	Matrix
193	I_1		
194	I_2		
195	I_3		
196	I_4		
197	I_5		
198	I_6		
199	I_7		
200	I_8		
201	I_9		
202	I_10		
203	I_11		
204	I_12		
205	I_13		
206	I_14		
207	I_15		
208	I_16		
209	I_17		
210	I_18		
211	I_19		
212	I_20		
213	I_21		
214	I_22		
215	I_23		
216	I_24		

Applied Biosystems/MDS SCIEX 4800 MALDI TOF/TOF™ Analyzer Sample Log Date _____ Bar Code _____ Plate Name _____ Spot Set _____			
Sample #		Sample	Matrix
217	J_1		
218	J_2		
219	J_3		
220	J_4		
221	J_5		
222	J_6		
223	J_7		
224	J_8		
225	J_9		
226	J_10		
227	J_11		
228	J_12		
229	J_13		
230	J_14		
231	J_15		
232	J_16		
233	J_17		
234	J_18		
235	J_19		
236	J_20		
237	J_21		
238	J_22		
239	J_23		
240	J_24		

Applied Biosystems/MDS SCIEX 4800 MALDI TOF/TOF™ Analyzer Sample Log
Date _____ Bar Code _____ Plate Name _____ Spot Set _____

Sample #		Sample	Matrix
241	K_1		
242	K_2		
243	K_3		
244	K_4		
245	K_5		
246	K_6		
247	K_7		
248	K_8		
249	K_9		
250	K_10		
251	K_11		
252	K_12		
253	K_13		
254	K_14		
255	K_15		
256	K_16		
257	K_17		
258	K_18		
259	K_19		
260	K_20		
261	K_21		
262	K_22		
263	K_23		
264	K_24		

Applied Biosystems/MDS SCIEX 4800 MALDI TOF/TOF™ Analyzer Sample Log Date _____ Bar Code _____ Plate Name _____ Spot Set _____			
Sample #		Sample	Matrix
265	L_1		
266	L_2		
267	L_3		
268	L_4		
269	L_5		
270	L_6		
271	L_7		
272	L_8		
273	L_9		
274	L_10		
275	L_11		
276	L_12		
277	L_13		
278	L_14		
279	L_15		
280	L_16		
281	L_17		
282	L_18		
283	L_19		
284	L_20		
285	L_21		
286	L_22		
287	L_23		
288	L_24		

Applied Biosystems/MDS SCIEX 4800 MALDI TOF/TOF™ Analyzer Sample Log Date _____ Bar Code _____ Plate Name _____ Spot Set _____			
Sample #		Sample	Matrix
289	M_1		
290	M_2		
291	M_3		
292	M_4		
293	M_5		
294	M_6		
295	M_7		
296	M_8		
297	M_9		
298	M_10		
299	M_11		
300	M_12		
301	M_13		
302	M_14		
303	M_15		
304	M_16		
305	M_17		
306	M_18		
307	M_19		
308	M_20		
309	M_21		
310	M_22		
311	M_23		
312	M_24		

Applied Biosystems/MDS SCIEX 4800 MALDI TOF/TOF™ Analyzer Sample Log

Date _____ Bar Code _____ Plate Name _____ Spot Set _____

Sample #		Sample	Matrix
313	N_1		
314	N_2		
315	N_3		
316	N_4		
317	N_5		
318	N_6		
319	N_7		
320	N_8		
321	N_9		
322	N_10		
323	N_11		
324	N_12		
325	N_13		
326	N_14		
327	N_15		
328	N_16		
329	N_17		
330	N_18		
331	N_19		
332	N_20		
333	N_21		
334	N_22		
335	N_23		
336	N_24		

Applied Biosystems/MDS SCIEX 4800 MALDI TOF/TOF™ Analyzer Sample Log Date _____ Bar Code _____ Plate Name _____ Spot Set _____			
Sample #		Sample	Matrix
337	O_1		
338	O_2		
339	O_3		
340	O_4		
341	O_5		
342	O_6		
343	O_7		
344	O_8		
345	O_9		
346	O_10		
347	O_11		
348	O_12		
349	O_13		
350	O_14		
351	O_15		
352	O_16		
353	O_17		
354	O_18		
355	O_19		
356	O_20		
357	O_21		
358	O_22		
359	O_23		
360	O_24		

Applied Biosystems/MDS SCIEX 4800 MALDI TOF/TOF™ Analyzer Sample Log Date _____ Bar Code _____ Plate Name _____ Spot Set _____			
Sample #		Sample	Matrix
361	P_1		
362	P_2		
363	P_3		
364	P_4		
365	P_5		
366	P_6		
367	P_7		
368	P_8		
369	P_9		
370	P_10		
371	P_11		
372	P_12		
373	P_13		
374	P_14		
375	P_15		
376	P_16		
377	P_17		
378	P_18		
379	P_19		
380	P_20		
381	P_21		
382	P_22		
383	P_23		
384	P_24		

