Lmax®

Microplate Luminometer Operator's Manual

Molecular Devices Corporation
1311 Orleans Drive
Sunnyvale, California 94089

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Molecular Devices Corporation

Lmax® Operator's Manual

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(408) 747-1700

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Conventions Used in this Manual

The names of keys that appear on the Lmax control panel and/or buttons in the SOFTmax PRO for Lmax software are shown in boxed Helvetica type. Example: Setup.

Italic and boldface type are used for emphasis. Examples: "Press *carefully* to engage," "**Do not overtighten.**"

NOTE: A note provides information that will help you properly execute an action or procedure.

⚠ **CAUTION:** Indicates an action or condition that could potentially damage the instrument or one of its components or could result in loss of data.

⚠ **WARNING:** Indicates a situation that could result in potential injury to a person working with the system.

☣ **BIOHAZARD:** Indicates a condition involving potentially infectious biological agents requiring that proper handling precautions be taken.

Safety Notices

This safety notice summarizes information that is basic to the safe operation of the equipment described in this manual. All safety instructions should be read and understood before installation, operation, maintenance, or repair of this instrument. Pay special attention to safety information presented throughout this manual. Observance of safety precautions will help to avoid actions that could damage or adversely affect the performance of the instrument or cause injury to persons operating the instrument.

Maintenance or service of the instrument other than that described in this manual should be performed only by Molecular Devices service technicians or their authorized representatives.

Safety During Installation and/or Maintenance

Any servicing of this equipment that requires removal of a cover can expose parts which involve the risk of electric shock or personal injury. Make sure that the power switch is turned off and the power cord is disconnected from the main power source and the instrument, and refer such servicing to qualified personnel.

Electrical Safety

To reduce the risk of electrical shock, this equipment uses a three-wire electrical cord and plug to connect this equipment to earth-ground. Make sure that the matching wall outlet receptacle is properly wired and earth-grounded. Check that the line voltage agrees with the voltage listed on the rating label affixed to the instrument.

Any maintenance procedures requiring removal of an instrument panel expose the operator to the possibility of electrical shock and/or mechanical injury. Such service procedures should be done only by trained, qualified personnel.

During measurement, the photomultiplier tube conducts high voltage. Always unplug the power cable from the instrument and the main power source before servicing the system.

Microplate Transport

The microplate will move into the reading chamber only when the instrument cover is closed. During measurement, the cover is locked by a mechanical device. Do not try to open the cover during the measurement process.

Tubing Systems and Fluid Bottles

Make sure that liquid does not get into the sample loading compartment or measurement chamber. If this happens, clean these parts immediately to prevent corrosion, conglutination, or other damage.

Maintenance and Cleaning

To ensure operator safety as well as the correct performance of the instrument, Molecular Devices suggests that you perform the maintenance routines described in detail in Chapter 4, "Maintenance." The injection system must be cleaned after every use. The air filter should be replaced at least once a month, depending upon how much dirt or dust it collects. The injector tips or the main fuse need to be replaced only when necessary. All necessary replacement parts can be obtained from Molecular Devices Corporation.

All maintenance and service routines beyond those mentioned in this manual must be performed only by service technicians authorized by Molecular Devices Corporation.

Transport

Transport the Lmax only in the original packaging since this packaging protects sensitive parts such as injector connections. The Lmax is heavy (approximately 90 lb/45 kg) and it is recommended that two people carry the instrument, holding it with both hands at the bottom edge.

Chemical and Biological Safety

Molecular Devices Corporation does not accept responsibility for the use of hazardous chemicals in the Lmax. In particular, Molecular Devices does not recommend the use of volatile organic solvents in the Lmax.

If pathogenic, toxic, or radioactive samples are to be used in this instrument, it is the responsibility of the user to ensure that all necessary safety regulations, guidelines, precautions, and practices are adhered to accordingly. Ask your laboratory safety officer to advise you about the level of containment required for your application and about proper decontamination or sterilization procedures to follow if fluids escape from containers.

- Observe all cautionary information printed on the original solution containers prior to their use.
- Instrument O-rings have not been designed as bioseals for aerosol or liquid containment.
- Handle body fluids with care because they can transmit disease. No known test offers complete assurance that they are free of microorganisms. Some of the most virulent—Hepatitis (B and C) and HIV (I–V) viruses, atypical mycobacteria, and certain systemic fungi—further emphasize the need for aerosol protection. Handle other infectious samples according to good laboratory procedures and methods to prevent spread of disease. Because spills may generate aerosols, observe proper safety precautions for aerosol containment. Do not run toxic, pathogenic, or radioactive materials in this instrument without taking appropriate safety precautions. Biosafe containment should be used when Risk Group II materials (as identified in the *World Health Organization Laboratory Biosafety Manual*) are handled; materials of a higher group require more than one level of protection.
- Dispose of all waste solutions according to appropriate environmental health and safety guidelines.

If pathogenic, toxic, or radioactive samples have been used in this instrument, is your responsibility to decontaminate the instrument and all accessories before requesting service by a Molecular Devices service representative.

Scope of This Manual

This manual is designed to familiarize users of this equipment with the components of the Lmax® Microplate Luminometer and how to use and maintain it properly. These instructions should be used in conjunction with the *SOFTmax PRO for Lmax User's Manual* for complete system operating procedures.

Chapter 1, "Instrument Description," presents an overview of the components that make up the Lmax and their specifications. Also included is a glossary of terms that may be new to you.

Chapter 2, "Installation," discusses the procedures necessary for proper installation of the Lmax instrument and connecting it to the computer that will be used with it.

Chapter 3, "Operation," describes the steps that are required to prepare the Lmax for a run.

Chapter 4, "Maintenance," discusses the procedures that are needed to keep the Lmax in proper running condition.

Chapter 5, "Troubleshooting," describes problems that may occur while using the Lmax and presents possible solutions.

Appendix A, "Cables and Accessories," lists the specifications and part numbers for cables and other accessories used with the Lmax.

Appendix B, "Applications," describes typical applications of the Lmax and provides examples of the options that can be programmed.

Chapter 1: Instrument Description

Introduction

The Lmax™ Microplate Luminometer is designed for bio- and chemiluminescence measurements using 96-well and 384-well microplates. Combining high sensitivity with extremely low crosstalk and programmable reagent injection, the Lmax is suitable for glow luminescence measurements in both 96- and 384-well plates. By making use of up to two injectors, it is also suitable for measuring flash luminescence in 96-well plates. The Lmax uses a special version of SOFTmax PRO for Lmax software from Molecular Devices which provides complete instrument control and powerful data processing capability.

Luminescence reactions with "flash-type" kinetics (as encountered, for example, with acridinium ester or luminol labels) require automatic injection of the trigger reagent into the sample while the sample is in the reading position. With reactions of "glow-type" luminescence (*e.g.*, the continuous glowing of dioxetane compounds over several hours), adjacent sample positions may be glowing at the same time, making the extremely low crosstalk of the Lmax very important.

Multi-phase measurements can be run as required (for example, in ATP measurements for sterility tests), taking into account an extraction time after the first injection.

The Lmax is well suited for the following applications:

- Luminescence-based immunoassays, including glow luminescence with dioxetane. See also the examples in Appendix B, sections B1 and B4.
- EIAs based on alkaline phosphatase or peroxidase activity.
- DNA hybridization assays for the detection of pathogenic microorganisms and viruses. See also the examples in Appendix B, section B3.
- All applications of the (coupled) ATP measurement via the luciferin-luciferase system. See also the examples in Appendix B, sections B5 and B7 (sequential reactions—for example, measurement of an internal standard).
- All applications of the (coupled) NAD(P)H measurement via bacterial luciferases.
- Reporter gene assays via the reporter gene: firefly luciferase, æquorin, β-galactosidase (lacZ), alkaline phosphatase or glucuronidase. See also the examples in Appendix B, section B8. Dual reporter gene assays can also be measured.
- Cellular luminescence (phagocytosis assay). See also the examples in Appendix B, section B2.

Detection System

The "Ultra-Fast Photon Counting" technology utilized in the Lmax, in conjunction with a special configuration of the photomultiplier reading head, allows the Lmax to achieve a reading sensitivity that matches the sensitivity of traditional tube luminometers.

Dependent only on the background caused by the reagent, the instrument is capable of detecting ATP quantities of less than 0.01 pg. The dynamic range covers 6 powers of ten. An optical filter may be inserted between sample and

photomultiplier to filter out specific wavelength ranges (see "Filter Installation for Light Detector" on page 2-8).

Injection System

The injection system can only be used with 96-well plates. The Lmax is equipped with two injectors with variable volumes: one (labeled "M" for measurement) injects directly in the reading position; the other (labeled "P" for pre-injection) injects into the preceding position. The injector volume can be set from 25 to 300 mL (determined in the Instrument Settings in SOFTmax PRO for Lmax). The precision of the injected volume is ±3 µL.

The Lmax allows free selection of the number and position of injections into the wells in 96-well plates. You can define delay times between injections and also between the last injection and the start of the reading.

Microplates

White, opaque microplates yield optimum results for most applications. The light reflection with white microplates provides a high signal-to-noise ratio with very low crosstalk from adjacent plate positions. Clear-bottom microplates also give low crosstalk values, although the signal efficiency is reduced in comparison to white microplates. *Transparent sample plates* ***can not be used*** *with the Lmax Microplate Reader due to extremely high crosstalk.*

Black microplates are superior when used with "glow-type" luminescence measurements since extremely high dynamic measuring range and minimum crosstalk are required. With black plates, the crosstalk from adjacent plate positions is undetectable. The reading efficiency and the signal-to-noise ratio are lower in black plates because of the decreased light reflection.

