user manual
proteomics

Ettan *MALDI-ToF*

Instrument Handbook

um 18-1144-00

Amersham Biosciences

Important user information

All users must read this entire manual to fully understand the safe use of Ettan™ MALDI-ToF.

WARNING!

The Warning sign highlights an instruction that must be strictly followed in order to avoid personal injury. Be sure not to proceed until the instructions are clearly understood and all stated conditions are met.

WARNING! High Voltage

The High Voltage sign highlights an instruction that must be strictly followed in order to avoid contact with lethal high voltage above 1 kV. Be sure not to proceed until the instructions are clearly understood and all stated conditions are met.

WARNING! Laser Radiation

The laser radiation sign highlights an instruction that must be strictly followed in order to avoid exposure to hazardous laser radiation. Be sure not to proceed until the instructions are clearly understood and all stated conditions are met.

Caution!

The Caution sign is used to call attention to instructions or conditions that must be followed to avoid damage to the product or other equipment. Be sure not to proceed until the instructions are clearly understood and all stated conditions are met.

Declaration of conformity

Safety standards

This product meets the requirements of the Low Voltage Directive 72/23/EEC through the harmonized standard EN 61 010-1:1993 + A2:1995.

EMC standards

This product meets the requirements of the EMC Directive 89/336/EEC through the harmonized standard EN 61 326-1:1997 + A1:1998.

WARNING!

This is a Class A product. In a domestic environment, this product may cause radio interference, in which case the user may be required to take adequate measures.

The **CE** symbol and corresponding declaration of conformity is valid for the instrument when it is:

- used as a stand-alone unit, or
- connected to other CE-marked Amersham Biosciences instruments, or
- connected to other products recommended or described in this manual, and
- used in the same state as it was delivered from Amersham Biosciences except for alterations described in this manual.

All goods and services are sold subject to the terms and conditions of sale of the company within the Amersham Biosciences group which supplies them. A copy of these terms and conditions is available on request.

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1 Introduction

The Ettan MALDI-ToF mass spectrometer is a bench-top research grade instrument incorporating a harmonic reflectron and high mass detector.

Ettan MALDI-ToF combines the protein knowledge of Amersham Biosciences with that of experts in protein identification (ProteoMetrics LLC) and mass spectrometry (Scientific Analysis Instruments Ltd.) to offer a MALDI-TOF mass spectrometer for fast, reliable protein identification.

MALDI-TOF
matrix-assisted laser desorption/ionization time-of-flight (see Section 1.4).

The compact and automated instrument features a high-resolution timed ion gate and a quadratic-field reflectron that can be used in post-source decay (PSD) studies of protein fragments.

Examples of applications:

- protein/peptide mass determination
- determining the indentity of a protein
- verifying a protein identity
- identification of interaction partners
- studies of various biomolecular problems

1.1 The Instrument Handbook

This handbook provides safety instructions, technical information and basic operating instructions for the Ettan MALDI-ToF instrument. In addition, maintenance schedules and instructions for user maintenance are included.

1.2 The Ettan MALDI-ToF system

The Ettan MALDI-ToF system comprises the Ettan MALDI-ToF instrument and the accompanying control and analysis software, which runs under Windows NT operating system on a PC. After the sample slide is introduced, the system is fully controlled via the software.

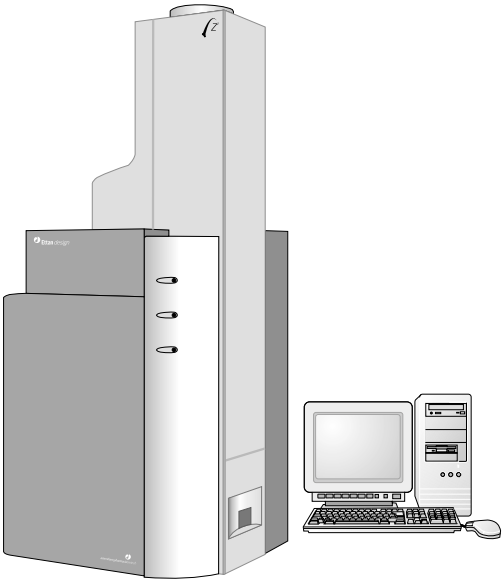


Fig 1-1. The Ettan MALDI-ToF system.

Samples are spotted or positioned onto a sample slide with a number of sample wells.

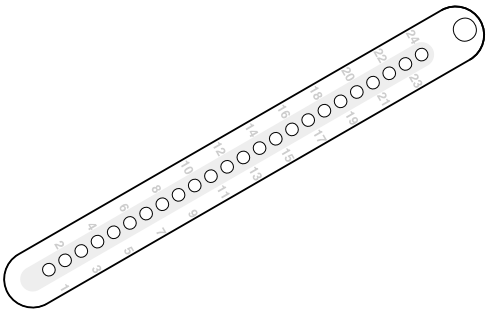
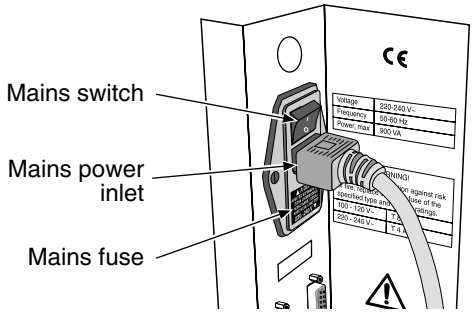


Fig 1-2. Typical sample slide.

Ettan MALDI-ToF allows selection of both positive and negative ion operation as standard. A detailed description of the Ettan MALDI-ToF instrument is provided in Chapter 6 of this manual.

1.2.1 Rating labels

The rating labels are located on the connector panel at the rear of the Ettan MALDI-ToF instrument, see Fig. 1-3. Pay attention to the component ratings. These ratings determine the electrical safety hazards in the equipment connected to the supply voltage. There are, however, other hazards, which may be more severe, see *Chapter 2 Safety instructions*.



Voltage	220-240 V~
Frequency	50-60 Hz
Power, max	900 VA

WARNING!	
For continued protection against risk of fire, replace only with fuse of the specified type and current ratings.	
100 - 120 V~	T 8 A
220 - 240 V~	T 4 A

Fig 1-3. Location and layout of rating labels.

1.3 Accessories

The Ettan MALDI-ToF instrument requires the use of specific sample slides, which can be ordered from Amersham Biosciences. In addition, MALDI-associated chemicals and calibration peptides are available. Ordering information for accessories is provided in Chapter 6.

1.4 Time-of-flight mass spectrometry

1.4.1 Basic principles

A time-of-flight mass spectrometer separates ions of different masses and produces signals that provide timing information for ions at each mass. The signals are accumulated and, by using statistics, the amount of ions at each mass can be represented in a diagram—the mass spectrum (see Fig. 1-4).

