

SpectraMax L

Luminescence Microplate Reader

User Guide



SpectraMax L Luminescence Microplate Reader User Guide

This document is provided to customers who have purchased Molecular Devices equipment, software, reagents, and consumables to use in the operation of such Molecular Devices equipment, software, reagents, and consumables. This document is copyright protected and any reproduction of this document, in whole or any part, is strictly prohibited, except as Molecular Devices may authorize in writing.

UK

CE

Software that may be described in this document is furnished under a non-transferrable license. It is against the law to copy, modify, or distribute the software on any medium, except as specifically allowed in the license agreement. Furthermore, the license agreement may prohibit the software from being disassembled, reverse engineered, or decompiled for any purpose.



Portions of this document may make reference to other manufacturers and/or their products, which may contain parts whose names are registered as trademarks and/or function as trademarks of their respective owners. Any such usage is intended only to designate those manufacturers' products as supplied by Molecular Devices for incorporation into its equipment and does not imply any right and/or license to use or permit others to use such manufacturers' and/or their product names as trademarks.

Each product is shipped with documentation stating specifications and other technical information. Molecular Devices products are warranted to meet the stated specifications. Molecular Devices makes no other warranties or representations express or implied, including but not limited to, the fitness of this product for any particular purpose and assumes no responsibility or contingent liability, including indirect or consequential damages, for any use to which the purchaser may put the equipment described herein, or for any adverse circumstances arising therefrom. The sole obligation of Molecular Devices and the customer's sole remedy are limited to repair or replacement of the product in the event that the product fails to do as warranted

For research use only. Not for use in diagnostic procedures.

The trademarks mentioned herein are the property of Molecular Devices, LLC or their respective owners. These trademarks may not be used in any type of promotion or advertising without the prior written permission of Molecular Devices, LLC.

Patents: http://www.moleculardevices.com/patents

Product manufactured by Molecular Devices, LLC.
3860 N. First Street, San Jose, California, 95134, United States of America.
Molecular Devices, LLC is ISO 9001 registered.
©2025 Molecular Devices, LLC.
All rights reserved.

Contents

| Safety Information | 5 |
|---|----|
| Warnings, Cautions, Notes, and Tips | 5 |
| Chapter 1: Introduction | 9 |
| Computer Integration | 10 |
| Instrument Features | 11 |
| PMT Technology | 11 |
| Chapter 2: Setting Up the Instrument | 13 |
| Unpacking the Instrument | 17 |
| Assembling the Injectors | 19 |
| Installing the Filters | 21 |
| Installing the Barcode Reader | 23 |
| Changing the Plate Aperture | 23 |
| Chapter 3: Getting Started | 25 |
| Software Ribbon | 25 |
| Instrument Calibration | 26 |
| Chapter 4: Protocols | 29 |
| Temperature Regulation | 29 |
| Injector Protocols | 29 |
| Protocol Settings | 31 |
| Powering Off the Instrument | 34 |
| Chapter 5: Maintenance | 35 |
| Cleaning the Instrument | 35 |
| Replacing Injector Tips | 36 |
| Replacing Injector Tubing | 38 |
| Chapter 6: Before You Move the Instrument | 43 |
| Packing the Instrument | 44 |
| Chapter 7: Opening the Drawer Manually | 45 |
| Obtaining Support | |
| Appendix A: Instrument Specifications | 47 |
| Flectromagnetic Compatibility | 49 |

Safety Information

Information about the safe use of the instrument from Molecular Devices includes an understanding of the user-attention statements in this guide, the safety labels on the instrument, precautions to follow before you operate the instrument, and precautions to follow while you operate the instrument.

Make sure that everyone involved with the operation of the instrument has:

- Received instruction in general safety practices for laboratories.
- Received instruction in specific safety practices for the instrument.
- Read and understood all Safety Data Sheets (SDS) for all materials being used.

Read and observe all warnings, cautions, and instructions. The most important key to safety is to operate the instrument with care.



WARNING! If the instrument is used in a manner not specified by Molecular Devices, the protection provided by the equipment might be impaired.

Warnings, Cautions, Notes, and Tips

All warning symbols are framed within a yellow triangle. An exclamation mark is used for most warnings. Other symbols can warn of other types of hazards such as biohazard, electrical, or laser safety warnings as are described in the text of the warning. Follow the related safety information.

The following user attention statements might be displayed in the text of Molecular Devices user documentation. Each statement implies the amount of observation or recommended procedure.



WARNING! A warning indicates a situation or operation that could cause personal injury if precautions are not followed.



CAUTION! A caution indicates a situation or operation that could cause damage to the instrument or loss of data if correct procedures are not followed.



Note: A note calls attention to significant information.



Tip: A tip provides useful information or a shortcut but is not essential to the completion of a procedure.

Symbols on the Instrument

Each safety label found on the instrument contains an alert symbol that indicates the type of potential safety hazard.

| Symbol | Indication |
|---|---|
| <u>^</u> | Consult the product documentation. |
| A | Potential biohazard. |
| | Potential lifting hazard. To prevent injury, use a minimum of two people to lift the instrument. |
| | Potential pinch hazard. |
| A | Electrostatic sensitive device (ESD). Observe precautions for handling electrostatic sensitive devices. |
| | Required in accordance with the Waste Electrical and Electronic Equipment (WEEE) Directive of the European Union. It indicates that you must not discard this electrical or electronic product or its components in domestic household waste or in the municipal waste collection system. For products under the requirement of the WEEE directive, contact your dealer or local Molecular Devices office for the procedures to facilitate the proper collection, treatment, recovery, recycling, and safe disposal of the device. |
| Info for USA only: California Proposition 65 WARNING Cancer to Reproductive Harm were P69Warnings.ca.gov | California Proposition 65 requires businesses to provide warnings to Californians about significant exposures to chemicals that cause cancer, birth defects, or other reproductive harm. |

Electrical Safety

To prevent electrical injuries and property damage, inspect all electrical equipment before use and report all electrical deficiencies. Contact Molecular Devices technical support for equipment service that requires the removal of covers or panels.



WARNING! HIGH VOLTAGE. Within the instrument is the potential of an electrical shock hazard existing from a high voltage source. Read and understand all safety instructions before you install, maintain, and service the instrument.

To prevent electrical shock, use the supplied power cord and connect to a properly grounded wall outlet.

To ensure sufficient ventilation and provide access to disconnect power from the instrument, maintain a 20 cm to 30 cm (7.9 in. to 11.8 in.) gap between the rear of the instrument and the wall.

Power off the instrument when not in use.

Chemical and Biological Safety

Normal operation of the instrument can involve the use of materials that are toxic, flammable, or otherwise biologically harmful. When you use such materials, observe the following precautions:

- Handle infectious samples based on good laboratory procedures and methods to prevent the spread of disease.
- Observe all cautionary information printed on the original containers of solutions before their use.
- Dispose of all waste solutions based on the waste disposal procedures of your facility.
- Operate the instrument in accordance with the instructions outlined in this guide, and take all the required precautions when using pathological, toxic, or radioactive materials.
- Splashing of liquids can occur. Take applicable safety precautions, such as using safety glasses and wearing protective clothing, when working with potentially hazardous liquids.
- Observe the applicable cautionary procedures as defined by your safety officer when using hazardous materials, flammable solvents, toxic, pathological, or radioactive materials in or near a powered-up instrument.



WARNING! Never use the instrument in an environment where potentially damaging liquids or gases are present.

Moving Parts Safety

The instrument contains moving parts that can cause injury. Under normal conditions, the instrument is designed to protect you from these moving parts.



WARNING! If the instrument is used in a manner not specified by Molecular Devices, the protection provided by the equipment might be impaired.

To prevent injury:

- Never try to exchange labware, reagents, or tools while the instrument is operating.
- Never try to physically restrict the moving components of the instrument.



WARNING! Do not attempt to access the interior of the instrument unless specifically instructed to do so. The moving parts inside the instrument can cause injury. Do not operate the instrument with any covers or panels removed.

Chapter 1: Introduction



The extreme flexibility and high sensitivity of the SpectraMax® L Luminescence Microplate Reader makes it ideal for applications within the fields of biochemistry, cell biology, immunology, molecular biology, and microbiology. Typical applications include luciferase reporter gene, ATP, Ca²⁺, aequorin, and ELISAs.

The instrument is a high-performance microplate reader that measures luminescence in 96-well and 384-well plates.

There are multiple instrument configurations that allow you to vary the components that are included in the instrument when it ships to you from the factory.

- Base one PMT, manual aperture
- Injectors up to four injectors
- PMTs one or two PMTs
- Dual Wavelength
- Automatic Aperture
- Filters
- Barcode Reader

In the SoftMax® Pro Data Acquisition and Analysis Software, select the Home tab and click **Instrument Configuration** to see the configuration of your instrument.