Component Description

The main components of the Lmax are:

- Reading chamber
- Microplate chamber and cover
- Injector pumps
- Rear panel

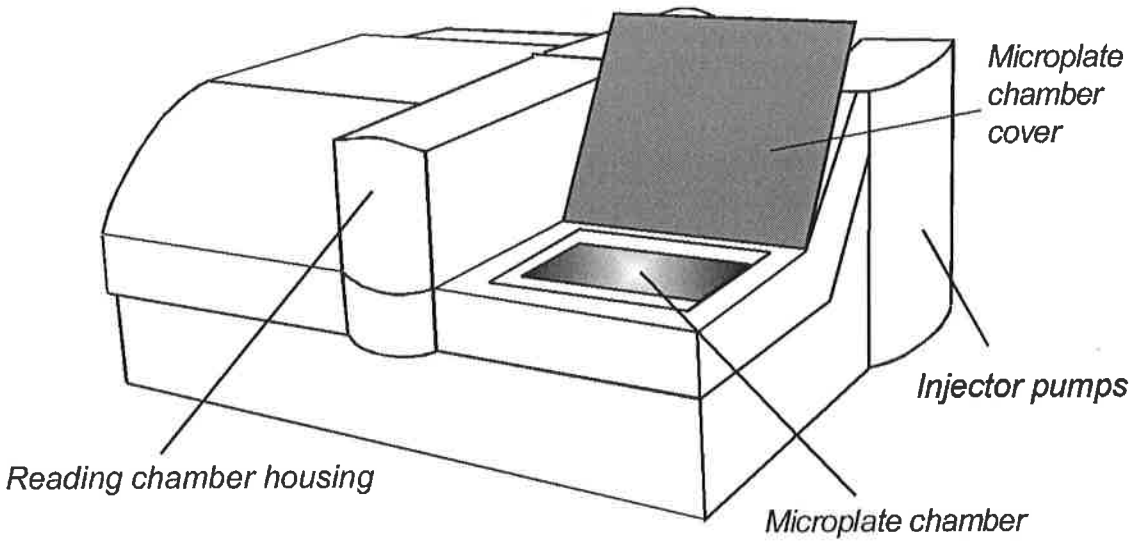

Figure 1.1: Front view of the Lmax.

Microplate Chamber

The microplate chamber is located under the cover on the right side of the instrument. A tray located inside the microplate chamber accommodates the microplate. When inserting a microplate, place it onto the tray with well A1 located at the upper left corner. You must close the cover before a reading can be initiated.

When the reading begins, the tray containing the microplate is transported into the reading chamber by its own microprocessor-controlled drive to the position defined by the Instrument Settings in SOFTmax PRO for Lmax.

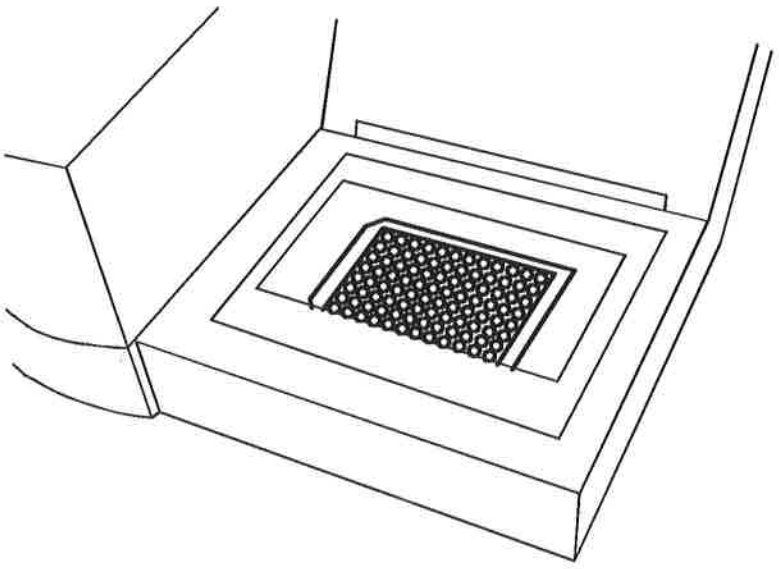

Figure 1.2: Open microplate chamber.

During measurement, the plate tray is moved from left to right and the reading head moves forward and backward (to be positioned over rows A through H). The injection and measurement take place from left to right in the topmost row first (row A, for example, assuming wells in row A are specified under Instrument Setup in SOFTmax PRO for Lmax; otherwise the top row of wells that are specified to be read). The plate then moves back to the starting position, the reading head moves over the next row, and process is repeated.

The microplate chamber is heated by two heating elements. One heater is located in the plate tray and comes into direct contact with the bottom of the microplate so that the microplate heats up quickly. The second heating element is located in the above the microplate to prevent a temperature gradient build-up and condensation in the sample chamber.

To protect the operator from injuries and to prevent instrument damage caused by incorrect operation of the Lmax, a double safety device is installed in the microplate chamber to ensure that readings cannot be made when:

- No sample plate is present.
  A light barrier in the sample loading chamber signals the Lmax if a sample plate is in the instrument at the start of a reading. If the light barrier is not interrupted (*i.e.*, no sample plate present or use of a transparent sample plate), the reading cannot be started.
- The cover is open.
  The lock at the bottom of the cover makes contact only when the cover is closed, which allows the read to proceed. During a reading, the cover is locked and cannot be opened.
- The transport safety device is not fully disengaged.
  A sensor detects whether or not the transport bar has been slid to the left and totally out of the way. If not, the instrument will not communicate with the computer.

⚠ **CAUTION:** Keep the microplate chamber clean and avoid spilling any fluid. In the event of contamination with debris or liquid, clean the microplate chamber immediately to prevent an adverse effect on the sample tray transport mechanism.

⚠ **CAUTION:** The bottom of the Lmax housing contains holes so that large amounts of liquid can exit. If the Lmax is located on a stainless steel surface, place a piece of non-reflective material between the bottom of the Lmax and the stainless steel surface to prevent reflection of light which can cause inaccurate readings.

Reading Chamber

The housing that covers the reading chamber protects the chamber from incident light that could adversely affect a reading or damage the photomultiplier tube. (Note that the Lmax has a safety feature in which the high voltage gain of the photomultiplier is automatically reduced to protect the user as well as the instrument.) The housing is secured by a Phillips screw and should be opened only when necessary:

- to engage or disengage the transport safety device prior to or after moving the instrument;
- to switch between 96-well and 384-well plate configuration;

- to verify the correct position of the reading head;
- for cleaning.

⚠ **WARNING:** Before removing the housing from the reading chamber, turn off and unplug the power cord from the instrument and the power source.

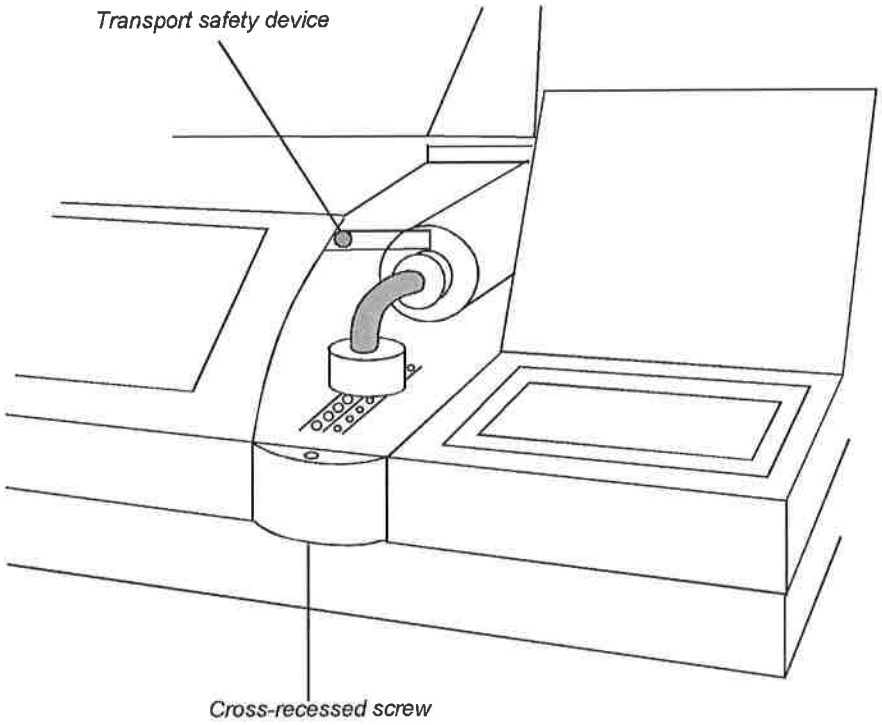

Figure 1.3: *Lmax with housing removed from the reading chamber.*

Transport Safety Device

The Lmax is shipped with a transport safety device that secures the reading head. Before operating the instrument, this device must be unlocked. If you move the instrument again later, you must lock it again to prevent damage to the reading head.

Reading Head

The reading head includes the following components:

- light guide that transmits the light pulses to the photomultiplier;
- injector tip M for injections in the reading position (*i.e.*, in a bore hole directly next to the light guide); and
- injector tip P for injections before the reading position (*i.e.*, in a bore hole one well before the light guide).

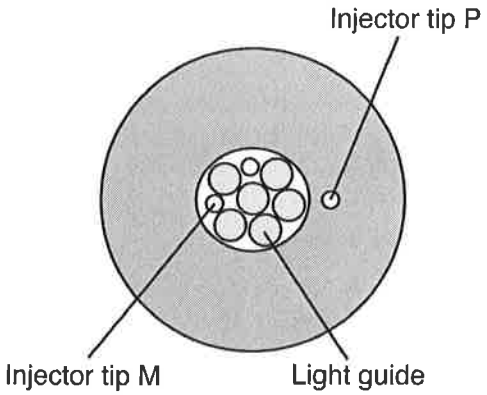

Figure 1.4: *Reading head—bottom view.*

The light guide is passed through the center of the reading head, the injector tip M sits in an oblique bore hole and comes out at the bottom of the reading head directly at the light guide. Thus, it is possible to start the measurement at the same time as the injection. The bore hole for injector tip P is located one well away from the light guide.

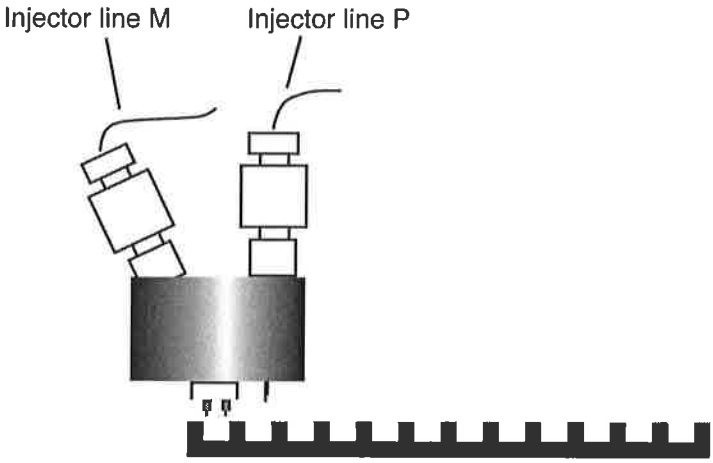

Figure 1.5: *Design of the reading head.*

The light guide extends approximately 2 mm below the reading head and fits into the small detachable guide. This guide piece moves within the guide rails and ensures that the reading head is positioned accurately relative to the microplate. In addition to guiding the reading head, the top plate and the guide piece also serve to suppress crosstalk of the light signal from adjacent well positions.