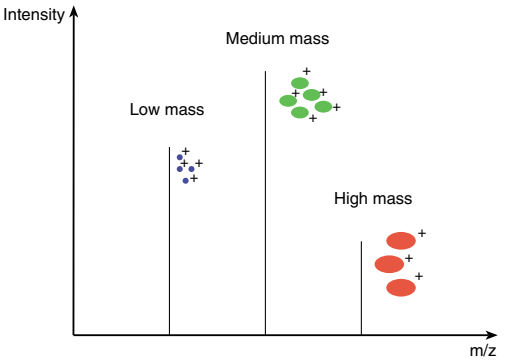


Fig 1-4. Example of a mass spectrum.

In a MALDI-TOF instrument, a laser pulse desorbs and ionizes the biomolecules incorporated in a matrix on the sample slide. The ions are accelerated by high voltage, acquiring a velocity that depends on the mass: light ions acquire a higher velocity than heavy ions (see Fig. 1-5).

Entering a field-free "drift region", each ion keeps the constant velocity until it hits the detector. Ions of different mass can thus be separated in time: heavy ions take longer than light ions.

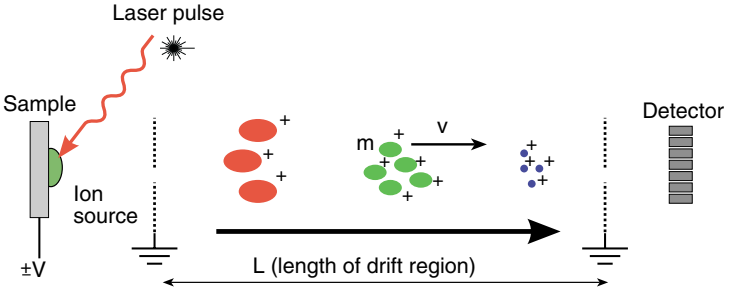
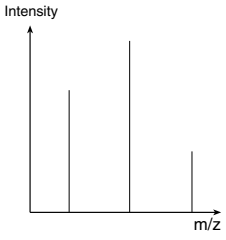


Fig 1-5. The principle of time-of-flight mass spectrometry.



In the mass spectrum, the accumulated signals are plotted against mass over charge (m/z), which usually corresponds to mass. The mathematics behind this conversion are described very briefly in the next section.

1.4.2 Simplified theory

The electric field in the ion source accelerates the ions into a field-free drift region. The kinetic energy of each ion is proportional to the charge of the ion (q) and the accelerating voltage (V).

The travel time in the drift region depends on the mass of each ion. As shown in Fig. 1-5, the ions will be separated so that ions with low mass reach the detector first.

The time-of-flight principle relies on the following basic equations:

$$qV = \frac{1}{2}Mv^2 \quad \text{, where q = ion charge, V = voltage, M = ion mass, v = velocity} \qquad (1)$$

$$t = \frac{L}{v} \quad \text{, where t = time of flight, L = length of the drift region (flight tube)} \qquad (2)$$

In this simplified model, the travel time for an ion is proportional to:

$$\sqrt{\frac{M}{q}} \qquad (3)$$

Considering that the ion mass and charge are proportional to the atomic mass of the ion and its charge state respectively,

$$M = 1,66\times10^{-27}m \quad \text{, where M = ion mass (kg), m = atomic mass (Da)} \qquad (4)$$

$$q = ez \quad \text{, where q = ion charge (C), e = constant } (1.60 \times 10^{-19}\ \text{C}), \text{ z = ion charge state (dimensionless)} \qquad (5)$$

we can express the travel time as:

$$t = k\sqrt{\frac{m}{z}} \quad \text{, where k = constant}$$

By detecting the number of ions arriving at different times, a mass spectrum for the sample can be calculated and displayed. The mass spectrum shows a number of peaks representing the intensities of ions of different mass-to-charge ratios (see Fig. 1-4). Since the charge state is usually z = +1 (or z= +2) in MALDI experiments, it is possible to identify peaks as representing certain masses.

1.4.3 Reflectron

The Ettan MALDI-ToF includes a harmonic reflectron that acts as an ion mirror (see Fig. 1-6). The reflectron improves resolution by extending the ion flight path. In addition, ions of equal mass having slightly different energy will be focused in time, thus giving more narrow peaks in spectrum, also contributing to higher resolution. See Section 6.5.2 for more details.

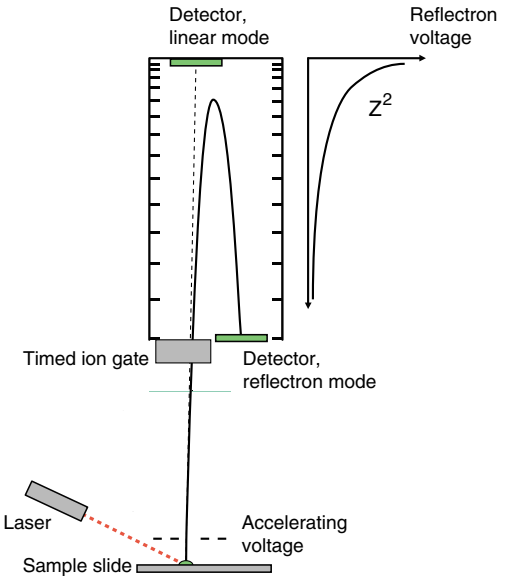


Fig 1-6. Harmonic reflectron principle. The reflectron voltage has a quadratic characteristic.

1.5 Associated documentation

The following documentation is included with the Ettan MALDI-ToF:

The *Ettan MALDI-ToF Site Preparation Guide* describes the requirements that must be fulfilled before the Ettan MALDI-ToF instrument can be installed.

The *Ettan MALDI-ToF User Manual* contains detailed operating instructions.

The *Ettan MALDI-ToF Method Handbook* provides practical guidelines and examples of specific applications.

The *Ettan MALDI ToF MS Software Online Help* contains detailed descriptions of all software menus and dialogues.

The Ettan MALDI-ToF manuals are delivered as spiral bound books or binders, as indicated in the margin.

2 Safety instructions

IMPORTANT! To avoid any risk of injury, the instrument should only be operated by properly trained personnel and always in accordance with the instructions provided.

The purpose of this chapter is to describe safety precautions and present all safety labels that are attached to the instrument. Built-in safety functions are described, and emergency and disposal procedures are included.

2.1 Safety precautions

1 Read this entire manual before using the Ettan MALDI-ToF instrument.

2 This instrument is designed for indoor use only.

3 The instrument must always be used with the protective earth lead of the power cord correctly grounded to earth at the mains outlet.

4 To permit sufficient cooling, ensure that the vents at the top, rear and bottom of the instrument are not covered.

5 Do not operate the instrument in extreme humidity (above 95%). Avoid condensation by letting the unit equilibrate to ambient temperature.

6 Keep the instrument dry and clean. Wipe regularly with a soft damp tissue. Let the instrument dry completely before use (see Chapter 4).

7 Any equipment connected to the instrument should meet the requirements of the EN 61 010-1 or other international safety standards.

