SpectraMax® L Luminometer

User Guide

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Molecular Devices

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Contents

Foreword

The foreword describes the intended audience for the manual. It defines the typographical conventions used in the manual and lists the related documentation.

Intended Audience

This manual is written for the SpectraMax® L Luminescence Microplate Reader user. It contains the information required to install, configure, and optimize the instrument, and to perform maintenance and troubleshooting procedures.

Conventions

Within the scope of this manual, the following typographical conventions are used:

WARNING! A warning indicates an operation that may cause personal injury if precautions are not followed.

CAUTION! Indicates an operation that may cause damage to the instrument, device, or data, if the precautions are not followed.

Tip! Provides useful information that helps apply the techniques and procedures in the text to your specific needs, and provides shortcuts, but is not essential to the completion of a procedure.

Note: Provides essential information for the completion of a procedure.

Related Documentation

The customer documentation for the SpectraMax L Luminescence Microplate Reader includes the *Formula Reference Guide for the SoftMax® Pro Software*, and the *SoftMax® Pro User Guide,* both included with the SoftMax Pro Software.

1 Introduction

The SpectraMax® L Luminescence Microplate Reader is a high-performance microplate reader that measures luminescence in 96 and 384-well microplates. The SpectraMax L Luminescence Microplate Reader with the variable-volume injectors option (10 µL to 100 µL) 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.

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

The extreme flexibility and high sensitivity of the SpectraMax L Luminescence Microplate Reader is ideal for applications within the fields of biochemistry, cell biology, immunology, molecular biology, and microbiology. Typical applications include luciferase reporter gene, ATP, Ca^{2+}, aequorin, and ELISAs

PMT Technology

The SpectraMax L Luminescence Microplate Reader uses high-sensitivity, low-noise PMTs to detect the emitted luminescent light. 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 SpectraMax L Luminescence Microplate Reader 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 SpectraMax L Luminescence Microplate Reader can read PMT values in four modes: Analog Only, Photon Counting, AutoRange, and MaxRange.

- Analog Only: Ideal for very bright signals above 1.6 x 10^7 RLU. PMT voltage is set low, and the measurement is based on current.
- Photon Counting: Ideal for very dim and medium signals below 25 x 10^6 RLU as it 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.
- AutoRange: Combines the benefits of both analog (at a higher range than is used for Analog Only) and Photon Counting modes. PMT signals are simultaneously converted to both analog current and digital pulses, achieving high sensitivity and wide dynamic range.
- MaxRange: Combines AutoRange and Analog Only modes to take advantage of the detection capability of each mode and maximixze the dynamic range (10 to 1 x 10^9 RLU). Detection occurs in AutoRange mode until the crossover point, then switches to MaxRange mode with its low PMT voltage setting when samples are extrememly bright.

The following table summarizes the benefits of each mode.

Table 1-1 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 sub-second delays if the signal is saturated.	Widest dynamic range and high sensitivity	10 to 1 x 10^9	Yes

Instrument Features

- Greater than nine orders of dynamic range
- Reads both 96 and 384-well microplates
- Dual injection into both 96 and 384-well microplates
- Programmable injection speeds
- AutoWash flushing of tubing and tips when the instrument is turned off
- StakMax® Microplate Handling Systemand robot compatible
- Landscape and portrait plate orientation
- Optional dual PMT configuration with filter positions for instrument wavelength dependent assays such as BRET
- Optional six PMT configuration for higher throughput
- Optional external injector modules for dual-PMT and six-PMT configurations
- Isothermal temperature control in the microplate chamber
- Windows and Macintosh compatible SoftMax® Pro Software
- Optional automatic aperture switching between 96 and 384-well microplates

Instrument Components

The main components of the SpectraMax L Luminescence Microplate Reader are:

- Top cover
- Microplate drawer
- Control Panel
- Injector Ports
- External injector modules (optional)
- Back panel