Reading Head Guide

Below the reading head, the metal plate contains guide rails between which are two rows of 8 holes and one row of 16 holes. For measurement, the microplate is transported under this hole matrix in the X-direction. The row of eight larger holes is for making measurements in a 96-well plate and for injections using the M injector. The row of eight smaller holes is for injection with the P injector before the reading position. The row of 16 holes is for taking readings in a 384-well plate. For cleaning or dismantling the reading head, it can be raised and taken out of the guide rail.

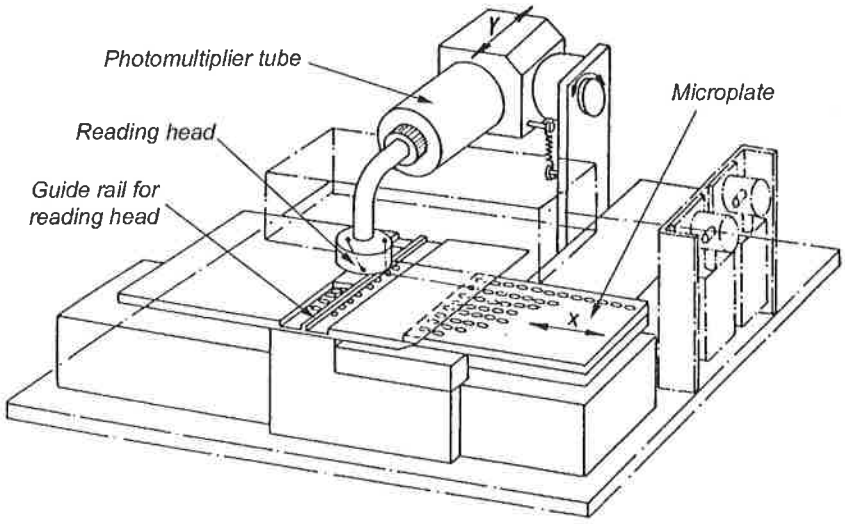

Figure 1.6: *Under the cover of the Lmax.*

Home Position of the Reading Head

When no measurements are being made, the reading head is in the home position, which is slightly behind the rows of holes. Below the home position are 2 additional holes (one each below the P and M injector) located above collection sinks. When priming or washing the injectors, the fluid is dispensed into these sinks and pumped to the outlet on the rear panel of the instrument. No fluid is dispensed into microplates during priming or washing.

The number of injections for priming or washing are specified by the user in the SOFTmax PRO for Lmax software.

Reading Head Movements

During measurement the reading head performs accurate forward and backward movements in the Y-direction; it can move to 8 different positions (16 positions for 384-well measurements). By combining the X-movement of the microplate with the Y-movement of the reading head, all positions of the microplate can be reached by the injector tips P and M and by the light detector. All required movements are carried out by step motors working under the control of the internal microprocessor. At the end of a measurement, the reading head returns to the home position.

Reagent Compartment

Two reagent supply bottles are located in a separate compartment on top of the instrument. The bottles are filled with their respective reagents and are connected to the supply tubings P and M. Inside the instrument, these tubings go to the injector and from there to the reading head, which includes an injection opening for each tubing.

The M injector ("M" stands for "measurement") injects fluid into the well in the reading position. The bottle connected here is also used for those reagents that are injected at the same time as the measurement or slightly before (often the trigger reagent).

The P injector ("P" stands for "pre-measurement") injects fluid into the position before the reading position. It is used for reagents that are injected a certain time before the measurement or the measurement injection.

Injectors

High-precision pumps are used to carry out the injections. The SOFTmax PRO for Lmax software allows selection of one or both injectors for any well of a 96-well plate (injections are not done with 384-well plates). Flexible delay times can be defined between the injections and also between the second injection and the start of the measurement.

The injectors were specifically designed for this purpose and ensure instantaneous sample mixing after injection and high precision of injection volume.

The injector pumps, located on the right-hand side of the instrument below the reagent bottles, are covered by a metal plate.

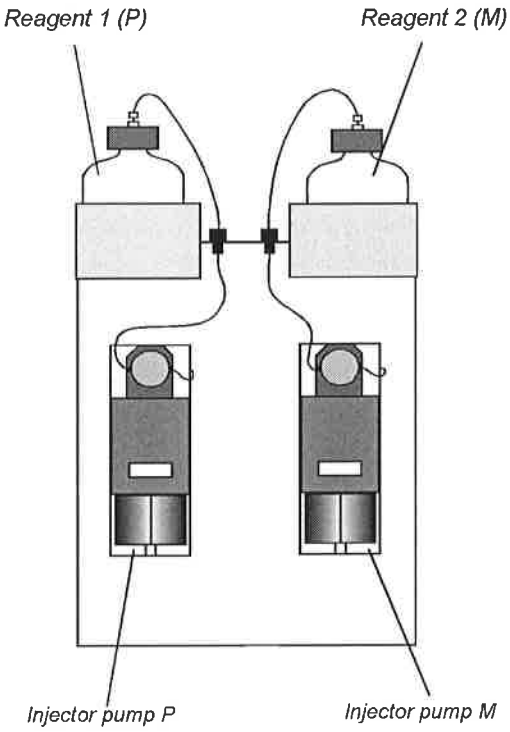

Figure 1.7: Injector pumps.

Injector Control

Prior to the Measurement

Before starting a measurement requiring injections, you must prime the injector(s) with the fluid that will be injected to ensure that the entire volume will be injected with the first injection. Before priming, launch SOFTmax PRO for Lmax and make sure that communication with the instrument is established. Prime the lines using SOFTmax PRO for Lmax (see the *SOFTmax PRO for Lmax User's Manual* for information on how to do this). Select the injector(s) to be primed, and the number of injections. The injection volume is approximately 300 µL and the default is 7 injections, which should be sufficient to ensure that the injection system is primed completely. All fluid dispensed is collected and automatically aspirated by the disposal pump. On the rear panel of the instrument you will find the outlet to connect the tubing to a waste bottle.

During Measurement

The reading head and sample tray move such that the first well to be read is positioned below injector tip P or M.

Example

You program a measurement for two injectors to start in well B4 of the microplate. Six columns each are to be measured in this and the next two rows, *i.e.*, the last reading position is D9.

First, the reading head moves forward to position B (2nd hole).

Column 4 of the sample plate is moved below injector tip P, while the light guide and injector tips M (large hole of hole matrix) are still above column 3. Following pre-injection, the plate is moved forward one column, so that injector M now injects into well position B4 and the measurement can be started. In the meantime, injection tip P is already above well B5 and performs the next pre-injection there. Depending on the selected delay between injections P and M, this P-injection can take place even during measurement of the preceding well. Such an interlaced injection procedure may reduce the total scan time of a microplate by up to 50% compared to microplate luminometers with only one common injector position.

If well B9 has been measured in the first row, the reading head moves down one row, while column 4 of the microplate is moved below injector tip P for pre-injection. In this manner, the microplate is injected and measured row by row from left to right in the defined measurement area.

The integrated software calculates and controls an interlaced measurement procedure for each reading protocol to ensure that with a given number of injections, delay times and reading times the entire measurement procedure will be completed in the shortest time possible.

After the Measurement

Upon completion of the measurement, the reading head and the microplate return to their home position. You should clean the lines and the injector system after each run (see "Cleaning" on page 4-3 for instructions on performing the cleaning procedure).

Sample Chamber Heating

The Lmax is equipped with a sample chamber heater to warm up the samples. The heater consists of three heating elements, one located below the microplate in the sample tray, the others directly to the left and right of the reading head guide rail. Heating from above and below allows quick warming up of the samples, and, at the same time, prevents the build-up of adverse temperature gradients.

Since the instrument is not equipped with a cooling system and produces a certain heat, you can only define temperatures which are about 5 degrees above room temperature. The setting accuracy is 0.1 degrees Celsius, the maximum value is 45°C.

The warm-up period for reaching the default temperature is de-pendent upon the defined value and the ambient temperature. The closer this value is to 45°C, the longer the warm-up period. The warm-up period is about 0.1°C per second.

Please note that the samples in the microplate will reach this temperature somewhat later, especially when the samples are still in the microplate chamber and are heated only from be-low.

The incubator is controlled by the SOFTmax PRO for Lmax program running on the external computer. To minimize the time needed to warm up a non pre-heated plate, it is advisable to pre-heat the read chamber before inserting the microplate.

Rear Panel

The rear panel of the Lmax contains:

- Power supply
- Main power switch
- Computer port (RS-232 interface)
- Fuses
- Outlet to waste collection bottle which will collect priming and washing solutions. You must connect tubing to the outlet and drain it into a waste bottle
- Fan (covered by a filter) for cooling the internal electronics

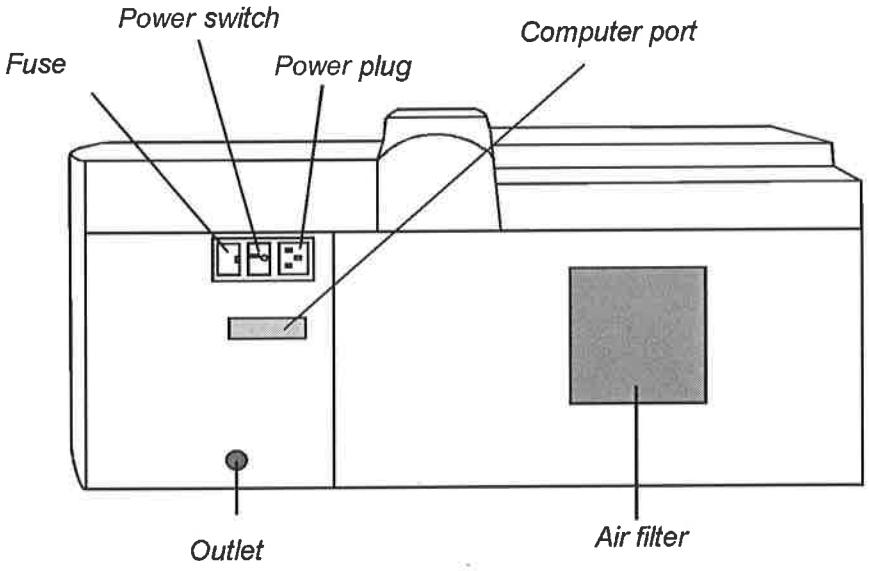

Figure 1.8: Rear panel of Lmax.

Fan

The Lmax is equipped with a fan for cooling heat generated by the electronic components (visible from back panel). Outside of it is an air filter to prevent dirt from getting inside the instrument. That filter is covered by a removable black cover which serves to minimize stray light from getting into the instrument. Depending on how dirty the filter is, you should blow it out, wash it, or replace it. To do this, take off the protective cover from the fan.