2.2 Safety labels

The following safety labels are attached to the Ettan MALDI-ToF instrument to warn the user of potentially hazardous conditions:

At the sample door

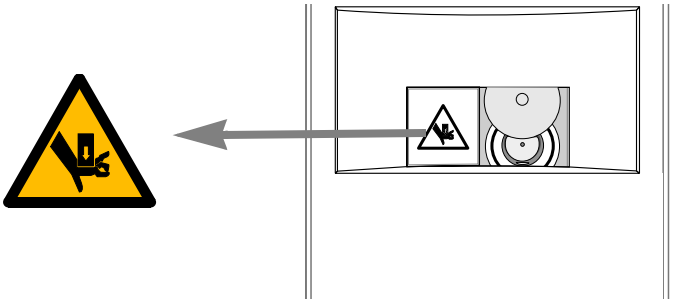


Fig 2-1. Pinch hazard warning at sample door.

WARNING! PINCH HAZARD. Make sure that nobody is working at the sample entry lock before choosing the **Process:Close Door** command in the Ettan MALDI Control software.

On the connector panel

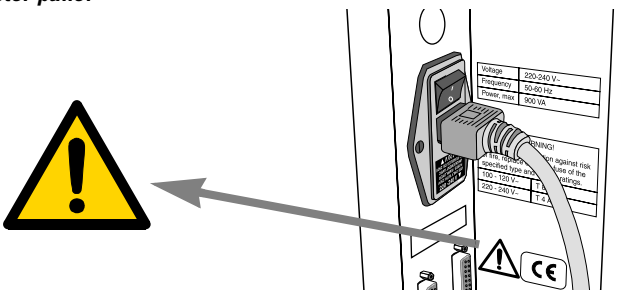


Fig 2-2. Safety label on the connector panel.

CAUTION! Read this manual before connecting or disconnecting any cables, or opening the covers of the Ettan MALDI-ToF instrument.

WARNING! NO SERVICEABLE PARTS INSIDE. Do not open covers. Service and maintenance should be performed by qualified personnel.

2.3 Warnings

WARNING! HIGH VOLTAGE. Always exercise extreme caution when handling this instrument. The Ettan MALDI-ToF contains a power supply producing lethal high voltage of more than 20 000 V DC inside the instrument. Always shut down the instrument (see Section 3.3, page 26) and disconnect the mains power cord before performing any maintenance operations.

WARNING! Do not use the Ettan MALDI-ToF in any other way than described in this Instrument Handbook. The protection provided by the instrument may be weakened if other operations are performed.

WARNING! INVISIBLE LASER RADIATION. AVOID DIRECT EXPOSURE TO BEAM. CLASS 3B LASER PRODUCT. Do not open instrument covers. Refer servicing to qualified service personnel.

WARNING! HEAVY OBJECT. The Ettan MALDI-ToF weighs 152 kg (335 lbs). Contact Amersham Biosciences before moving the instrument.

2.4 Built-in safety functions

Ettan MALDI-ToF is certified in accordance with the EN 61 010-1 safety standard. To protect the user, the instrument also has built-in interlocks.

2.4.1 Interlocks

WARNING! Never operate the Ettan MALDI-ToF with any interlock disabled.

High voltage and laser interlocks

The high voltage power supplies and the laser are powered by a 24 V DC supply. The mains input to this supply is switched on by a relay when a number of conditions are met.

Table 2-1 lists the interlock conditions that must be fulfilled before the high voltage power supplies and the laser are enabled.

Interlock condition	*Type of interlock*
Magnetron gauge above trip level (main chamber pressure)	Hardware (magnetron gauge, comparator, relay)
Magnetron gauge fault	Hardware (magnetron gauge, comparator, relay)
Pirani gauge above trip level (backing line pressure)	Hardware (pirani gauge, comparator, relay)
Reflectron turbo vacuum pump at speed	Hardware (turbo pump controller, relay)
Sample inlet door closed	Hardware (microswitch)
Cables to door, sample motor, vacuum pumps connected	Hardware (loop in connector)
Software high voltage enable request	Software/hardware logic
Source turbo vacuum pump at speed	Hardware (turbo pump controller, relay)
Tube cover in position (rear panel)	Hardware (microswitch)

Table 2-1. Interlock conditions for high voltage supplies and laser.

2.5 Emergency procedures

2.5.1 Emergency shutdown

CAUTION! Do not switch off mains power unless there is an immediate risk of physical injury. The Ettan MALDI-ToF may be damaged by a sudden loss of power.

In a situation where there is a risk of physical injury, turn off the mains switch at the connector panel of the Ettan MALDI-ToF. If the sample inlet door is moving, it will immediately stop when power is interrupted.

2.5.2 Power failure routine

In the event of a power failure, the experiment is interrupted in an undefined state. However, the data collected up to the power interruption is saved. To protect the flight tube from diffusing of vacuum pump oil, a valve is opened and the flight tube is vented to atmosphere, or if connected, to a nitrogen supply.

When power returns:

1 The **Power** indicator on the instrument lights.

2 The fans and the vacuum pumps start.

3 The PC starts and displays the log in dialogue.

2.5.3 Restart Procedure

In the event of system shutdown due to power failure, emergency stop or process interruption, any malfunctions must be rectified before the Ettan MALDI-ToF is restarted.

To restart the Ettan MALDI-ToF, follow the Start-up instructions provided in *Chapter 3*.

2.6 Disposal procedures

The sample slides should be disposed of as metal waste. To ensure that your samples are not contaminated, always use new sample slides.

2.7 Recycling

The Ettan MALDI-ToF contains the following materials:

- Stainless steel
- Aluminium
- Ceramic
- Plastic: Acetal (POM), Polyetheretherketone (PEEK)
- Fluoric rubber (Viton)
- Electronics components
- Vacuum oil

When taking the Ettan MALDI-ToF out of service, the different materials must be separated and recycled according to local regulations.

3 Basic operation

This chapter provides basic operating instructions for the Ettan MALDI-ToF instrument, including start-up, acquisition, introducing samples, and shutdown. Refer to the *Ettan MALDI-ToF User Manual* for instructions on performing analyses.

The start-up instructions provided in this chapter assume that the instrument and the PC have been correctly installed by Amersham Biosciences personnel.

When planning analyses, bear in mind that the instrument needs at least 24 hours from power-on to establish the required vacuum. If the flight tube is filled with nitrogen, the down-pumping time may be somewhat reduced, due to the low water content of the nitrogen.

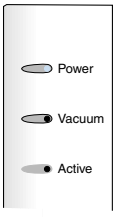
3.1 Starting the instrument

WARNING! High voltage is present within the Ettan MALDI-ToF instrument during an experiment.

CAUTION! Do not move the Ettan MALDI-ToF with power on as this may damage the vacuum pumps.