Using the Control Pad and Handheld Bar Code Scanner

F

This appendix contains the following:

Using the Control Pad

Overview

When working in Interactive mode, you can use the control pad (Figure F-1) provided with the 4800 MALDI TOF/TOF™ Analyzer to:

- Adjust sample position under the laser beam
- Start and stop acquisition
- Adjust laser intensity
- Save a spot (methods, spectrum, and peak list) to the database

Figure F-1 Control pad

When using the Plate Alignment wizard, Button 1 displays the laser position in Video Viewer. Button 4 allows you to use the left joystick to center the laser target (crosshairs) within the laser spot. See the *4000 Series Explorer™ Software Online Help*, Aligning the Laser Target in the Video Viewer.

Using the Handheld Bar Code Scanner

LASER HAZARD. Class 2 (II) lasers can cause damage to eyes. Avoid looking into a Class 2 (II) laser beam or pointing a Class 2 (II) laser beam into another person's eyes.

The Opti-TOF™ plates provided with the 4800 MALDI TOF/TOF™ Analyzer have a bar code label on the bottom of the insert. When the Opti-TOF insert is in the magnetic holder, you can scan the bar code through the hole in the bottom of the holder.

You can scan bar codes in two ways:

- Holding the scanner by hand
- With the scanner mounted in the holder

Scanning by Hand

To scan by hand:

1. Hold the scanner about 23 cm (9 in.) from the plate.
2. Point the scanner at a slight angle to the bar code.
3. Press the trigger. The laser beam must illuminate the entire length of the bar code to correctly read the bar code. A beep indicates a successful read.

Scanning in the Holder

The bar code scanner must be in the AutoSense® scan mode to scan when mounted in the holder. To enable the AutoSense® scan mode, refer to the *QuickScan® 6000/6000plus AutoSense® Stand Instructions* included with the scanner.

Note: The scanner must be approximately 23 cm (9 in.) from the plate to properly read the bar code.

The trigger is automatically depressed when the scanner is mounted in the holder. To scan a bar code:

1. Slide the plate onto the base of the scanner. Do not hold the plate at an angle.
2. Pass the bar code label below the scanner laser beam.

Note: When the laser beam illuminates the entire length of the bar code, tip the plate *very slightly.* The scanner is sensitive; you may need to move the plate *slightly* under the beam to get the scanner to read the bar code. A beep indicates a successful read.

Maintenance Log Sheet

G

This following page is for recording the maintenance performed on your 4800 MALDI TOF/TOF™ Analyzer. Copy the log sheet and keep the copy by your 4800 MALDI TOF/TOF™ Analyzer.

Instructions for performing maintenance procedures are in Chapter 5.

Maintenance Log for 4800 MALDI TOF/TOF™ Analyzer

Serial Number _____ Year _____

Record the date and your initials when you perform maintenance procedures.

Every Month	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
Drain oil mist filter												
Check roughing pump oil level												
Every Three Months	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**
Clean Fan Filters												
Every Six Months	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**
Hardware maintenance performed by an Applied Biosystems service representative												
Regularly	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**
Archive and Purge Data												
Back Up Data												

Warranty and Service Information

H

This appendix contains the following:

Computer Configuration

Applied Biosystems/MDS SCIEX supplies or recommends certain configurations of computer hardware, software, and peripherals for use with its instrumentation. Applied Biosystems/MDS SCIEX reserves the right to decline support for or impose extra charges for supporting nonstandard computer configurations or components that have not been supplied or recommended by Applied Biosystems/MDS SCIEX. Applied Biosystems/MDS SCIEX also reserves the right to require that computer hardware and software be restored to the standard configuration prior to providing service or technical support. For systems that have built-in computers or processing units, installing unauthorized hardware or software may void the Warranty or Service Plan.

Limited Product Warranty

Limited Warranty

Applied Biosystems/MDS SCIEX warrants that all standard components of its **4800 MALDI TOF/TOF™** Analyzer will be free of defects in materials and workmanship for a period of one (1) year from the date the warranty period begins. Applied Biosystems/MDS SCIEX will repair or replace, at its discretion, all defective components during this warranty period. After this warranty period, repairs and replacement components may be purchased from Applied Biosystems/MDS SCIEX at its published rates. Applied Biosystems/MDS SCIEX also provides service agreements for post-warranty coverage. Applied Biosystems/MDS SCIEX reserves the right to use new, repaired, or refurbished instruments or components for warranty and post-warranty service agreement replacements. Repair or replacement of products or components that are under warranty does not extend the original warranty period.