Functional Description

The Lmax is designed to be operated using a special version of SOFTmax PRO for Lmax software running on a computer connected to the instrument. The information contained in this section provides an overview of the instrument capabilities. For a complete description of the modes of operation, how to choose instrument settings, etc., refer to the *SOFTmax PRO for Lmax User's Manual*.

Modes of Operation

The Lmax can perform four modes of operation: endpoint, dual read endpoint, fast kinetics, and long kinetics. Instrument setup parameters for each read mode are discussed in the *SOFTmax PRO for Lmax User's Manual* that accompanies the Lmax.

- **Endpoint:** Allows a integration read of samples in 96- or 384-well plates with two injections possible, if desired, in any or all wells of a 96-well plate (no injection possible in 384-well plates). The minimum integration time is 0.1 second. A pre-read of the microplate may be made before injection of buffer or reagent and/or reading of the samples. The signal is integrated for each well over a user-selectable reading time (maximum 1000 seconds). You may program a delay after each injection, if desired (from 1.6 to 1000 seconds for the M injection and from 0 to 1000 seconds for the P injection) and you can read all or only some of the wells in the plate.
- **Dual Read:** Also an endpoint reading, this mode is designed to perform two separate integrations, one after each of two possible injections in any or all wells of a 96-well microplate (no injection possible in a 384-well plate). Integration times for both readings must be the same (minimum integration time is 0.1 second; maximum is 1000 seconds). Pre-reading of the microplate is not possible with this mode. You may program a delay after each injection, if desired (from 1.6 to 1000 seconds for the M injection and from 0 to 1000 seconds for the P injection), and you can read all or only some of the wells in the plate. Pre-reading of the microplate is not possible with this mode. Default values reported are relative luminescence units (RLU).
- **Long Kinetics:** Allows repeated readings of one or more wells of a 96- or 384-well microplate. The minimum integration interval is 2:00. Readings are made for all wells in the microplate and are repeated for as many readings as determined by the software settings. One or two injections can be made in any or all wells of a 96-well plate at the beginning of the reading (no injection possible in 384-well plates). Pre-reading of the microplate is not possible with this mode. Default data reductions for long kinetics data are Vmax per Sec, Vmax, Time to Vmax, or Onset Time. An important application for this mode is cellular luminescence (see page B-5).
- **Fast Kinetics:** Allows repeated readings of one or more wells of a 96- or 384-well microplate up to a 100-point maximum integration. All readings of a single well are made, after which the next well is read, and so on. One or two injections can be made in each well of a 96-well plate at the start of the reading (no injection possible in 384-well plates). Pre-reading of the microplate is not possible with this mode. Integration time can be set from 1 to 100 seconds. Default data reductions for kinetic data are Vmax per Sec, Vmax, Time to Vmax, or Onset Time.

Temperature Regulation

Upon power up, when the incubator is off, the temperature in the Lmax microplate chamber is ambient and isothermal. Turning on the incubator (via SOFTmax PRO for Lmax software) will cause the Lmax to begin warming the microplate chamber. The temperature set point defaults to 37.0°C at start-up. With the incubator on, the temperature of the microplate chamber can be set and regulated from 5°C above ambient to 45°C.

NOTE: If the temperature set point is lower than the ambient temperature, the temperature in the microplate chamber will remain at ambient. Temperature regulation is controlled by heaters only and, therefore, cannot cool the temperature to a setting lower than ambient.

The temperature is maintained at the set point until you turn temperature regulation off.

Computer Control

The Lmax is equipped with an RS-232 serial port through which the computer communicates with and controls the instrument (for specific information about the cable connection, see Appendix A, "Cables and Accessories"). SOFTmax PRO for Lmax software is required to control the Lmax.

Specifications

NOTE: Technical specifications are subject to change without notice.

General

Microplate formats	96- and 384-well standard microplate (not transparent) in compact or strip-rack format
Detector	Ultra-fast photon counter with overload detection; spectral range 380–630 nm with a dark count rate of less than 250 cps at 20°C ambient temperature
Sensitivity	Higher than 20 attomole ATP
Crosstalk	Less than 0.01%
Read time	0.1 up to 1000 sec in steps of 0.1 sec
Dynamic range	More than 6 decades
Temperature control (chamber)	Ambient +5°C to 45°C in steps of 0.1°C; optional, three heating zones, microprocessor controlled
Injectors	Two: one in the reading head and the other directed to the preceding well
Injection volumes	Freely selectable between 25 and 300 µL for injectors P ("pre-injection") and M (reading or "measurement" position); precision ±3 µL
Delay	Delay between injection in reading position and measurement: adjustable from 0 to 1000 sec; between injection before the reading position and further steps: 1.6 to 1000 sec for P-injector; 0 to 1000 sec for M-injector; each adjustable in steps of 0.1 sec
Injection/Read wells	Rectangular from 1 to 96 or 1 to 384 wells. For 96-well plates, any well or combination of wells may be designated for one, two, or no injections (injection cannot be used with 384-well plates).
Ports	Serial RS232 port for computer connection
Waste disposal	Automatic to waste bottle

Software	
SOFTmax PRO for Lmax	Windows 95/98/2000/NT compliant Macintosh OS compliant (fat binary)

Environmental	
Operating conditions	15 to 30°C ambient temperature
Operating humidity	10 to 90% non-condensing
Storage temperature	0 to 40°C

Physical	
Size (h × w × d)	11.4" (290 mm) × 23.6" (600 mm) × 19.8" (502 mm)
Weight	90 lb (45 kg)
Power consumption	181 VA maximum
Line voltage and frequency	115 V, 60 Hz or 230 VAC, 50 Hz

Glossary of Terms

Incubator

Choosing **Incubator** from the **Control** menu or clicking the incubator button opens a dialog box allowing you to start or stop temperature regulation and to select an elevated temperature for the microplate chamber.

Instrument Setup

(In SOFTmax PRO for Lmax software) Defines the parameters (mode, run time, read interval, etc.) used to read the microplate.

Luminescence

The emission of light by processes that derive energy from essentially non-thermal changes, the motion of subatomic particles, or the excitation of an atomic system by radiation.

Read Mode

The method used to read the microplate: endpoint, dual read, or kinetic.

SOFTmax PRO for Lmax

An integrated software program (from Molecular Devices Corporation) that is used to control and collect data from the Lmax instrument.

Chapter 2 Installation

Installation Safety

Always make sure the power switch on the instrument is in the OFF position and remove the power cord from the back of the instrument prior to any installation or relocation of the instrument.

Do not operate the instrument in an environment where potentially damaging liquids or gases are present.

Do not touch or loosen any screws or parts other than those specifically designated in the instructions. Doing so might cause misalignment and could void the instrument warranty.

Make sure the Lmax is set to the correct voltage. Verify that the voltage specified on the label matches local the voltage of your local power provider.

NOTE: "230 V" allows operation from 220 V -15% to 240 V +10% at a frequency range of 47–62 Hz. "115 V" permits operation from 110 V -15% to 120 V +10% at a frequency range of 47–62 Hz.

Use properly grounded electrical outlets.

Make sure your instrument is protected from electrostatic discharges caused by carpeting since such discharges could damage sensitive parts of the instrument.

Unpacking

The Lmax is packed in a specially designed carton. Please retain the carton and the packing materials. If the unit should need to be returned for repair, you must use the original packing materials and carton for shipping. If the carton has been damaged in transit, it is particularly important that you *retain it for inspection by the carrier in case there has also been damage to the instrument.*

⚠ **WARNING:** The Lmax weighs approximately 90 pounds (45 kg) and should be lifted with care. It is recommended that two persons lift the instrument together, taking the proper precautions to avoid injury.

After examining the carton, place it on a flat surface in the upright position. Open the top of the box and lift the Lmax, along with the packing materials around the ends, up and out of the shipping box. Remove the packing material from both ends of the instrument and set the instrument down carefully. The packing list that accompanies the instrument describes all components that should have been placed in the packing carton. Make sure all these items are present before proceeding.

Setting Up the Instrument

1) Place the Lmax on a *level surface, away from direct sunlight, dust, drafts, vibration, magnetic fields, and moisture.* Ambient temperature should be between 15 and 30°C; relative humidity should be between 10 and 90% (non-condensing). Minimum clearance between the back panel of the instrument and the wall should be at least 10 cm (4 inches) to allow adequate air circulation for cooling.
2) The Lmax is shipped with a transport safety device that secures the reading head. Before operating the instrument, this device must be unlocked. If you move the instrument again later, you must lock it again to prevent damage to the reading head. To unlock the transport safety device, follow these steps:
    1) Loosen the cross-headed screw beneath the reading chamber cover (shown in Figure 2.1).
    2) The transport safety device consists of a metal plate that can be pushed into the reading chamber by means of a knurled screw. When you *loosen* ***(but do not remove)*** the knurled screw, you can push the metal plate all the way to the left and lock it again by tightening the knurled screw.

⚠ **CAUTION:** When opening the transport safety device, ensure that the reading head with the guide piece sits properly in the guide rail of the top plate.

    3) Lower the center cover plate again secure it with the cross-headed screw.

1) **NOTE:** During the power-up sequence, the Lmax checks the correct home position of the sample tray and the reading head. If the transport safety device is not unlocked and moved completely to the left, the instrument will not operate.

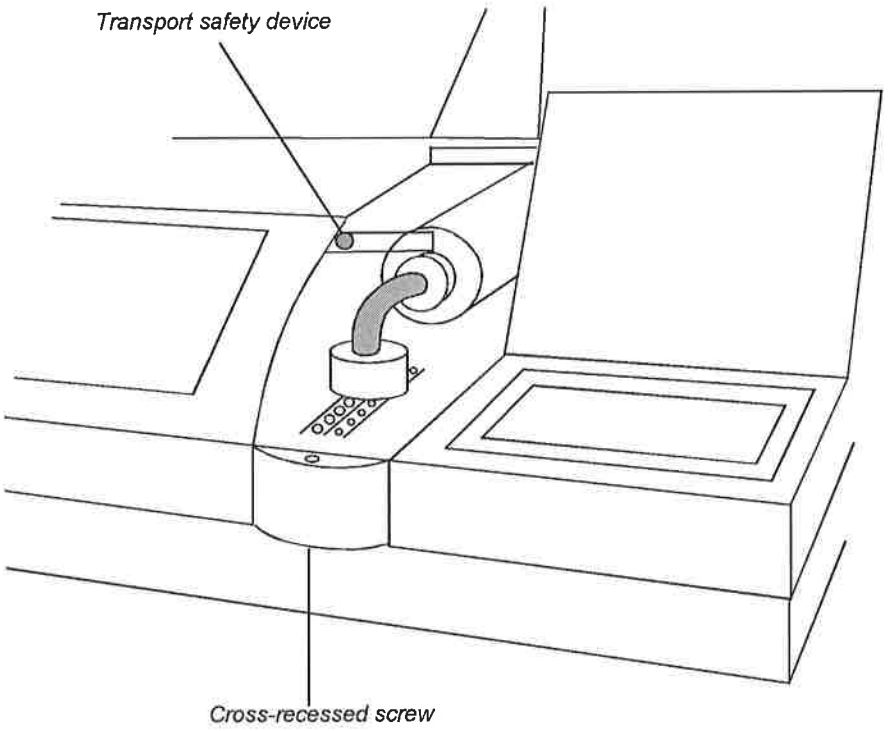

Figure 2.1 Lmax with open counting chamber.