To start the Ettan MALDI-ToF system:

1 Turn on mains power to the Ettan MALDI-ToF with the **Mains** power switch at the connector panel.



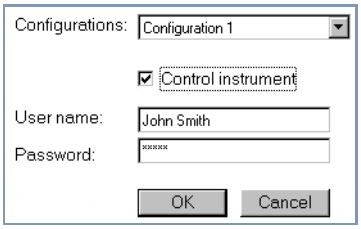
2 Check that the Ettan MALDI-ToF is operating:

- the **Power** lamp should light.
- the fans should operate, producing a cooling air flow that exits at the rear and top of the instrument.

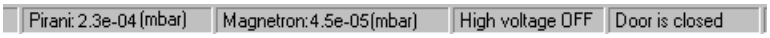
3 Switch on mains power to the PC.

4 Start Ettan MALDI Control software by selecting **Programs:MALDI Software:Ettan MALDI Control** from the Windows **Start** menu.

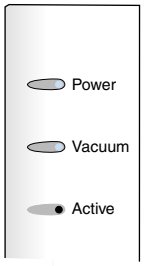
5 Log in to the Ettan MALDI Control software. The **Control instrument** option must be checked.



6 When the Ettan MALDI Control software is running, check the vacuum levels displayed in the status bar at the lower end of the screen:



7 The **Pirani (mbar)** and **Magnetron (mbar)** pressure indications should be decreasing. Operational vacuum below 1×10^{-6} mbar should be reached within 24 hours.



8 Before starting an experiment, check that the **Vacuum** indicator lights, indicating stable vacuum within specifications.

9 During an experiment, the **Active** indicator lights to indicate that data is being transferred between the Ettan MALDI-ToF and the controlling computer.

3.2 Introducing samples

Before introducing samples into the Ettan MALDI-ToF instrument, they must be purified and incorporated into a suitable matrix that allows the laser beam to desorb and ionize molecules. The samples must be dry before introducing into the instrument, otherwise the pump-down time will be extended. General guidelines for sample preparation and examples of specific applications are provided in the*Ettan MALDI-ToF Method Handbook*.

Note: *Use lint-free gloves or a pair of tweezers when handling sample slides.*

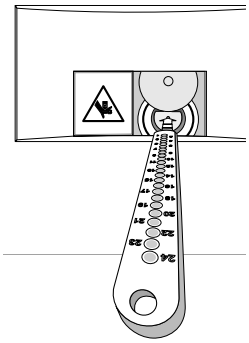
The prepared samples should be dispensed into the numbered wells on a sample slide for use with the Ettan MALDI-ToF.

CAUTION! Always use sample slides from Amersham Biosciences. Avoid depositing materials on the sample slide, that may stick to surfaces of the flight tube, e.g. syrup.

To introduce samples into the instrument:

1 Choose **Process:Open door** to open the sample door.

2 The slide tray is moved from the target position to the sample entry lock, which is then vented to atmosphere. This takes about 30 seconds. The **Vacuum** indicator will go off as the vacuum in the sample entry lock is lost. In the **Instrument Status** window, **Sample is Exchanging** will be checked.



3 When the door to the sample entry lock has opened, remove any sample slide already in the instrument.

4 Insert the new sample slide into the sample entry lock. Press the slide firmly but gently until it stops.

Note: *To minimize moisture in the flight tube, do not leave the door open for long periods of time. Close the door even if a sample slide is not inserted.*

WARNING! Make sure that nobody is working at the sample entry lock when closing the sample inlet door.

5 Choose **Process:Close door** to close the sample door. The following will happen (the closing procedure takes 1-2 minutes):

- The door closes.
- The sample entry lock is evacuated by the vacuum system. If operational vacuum is not reached within 2 minutes, an error message is displayed and the door opens, see Chapter 5 for details.
- The **Vacuum** indicator is lit.
- The sample slide is moved into the target position. A sound from the sample exchange motor is heard.

Further operating instructions are provided in the *Ettan MALDI-ToF User Manual*.

3.3 Closing down the system

The instrument should normally be connected to mains power continuously to preserve operating vacuum. When finishing the day's work, you only need to close down the Ettan MALDI Control software.

3.3.1 Closing down the Ettan MALDI Control software

1 Make sure that your analysis data have been saved.

2 Check that no experiment is running, the following conditions should be indicated in the status bar:

- **Instrument status:Idle**
- **High voltage OFF**
- **Door is closed**

3 Check that the **Active** lamp is off.

4 Close down Ettan MALDI Control software by selecting **Exit** from the **File** menu.

3.3.2 Long-term shutdown

If the Ettan MALDI-ToF will not be used for a longer period of time:

- Close down the instrument
- Cover the instrument to protect it from dust
- Keep a steady temperature in the room
- Do not move the instrument unnecessarily

If nitrogen is used, check that the nitrogen pressure is within specifications (see Section 6.3) before turning off instrument power. The nitrogen gas is used to fill the flight tube instead of venting it to air, as this may cause adsorption of water to surfaces within the instrument.

To close down the Ettan MALDI-ToF instrument:

1 Close down the Ettan MALDI Control software as described in Section 3.3.1.

2 Switch off mains power to the PC.

3 Switch off mains power at the connector panel on the Ettan MALDI-ToF instrument.

4 Maintenance

4.1 Introduction

Regular maintenance is important for safe and trouble-free operation of the Ettan MALDI-ToF. The user should perform daily and monthly maintenance. Preventive maintenance should be performed on a yearly basis by qualified service personnel.

This chapter provides instructions for user maintenance, a schedule for preventive maintenance, and instructions for replacement of the mains fuse.

Contact your Amersham Biosciences representative for more service information.

WARNING! HIGH VOLTAGE. Always exercise extreme caution when handling this instrument. The Ettan MALDI-ToF contains a power supply producing lethal high voltage of more than 20 000 V DC inside the instrument. Always shut down the instrument and disconnect the mains power cord before performing any maintenance operations.

WARNING! NO SERVICEABLE PARTS INSIDE. Do not open covers. Service and maintenance should be performed by qualified personnel.

WARNING! Do not touch or inhale the thermal breakdown products of fluorinated materials, which may be present if the rotary pump has been heated to 260 °C or more. The pump may be overheated in cases of misuse or malfunction. Fluorinated materials can be present in oils, greases and seals.

4.2 User maintenance schedule

Table 4-1 lists the maintenance operations that should be performed by the user at regular intervals.

Interval	*Action*	*Instructions/reference*
Daily or when required	Clean the instrument	Wipe the instrument cover with a soft damp tissue. Let the instrument dry completely before use. See Section 4.5.1.
Daily	Check fan operation	Check that cooling air flows through the instrument, exiting at the rear and top of the instrument. See Section 4.6
Monthly	Clean the O-ring at the sample inlet door and check for cracks or other damage	See Section 4.8
Every 6th month	Replace the O-ring at the sample inlet door	See Section 4.8

Table 4-1. User maintenance.

4.3 Spare parts

CAUTION! Only use spare parts supplied or specified by Amersham Biosciences.

Table 4-2 lists the spare parts that are needed for user maintenance operations.