Applied Biosystems/MDS SCIEX warrants that all optional accessories supplied with its 4800 MALDI TOF/TOF™ Analyzer, such as peripherals, printers, and special monitors, will be free of defects in materials and workmanship for a period of ninety (90) days from the date the warranty begins. Applied Biosystems/MDS SCIEX will repair or replace, at its discretion, defective accessories during this warranty period. After this warranty period, Applied Biosystems/MDS SCIEX will pass on to the buyer, to the extent that it is permitted to do so, the warranty of the original manufacturer for such accessories.

With the exception of consumable and maintenance items, replaceable products or components used on or in the instrument are themselves warranted to be free of defects in materials and workmanship for a period of ninety (90) days.

Applied Biosystems/MDS SCIEX warrants that chemicals and other consumable products will be free of defects in materials and workmanship when received by the buyer, but not thereafter, unless otherwise specified in documentation accompanying the product.

Applied Biosystems/MDS SCIEX warrants that for a period of ninety(90) days from the date the warranty period begins, the tapes, diskettes, or other media bearing the operating software of the product, if any, will be free of defects in materials and workmanship under normal use. If there is a defect in the media covered by the above warranty and the media is returned to Applied Biosystems/ MDS SCIEX within the ninety (90) day warranty period, Applied Biosystems/MDS SCIEX will replace the defective media.

Applied Biosystems/MDS SCIEX does not warrant that the operation of the instrument or its operating software will be uninterrupted or error free.

Warranty Period Effective Date

Any applicable warranty period under these sections begins on the earlier of the date of installation or ninety (90) days from the date of shipment for hardware and software installed by Applied Biosystems/ MDS SCIEX personnel. For all hardware and software installed by the buyer or anyone other than Applied Biosystems/MDS SCIEX, and for all other products, the applicable warranty period begins the date the product is delivered to the buyer.

Warranty Claims

Warranty claims must be made within the applicable warranty period, or, for chemicals or other consumable products, within thirty (30) days after receipt by the buyer.

Warranty Exceptions

The above warranties do not apply to defects resulting from misuse, neglect, or accident, including without limitation: operation with incompatible solvents or samples in the system; operation outside of the environmental or use specifications or not in conformance with the instructions for the instrument system, software, or accessories; improper or inadequate maintenance by the user; installation of software or interfacing, or use in combination with software or products, not supplied or authorized by Applied Biosystems/MDS SCIEX; and modification or repair of the product not authorized by Applied Biosystems/MDS SCIEX.

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Warranty Limitations

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NO AGENT, EMPLOYEE, OR REPRESENTATIVE OF APPLIED BIOSYSTEMS/MDS SCIEX HAS ANY AUTHORITY TO MODIFY THE TERMS OF THIS LIMITED WARRANTY STATEMENT OR TO BIND APPLIED BIOSYSTEMS/MDS SCIEX TO ANY AFFIRMATION, REPRESENTATION, OR WARRANTY CONCERNING THE PRODUCT THAT IS NOT CONTAINED IN THIS LIMITED WARRANTY STATEMENT, AND ANY SUCH MODIFICATION, AFFIRMATION,

REPRESENTATION, OR WARRANTY MADE BY ANY AGENT, EMPLOYEE, OR REPRESENTATIVE OF APPLIED BIOSYSTEMS/MDS SCIEX WILL NOT BE BINDING ON APPLIED BIOSYSTEMS/MDS SCIEX, UNLESS IN A WRITING SIGNED BY AN EXECUTIVE OFFICER OF APPLIED BIOSYSTEMS/MDS SCIEX.

THIS WARRANTY IS LIMITED TO THE BUYER OF THE PRODUCT FROM APPLIED BIOSYSTEMS/MDS SCIEX AND IS NOT TRANSFERABLE.

Some countries or jurisdictions limit the scope of or preclude limitations or exclusion of warranties, of liability, such as liability for gross negligence or wilful misconduct, or of remedies or damages, as or to the extent set forth above. In such countries and jurisdictions, the limitation or exclusion of warranties, liability, remedies or damages set forth above shall apply to the fullest extent permitted by law, and shall not apply to the extent prohibited by law.

Damages, Claims, and Returns

Damages

If shipping damage to the product is discovered, contact the shipping carrier and request inspection by a local agent. Secure a written report of the findings to support any claim. Do not return damaged goods to Applied Biosystems/MDS SCIEX without first securing an inspection report and contacting Applied Biosystems/MDS SCIEX Technical Support for a Return Authorization (RA) number.

Claims

After a damage inspection report is received by Applied Biosystems/MDS SCIEX, Applied Biosystems/MDS SCIEX will process the claim unless other instructions are provided.

Returns

Do not return any material without prior notification and authorization.

If for any reason it becomes necessary to return material to Applied Biosystems/MDS SCIEX, contact Applied Biosystems/MDS SCIEX Technical Support or your nearest Applied Biosystems/MDS SCIEX subsidiary or distributor for a return authorization (RA) number and forwarding address. Place the RA number in a prominent location on the outside of the shipping container, and return the material to the address designated by the Applied Biosystems/MDS SCIEX representative.

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