2) Turn the instrument around so that the back of the instrument is facing you as shown in Figure 2.2.

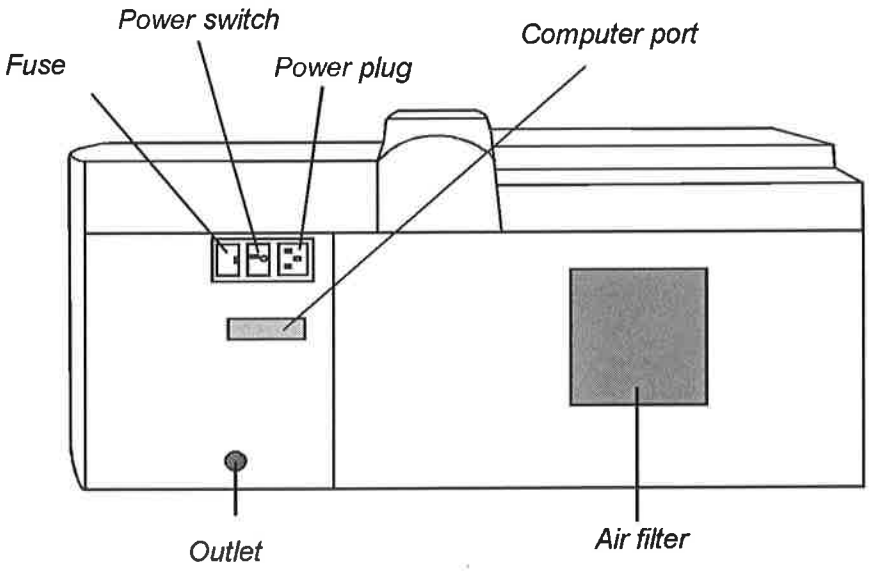

Figure 2.2 : View of Rear Panel

3) Insert the female end of the power cord into the power receptacle at the rear of the Lmax. Connect the male end to a grounded power outlet of the appropriate voltage. Molecular Devices recommends that you use a surge protector between the power cord and the grounded power outlet.

4) Insert the 25-pin computer connection cord into the RS-232 serial port receptacle on the back panel of the instrument. Attach the other 9-pin end to your computer (see Appendix A for more information).
5) Turn the Lmax around so that the control panel now faces you. Be sure no cables run beneath the instrument. Leave at least three inches between the back of the instrument and the nearest objects or surfaces to ensure proper ventilation and cooling.

Connect the Reagent Bottles

1) Place the reagent bottles into the compartment at the right-hand side of the instrument.
2) Connect the tubings from the bottles to the ports labelled "P" and "M" on the Lmax. Use the supplied transparent tubings which are provided with KEL/F screw fittings at both ends, and screw these into the cap of the bottle and into the two screw nuts P and M on the instrument.
3) Connect the Lmax to the power outlet using the power cord supplied with the instrument. The power socket is on the rear of the instrument.
4) Transparent outlet tubing with 3 mm internal diameter is supplied with the instrument which is connected to the outlet by the following procedure:
5) Unscrew the spigot nut from the outlet and slip the spigot nut including pinch ring over the tubing end.
6) Place this tubing end onto the spigot in the outlet opening and screw the spigot nut onto the threading.
7) Connect the other end of the tubing to a collection bottle of adequate size.

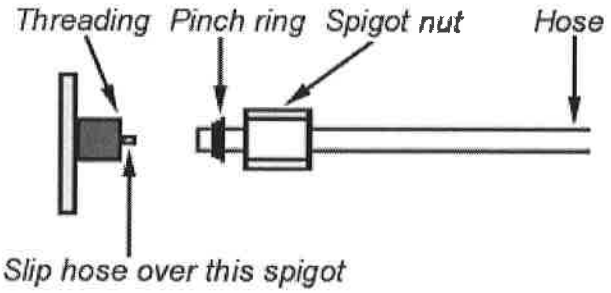

***Figure 2.3** Connecting the outlet tubing.*

Changing the Microplate Format from 96- to 384-Well

⚠ **CAUTION:** Turn off power to the instrument and disconnect the power cord before changing the plate format from 96- to 384-well and vice versa.

1) Open the reading chamber by unscrewing the cross-headed screw below the reading chamber cover
2) Raise the sample compartment cover and then the reading chamber cover plate.
3) Loosen (but preferably do not remove) the knurled screw of the transportation safety device. Raise the read head and push the transport safety bar all

the way to the right (beneath the read head). Tighten the knurled screw in the new position.

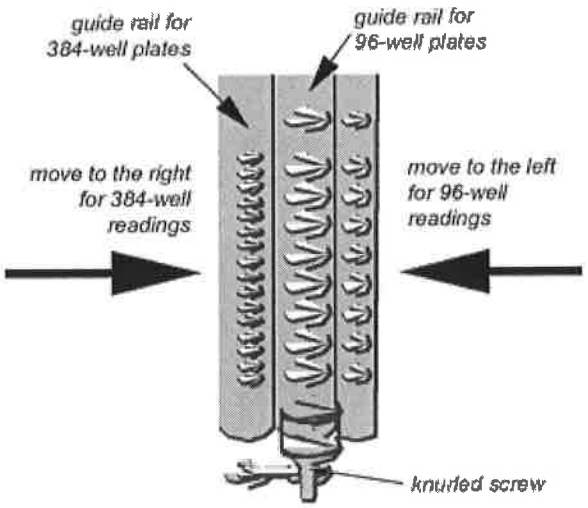

Figure 2.4 *Perforated plate with two guide rails for 96- and 384-well plates*

4) In front of the guide rails for the counting head there is an additional knurled screw. Loosen this screw until the perforated plate can be moved to the right or left. Move the perforated plate to the left for measuring 384-well plates, move it to the right for measuring 96-well microplates. After sliding the plate into the desired position, tighten the knurled screw (Figure 3-4).

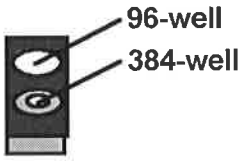

Figure 2.5 *Slider with two positions.*

5) Pick up the slide/guide piece from below the read head, rotate it and insert it into the other track. For measuring 96-well plates the larger 9 mm diameter hole is used, for measurement of 384-well microplates the smaller of 3 mm hole is used (Figure 3-5). For measuring 96-well plates, the slide/guide piece must be in the track containing the 8 large holes and it must be oriented such that the large hole is toward the rear of the instrument. For measuring 384-well plates, the slide/guide piece must be in the track containing the 16 small holes and it must be oriented such that the small hole is toward the rear of the instrument.
6) Loosen the knurled screw of the safety device, lift up the read head, and move the safety device all the way to the left. Carefully place the counting head together with the slider into the corresponding guide rail. Make sure that the slider lies flat inside the guide rail to minimize crosstalk from neighboring wells. Tighten the knurled screw of the transportation device
7) Replace the center cover plate with the crossheaded screw.
8) Specify the new plate type in SOFTmax PRO for Lmax.

Filter Installation for Light Detector

An optical filter may be installed between the sample and photomultiplier to extend the dynamic measurement range of the instrument.

NOTE: Sensitivity decreases as a result of using such filters to achieve increased measurement range.

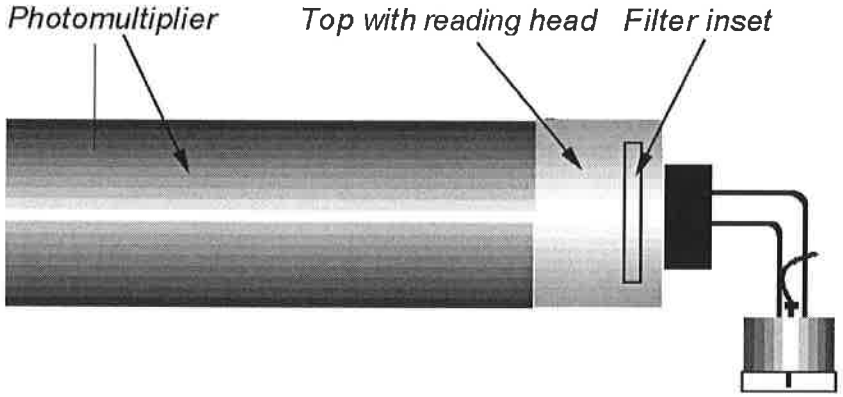

***Figure 2.6** Light filter installation*

1) With the instrument closed, move the counting head to one of the first positions by moving the read head to the service position from within SOFTmax PRO for Lmax.
2) With the instrument turned off, remove the housing from the reading chamber.
3) Unscrew the top of the counting head from the photomultiplier.
4) Insert the light filter. Please be careful not to damage the injector tubings.
5) Attach the top of the counting head again.
6) Position the counting head with the guide piece exactly in the guide rail with the big holes.
7) Close the instrument and turn it on again. The counting head returns to its home position.

Chapter 3 Operation

Prepare for a Reading

Turn the Instrument and Computer On

The power switch for the Lmax is located on the back panel. Press the rocker switch to the on position. The instrument will automatically perform diagnostic checks to ensure that it is functioning correctly. Turn the computer on at this time also and start the SOFTmax PRO for Lmax software program.

Set the Temperature

If elevated temperature within the microplate chamber is desired, you should turn on the incubator first, allowing enough time for the temperature to reach the set point before performing a reading. When you first turn the instrument on, up to 30 minutes may be required for the temperature within the chamber to reach the set point. Turning on the incubator and choosing a temperature set point is done using the SOFTmax PRO for Lmax software.

NOTE: Temperature cannot be regulated at a set point that is lower than 1°C above the ambient temperature.

The microplate chamber temperature will be maintained at the set point until you disable temperature control. When the incubator is off, the temperature within the microplate chamber will begin returning to ambient.

NOTE: Should you turn the incubator back on after a momentary shutdown, allow about ten minutes for the control algorithm to fully stabilize the microplate chamber temperature.

Read the Microplate

BIOHAZARD: The underside of the microplate must be dry prior to placing it in the microplate chamber. If the microplate has fluid on the underside, dry it using a paper towel (or equivalent) before placing it in the Lmax.