Item	*Code no.*
O-ring 42,3 × 3 (Viton)	18-1150-37

Table 4-2. Spare parts required for user maintenance.

4.4 Tools and accessories

In addition to the spare parts, the following tools and accessories are needed for maintenance operations:

- Gloves, powder and lint-free
- Ethanol, 99.5%
- Mild detergent
- Aluminium foil for covering the sample door opening
- A pair of plastic tweezers
- Lint-free tissues

4.5 Cleaning procedures

4.5.1 Cleaning the instrument

For proper function, the instrument should be kept clean and dry. Chemical stains and dust should be removed.

WARNING! Do not drop any liquid into the air vents at the back and top of the instrument. If this has happened, shut down the Ettan MALDI-ToF (Section 3.3) and disconnect the mains power cable. Let the instrument dry completely before reconnecting to mains power.

1 Wipe the instrument cover with a soft damp tissue.

2 If needed, use a mild detergent to remove stains.

4.6 Fan maintenance

Check that cooling air flows through the instrument, exiting through the vents at the rear and top of the instrument.

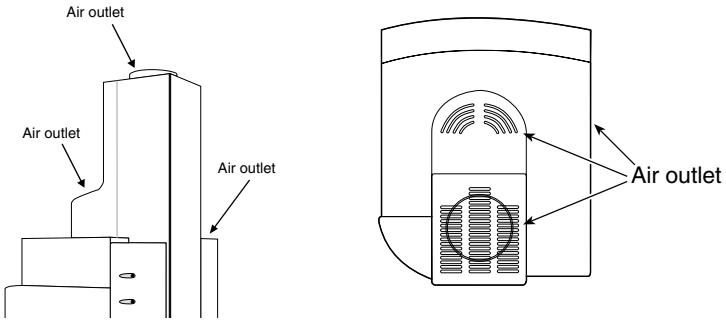
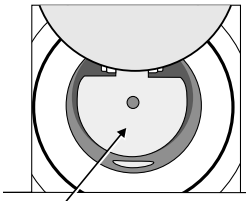


Fig 4-1. Checking the air flow.

If the fans are noisy, service may be required. Call your Amersham Biosciences service representative.

4.7 Working with the vacuum system

CAUTION! Cleanliness is important when working with the vacuum components. Any contaminants or moisture inside the flight tube may increase down-pumping time or reduce the end vacuum.
Always wear powder-free gloves when working with the vacuum system.



The sample entry lock

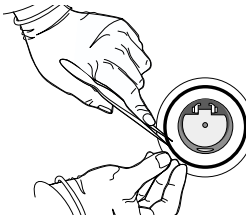
4.7.1 Sample entry lock

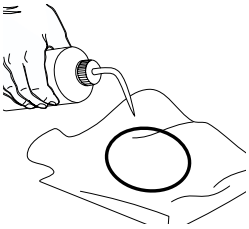
The sample entry lock enables samples to be exchanged without venting the flight tube. However, the sample entry lock must be evacuated when the sample door is closed, before the sample can be moved to the target position.

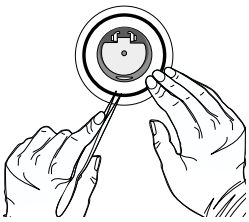
Avoid leaving the sample inlet door open for long periods of time (not more than 2 minutes), since water molecules from the surrounding air may enter the sample entry lock. The desiccant used to dry the vent gas will also be unnecessarily saturated with water. Cover the sample inlet door opening with aluminium foil if the door must be open for more than five minutes.

4.8 O-ring maintenance

CAUTION! Damaging the O-ring groove may cause vacuum leakage. Use plastic tweezers when removing the O-ring. Take extreme care not to touch the bottom of the groove.







1 Place a sheet of aluminium foil on the bench in front of the sample entry door.

2 Open the sample inlet door.

3 Remove the O-ring carefully using a pair of plastic tweezers.

4 Check for cracks or other damage to the O-ring. Replace the O-ring if necessary and every 6th month.

5 Wipe the O-ring with a tissue to remove dust.

6 If needed, clean the O-ring with ethanol.

7 Clean the O-ring seat and the surrounding area at the sample inlet opening with ethanol.

8 Re-insert the O-ring, pressing it into the groove around the sample inlet opening. Make sure that it is properly seated in the groove.

9 Close the sample inlet door.

4.9 Preventive maintenance

Table 4-3 lists the preventive maintenance (PM) operations that should be performed by qualified service personnel during the yearly service visit.

Component	*Maintenance action*
Ettan MALDI-ToF system	Perform entry test
Connections	Check all internal and external cable connections
Mechanics	Inspect and lubricate door mechanism
	Replace the Viton O-ring
	Check function of the sample mechanism
	Check the laser focus mechanism
Optics	Check the mirror and focal lenses
Laser	Measure laser power and detect signal
	Adjust laser
Electronics and power supplies	Measure pulser signals and high voltages
Turbo molecular pumps	Check bearings
Rotary vacuum pump	Clean the pump motor cover
	Replace the pump oil
	Inspect and clean the inlet filter
	Clean or replace the gas ballast O-ring
	Clean the pump motor cover
	Test the motor condition
	Fit new blades
Vacuum system	Measure down-pumping time and final vacuum levels

Component	*Maintenance action*
Vacuum system	Measure time to open and close the sample inlet door
Fans	Clean cover and check operation of fans
Air filter	Replace filter
Ettan MALDI-ToF system	Perform final test

Table 4-3. Preventive maintenance schedule.

4.10 Replacing spare parts

CAUTION! Only use spare parts supplied or specified by Amersham Biosciences.

In addition to the spare parts for user maintenance, the parts listed in Table 4-4 can be replaced by the user.

Item	*Specification*
Mains fuse, 230 V	T 4 A/250 V
Mains fuse, 115 V	T 8 A/125 V

Table 4-4. Spare parts replaceable by the user.

4.10.1 Replacing the mains fuses

WARNING! Always disconnect the mains supply before removing the mains fuses. For continued protection against risk of fire, replace only with fuses of the same type and rating.

Replace the mains fuse as follows:

1 Disconnect the mains power cable from the Ettan MALDI-ToF.

2 Insert a small screwdriver in the slot at the fuse cover.

3 Pull out the fuse holder.

4 Replace the fuse.

5 Insert the fuse holder. Note that the arrow beside the selected voltage range must point at the white line on the mains connector.

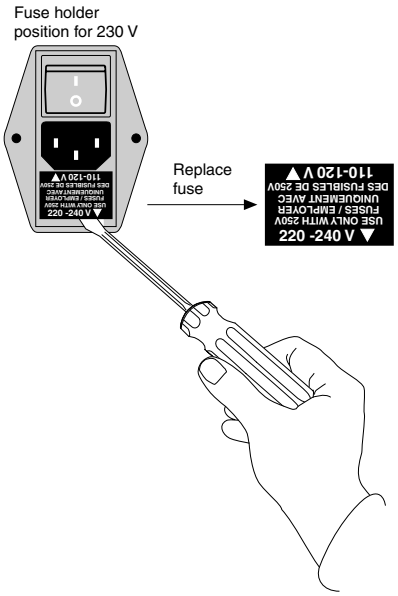


Fig 4-2. Replacing the mains fuse.