Read Modes

The components included in your instrument configuration determine which read modes are available:

- Lumi (Luminescence): For all instrument configurations, you can select the Lumi read mode.
 When the instrument has two PMTs and no filters, the instrument runs Lumi protocols that use both PMTs to read 2 wells simultaneously and it assumes the readings of the PMTs are comparable.
- Lumi2W (Luminescence Dual Wavelength): When the instrument has two PMTs and the PMTs are equipped with filters, you can select the Lumi2W read mode to use the PMTs one at a time to detect the two different wavelengths.

Read Types

For all instrument configurations, you can select the following read types:

- Endpoint: A read of each well is taken in the center of each well, either at all wavelengths if filters are not used, or at specified wavelengths if filters are used. Raw data values are reported as relative light units (RLU). Each well can receive up to two injections and then is read once. You can set a delay time after each injection, from 0.1 to 3,600 seconds for the P-injection, and from 0 to 3,600 seconds for the M-injection.
- **Dual Read:** Each well receives two injections, each followed by a read. The first injection is the P-injection with a subsequent read. The second injection is the M-injection with a subsequent read. You can set a delay time between injection and read, from 0.1 to 3,600 seconds for the P-injection, and from 0 to 3,600 seconds for the M-injection.

- Kinetic: The instrument collects data over time with multiple reads taken in the center of
 each well at regular intervals. Each well can receive multiple injections and reads. You can
 specify single injections, followed by single reads, for multiple time points. The minimum
 integration interval is dependent on the number of wells you select, the integration time you
 select, and the number of injections you select.
- Fast Kinetic: The instrument performs repeated reads of each well up to a 200 point maximum integration. All reads of a single well are made before the next well is read. Prereading a plate is not possible in Fast Kinetic read type. Each well can receive multiple injections and reads. You can specify multiple injections and multiple reads, for multiple times. Integration time can be set from 0.01 to 3600 seconds.

When the instrument configuration includes the variable-volume injectors option, it can measure both flash and glow luminescence. High-precision peristaltic pumps ensure precise injection volumes and rapid sample mixing. Continuous monitoring between injection and measurement provides for extremely fast reactions and reduced delay time.

- 96-well Standard plates 10 μL to 200 μL
- 384-well and 96-well half area plates 10 μL to 80 μL

Optional multiple photomultiplier tubes (PMTs) and multiple injector configurations allow increased throughput and wavelength-dependent assays such as BRET1 and BRET2.

Computer Integration

Each Molecular Devices microplate reader ships with a license key for the SoftMax® Pro Data Acquisition and Analysis Software. You install the SoftMax Pro Software on the computer that you use to operate the instrument to provide integrated instrument control, data display, and statistical data analysis.

You should install the SoftMax Pro Software on the computer before you set up the instrument. Please be aware that some updates to the SoftMax Pro Software require a purchase. Contact Molecular Devices before you update the software.

For information about the computer specifications required to run the software, the software installation and licensing instructions, and the directions to create the software connection between the computer and the instrument, see:

- SoftMax Pro Data Acquisition and Analysis Software Standard Edition and MiniMax Imaging Edition - Installation Guide
- SoftMax Pro Data Acquisition and Analysis Software Installation Guide for the Multi Computer Setup
- SoftMax Pro Data Acquisition and Analysis Software Installation Guide for the Single Computer Setup

When you connect the microplate handler to a compatible microplate reader, the StakMax Software runs with the SoftMax Pro Software that controls the microplate reader.

When you connect the microplate handler to the AquaMax Microplate Washer, the AquaMax Software controls the StakMax Microplate Handler. See the *AquaMax 2000/4000 Microplate Washer User Guide*.

Instrument Features

The instrument can have the following features:

- Greater than nine orders of dynamic range
- Reads both 96-well and 384-well plates
- Dual injection into both 96-well and 384-well plates
- Programmable injection speeds
- AutoWash flush of tubing and tips when you power off the instrument
- StakMax® Microplate Handling System and robot compatible
- Landscape and portrait plate orientation
- Optional dual PMT configuration with filter positions for wavelength dependent assays such as BRET
- Optional dual PMT configuration for higher throughput
- External injector module for dual-PMT, four injector configurations
- Isothermal temperature control in the plate chamber
- SoftMax Pro Software
- Optional automatic aperture switching between 96-well and 384-well plates
- Optional barcode reader to read labels on plates

The instrument can accommodate SBS-standard 96-well and 384-well plates with dimensions of 128.2 mm long, 86.0 mm wide, and heights from 14 mm to 15 mm.

PMT Technology

The instrument uses high-sensitivity, low-noise PMTs to detect emitted luminescence. PMTs convert incident photons into electrons through the photoelectric effect. When an incident photon impinges on the active surface of the PMT (the photocathode), an electron is generated. The electron flows through a series of electron multipliers (dynodes) to the anode. The amount of current that flows from the anode is directly proportional to the number of photons at the photocathode.

The amount of amplification that a PMT can produce depends on the number of dynodes in the PMT, and the voltage that is applied to it.

The PMTs in the instrument meet two essential criteria for high-quality detection. First, since different photocathodes have varying sensitivities to specific wavelengths of light (quantum efficiency), the PMTs have been optimized for the typical wavelengths of luminescent emission light. Second, the PMTs have exceptional reliability and optimal signal-to-noise performance.

PMT Modes

The instrument can read PMT values in the following modes:

Operating Modes

| Mode | Recommended Use | Benefits | Dynamic Range (RLU) | Target Calibration Wavelength Required |
|--------------------|---|--|--|---|
| Analog Only | Very bright assays. | Uses low PMT voltage to prevent PMT saturation | 1 x 10 ⁷ to 1 x 10 ⁹ | Yes |
| Photon Counting | Assays requiring high sensitivity with an average dynamic range. Use when non-calibrated data is desired. | Excellent discrimination between signal and background noise | 10 to 25 x 10 ⁶ | No |
| AutoRange | Assays requiring high sensitivity with a wide dynamic range. | High sensitivity and wide dynamic range | 10 to 2 x 10 ⁸ | Yes |
| MaxRange | Assays with massive dynamic range that can tolerate subsecond delays if the signal is saturated. | Widest dynamic range and high sensitivity | 10 to 1 x 10 ⁹ | Yes |

Chapter 2: Setting Up the Instrument



Before you unpack and set up the SpectraMax L, prepare a dry, flat work area away from direct sunlight, dust, drafts, vibration, and moisture. Remember to leave sufficient space for the instrument, computer, and required cables. See Instrument Specifications on page 47.

The package contains the instrument plus one or more boxes that contain accessories.

Package Contents for Base Instrument

| Illustration | Part Number | Description |
|----------------------|------------------------------|---|
| SoftMax Pro Software | Latest Version | SoftMax Pro Software installation guide with Product Key |
| | 4400-0002 or 4400-0036 | Region Specific Power cord, 115 V or Power cord, 230 V |
| F | 4200-0076 | Power supply adapter |
| 72 | 0700-0861 1-2100-0926 | USB cable, 2 meter (6.56 foot) |
| | 8500-1229 | Drawer pull tool |
| | 5060689 | Flat head screw driver |

Package Contents for Base Instrument Continued

| Illustration | Part Number | Description |
|--|-------------|--|
| | 8500-1559 | M2 Hex Wrench |
| Entered Section 1997 (1997) (1 | 9000-0133 | SpectraMax Mouse Pad |
| M00-0872 | 9000-0972 | Package of 5 sample microplates |
| | 2400-2464 | Dust Cover |
| | 5000505 | CE Declaration of Conformity |
| | 5019680 | Card, User Guide Download |
| | 0120-1319 | Datasheet, Innovative Solutions |
| | 0120-1437 | Datasheet, StakMax Microplate Handling System |
| | 0120-1477 | Datasheet, Microplate Reader Validation and Compliance |

Additional Package Contents for Other Instrument Configurations

| Additional Package Contents for Other | Part Number | Description |
|---------------------------------------|---------------------------|--|
| | 4400-0002 or 4400-0036 | Injector module Power cord, 115 V or Power cord, 230 V |
| | 4200-0900 | Injector module Power supply adapter |
| | 0310-5238 | Waste drain hose |
| 3, | 0700-0861 | Injector module communication cable |
| | 9000-0964 | 2 Reagent bottles, amber |
| | 9000-0965 | 2 Wash bottles, natural |
| | 8500-1072 | Wrench, Fitting, Upchurch |
| | 5004193 | 4 Set screws, 1/4-28 x 1/4", oval point |

Additional Package Contents for Other Instrument Configurations (continued)

| Illustration | Part Number | Description |
|--------------|-------------|------------------------------------|
| | 0310-5705 | Tip extractor |
| | 5000592 | Diagram, Assembly, Canopy and Tray |
| | 0310-5677 | Barcode reader |

For a complete list of the contents of the package, see the enclosed packing list.