Insert the filled microplate into the microplate chamber, making sure well A1 is located in the upper left corner. Make sure the microplate rests flat on the bottom of the microplate chamber.

You must use SOFTmax PRO for Lmax software running on a computer (properly connected to the Lmax) in order to perform instrument setup, reading, and to analyze the data that is collected.

When reading is complete, you will be able to open the cover over the microplate in order to remove it.

Operation Overview

The following steps provide a quick reminder of the basic operating procedures required to perform an assay using the Lmax.

1) Turn on the power switch of the Lmax (located on the back panel).
2) Select the desired instrument settings (temperature, read mode, type of analysis, template, etc.) using SOFTmax PRO for Lmax software on the external computer.
3) Install the proper reagents and/or buffers into the bottles and prime the lines.
4) Load the prepared microplate into the chamber (position well A1 in the upper left-hand corner and make sure the plate sits flat).
5) Using SOFTmax PRO for Lmax, set up the appropriate protocol and start the reading.

Chapter 4 Maintenance

Technical Support

Molecular Devices Corporation is a leading worldwide manufacturer and distributor of analytical instrumentation. We are committed to the quality of our products and to fully supporting our customers with the highest possible level of technical service. In order to fully benefit from our technical services, please complete the registration card and return it to the address printed on the card.

If you have any problems using the Lmax Microplate Luminometer, in the U.S., contact our Technical Services group at 1-800-635-5577; elsewhere contact your local representative.

BIOHAZARD: Wear gloves during any cleaning procedure that could involve contact with either hazardous or biohazardous materials or fluids.

WARNING: All maintenance procedures described in this manual can be safely performed by qualified personnel. Maintenance not covered in this manual should be performed only by a Molecular Devices representative.

WARNING: Turn the power switch off and disconnect the power cord from the main power source before performing any maintenance procedure that requires removal of any panel, cover, or disassembly of any interior instrument component.

WARNING: Removal of protective covers that are marked with the High Voltage warning symbol shown below can result in a safety hazard.

Cleaning

Washing the Lines and Injector System

BIOHAZARD: Wear gloves during any cleaning procedure that could involve contact with either hazardous or biohazardous materials or fluids.

You should wash the lines and injector system after every run. Select the **Wash** command from the **Control** menu in SOFTmax PRO for Lmax. Specify the injector(s) and number of desired injections, fill the bottle(s) with wash fluid and start the wash process. The wash fluid exits in the home position of the reading head and is automatically aspirated.

CAUTION: Excess liquid overflowing from the sample plate into the microplate chamber does not drain out. Wipe it out immediately to avoid deteriorating performance of the Lmax due to dirt an accumulation of particles in the instrument.
To remove dirt from under the top plate (above the microplate), wrap a paper tissue around the microplate and run a measurement without injections. Because the microplate and the top plate come into direct contact during measurement, the bottom of the top plate will be wiped off with this procedure. If necessary, repeat this step several times.

Exterior Cleaning

Periodically, you should clean the *outside* surfaces of the Lmax using a cloth or sponge that has been dampened with water. Do not use abrasive cleaners. If required, clean the surfaces using a mild soap solution diluted with water or a glass cleaner and then wipe with a damp cloth or sponge to remove any residue. Do not spray cleaner directly onto the instrument.

If needed, clean the microplate chamber using a cloth or sponge that has been dampened with water.

Should fluids spill into the microplate chamber, wipe them up immediately. Clean only the exterior of the unit (and the microplate chamber if necessary). **Never clean the inside of the instrument.** Do not allow excess water or other fluids to drip inside the instrument.

Cleaning the Fan Filter

The fan filter on the bottom of the instrument requires periodic cleaning. The frequency of the cleaning depends on how dusty your particular lab is and could range from once a month to once every six months.

1) Turn power to the instrument OFF and then remove the power cord and cables from the back of the instrument.
2) Remove any plate from the microplate chamber. Turn the instrument over so that it rests flat on the bench.
3) Remove the black fan cover (pull gently until it comes off) and remove the filter.
4) Clean the filter by blowing clean, canned air through it or by rinsing it—first with water and then with alcohol. Allow it to dry completely.
5) Place the clean, dry filter over the fan and replace the black cover.
6) Turn the instrument back over. Reconnect the power cord and cables to the instrument.

Replacing the Injector Tips at the Reading Head

1) Close the instrument cover. Choose "Move to Service Position" from the Control menu in SOFTmax PRO for Lmax.
2) With the instrument turned off, remove the housing of the reading chamber by loosening the cross-headed screws on the front panel (see Figure 1.3).
3) Unscrew the fittings of the supply tubings at the reading head completely. (A small wrench is included with the accessories.)
4) Unscrew the injector tip adapters.

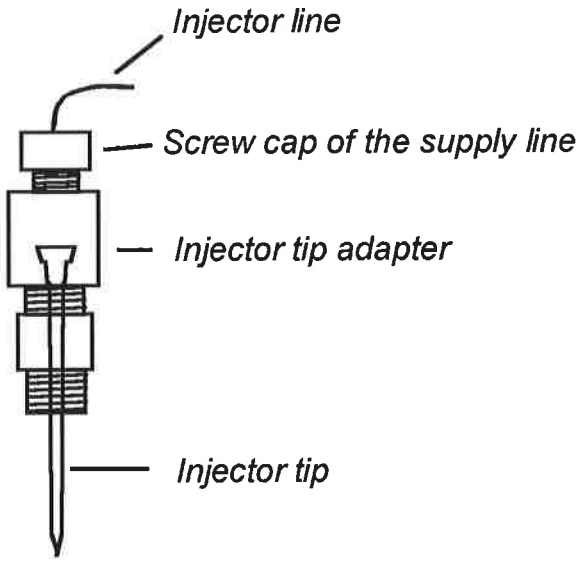

Figure 4.1: *Design of the injector tip holder in the reading head*

5) Taking care that the conical seals inside are not lost, unscrew the adapters and the injector tips.
6) Remove the seals from the old tips and insert them into the new tips. Screw the injector tip adapters together and then screw them into the threaded bore hole of the reading head by hand. Connect the supply tubings via the screw-type fittings.
7) Check to make sure that the reading head sits properly in the metal slider/guide piece which, in turn, slides within the guide rails.
8) Close the reading chamber door and secure it by tightening the Phillips screw.
9) Close the instrument cover and turn it on. The system performs an instrument test, checking the home position of the plate tray and the reading head.

Changing the Fuses

Fuses burn out occasionally and must be replaced. If the instrument does not seem to be getting power after switching it on, first check to see whether the power cord is securely plugged in to a functioning power outlet and to the receptacle at the rear of the Lmax. If power failed while the Lmax was already on, check that the power cord is not loose or disconnected and that power to the power outlet is functioning properly. If these checks fail to remedy the loss of power, follow the steps listed below to replace the fuses. Spare fuses are shipped with the instrument (they may be taped to the back of the Lmax).

If you no longer have spare fuses, you may obtain new ones from Molecular Devices (part numbers: 4601-0043 for 2A 115V fuse and 4601-0044 for 1A 230V fuse, or you can obtain them from a local hardware store. Make sure fuses are rated time-delay).

To change fuses, follow the steps below.

1) Disconnect the power cord from the instrument and power source before replacing any fuses.
2) The main fuses are located on the rear panel of the Lmax. The fuse carrier and the main power connection socket are one unit.

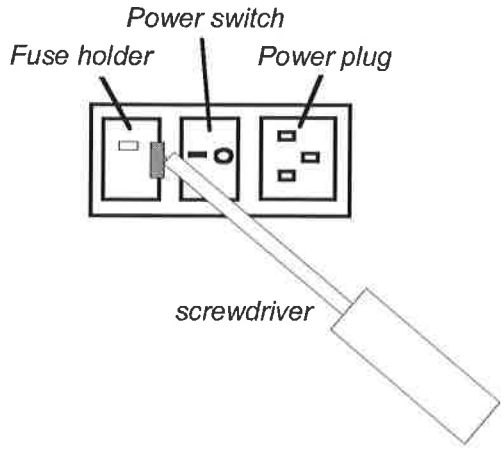

Figure 4.2 *Replacing the main fuses.*

3) Using a screwdriver, push against the clip holder, pull out the fuse link from the right, and then insert new fuses: 230 V, 1-Amp time-delay; 115 V, 2-Amp time delay.

⚠ **CAUTION:** Use only these fuse values for replacement. The 1-Amp fuse must be IEC-approved. The 2-Amp fuse must be UL-listed (please observe respective labels).

4) If the fuses blow again after power on, call the Molecular Devices service department.

Moving the Lmax

If you need to relocate the Lmax, follow these steps.

⚠ **WARNING:** The Lmax weighs approximately 90 pounds (45 kilograms). To avoid injury, it is recommended that two people lift the instrument together, using proper lifting techniques.

1) Place an unfilled microplate in the reading compartment (this helps to prevent any jarring to the heater element located beneath the microplate).
2) Turn off the power switch and unplug the power cord from the source and from the receptacle on the back of the instrument.
3) Depending on the distance that you will be moving the instrument, you may wish to repackage the Lmax in its original shipping carton. Otherwise, carry the instrument or place it on a rolling cart to transport it.

4) Ensure that the new location meets the proper specifications as described in Chapter 2, "Installation."

Transport Safety Device

The Lmax is shipped with a transport safety device that secures the reading head. Before operating the instrument, this device must be unlocked. If you move the instrument again later, you must lock it again to prevent damage to the reading head.

Opening and Securing the Transport Safety Device

To unlock the transport safety device, loosen the Phillips screw beneath the reading chamber cover (see Figure 2.1).

The transport safety device consists of a metal plate that can be pushed into the reading chamber by means of a knurled screw. When you *loosen* ***(but do not remove)*** the knurled screw, you can push the metal plate all the way to the left and lock it again by tightening the knurled screw.

⚠ **CAUTION:** When opening the transport safety device, ensure that the reading head with the guide piece sits properly in the guide rail of the top plate.

When you secure the transport safety device again, you also must make sure that the reading head is in the home position—the counting head is in the slider and the slider is in the guide rail—since the reading head is protected by the transport bar against any movement in this position only.

NOTE: Before transporting the Lmax, place a clean microplate in the microplate chamber as this helps to avoid jarring the heater mechanisms in the chamber.

When the transport safety device is in the desired position and you have installed a clean microplate in the chamber, you may move the Lmax.

After the move when you unlock the transport safety device, close the cover, reconnect the power cord, and turn instrument power on, the Lmax checks the correct home position of the sample tray and the reading head.

If the transport safety device is not unlocked and moved completely to the left, the instrument will not be able to communicate with the computer.