5 Troubleshooting

This chapter provides basic diagnostic and troubleshooting guides. The diagnostic guide is based on the error messages that are displayed by the Ettan MALDI Control software. The basic troubleshooting guide focuses on error symptoms related to instrument operation.

For errors related to the appearance of mass spectra:

- If you have a problem with sample preparation, refer to the *Ettan MALDI-ToF Method Handbook*.
- The instrument may need calibrating, see the *Ettan MALDI-ToF User Manual* for instructions.

5.1 Diagnostics

Error message	***Possible cause***	***Corrective action***
Moist in sample	Incorrect sample preparation	Refer to *Ettan MALDI-ToF Method Handbook*.
Leakage	Dust or other object obstructing the movement of the sample inlet door	Check for foreign objects at the sample inlet door. Clean the O-ring if necessary. See Section 4.8.

Table 5-1. Diagnostic guide.

5.2 Troubleshooting

Error symptom	*Possible cause*	*Corrective action*
Insufficient vacuum, or long down-pumping time after sample exchange	Leakage at sample inlet door	Open sample inlet door. If Pirani and magnetron pressures stay within specifications, check for foreign objects at the sample inlet door. Clean the O-ring if necessary. See Section 4.8.
	Leakage at vacuum connections	Call APBiotech service
Oil on the floor below the instrument	Leakage at the rotary pump	Call APBiotech service
Can not start a run	Operational vacuum not yet established	Wait until Vacuum indicator lights before starting the run
Apparent low sensitivity	Worn-out detector or laser	Run standard sample. Call APBiotech service if problem persists.

Table 5-2. Troubleshooting guide.

6 Reference information

6.1 Description

6.1.1 The Ettan MALDI-ToF instrument

The Ettan MALDI-ToF is a mass spectrometer that can operate in linear mode or in reflectron mode, using a harmonic reflectron that increases resolution and sharpens time focusing.

Samples are placed on slides and introduced via the sample entry door. A sample entry lock ensures that the flight tube vacuum is preserved during sample exchange.

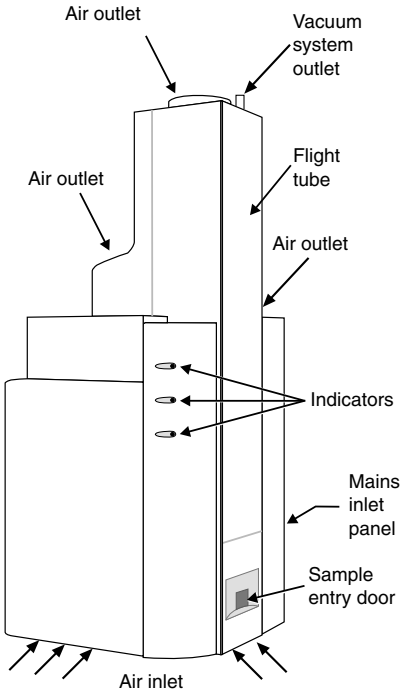


Fig 6-1. The Ettan MALDI-ToF instrument.

6.1.2 Cooling

A number of fans mounted in the instrument provide a flow of cooling air that exits through the vents at the rear and top of the instrument. These air inlets or vents must not be blocked—do not place papers underneath the instrument.

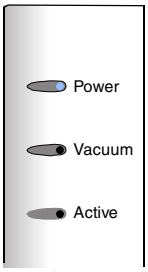
6.1.3 Vacuum system outlet

To protect users and the electronics from any oil mist, a vacuum system outlet is situated at the top of the instrument.

CAUTION! The vacuum system outlet should be vented into a fume hood, evacuating ambient air.

6.1.4 Indicators on the front panel

The front panel of the Ettan MALDI-ToF contains the following indicators:



Indicator	*Colour*	*Lights when*
Power	Blue	mains power is connected to the instrument and the mains switch is on
Vacuum	Blue	the vacuum has reached the operational level below 1.4×10^{-6} mbar
Active	Blue	the high voltage supply is on, an experiment may be running

Table 6-1. Indicators on the Ettan MALDI-ToF front panel.

6.1.5 Connectors

The connector panel is located at the rear of the instrument. The mains power inlet, with the mains switch and mains fuse, is located on the connector panel (see Fig. 6-2).

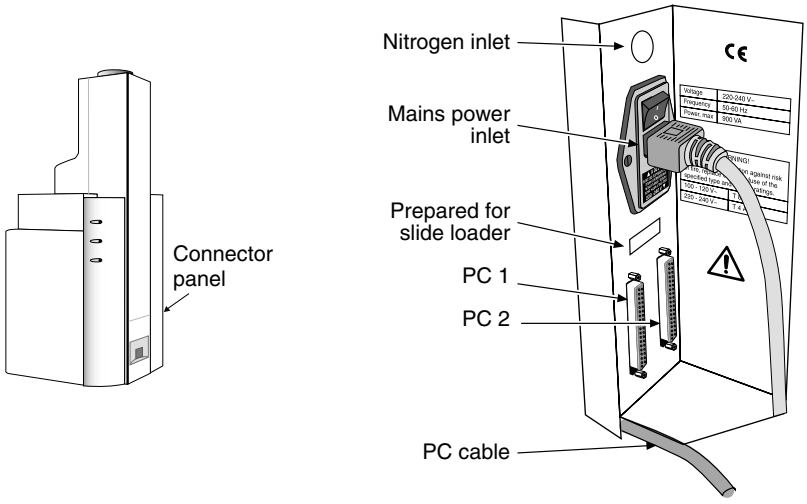


Fig 6-2. Connector panel with mains switch.

The communication ports **PC 1** and **PC 2** are intended for service purposes only. The PC used for acquisition of mass data is connected via a specially matched cable, which is permanently installed in the Ettan MALDI-ToF instrument.

CAUTION! Do not, under any circumstances, connect any equipment to the **PC 1** or **PC 2** port.

6.1.6 Sample entry lock

The sample entry lock enables the vacuum in the flight tube to be maintained during sample exchange. When **Process:Open door** is selected in the Ettan MALDI Control software, a sample manipulator moves the sample holder from the target area to the sample entry lock. Solenoid valves then open and vent the sample entry lock to atmosphere. Finally, the sample door opens and the sample slide can be exchanged.

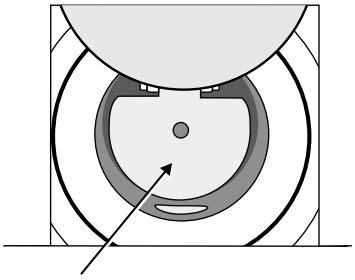


Fig 6-3. The sample entry lock.