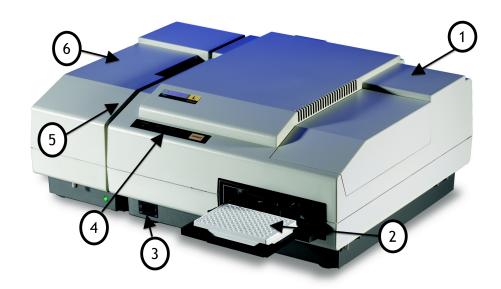
Unpacking the Instrument

The packaging is designed to protect the instrument during transportation. Retain the container and the packing materials for future transport needs. Do not use tools that can damage the packaging or the instrument.



CAUTION! When transporting the instrument, warranty claims are void if improper packing results in damage to the instrument.

The main instrument components are:



Instrument Components

| ltem | Name | Description |
|------|-----------------|---|
| 1 | Top cover | Cover for base configuration |
| 2 | Plate drawer | Houses the plate in landscape or portrait orientation |
| 3 | Power switch | Powers the instrument on and off |
| 4 | Control panel | Contains the instrument status lights and the Drawer button |
| 5 | Injector ports | Two-injector configuration |
| 6 | Injector module | Factory-installed injector module four-injector configuration |



WARNING! LIFTING HAZARD. To prevent injury, use a minimum of two people to lift the instrument.



CAUTION! Do not touch or loosen any screws or parts other than those specifically described in the instructions. Doing so may cause misalignment and voids the instrument warranty.

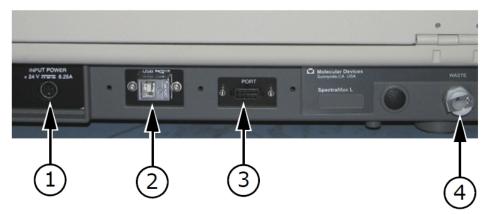
To unpack the instrument:

1. Check the box for visible damage that occurred during transportation. In case of damage, inform the supplier immediately and keep the damaged packaging.



CAUTION! Keep the box upright. Do not tip or tilt the box or place it on its side.

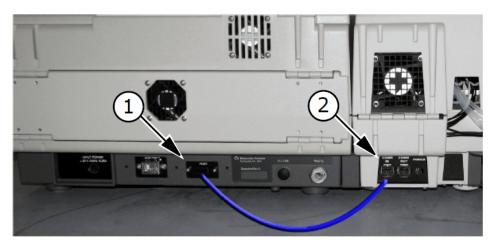
- 2. With the box facing up as indicated on the package, use a box cutter to carefully cut open the top of the box.
- 3. Remove the accessory boxes and lift the cardboard and foam packing materials above the instrument.
- 4. Lift the instrument, along with the plastic bag, up and out of the shipping box.
- 5. Remove the plastic bag and place the instrument on a level surface, near the controlling computer, with the back panel facing you.



Back Panel

| ltem | Name |
|------|------------------------------------|
| 1 | Electrical power port |
| 2 | Computer communication USB port |
| 3 | Injector module communication port |
| 4 | Injector waste port |

- 6. Insert the USB cable in the USB port on the back of the instrument and insert the other end in the USB port on the control computer.
- 7. Insert one end of the instrument power supply into the instrument power cord port and the other end into the power cord. Then plug the power cord into a grounded electrical outlet.
- 8. If the instrument configuration includes an injector module, insert one end of the injector module power supply into the injector module power port on the back of the injector module and the other end into the power cord. Then plug the power cord into a grounded electrical outlet.
- 9. Insert the square end (release tab up) of the injector module communication cable into the PORT on the back panel of the instrument and then insert the round end into the COMM IN port on the rear of the injector module.



Communication Cable Connection for Injector Module

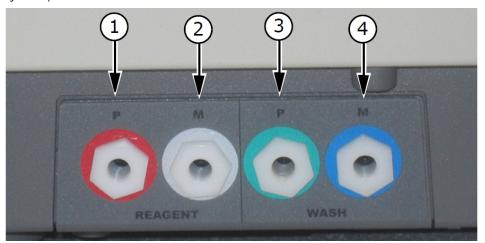
| Item | Name |
|------|---|
| 1 | PORT - Injector module communication port on instrument |
| 2 | COMM IN - Communication in port on injector module |

10. Turn the instrument around so that the control panel faces you. Ensure no cables run beneath the instrument. Leave sufficient space between the back of the instrument and the nearest objects or surfaces to allow you to lift the instrument hood and injector hood and to ensure proper ventilation and cooling.

Assembling the Injectors

The base instrument configuration does not include injectors. The instrument configuration can have several factory installed injector setups. For all instrument configurations that include injectors, the wash bottles and reagent bottles remain outside the instrument.

When the instrument configuration includes two injectors there is one pre-injector (P-injector) and one measurement injector (M-injector). The injectors are built into the instrument with injector ports located on the left side of the instrument.



Injector Ports - Base Configuration Instrument

| Item | Name | Description |
|------|-----------|--|
| 1 | P-Reagent | Connection for tubing from the P-reagent bottle. Controlled by P-injector commands in the SoftMax Pro Software. If plate settings include both a P-injection and a M-injection, the P-injection occurs before the M-injection. |
| 2 | M-Reagent | Connection for tubing from the M-reagent bottle. Controlled by M-injector commands. |
| 3 | P-Wash | Connection for tubing from P-wash bottle. Washes the P-injector from injector port through injector tip. Controlled by the P-Wash commands and automatically when you turn the instrument off. |
| 4 | M-Wash | Connection for tubing from M-wash bottle. Washes the M-injector from injector port through injector tip. Controlled by the M-Wash commands and automatically when you turn the instrument off. |

When the instrument configuration includes four injectors, the instrument has a factory-installed injector module on the left side. The injector module tubing is grouped into P-reagent, M-reagent, P-wash, and M-wash bundles. Injector module tubing exits the injector module through the rear of the instrument.

- 1. Before you connect the tubing, clean the wash bottles with an appropriate solvent.
- 2. For the base instrument configuration, connect the tubing to the P-Wash injector port on the instrument.
- 3. For the base instrument configuration, connect the tubing to the M-Wash injector port on the instrument.
- 4. For the base instrument configuration, connect one piece of tubing to the P-Reagent port and the other to the M-Reagent port on the instrument.
- 5. Connect the waste line drain hose to the Waste port on the instrument back panel. Place the other end of the waste line in a suitable waste container.



Note: Make sure the waste container is in a secure location and cannot spill.

Installing the Filters

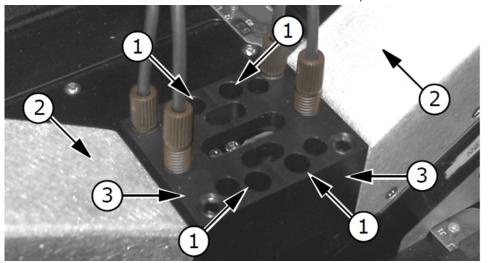
If you intend to run wavelength-dependent assays, perform the following steps to install filters in the instrument.

Required tools:

- M2 hex wrench
- Slotted screwdriver
- Powder-free latex or nitrile gloves

To install the filters:

- 1. Power off the instrument.
- 2. Loosen the captive screws on the front of the instrument and then raise the top cover up and over the instrument.
- 3. Remove the two hex screws that secure each detector to the instrument chassis. The screws are located inside the access holes in the detector adapter.



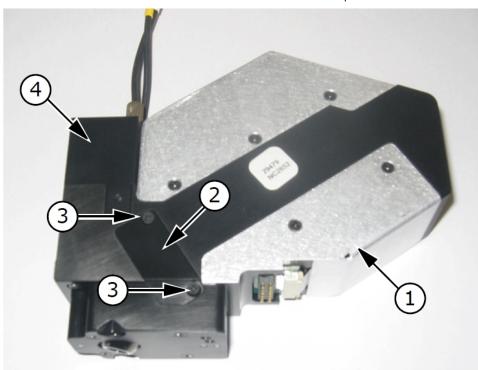


CAUTION! You must keep the PMT away from any direct light source. Light can damage the PMT and cause temporarily high background reads.

Detector Attached to Instrument Chassis

| Item | Name |
|------|-------------------------|
| 1 | Access holes for screws |
| 2 | Detector |
| 3 | Detector adapter |

4. Pull the detector away from the instrument chassis and then place it on a clean surface away from any direct source of light.



5. Loosen the two flat head screws that secure the filter cover plate to the detector.

Detector With Filter Cover Plate Attached

| Item | Name |
|------|--------------------|
| 1 | Detector |
| 2 | Filter cover plate |
| 3 | Flat head screws |
| 4 | Detector adapter |

- 6. Lift the filter cover plate off the detector and then remove the filter (or silver open ring) from inside the detector.
- 7. Insert the new filter into the detector, position the filter cover plate on the detector, and then tighten the captive screws.
- 8. Position the detector on the instrument chassis and then replace the screws that secure the detector to the instrument chassis.
- 9. Lower the top cover and then tighten the captive screws on the front of the instrument.