Long-Term Shutdown

If you will not be using the Lmax for an extended period of time, clean the *external* surfaces of the instrument and make sure no deposits are left inside the sample compartment.

Chapter 5 Troubleshooting

Introduction

This chapter lists errors that may occur while using the instrument, followed by their most likely causes and remedies. Maintenance procedures are described in Chapter 4, "Maintenance." For problems with the Lmax that are not listed here, in the U.S., contact Molecular Devices Technical Services group at 1-800-635-5577; elsewhere, call your local representative.

BIOHAZARD: It is your responsibility to decontaminate the instrument, as well as any accessories, before requesting service by Molecular Devices representatives and before returning the instrument or any components to Molecular Devices Corporation.

Table 5.1: : Errors and Possible Resolution

Error	Resolution
General Errors	
No power to instrument	Check power cord connection. Check fuses.
Power on but no communication with the computer	Slide the transport bar all of the way to the retracted position.
Fluctuating Measured Values	
Fluctuating injection quantity	Replace blocked reagent filters.
Leaks in reagent tubing	Check for leaks. Replace tubing as necessary.
Bad mixing of reagents with sample	Replace injector tips.
Poor counting statistics	Increase measurement time.
Electrostatic charges building up on injector tips/microplate	Increase humidity around instrument.
Elevated Backgrounds	
Fan defective (causing internal heating)	Call Molecular Devices Technical Services.
Missing fan cover	Replace cover.
Blocked dust filter (causing internal heating)	Clean or replace filter.
Injection system contaminated	Flush system with filtered deionized water or 20% ethanol. Replace tubing/injector tips if cleaning fails to solve the problem.

Table 5.1: : Errors and Possible Resolution

Error	Resolution
High crosstalk between adjacent wells	Do not use clear plates. When measuring high light output samples, some crosstalk is evident. Crosstalk should be less than 3 x 10-5 between adjacent wells. If this figure is exceeded, PMT/light guide/foot assembly alignment may be necessary. Call Molecular Devices Technical Services. Note: It is essential to flush the system after instrument use to ensure that no deposits or dried reagents remain on the injector tips.
Splashing of reagents or wet microplate	1. Damaged injector tips—replace them. 2. Air leaks in reagent lines—check tubing/tee seals and connections. 3. Insufficient number of prime cycles. 4. Incorrectly adjusted perforated plate/mechanical alignment.
Incorrect injection volumes or incorrect reagent consumption	Injectors are in need of recalibration. Call Molecular Devices Technical Services.
Erratic injection volumes	Replace reagent filters.

For all other instrument problems, please contact your local Molecular Devices Technical Services representative for assistance.

Appendix A Cables and Accessories

Cables

Molecular Devices recommends that you use high-quality, double-shielded cables to connect the Lmax to the computer. Choose cables that meet the following requirements:

Serial Interface Cable (Null Modem Cable)

The Lmax communicates with the computer via a serial RS-232 interface using a null-modem cable with 9-pin type connection on one end for the computer and a 9- to 25-pin type adapter on the other for the Lmax. Only instruments with interfaces classified as safely separated according to IEC 950 may be connected.

The data transfer parameters are:

9600 baud; 8 data bits; 1 stop bit; parity bit: even (RS-232 level).

The following data and control signals are used
(25-pin CANNON connector DB 25 P):

Pin 1		cable shield
Pin 2	TxD	transmit data
Pin 3	RxD	receive data
Pin 4	RTS	request to send (always "on")
Pin 5	CTS	clear to send
Pin 6	DSR	data set ready
Pin 7		signal ground
Pin 8	DCD	data carrier detect
Pin 20	DTR	data terminal ready

On the output side, a handshake mode is foreseen using the control lines CTS, DSR, and DCD. All three lines must be in the ON state for data transfer.

Items Available for Use with the Lmax

Cable, RS-232 (Lmax to computer) 9000-0530
Power Cord for 115-volt operation 9000-0540
Power Cord for 230-volt operation 9000-0539
Fuse, 2-Amp Time Delay for 115-volt operation 4601-0043
Fuse, 1-Amp Time Delay 4601-0044
Fuse, 2-amp Time Delay for 115-volt operation 4601-0043

Appendix B Applications

General Information about Application Examples

This section describes some typical applications of the Lmax and illustrates the various options that can be programmed. Each section following this describes an individual application. The first part of each of these sections describes the chemistry of the particular application, followed by the operating procedure, from setting up a reading protocol to starting the reading.

The following applications are discussed:

- Section B1Chemiluminescent Immunoassays (CLIA)
- Section B2Cellular Luminescence
- Section B3DNA-Probe Assays
- Section B4Dioxetane-Based Glow Luminescence
- Section B5Free ATP
- Section B6Microbial ATP in the Absence of Somatic Cells
- Section B7Microbial ATP in the Presence of Somatic Cells
- Section B8Luciferase Reporter Gene Assay

B1: Chemiluminescent Immunoassays (CLIA)

Introduction

These assays usually involve reactions of acridinium compounds or luminol derivatives that produce luminescence. The chemistry of these reactions is normally characterized by fast kinetics (in seconds) and is often referred to as "flash" luminescence.

Luminometers usually are operated with two injectors and typical injection volumes of 100 µL. The reagents for this reaction include the following substances:

Label	Reagent 1 1st Injection	Reagent 2 2nd Injection
Acridinium Ester	Acidic H_2O_2	NaOH
Luminol	Catalyst*	H_2O_2 + NaOH

* The catalyst can be a variety of molecules from simple transition metals to macromolecules such as horseradish peroxidase.

Procedure

Reading Protocol: Endpoint

Define both injectors as being active. In most cases, a minimum delay of 1.6 s suffices between the injections. The read time is typically 1 to 2 s.

Since these measurements usually take place at ambient temperatures, the incubator should be off.

If necessary, the pre-read plate blank can be activated.

Install the Reagent Bottles

Connect the bottle containing the first reagent to be injected to the injector labelled "P" and the bottle containing the second reagent to the injector labelled "M."

Start the Reading

Prime the injector system with reagents. Open the desired reading protocol and select the wells to be read.

Start the reading.

B2: Cellular Luminescence

Introduction

A basic example of cellular luminescence is the measurement of the phagocytosis activity of monocytes when examining the immune systems in patient blood. Through chemical stimulation (e.g., opsonized zymosan), the cells of the immune systems are stimulated for phagocytosis (i.e., they start to devour the foreign particles). So-called oxygen radicals are produced during a time period of 15 minutes to 2 hours. To what extent and in which time period this production of oxygen radicals takes place are the criteria that provide information about the immune system and possible inflammations, etc.

The light generated by the production of oxygen radicals is often not sufficient to be measured directly. Therefore, a luminescence enhancer (e.g., luminol or lucigenin) is added to the sample, increasing the light efficiency by a factor of more than 1000. The light emission measured at the photomultiplier (in RLU/s) corresponds to the actual production of the oxygen radicals and its kinetics is measured over a time period of approximately 45 minutes.

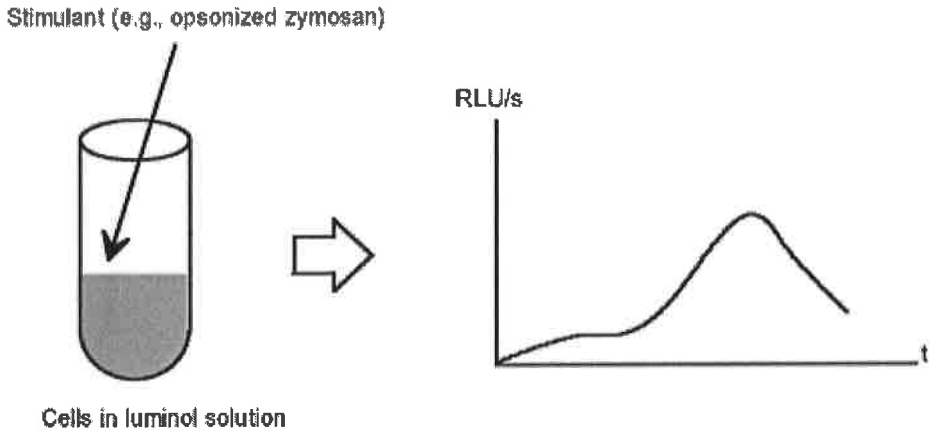

Figure B.1: Phagocytosis — the light emission after addition of the stimulant continues for 15 minutes up to approximately 2 hours.

Procedure

Quasi-Parallel Reading in the Long Kinetics Mode

Once the isolated cells have been diluted to an adequate level and the luminol solution has been added, the stimulant (e.g., opsonized zymosan) is injected. The samples are not read successively but rather in quasi-parallel. This means that each sample is briefly measured once per cycle. Each further cycle of the microplate yields another data point for each sample. The curve for each sample consists of the data points of the cycles.

The number of cycles is defined in the reading protocol. This quasi-parallel reading allows a high sample throughput in a short time. It is dependent upon the length of the reading of the single samples and the entered total reading time (reasonable maximum: length of the chemical reaction). The program calculates the

number of data points of the curve from the entered total read time, the read time of one well, and the number of wells.

Procedure

Reading Protocol: Long Kinetics Mode

Set up a reading protocol in the Long Kinetics mode using SOFTmax PRO for Lmax software. Typically, one should select an incubator temperature of 37°C.

In most cases, it is best to work with one injector that injects the stimulant shortly before the reading. Another injector can be used, if desired, e.g., to inject a buffer for changing the pH value.

Starting the Reading

Prior to the actual start of the reading, it is advisable to preheat the reading chamber to the selected assay temperature (incubator control is done using SOFTmax PRO for Lmax software). When the injector system is primed with reagents, activate the desired reading protocol. Select the number of wells to be read.

Start the reading.

B3: DNA Probe Assays

Introduction

These assays involve the use of an acridinium ester or other luminescently labelled DNA or RNA probes. These labelled nucleic acid probes hybridize to specific RNA sequences of a particular organism (*Chlamydia*, for example) and, thus, detect its presence.

The quantity of light emitted identifies the quantity of the specific sequences present and, as a result, the presence or absence of the organism being tested for. The presence of the organism is determined by comparing light emission in patient samples to light emission values in known standards. These assays are usually performed using a kit supplied by a clinical reagent manufacturer.

Procedure

Reading Protocol: Endpoint

Define both injectors as being active. In most cases, a minimum delay of 1.6 s suffices between the injections. The reading time is typically 1 to 2 s.

Since the readings usually take place at ambient temperatures, the incubator should be off.

If necessary, enable pre-read plate blanking.

Install Reagent Bottles

Connect the bottle containing the first reagent to be injected to the injector labelled "P" and the bottle containing the second reagent to the injector labelled "M."