6.2 Sample slides

Samples are spotted onto sample slides, which are inserted into the sample entry lock on the Ettan MALDI-ToF. One type is currently available, the Etched well sample slide:

Samples are applied onto smooth spots surrounded by an etched trough. Excess sample can drain into the trough. This slide is recommended for proteins and peptides.

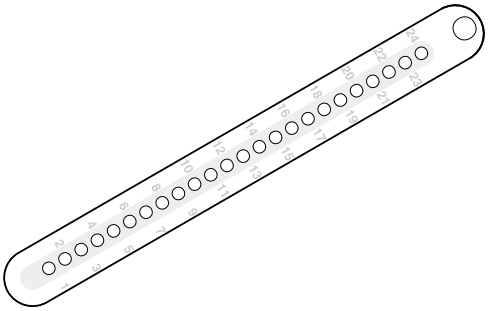


Fig 6-4. Etched well sample slide.

6.3 Specifications

6.3.1 Technical specifications

Mains voltage	Preset from factory: 100-120 V~ ±10%, 50/60 Hz, or 220-240 V~ ±10%, 50 Hz, single phase (Installation category II)
Power consumption	Max. 900 VA
Operating temperature	15-27 °C
Dimensions (including stand)	153 × 53 × 66 cm (H × W × D)
Length of flight path	1.2 m (linear), 2 m (reflectron)
Weight	152 kg
Laser	Pulsed UV 337 nm (nitrogen), Class 3A
Laser frequency	1 or 8 Hz
Nitrogen supply	Max. 0.1 bar (10 kPa)

Table 6-2. Ettan MALDI-ToF technical specifications.

6.3.2 Regulatory requirements

Ettan MALDI-ToF meets the standards listed in Table 6-3 .

Safety	IEC 61 010-1:1990, IEC 61 010-1/A1:1992, and IEC 61 010-1/A2:1995
Laser	IEC 60 825-1:1993, and IEC 60 825-1/A1:1997
EMC	IEC 61 326-1:1997, and IEC 61 326-1/A1:1998

Table 6-3. Regulatory requirements.

6.4 Ordering information

To order accessories for Ettan MALDI-ToF, quote the designation and Code no. when contacting your Amersham Biosciences representative.

Product	*Code no.*
Sample Slide Kit	18-1147-18
Matrix chemicals: α-cyano-4-hydroxycinnaminic acid (4-HCCA) (2.5 g)	17-6002-80
2,5-dihydroxybenzoic acid (gentisic acid or DHB) (2.5 g)	17-6002-81
Sinapinic acid (sinapic acid: *trans*-3,5-dimethoxy-4-hydroxycinnamic acid) (2.5 g)	17-6002-82
Calibration peptides: (ile^7)-Angiotensin III acetate salt (1mg)	17-6002-83
hACTH, fragment 18-39 (adrenocorticotropic hormone) (0.5 mg)	17-6002-84
hACTH, fragment 7-38 (adrenocorticotropic hormone) (0.5 mg)	17-6002-85
Calibration protein: Albumin, bovine (10 g)	17-6002-86
O-ring 42,3 × 3 (Viton)	18-1150-37

Table 6-4. Ordering information.

6.5 Technical description

6.5.1 Operating principles

The basic theory behind the operation of the Ettan MALDI-ToF is briefly described in Sections 1.4.1 to 1.4.3.

A laser pulse ionizes the sample and desorbs the ions into the vacuum. The high voltage accelerates the ions, which then enter the field-free drift region (flight tube), travelling at constant velocity until they hit the detector, or, in reflectron mode, until they enter the reflectron.

In reflectron mode, the ions are retarded, finally reversed and accelerated again towards the second (reflectron) detector. See separate description in Section 6.5.2.

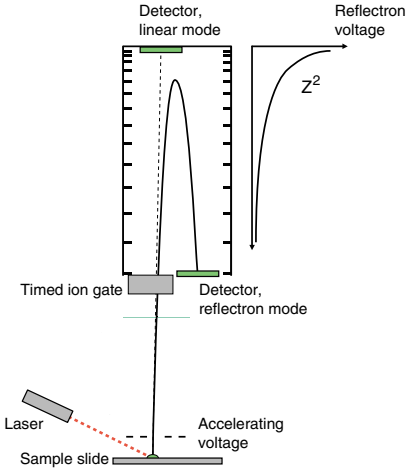


Fig 6-5. Basic operating principle of the Ettan MALDI-ToF.

6.5.2 The reflectron

Ettan MALDI-ToF contains a unique reflectron, based on patented Z^2 technology. By applying a quadratically increasing voltage, it produces a linearly increasing electrical field (see Fig. 6-6). This type of reflectron is also referred to as “harmonic”, due to the fact that the ions observe one half cycle of harmonic motion.

The reflectron has three main properties, all contributing to a high resolution:

- The ion flight path is extended, compared with a linear ToF
- Ions of the same m/z ratio, having different energies, arrive at the same time at the detector (time-focusing)
- Ions of different m/z ratios are separated in time, even if the have the same velocity when entering the reflectron

Time focusing

The ability to time-focus ions is illustrated in Fig. 6-6. Two ions of the same m/z have slightly different energy after the initial acceleration, and thus separate during the flight in the drift region. In the reflectron, the ion having the higher energy travels further before turning back and both ions arrive to the detector at the same time.

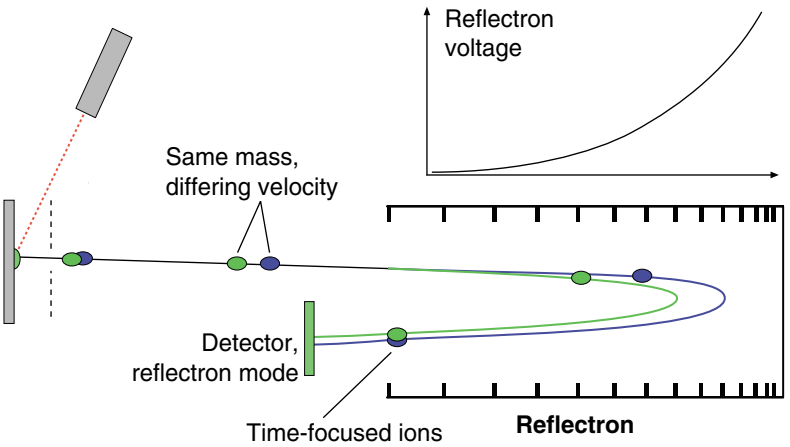


Fig 6-6. Principle of the quadratic-field reflectron.

Post Source Decay (PSD):
See *Ettan MALDI-ToF User Manual* for more information.

Separating ions having the same velocity

In a PSD experiment, the ability to separate ions having the same initial velocity is used to analyse fragments from a precursor ion. When formed, the fragment ions keep the total kinetic energy of the precursor, i.e. they continue at the same velocity towards the entrance to the

reflectron. In the reflectron, they are separated due to their mass differences and a fragment ion spectrum can be obtained. Intact precursor ions also give rise to a peak in the spectrum. Because of the Z^2 technology, the complete PSD spectrum is acquired in a single run.