Installing the Barcode Reader

When your instrument configuration includes a barcode reader, the label on the plate must be in the landscape position.

To install the barcode reader:

- 1. Power off the instrument.
- 2. Loosen the captive screws on the front of the instrument and then raise the top cover up and over the instrument.
- 3. Insert the barcode reader ribbon into the slot on the right of the filters. Of the two available slots, insert the ribbon in the slot toward the front of the instrument.
- 4. Place the barcode reader into the opening in the instrument insulation such that the reader can read the plate in the drawer.
- 5. Lower the top cover and then tighten the captive screws on the front of the instrument.

Changing the Plate Aperture

The plate drawer is on the front right of the instrument. The instrument control panel has a DRAWER icon to open and close the plate drawer. You can also open and close the plate drawer from within the SoftMax Pro Software. See Opening the Drawer Manually on page 45.

The instrument can accommodate SBS-standard 96 and 384-well plates with dimensions of 128.2 mm long, 86.0 mm wide, and heights from 14 mm to 15 mm.

The instrument configuration has either an automatic plate aperture or a manual plate aperture to switch between 96-well plates and 384-well plates.

Automatic Aperture

For instruments with the automatic aperture, you do not need to change the aperture when you switch between 96- and 384-well plates. When you start a plate read the instrument automatically changes the aperture to match the plate type you select.

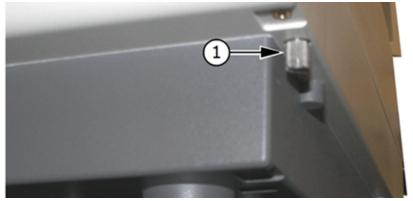
Manual Aperture

For instruments with the manual aperture, you must use the SoftMax Pro Software Change Aperture command when you switch between 96-well plates and 384-well plates and from 384-well plates to 96-well plates. The command instructs the reader to check whether a plate is in the drawer. If no plate is present, the plate carriage moves away from the read head so that you can manually change the aperture.

To manually change the aperture:

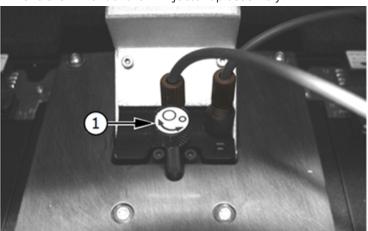
- 1. In the SoftMax Pro Software, select the Operation tab and click **Change Aperture** to display a message.
- 2. Remove any plate from the plate drawer.

3. Loosen the captive screws on the front of the instrument and raise the top cover.



Captive Screw Below Front of Instrument

- 4. Locate the aperture switch near the M-injector tip assembly. The position of the switch is indicated by the size of the circle directly in front of the M-injector tip assembly. The large circle on the dial of the switch is for 96-well plates; the smaller circle is for 384-well plates.
 - To select the 384-well plate, push down on the dial and rotate counter-clockwise until the small circle is in front of the M-injector tip assembly.
 - To select the 96-well plate, push down on the dial and rotate clockwise until the large circle is in front of the M-injector tip assembly.



Aperture Switch

5. Lower the top cover and tighten the captive screws.

Chapter 3: Getting Started



Now that you have unpacked the instrument, connected the injectors, and connected the instrument to the computer that runs the SoftMax Pro Software, you are ready to get started.

- 1. Power on the instrument switch located on the front panel of the instrument. All three LEDs on the instrument control panel illuminate and then one remains lit.
 - Green The startup was successful and the instrument is in the idle state.
 - Yellow The instrument is in busy state.
 - Red Fault condition. Turn the power switch off and then on again to reset the instrument. If the red LED remains lit, contact Molecular Devices Technical Support.
- 2. Power on the computer that runs the SoftMax Pro Software.
- 3. Connect the SoftMax Pro Software to the instrument. See the *SoftMax Pro Data Acquisition* and *Analysis Software User Guide* for more information.

Software Ribbon

In the SoftMax Pro Software, the SpectraMax L has a unique set of icons on the Ribbon. For a description of each setting, see the SoftMax Pro Software application help.

Home Tab

- Simulator Configuration When you operate the instrument in Offline or Simulator mode, use the Simulator Configuration dialog to define the instrument configuration for simulated protocols. You can use Offline and Simulator mode to define protocols that you save for use later. The settings for the protocols you save must be compatible with the instrument configuration of the instrument on which you intend to run the protocol.
- Instrument Configuration When you connect the computer to the instrument, use the Instrument Configuration dialog to view the instrument components that are available for protocol settings.

Operations Tab

- Wash Injectors Displays the Wash Injectors dialog. See Washing Injectors on page 29.
- Prime Injectors Displays the Prime Injectors dialog. See Priming Injectors on page 30
- Reverse Injectors Displays the Reverse Injectors dialog. See Reversing Injectors on page 30.
- **Injector Tubing** Displays the Injectors Pump Tubing dialog that you use to determine when to replace injector tubing and to calibrate the injectors. See Replacing Injector Tubing on page 38.
- Change Aperture Displays a message when the instrument is ready for you to change the plate aperture from 96-well plates to 384-well plates or vice-versa. The instrument configuration has either a manual aperture or an automatic aperture. For the manual aperture configuration you must use the Change Aperture command when you switch plate formats. See Changing the Plate Aperture on page 23.
- Shake Settings Displays the Shake Plate Settings dialog you use to custom define how to shake a plate. This shake process is independent of a protocol. When you create a protocol, you can define shake parameters on the Settings dialog.



Note: The Shake Settings icon on the Operations tab is independent of the Shake icon on the Home tab.

Instrument Calibration

Calibrate the instrument once a year for maximum performance. In the SoftMax Pro Software, select the **Operations** tab and click **Calibration** to display the Calibration dialog. There are two calibration methods:

- Select **Internal Light Emitting Diodes** to calibrate using the instrument's onboard LEDs. This method does not require sample preparation.
- Select Custom Multi-PMT Normalization Microplate when the instrument has multiple PMTs
 and you want to use the light spectrum from your own assays to calibrate the instrument,
 rather than the light spectrum from the factory configured LEDs.

Calibrating with Internal Light Emitting Diodes

To calibrate using the internal light emitting diodes:

- 1. Remove any plate from the plate drawer.
- 2. In the SoftMax Pro Software, select the **Operations** tab and click **Calibration** to display the Calibration dialog.
- 3. Select the Internal Light Emitting Diodes Calibration Method option.
- 4. Click **OK**. Calibration is done automatically.

Custom Multi-PMT Normalization Microplate

Create your own custom calibrations if you prefer to calibrate using the light spectrum from your assays rather than the light spectrum from the factory configured LEDs. Custom calibrations are used from the Settings dialog in the Sensitivity category. See Protocol Settings on page 31.

There are two different PMT Calibration Factor options:

- Select **Auto Calculation** to use your own assay materials for calibration and you want the software to automatically calculate a PMT calibration factor.
- Select **Manual Set** to use your own assay materials for calibration and you want to manually calculate the PMT calibration factor based on the signal obtained from each PMT.

Auto Calculation

The Auto Calculation option allows you to use your own assay materials for calibration and have the software automatically calculate a PMT calibration factor.

To use auto calculation:

- 1. Remove any plate from the plate drawer.
- 2. In the SoftMax Pro Software, select the **Operations** tab and click **Calibration** to display the Calibration dialog.
- 3. Select the Custom Multi-PMT Normalization Microplate Calibration Method option.
- 4. Click the **Custom Calibration #** drop-down and select the custom calibration to define. You can define two different custom calibrations.
- 5. Select the **Auto-Calculation** PMT Calibration Factor option.
- 6. In the **Dark Adapt Delay** field, enter the number of seconds to allow the sample to adapt to the dark interior of the plate drawer before the calibration starts. This allows the plate autophosphorescence to subside.
- 7. Click OK.
- 8. A message displays to tell you to insert a 96-well plate into the instrument with material of greater than 100k RLU in wells B2, G2, B11, and G11. After you insert the plate, click **OK**.

Manual Set

The Manual Set option allows you to use your own assay materials for calibration and manually calculate the PMT calibration factor based on the signal obtained from each PMT. You can determine these values by reading all or part of a plate that contains your assay material (should be greater than 100k RLU) and then determining a factor, or multiplier, for each PMT that equalizes the RLU values for each PMT.