Start the Reading

When the injector system is primed with reagents, open the desired protocol and select the wells to be read.

Start the reading.

B4: Dioxetane Based Glow Luminescence

Introduction

Some immunoassays use alkaline phosphatase bound to Ag/Ab compounds or DNA hybrids as labels. The concentration of the enzyme is determined by dephosphorization of certain 1.2-dioxetane compounds that are typically injected into the tubes from 10 to 20 minutes prior to the reading. To keep the delay between the injection and the actual reading for each sample constant, the reagent may be added in a preceding "pseudo" reading by the Lmax.

Because the light emission is occasionally very high, it may be necessary to install a neutral density filter (1:100) in the Lmax (this filter can be ordered from Molecular Devices Corporation—filter installation is described in Chapter 2, "Installation.")

Procedure

Reading Protocols

Define two reading protocols, the first working with injector M, the second without injectors **(important: the reading time must be greater than 1.2 s)**. Depending on the enzyme reaction, select the optimum temperature and, in the second protocol, a possible replicate calculation.

Start the Reading

Start the enzyme reaction by running the first reading protocol with injector M (at the same time, start the stopwatch). Reject this data acquired first. When the incubation period (e.g., 20 min) is over, start the second reading protocol. Each sample is now read with the same delay used in the preceding injection.

B5: Free ATP

Introduction

The visible "glowing" of the firefly *Photinus pyralis* is based on the ATP-dependent oxidation of the substrate luciferin in the presence of the luciferase enzyme. By selecting a suitable starting concentration for the above-mentioned reaction participants, this bioluminescence reaction can be used to measure an unknown ATP concentration directly.

The injection of Luciferin/Luciferase into the unknown ATP solution triggers the chemical reaction and, thus, the light emission. Typically, it is measured over the first 10 to 30 seconds and yields an integral value determined over this time.

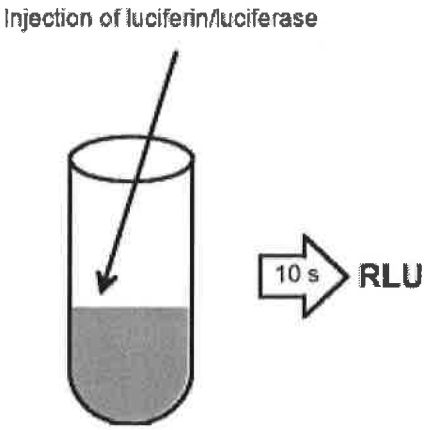

Figure B.2: *Measurement of free ATP*

Procedure

Reading Protocol: Endpoint

Define injector M as the active injector, define the reading time and, if necessary, the replicates.

Since these measurements usually take place at ambient temperatures, the incubator should be off.

If necessary, enable pre-read plate blanking.

Install the Reagent Bottles

Connect the reagent bottle containing the trigger reagent to the injector labelled "M".

Start the Reading

When the injector system is primed with reagents, open the desired protocol and enter the wells to be read.

Start the reading.

B6: Microbial ATP without Somatic Cells

Introduction

ATP, which is a ubiquitous molecule in all microorganisms, can be used for the indirect, semi-quantitative measurement of microbial contaminations (for example, in drinking water).

Microbial ATP must first be released through injection of an extraction reagent (extraction time: about 5 to 10 seconds). After this extraction time, luciferin/luciferase is injected, and the light emission measured.

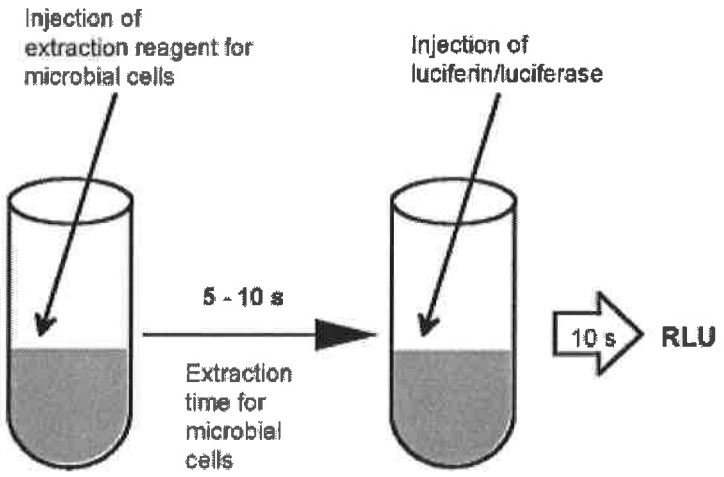

Figure B.3: Measurement of microbial contamination via an ATP measurement

Procedure

Reading Mode: Endpoint

Define both injectors as active, define the delay between the injections, the reading time, and the replicates.

Since these measurements usually take place at ambient temperatures, the incubator should be off.

Set the delay between injectors M and P to 10 s. A pre-read plate blank should suffice for most standard applications.

Install the Reagent Bottles

Prime injector P with the extraction reagent for microbial cells and injector M with the luciferin/luciferase reagent. First, prime the detergent containing extraction agent, since this process may require more injections.

Start the Reading

When the injector system is primed with reagents, open the desired protocol and select the wells to be read.

Start the reading.

B7: ATP from Microbial Cells in the Presence of Somatic Cells

Introduction

The measurement of microbial contamination described in Section B6 in foodstuffs (e.g., fruit juices) requires the preceding destruction of the "natural" ATP from somatic cells (e.g., fruit flesh).

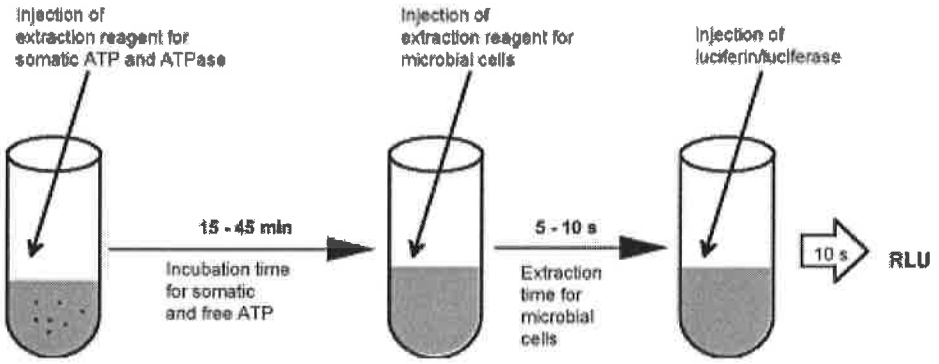

Figure B.4: *Measurement of samples containing contaminating microorganisms in the presence of free/somatic ATP*

Three injections are required. First, a mild extraction reagent for somatic cells mixed with ATPase is injected. The time period for the degradation of somatic ATP, the so-called incubation time, is between 15 and 45 minutes. A second injection lyses the microbial cells. Following an extraction time of 5 to 10 seconds (see Section B6), luciferin/luciferase is injected and the light emission is measured.

Procedure

The procedure is essentially the same as the measurement of microbial ATP in the absence of somatic cells described in Section B6. The only (important) difference is the preceding injection of the ATPase-containing extraction reagent for somatic ATP.

The following differences from the procedure described in Section B6 should be borne in mind: since the Lmax has only two injectors, the extraction reagent must be injected ahead. An additional reading protocol must be used, defining only one injection per sample. After the required incubation time, the second reading protocol can then be started as described in Section B6.

Parameter Protocol 1 for Extraction Reagent: Endpoint

Define injector P for the extraction reagent for somatic ATP for all samples. Enter the same reading time as in the second reading protocol.

Connect the Reagent Bottle

Connect the reagent bottle to the injector labeled "P."

Start the Reading

When the injector system is primed with extraction reagents for somatic ATP, open the desired protocol and select the wells that are to be injected.

Start the reading **and start the stopwatch.**

Wash the Injector Used

After the injection, the injector must be cleaned thoroughly (via the Control menu in SOFTmax PRO for Lmax).

Parameter Protocol 2: Endpoint

Define both injectors as active, the delay between the injections, the reading time, and the replicates.

Since these reads usually take place at ambient temperatures, the incubator should be off.

Set the delay between injections, for example, to 10 s. Pre-read plate blanking should suffice for standard applications.

Install the Reagent Bottles

First, prime the detergent-containing extraction agent, since this process may require more injections. Prime injector P with the extraction reagent for microbial cells and injector M with the luciferin/luciferase reagent.

Starting the Reading

At the end of the desired incubation time, open the second reading protocol and select the wells to be read.

Start the reading.

B8: Luciferase Reporter Gene Assay

Introduction

In experiments examining gene regulation, DNA constructs consisting of the regulatory element to be examined and the coding region of the luciferase gene are transferred into the cells. After a certain time required for the expression of this fusion gene in the cells, the cells are harvested and the concentration of the synthesized luciferase in the cell extract is determined. The enzyme-dependent light emission represents a direct measure for the activity of the examined gene regulatory elements.

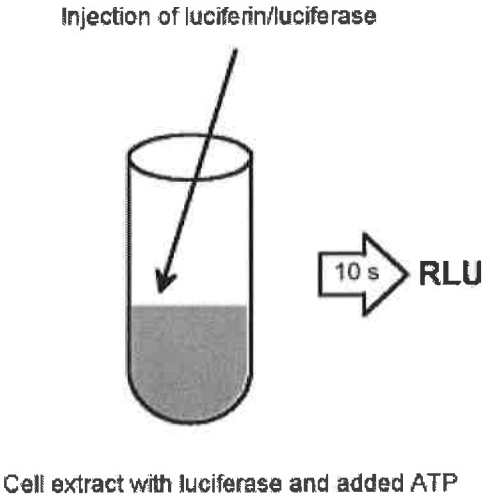

Figure B.5: The trigger reagent (luciferin) is injected into the cell extract containing ATP and the light emission is measured over approximately 10 seconds.

Procedure

Reading Protocol: Endpoint

Define the active injector, the reading time and, if necessary, the replicates.

Since these measurements usually take place at ambient temperatures, the incubator should be off.

If necessary, use a pre-read plate blank.

Installation of Reagent Bottles

Connect the bottle containing the reagent to be injected either to the injector labelled "P" or "M," as specified in the reading protocol.

Starting the Reading

When the injector system is primed with reagents, open the desired reading protocol and select the wells to be read.

Start the reading.

NOTE: The recently introduced reporter gene assays for β-galactosidase, glucuronidase, or secreted alkaline phosphatase can all be measured on the Lmax. Enter the delays between the injections and/or the measurement as described in various published methods or according to the kit manufacturer's instructions. If longer incubation times are needed, as for example with the β-galactosidase assay, we recommend using two reading protocols, as described in Sections B4 and B7.