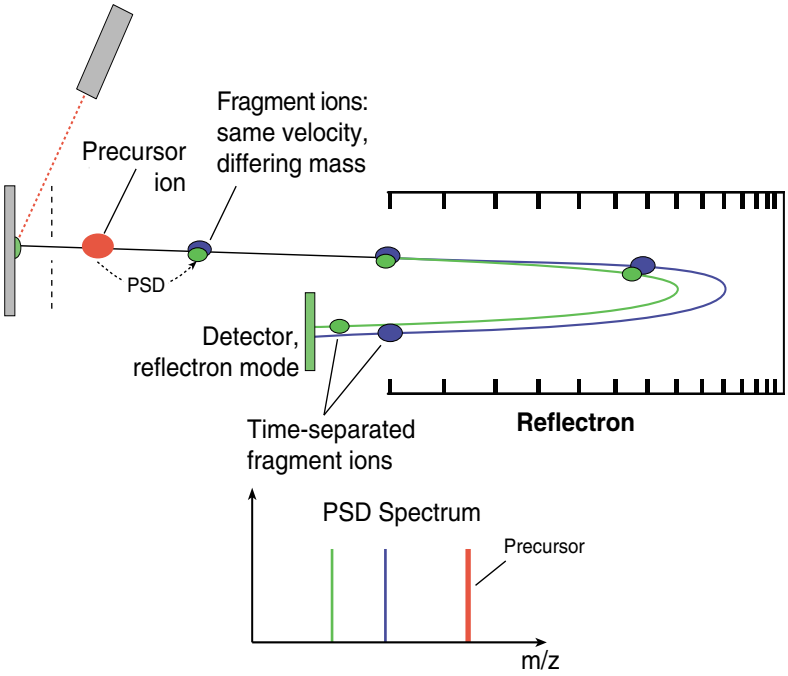


Fig 6-7. Time-separating ions created by post-source decay of a precursor ion.

Timed ion gate

The Ettan MALDI-ToF is equipped with a timed ion gate, which is used in PSD experiments. The m/z for a selected peptide ion can be defined and only this ion and its fragment ions will pass the gate. The fragment ions can then be analysed to obtain sequence information.

6.5.3 Block diagram

The block diagram in Fig. 6-8 shows the main subsystems of the Ettan MALDI-ToF instrument.

A high vacuum is required for proper function of the instrument. As indicated in the block diagram, the vacuum system evacuates the ion source and the flight tube, including the ion gate, the reflectron and the detectors.

An external computer supervises the electronics of the instrument, and controls the transfer of data from the Ettan MALDI-ToF. The instrument hardware controls the sample exchange and the acquisition of mass data.

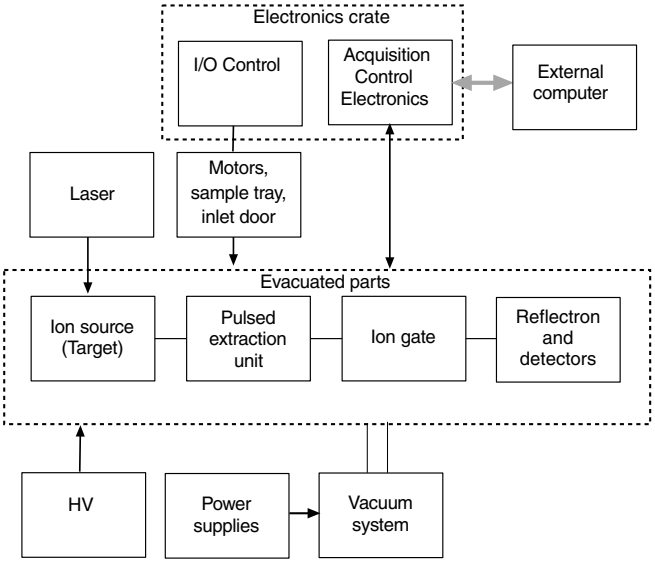


Fig 6-8. Block diagram of the Ettan MALDI-ToF instrument.

6.5.4 Vacuum system

The vacuum system operates continuously while mains power is connected to the instrument. Refer to the block diagram in Fig. 6-9 for the following description.

The rotary pump forms the first stage of the vacuum system, backing the turbo pumps and evacuating the sample entry lock (inlet chamber). Vacuum valves are used to enable the rotary pump to evacuate different parts of the system at different stages.

The turbo pumps comprise the second stage of the vacuum pumping system, providing operating vacuum in the flight tube and the reflectron.

When a sample is to be inserted, the inlet chamber is vented to atmospheric pressure. When the sample door has closed, the inlet valve opens and the backing pump reduces the pressure in the inlet chamber.

When the inlet chamber pressure has reached about 1×10^{-2} mbar, the sample slide moves into the flight tube and the inlet valve closes. When the pressure in the flight tube has reached 1.4×10^{-6} mbar, the **Vacuum** indicator lights and an analysis can be started.

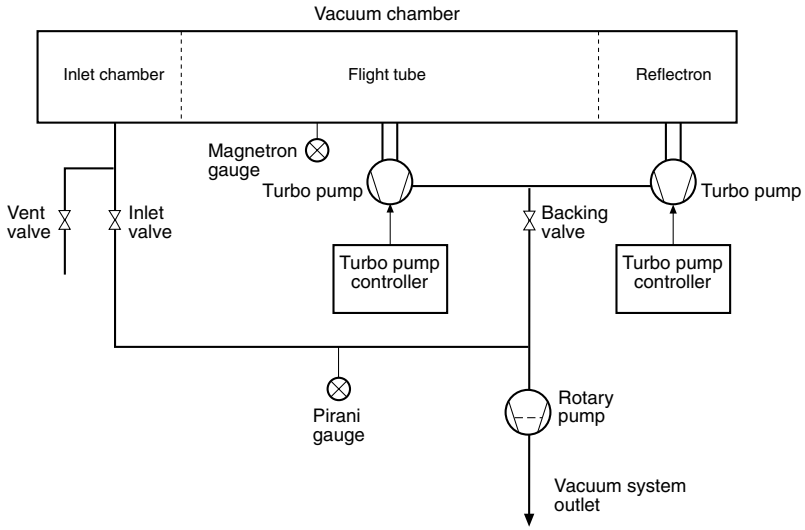


Fig 6-9. The vacuum system.

To cover the entire measuring range from atmosphere to operating vacuum, two vacuum gauges are used in the vacuum system. The Pirani gauge monitors the backing pressure of the rotary pump in the range 1 bar to 10^{-3} mbar (0.1 MPa to 0.1 Pa), and the magnetron gauge measures the high vacuum range down to 10^{-8} mbar (10^{-6} Pa).

For further details on the vacuum components, refer to the manufacturer's manuals.

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