To use manual calculation:

- 1. In the SoftMax Pro Software, select the **Operations** tab and click **Calibration** to display the Calibration dialog.
- 2. Select the Custom Multi-PMT Normalization Microplate Calibration Method option.
- 3. Click the **Custom Calibration #** drop-down and select the custom calibration to define. You can define two different custom calibrations.
- 4. Select the Manual Set PMT Calibration Factor option.
- 5. Enter the values for each available PMT from your calculations. Available fields are dependent the instrument configuration.
- 6. Click OK.

Chapter 4: Protocols



Use the SoftMax Pro Software to define the settings to run protocols on the instrument. The following is a high-level description of the basic workflow to read a plate in a SpectraMax L. For details about the SoftMax Pro Software, see the application online help.

Temperature Regulation

Use the SoftMax Pro Software to adjust the temperature of the plate chamber from 5° C to 45° C above the ambient room temperature. The temperature sensors detect the temperature of the air inside the chamber, not the temperature of the samples in the plate. If you use the instrument to warm the samples, use a seal on the plate to prevent evaporation of the sample. The seal also helps to maintain a uniform temperature. It can take an hour or more for a prepared sample to equilibrate inside the plate chamber. To speed up equilibration, warm the sample and the assay reagents before you place the plate in the chamber. Validate the data quality to determine whether the seal can stay on the plate for the read.

To change the plate chamber temperature:

- 1. In the SoftMax Pro Software, select the **Operations** tab and click **Temperature** to display the Temperature Control dialog.
- 2. Select **On** to turn on the incubator and enter the temperature.
- 3. Click OK.

Injector Protocols

Injector protocols typically require you to prime the injector, wash the injector, and reverse the injector. Injectors also require maintenance. See Replacing Injector Tips on page 36 and Replacing Injector Tubing on page 38.

Before you run injector protocols, follow the steps to set up the injectors. See Assembling the Injectors on page 19.

Washing Injectors

To wash the injectors:

- 1. In the SoftMax Pro Software, select the **Operations** tab and click **Wash Injectors** to display the Wash Injectors dialog.
- 2. Select a **Setting** option:
 - Select Auto and then select the check box for the Pre-Measurement Injectors (P)
 and/or the Measurement Injectors (M) to automatically wash all P-injectors and/or Minjectors.
 - Select Manual and then select the check box for each P-injector and each M-injector to wash
- 3. In the # of Injections Per Injector field, enter the number of 100- μ L injections to do per wash.
- 4. Select an option:
 - Select Wash From Wash Bottle to draw the wash liquid from the wash tubing.
 - Select Wash From Reagent Bottle to draw the wash liquid from the reagent tubing.
- 5. Select the **Empty Lines After Wash** check box to drain the wash lines.
- 6. Click **Wash** to flush the injector tubing and tips with fluid. The instrument shuts off after the wash completes.

Priming Injectors

Use the Prime Injectors dialog to fill the tubing with reagent, to remove air bubbles, and prepare the system for injection.

To prime the injectors:

- 1. In the SoftMax Pro Software, select the **Operations** tab and click **Prime Injectors** to display the Prime Injectors dialog.
- 2. Select a Setting option:
 - Select Auto and then select the check box for the Pre-Measurement Injectors (P)
 and/or the Measurement Injectors (M) to automatically prime all P-injectors and/or Minjectors.
 - Select Manual and then select the check box for each P-injector and each M-injector to prime.
- 3. In the **# of Injections Per Injector** field, enter the number of 100-μL injections to do per prime.
- 4. In the **P-Inj Prime Speed** field, enter the desired injector speed in μ L/second. M-injector prime speed is fixed at 230 μ L/s when you choose to prime the M-injectors.
- 5. Click Prime.

Reversing Injectors

Use the Reverse Injectors dialog to recover the reagent from the injector tubing. Reversing the injectors makes sure there is no reagent fluid left in the injector tubing, valves, and tips of the injection flow path. The reversal process also recovers reagent for future use.

To reverse the injectors:

- 1. In the SoftMax Pro Software, select the **Operations** tab and click **Reverse Injectors** to display the Reverse Injectors dialog.
- 2. Select a Setting option:
 - Select **Auto** and then select the check box for the **Pre-Measurement Injectors (P)** and/or the **Measurement Injectors (M)** to automatically reverse all P-injectors and/or M-injectors.
 - Select **Manual** and then select the check box for each P-injector and each M-injector to reverse.
- 3. In the **# of Injections Per Injector** field, enter the number of reverse 100-µL injections to do per injector.
- 4. Click **Reverse** to reverse the injection cycle pumps and return the reagent from the tubing to the reservoir.

Protocol Settings

Use the Settings dialog in the SoftMax Pro Software to define the instrument settings to read the plate. See the SoftMax Pro Software application help for details.

To define protocol settings:

- 1. Start the SoftMax Pro Software.
- 2. Select the SpectraMax L instrument.



Note: When you do not physically connect the computer to an instrument, you can select Offline or Simulator On to create documents for later use. Document settings are specific to the instrument that you select. If you change instruments, some settings may be reset to the default protocol settings.

- 3. In the Navigation Tree, select a Plate section.
- 4. Click in the Settings Information area of the Workspace or select the Home tab and click **Acquisition Settings** to display the Settings dialog.
- 5. Select the Lumi or Lumi2W read mode.
- 6. Select the **Endpoint**, **Dual Read**, **Kinetic**, or **Fast Kinetic** read type.
- 7. Select the **Plate Type** category.
 - a. Click the Plate Format drop-down and select 96-Wells or 384-Wells.
 - b. In the **Select Specific** list, select the plate.
- 8. Select the Read Area category.
 - a. Select the wells to read. Wells that will be read appear shaded dark.
 - To read all the wells in the plate, select the **Select All** check box.
 - To select a contiguous region, click and drag the cursor over the wells to read.
 - To select non-contiguous wells, press Ctrl and click the wells to read.
 - b. Click a well a second time to exclude the well from the read.

- 9. Select the **Sensitivity** category.
 - a. Click the PMT Settings drop-down:
 - Select Auto Range to combine the benefit of both Analog Only (at a higher range) and Photon Counting modes. PMT signals are simultaneously converted to both analog current and digital pulses to achieve high sensitivity and wide dynamic range.
 - Select **Analog Only** for very bright signals above 1.6 x 10⁷ RLU. PMT voltage is set low, and the measurement is based on current.
 - Select **Photon Counting** for very dim and medium signals below 25 x 10⁶ RLU. This allows discrimination between the signal and the noise generated in the dynode chain. PMT signals are converted into digital pulses, which can be measured above a set threshold.
 - Select **Max Range** to combine Auto Range and Analog Only to maximize the dynamic range (10 to 1 x 10⁹ RLU). Detection occurs in Auto Range mode until the crossover point, then switches to Max Range mode with its low PMT voltage setting when samples are extremely bright. Select for assays with massive dynamic range.
 - b. If you select Auto Range, Analog Only, or Max Range in the previous step, you must select the **Target Calibration Wavelength** option that most closely corresponds to the peak emission wavelength of your assay:



Note: The sensitivity of the photomultiplier tube (PMT) depends on the wavelength of the light. The instrument is calibrated at several wavelengths. Select the Target Calibration Wavelength that is close to your sample's emission wavelength for best RLU comparability. Note that if you want only specific wavelengths to be detected, you must install optical filters. See Installing the Filters on page 21.

- Select 395 nm (Acridinium Ester, DeepBlueC)
- Select 470 nm (Dual Firefly/Renilla Luciferase, Renilla Luciferase, CSPS, CDPstar, Aequorin, Vibrio Luciferase, AMPPD, Luminol, Coelentrazine
- Select 527 nm (Lumi Phos 530)
- Select **570 nm (Firefly Luciferase)**
- c. If you select Photon Counting, select a Correction Option:
 - Select **No Correction** if you do not want to apply a custom calibration.
 - Select **Custom Calibration** and click the **Custom Calibration** # drop-down and select **1** or **2**. One and two correspond to the user-defined custom calibrations that you define on the Calibration dialog. See Instrument Calibration on page 26.
- 10. Select the **Timing** category.
 - For the Endpoint read type, in the **Integration Time** field, enter the number of seconds required for well measurement.
 - For the Dual Read type, select Normal if the integration times for the read following the P-injection and the M-injection are the same and enter the number of seconds required for well measurement or select Separate if the integration times for the read following the P-injection and the M-injection are different.
 - For the Kinetic read type, enter the integration time, the total run time, and the interval.
 - For the Fast Kinetic read type, enter the integration time and the number of reads per well.

- 11. Select the **Shake** category.
 - a. Select the Before First Read check box to shake the plate after the injection and prior to the read.
 - b. Click the **Shake Type** drop-down.
 - Select **Classic** to shake the plate along the X-axis, alternating between fast and slow speed.
 - Select **Single** to shake the plate along the X-axis at a constant rate.
 - Select **Dual** to shake the plate in an L pattern.
 - Select **Orbital** to shake the plate in a circular pattern.
 - c. In the **Shake Duration** field, enter the number of seconds to shake the plate between 1 and 999 seconds.
 - d. In the **Shake Speed** field, enter how fast the plate is to shake in millimeters per second.
 - e. In the **Shake Diameter** field, enter the size of the shake diameter.
- 12. Select the Injection & Delay category.
 - a. Select the **P-Injection** check box to inject using P-injectors and/or select the **M-Injection** check box to inject using M-injectors. When you select either check box, an Injection Wells category appears in the category list.
 - b. For each injection you select, in the **P/M-Injector Volume** field, enter the volume of fluid to inject.
 - c. In the **Post Injection Delay** field, enter the number of seconds to delay between injection and reading.
 - d. In the **Injection Speed** field, enter the number of μL to dispense per second. The recommended injection speed for injections is at least 230 $\mu L/s$.
 - e. In the **Shake After Injection** field, enter the number of seconds to shake the plate after the injection and prior to measurement.
- 13. Select the **Injection Wells** category to select the wells to receive a P-injection and/or an M-injection.
 - a. Select **P Injector** and then select the wells to receive a P–injection. Click a well again to clear it.
 - b. Select **M Injector** and then select the wells to receive a M-injection. Click a well again to clear it.
- 14. Select the **PMT Selections** category and then select an option:
 - Select Default PMT Selection to use all onboard PMTs to do the read.
 - Select Manual PMT Selection and then select the check box for each PMT to use for the read.
- 15. Select the **More** category.
 - a. Select the **Dark Adapt** check box to delay the read for the number of minutes you enter in the **Delay** field. This allows the plate autophosphorescence to subside before the read begins, reducing background.
 - b. When the instrument has a barcode reader, the following options are available:
 - Select the Append Barcode to Section Name check box to add the barcode to the Plate section name in the results.
 - Select the **File Name Options** check box to include the barcode in the file name and then select one of the following options:
 - Select Append Barcode to File Name to append the barcode to the file name.
 - Select **Overwrite File Name with Barcode** to overwrite the file name with the barcode.
- 16. After you define the protocol settings, click **OK** to close the Settings dialog.

- 17. Create a template, if applicable.
- 18. Set Data Reduction parameters, if applicable.
- 19. Set Display options, if applicable.
- 20. Click .
- 21. Save the data to a data file and save the settings to a protocol file.
- 22. After you complete an injector protocol:
 - a. Reverse the injectors. See Reversing Injectors on page 30.
 - b. Place the tubing from the reagent bottles in a container of deionized water.
 - c. Wash the injectors as recommended by the assay vendor. See Washing Injectors on page 29.

Powering Off the Instrument

When you power off the instrument, the shutdown process automatically clears the pumps and fluid lines to prepare the instrument for future use.

- The injectors are reversed. This makes sure there is no reagent fluid left in the injector tubing, valves, and tips of the injection flow path.
- The instrument performs an AutoWash to flush wash fluid through the injectors and fluid lines, if wash bottles are attached.
- The plate drawer is placed in the lock position to prevent movement of the drawer if you relocate the instrument.

Chapter 5: Maintenance



Perform only the maintenance tasks described in this guide. Contact a Molecular Devices service engineer to inspect and perform a preventive maintenance service on the instrument each year.

Before operating the instrument or performing maintenance operations, make sure you are familiar with the safety information in this guide. See Safety Information on page 5.

Keep the plate drawer closed when the instrument is not in use. Always close the drawer before you power off the instrument.



CAUTION! Maintenance procedures other than those specified in this guide must be performed by Molecular Devices. When service is required, contact Molecular Devices technical support.



CAUTION! Never touch the optic mirrors, lenses, filters, or cables. The optics are extremely delicate, and critical to the function of the instrument.

Cleaning the Instrument



WARNING! BIOHAZARD. It is your responsibility to decontaminate components of the instrument before you request service by a service engineer, or you return parts to Molecular Devices for repair. Molecular Devices does not accept items that have not been decontaminated where applicable to do so. If parts are returned, they must be enclosed in a sealed plastic bag that states that the contents are safe to handle and are not contaminated.



WARNING! BIOHAZARD. Always wear gloves when operating the instrument and during cleaning procedures that could involve contact with either hazardous or biohazardous materials or fluids.

Do the following before you clean equipment that has been exposed to hazardous material:

- Contact the applicable Chemical and Biological Safety personnel.
- Review the Chemical and Biological Safety information contained in this guide. See Chemical and Biological Safety on page 7.

Always turn the power switch off and disconnect the power cord from the main power source before using liquids to clean the instrument.

Replacing Injector Tips

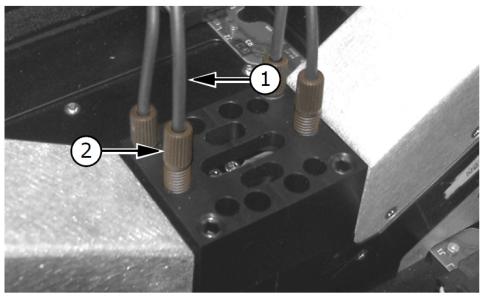
Replace the injector tips if performance deteriorates or you suspect contamination. The instrument can use either metal or plastic tips.

Required tools: Tip Extractor



Tip: Instruments with the automatic aperture switch option can use either a metal or plastic injector tip. The metal tip is recommended unless the assay requires a metal-free path.

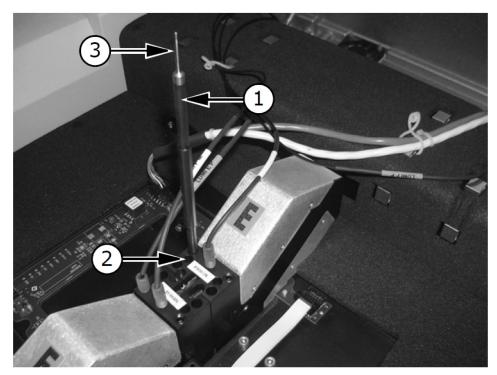
- 1. Power off the instrument.
- 2. Loosen the captive screws on the front of the instrument and raise the top cover up and over the instrument.
 - **Tip:** If the captive screws are too tight to loosen by hand, use a slotted screwdriver.
- 3. Locate the tubing for the injector, unscrew the fitting by turning it counter-clockwise, and then twist the tubing assembly off the adapter.



Injector and Tubing Assembly

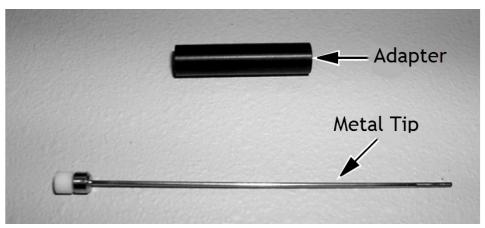
| ltem | Name |
|------|------------------|
| 1 | Injector tubing |
| 2 | Injector fitting |

- 4. Remove the injector tip.
 - Metal Insert the magnetic end of the tip extractor into the injector port and then pull the injector tip and attached adapter out of the port.
 - Plastic Insert the pointed end of the tip extractor into the injector port and then pull the
 adapter out of the port. Insert the tip extractor into the injector port a second time to
 remove the tip.



Metal Injector Tip and Adapter

| Item | Name |
|------|---|
| 1 | Tip extractor – end for metal tip inserted in adapter |
| 2 | Injector port |
| 3 | Pointed end of tip extractor to remove plastic tips |

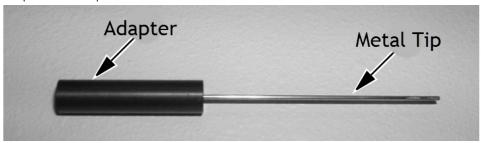


Metal Tip and Adapter



Plastic Tip and Adapter

- 5. Replace the injector tip.
 - Metal Install the replacement injector tip in the adapter. Make sure the white end of the injector tip fits inside the injector adapter.
 - Plastic Insert the plastic injector tip into the injector port, then insert the injector adapter into the port.



Assembled Metal Tip and Adapter

- 6. Tighten the fitting in the injector port. It might be necessary to grip the injector tubing while tightening the fitting because there is friction between the two.
- 7. Lower the top cover and tighten the captive screws.

Replacing Injector Tubing

To deliver reagent, the injectors use peristaltic pumps. Peristaltic pump systems use rotors with rollers that compress and relax flexible tubing. Reagent enters the relaxed tubing and is then pushed through as the rotating roller compresses the tubing. Alternate relaxing and compressing of the tubing results in a continuous stream of reagent passing through the tubing.



Note: Over long periods of injector use, the elasticity of the tubing may decline and the volume delivered per pump turn may become smaller.

The dispense accuracy should be periodically verified by running the gravimetric tubing calibration procedure. When you replace worn out tubing, you should perform a before and after calibration to ensure consistent results.

When not in use for long periods of time, open the stabilizer lids on top of the pumps to relieve compression on the tubing. This helps to retain uniformity of the tubing and ensure repeatability. Before use, make sure you close the stabilizer lids.

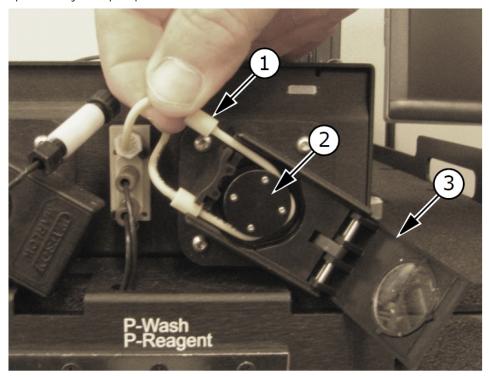
The SoftMax Pro Software records the volume injected by each injector. Select the Operations tab and click **Injector Tubing** to display the Injector Pump Tubing dialog. At 75,000,000 μ L (approximately 3,800 96-well plates) the SoftMax Pro Software displays a message to recommend replacement of the injector tubing.

Required equipment:

- Precision electronic scale
- 96 and 384-well plates
- Filtered deionized water
- Two clean laboratory bottles

To replace the injector tubing:

- 1. Loosen the captive screws on the front of the instrument and raise the top cover up and over the instrument.
- 2. Open the injector pump access door.

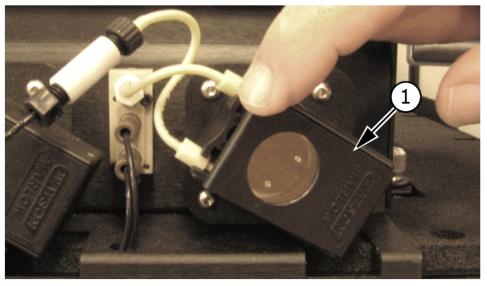


Injector Pump With Access Door Opened

| ltem | Name |
|------|---------------------------|
| 1 | Retaining stop |
| 2 | Pump wheel |
| 3 | Injector pump access door |

- 3. To remove the tubing from the pump housing, pull the retaining stop away from the injector, and then disconnect the ends of the tubing from the barbed fittings.
- 4. Loop the portion of the tube located between the retaining stops around the pump wheel. Stretch the pump tubing so that it is held in place by the retaining stops. Make sure the tube is completely around the pump wheel.

5. Close the injector pump access door.



Injector Pump With Access Door Closed

- 6. Connect the tubing to the barbed fittings.
- 7. Lower the top cover, and then tighten the captive screws.

Prepare the Instrument

To prepare the instrument after you replace the injector tubing:

- 1. Connect the tubing for the P–reagent bottle and M–reagent bottle to the ports on the left side of the instrument.
- 2. Fill a bottle with filtered deionized water, and then place the other ends of the tubing into the bottle.



CAUTION! Do not mix the waste and the input water bottles. Doing so can contaminate the instrument injector plumbing.

- 3. Connect the waste line to the Waste Port on the back panel. Place the other end of the line in a waste bottle.
- 4. Power on the instrument.
- 5. Power on the control computer.
- 6. Click Start > Programs > Molecular Devices > SoftMax Pro.
- 7. Prime the injectors. See Priming Injectors on page 30.
- 8. Prime the injectors two more times to check for leaks at the reagent ports.

Injector Accuracy Test

This gravimetric method tests the accuracy of the injection volume. The method is based on the weight of water dispensed from the injector.

Weigh the plate using an electronic balance with an accuracy of 0.01 grams. Because water injected into the plate evaporates, the plate should be sealed following the injection process. Seal the plate with a commercially available plate sealing tape. Label tape can be used if plate sealing tape is not available.

To test injector accuracy:

- 1. Prime the injectors. See Priming Injectors on page 30.
- 2. Prime the injectors two more times.
- 3. Measure and record the weight of an empty 96-well plate. Include the plate sealing material in the weight.
- 4. Put the weighed plate into the instrument.
- 5. In the SoftMax Pro Software, select a Plate section in the Navigation Tree and click who display the Settings dialog.
- 6. Select the **Endpoint** read type.
- 7. Select the **Plate Type** category and select the **96-well Standard** plate.
- 8. Select the **Read Area** category and select the **Select All** check box.
- 9. Select the Integration Time category and enter an Integration Time of 1 second.
- 10. Select the **Sensitivity** category.
- 11. Click the **PMT Setting** drop-down and select **AutoRange**.
- 12. For the Target Calibration Wavelength, select the 395 nm option.
- 13. Select the **Injection & Delay** category.
- 14. Select the **M–Injection** check box and enter the following injection parameters.
 - M-Injector Volume (µL) 50
 - Injection Speed (µL/s) 230
- 15. Click **OK** to close the Settings dialog.
- 16. Select the Home tab and click to start the M-injections.
- 17. When the injections finish, remove, seal, and weigh the plate. Record the weight and then determine the weight difference between the empty plate and the plate after the injections. The weight difference must be 4.80 grams +/- 0.05 grams. If the measured weights recorded from the above steps are not within 4.80 grams +/- 0.05 grams, you should calibrate the injectors.
- 18. Repeat the above steps for P-injection.
 - Again, the weight difference must be 4.80 grams +/- 0.05 grams. If the measured weights recorded from the above steps are not within 4.80 grams +/- 0.05 grams, you should calibrate the injectors.

Calibrate Injectors

To calibrate the injectors:

- 1. In the SoftMax Pro Software, select the Operations tab and click **Injector Tubing** to display the Injectors Pump Tubing dialog.
- 2. Click the **Associated PMTs** drop-down and select PMT pair to display the following information.
 - Remaining Life % Displays the life left before recommended tubing replacement.
 - Steps Per μL Displays the specific value for each peristaltic tube, calculated by completing step 3 below.
 - Recommended Life Vol μL Displays the fixed value determined by the SoftMax Pro Software.
 - Delivered Vol µL Displays the volume of fluid injected since the last reset.
 - Reset to 0 µL Click to reset the Delivered Vol to 0.
- 3. Calculate the Steps per μ L for each injector using the formula Steps per μ L = 4.8 grams / actual weight (determined from the injector accuracy test above).
- 4. Enter the Steps Per μ L for the M-injectors and/or the P-injectors as calculated in the previous step.
- 5. Click **Reset to 0** μ **L** for the M-injectors and/or the P-injectors.
- 6. Click OK.
- 7. Close the SoftMax Pro Software.
- 8. Power off the instrument, wait five seconds, and then power on the instrument.

Chapter 6: Before You Move the Instrument



When you move the instrument from one location to a new location, there are several things you must do to prevent damage to the instrument.



WARNING! BIOHAZARD. It is your responsibility to decontaminate components of the instrument before you request service by a service engineer or you return parts to Molecular Devices for repair. Molecular Devices does not accept items that have not been decontaminated where applicable to do so. If parts are returned, they must be enclosed in a sealed plastic bag that states that the contents are safe to handle and are not contaminated.



WARNING! LIFTING HAZARD. To prevent injury, use a minimum of two people to lift the instrument.



CAUTION! When transporting the instrument, warranty claims are void if improper packing results in damage to the instrument.

Before you power off the instrument, do the following to prepare the instrument for a move:

1. Remove any plate from the plate drawer, if present.



CAUTION! Always turn off power to the instrument using the power switch. Do not shut down power by unplugging the main power cable from the wall outlet. Doing so can damage the instrument.

2. Use the switch on the front of the instrument to power off the instrument.



Note: During the shutdown sequence the instrument carriage is locked to prevent damage during transit.

- 3. Make sure that the SoftMax Pro Software is not running and turn off the connected computer. Then disconnect the computer cable from the rear of the instrument and from the computer.
- 4. Unplug the power cord from the rear of the instrument and from the wall outlet.
- 5. Remove the waste line.
- 6. Remove the communication cable from the injector module.
- 7. Store the power cord, cables, and waste tubing in the accessories tool box.
- 8. If you plan to store the instrument, ship the instrument, or transport the instrument to a different building, pack the instrument in the original packaging. See Packing the Instrument on page 44.
- 9. Make sure that the new location is a dry, flat work area that has sufficient space for the instrument and required cables. See Instrument Specifications on page 47.

Packing the Instrument

To minimize the possibility of damage during storage or shipment, the instrument should be repacked in the original packaging materials.



CAUTION! When transporting the instrument, warranty claims are void if improper packing results in damage to the instrument.

Do all steps in Before You Move the Instrument on page 43 before you proceed with the following procedures. Correct packaging of the instrument also includes applicable decontamination procedures.



CAUTION! Keep the box upright. Do not tip or tilt the box or place it on its side.

The instrument should be stored in a dry, dust-free, environmentally controlled area. For more information about acceptable storage environments, see Instrument Specifications on page 47.



WARNING! LIFTING HAZARD. To prevent injury, use a minimum of two people to lift the instrument.

Do the following to pack the instrument:

- 1. Store the power cords, USB cable, injector module communication cable, and waste tubing in the instrument accessories box.
- 2. Wrap the instrument in static-free plastic.
- 3. Lift the instrument and place it into the shipping container.



CAUTION! Keep the instrument upright and level when lifting. Do not tip or shake the instrument to prevent damage to the moving components inside the instrument.

- 4. Replace the molded foam packaging and then the cardboard insert with additional foam packaging above the instrument.
- 5. Place the accessories tool box in the space in the foam packaging above the instrument.
- 6. Close the box and seal it with packing tape.

Chapter 7: Opening the Drawer Manually



Do the following if the plate drawer fails to open when you press the Drawer button or when you click Open Drawer in the SoftMax Pro Software.

Required tool: Carriage puller

- 1. Power off the instrument, wait five seconds, and then power on the instrument. If the plate drawer does not open, complete the following steps.
- 2. Power off the instrument.
- 3. Loosen the captive screws on the front of the instrument, and then raise the top cover up and over the instrument.
- 4. Turn the plate carriage release knob clockwise until it stops. The plate carriage-release knob is located at the right rear of the instrument.

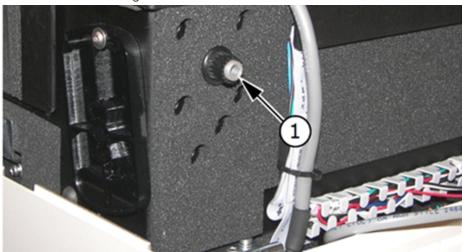


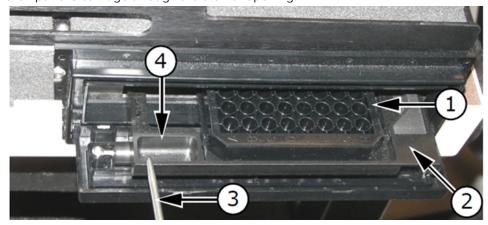
Plate Carriage Release Knob

5. Gently push on the bottom of the drawer, and then insert the carriage puller between the top of the drawer and the instrument. Pull the drawer open enough to insert the carriage puller on the left side of the drawer.



CAUTION! Do not hook the carriage puller onto the plate itself.

6. Insert the end the carriage puller in the pull point on the left side of the plate carriage, and then pull the carriage through the drawer opening.



Pull Point On Plate Carriage

| ltem | Name |
|------|-----------------|
| 1 | Plate |
| 2 | Plate drawer |
| 3 | Carriage puller |
| 4 | Pull point |



Note: If you are unable to open the plate drawer after you perform this procedure, contact your local Molecular Devices service representative.

7. Lower the top cover and tighten the captive screws.

Obtaining Support

Molecular Devices is a leading worldwide manufacturer and distributor of analytical instrumentation, software, and reagents. We are committed to the quality of our products and to fully supporting our customers with the highest level of technical service.

Our Support website, support.moleculardevices.com, has a link to the Knowledge Base, which contains technical notes, software upgrades, safety data sheets, and other resources. If you still need assistance after consulting the Knowledge Base, you can submit a request to Molecular Devices Technical Support.

You can contact your local representative or Molecular Devices Technical Support at 800-635-5577 x 1815 (North America only) or +1 408-747-1700. In Europe call +44 (0) 118 944 8000.

To find regional support contact information, visit www.moleculardevices.com/contact.

Appendix A: Instrument Specifications



Technical specifications are subject to change without notice. Molecular Devices provides validation documentation for software and hardware, as well as absorbance, fluorescence, and luminescence detection test tools with its SpectraTest® solutions. The SpectraTest line of microplate reader validation packages provide automated and comprehensive validation of a microplate reader's optical performance.

Validation Packages Part Numbers

| Part Number | Item Name | Compatible Instruments |
|----------------|---|---|
| 0200- 6191 | SpectraTest ABS2 Absorbance Validation Plate | FlexStation 3, SpectraMax ABS, SpectraMax ABS Plus, SpectraMax i3, SpectraMax i3x, SpectraMax iD3, SpectraMax iD3s, SpectraMax iD5, SpectraMax iD5e, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, SpectraMax M5e, SpectraMax Plus 384 |
| 0200- 5060 | SpectraTest FL1 Fluorescence Validation Plate | FlexStation 3, Gemini EM, Gemini XPS, SpectraMax i3, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5s, SpectraMax iD5e, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, SpectraMax M5e |
| 0200- 6186 | SpectraTest LM1 Luminescence Validation Plate | FlexStation 3, SpectraMax i3, SpectraMax i3x, SpectraMax iD3, SpectraMax iD3s, SpectraMax iD5, SpectraMax iD5e, SpectraMax L, SpectraMax M3, SpectraMax M4, SpectraMax M5, SpectraMax M5e |
| 0200- 2420 | Cuvette Absorbance Validation Set | SpectraMax ABS Plus, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, SpectraMax M5e, SpectraMax Plus 384 |
| 0200- 7200 | Multi-Mode Validation Plate | FilterMax F3, FilterMax F5, SpectraMax i3*, SpectraMax i3x*, SpectraMax iD5*, SpectraMax iD5e*, SpectraMax Paradigm * Specific read modes or cartridges. |

Table A-1: Instrument Specifications

| Performance | Base Configuration | Multiple Detectors |
|--------------------------------|--|--|
| Environment | Indoor use only | Same as base configuration |
| Altitude Restrictions | Up to 2000 m (6562 ft) | Same as base configuration |
| Detector | Low-noise photomultiplier tube with simultaneous photon counting and analog mode | Same as base configuration |
| Spectral range | 380-630 nm | Same as base configuration |
| Limit of detection | < 20 attomol ATP per well | Same as base configuration |
| Dynamic range | > 9 orders of magnitude | Same as base configuration |
| Crosstalk | 96-well < 10 ⁻⁶ (black plate), < 3 × 10 ⁻⁵ (white plate) | 96-well < 10 ⁻⁶ (black plate), < 5 × 10 ⁻⁵ (white plate) |
| Heater temperature range | Ambient + 5° C to 45° C (41° F to 113° F) | Same as base configuration |
| Ambient operating temperature | 15° C to 40° C (59° F to 104° F) | Same as base configuration |
| Humidity restrictions | 0% to 70% (non-condensing) | Same as base configuration |
| Plate Orientation | Landscape and Portrait | Same as base configuration |
| Read modes | Endpoint, Dual Read, Kinetic, Fast Kinetic, Dual Wavelength | Same as base configuration |
| Compatibility | Base Configuration | Multiple Detectors |
| Microplates | All 96 and 384-well plates with standard SBS footprints (128.2 mm by 86.0 mm) and heights from 14 mm to 15 mm | Same as base configuration |
| Injectors | Base Configuration | Multiple Detectors |
| Injectors | Can optionally include 2 variable-volume, Watson-Marlow 400F/A pumps with spring loaded tracks for precision, extended tube life, and easy Santoprene tube loading | Same as base configuration with total of 4 injectors |
| Volume | 10 to 100 μL in 1 μL increments | Same as base configuration |
| Accuracy | ±1 µL all deliveries of requested volume | ±1 µL ± 2 % |

Table A-1: Instrument Specifications (continued)

| Performance | Base Configuration | Multiple Detectors |
|-------------------------------|--|--|
| Precision | $\pm 1~\mu L$ all deliveries of requested volume | ±1 µL ± 2 % |
| Physical | Base Configuration | Multiple Detectors |
| Size | 22 cm (8.75 inches) high by 42 cm (16.5 inches) wide by 43 cm (17 inches) deep | 57 cm (22.5 inches) wide for two (2) PMTs with injectors |
| Weight | 16.4 kg (36 lb) | 18.2 kg (40 lb) for two (2) PMTs with injectors |
| Line voltage and frequency | Input Adapter: 100–240VAC, 2A, 50/60Hz Output: 24VDC, 6.25A | Additional input adapter for injector module Output: 24VDC, 1.5 A |

Electromagnetic Compatibility

Regulatory Information for Canada (ICES/NMB-001:2020)

This ISM device complies with Canadian ICES-001.

Cet appareil ISM est confomre à la norme NMB-001 du Canada.

ISM Equipment Classification (Group 1, Class A)

This equipment is designated as scientific equipment for laboratory use that intentionally generate and/or use conductively coupled radio-frequency energy for internal functioning, and are suitable for use in all establishments, other than domestic and those directly connected to a low voltage power supply network which supply buildings used for domestic purposes.

Contact Us

Phone: +1-800-635-5577
Web: moleculardevices.com
Email: info@moldev.com

Visit our website for a current listing of worldwide distributors.