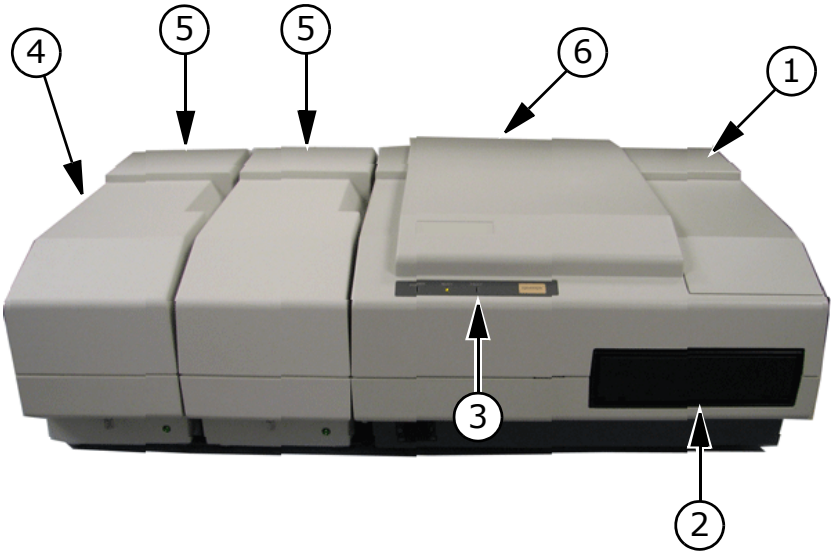


Figure 1-1 Instrument components

Item	Name	Description
1	Top cover	Cover for base configuration
2	Microplate drawer	Houses the microplate in landscape or portrait orientation
3	Control panel	Contains the instrument status lights and the Drawer button
4	Injector ports	Located on the left side of the instrument. Contains the wash bottle and reagent ports (base configuration instruments only)
5	External injector module (optional)	Contains optional additional injectors
6	Back panel	Contains the power and communication connections, the waste port, and the power switch

WARNING! Shock Hazard! Do not remove the top cover. Operating the instrument with the top cover removed exposes the user to high voltage.

The SpectraMax L Luminescence Microplate Reader can accommodate SBS-standard 96 and 384-well microplates with dimensions of 128.2 mm long, 86.0 mm wide, and heights from 14 mm to 15 mm.

Control Panel

The control panel houses the instrument status LEDs and the microplate DRAWER button.

Table 1-2 Instrument status LEDs

Item	Description
Green LED	The instrument is in idle state.
Yellow LED	The instrument is in busy state.
Red LED	Fault condition. The instrument must be reset by turning the power switch off and on again. If the red LED remains lit following the reset, contact Molecular Devices Technical Support.
DRAWER button	Opens and closes the microplate drawer when the instrument is in idle state. The microplate drawer will not operate when the yellow LED is lit.

Injector Ports

The injector ports, located on the left side of the instrument (base configuration), are grouped into Reagent ports and Wash ports. Each group includes a pre-injection (P) and a measurement (M) port. See Figure 2-4 on page 20.

Table 1-3 Reagents ports

Port	Description
P	Connection for tubing from P–reagent bottle
M	Connection for tubing from M–reagent bottle

Table 1-4 Wash ports

Port	Description
P	Connection for tubing from P–wash bottle
M	Connection for tubing from M–wash bottle

External Injector Modules

The SpectraMax L Luminescence Microplate Reader can be configured with one or two optional external injector modules. The modules are located adjacent to the instrument on the left side. They provide the additional space necessary to house the injector pumps. With external injector modules, tubing is grouped into P-reagent, M-reagent, P-wash, and M-wash bundles.

Back Panel

The back panel contains the power and communication ports, and the connection for the waste tube. See Figure 2-1 on page 16.

The panel includes a label providing the SpectraMax L Luminescence Microplate Reader serial number and line voltage. The serial number is required when contacting Molecular Devices Technical Support.

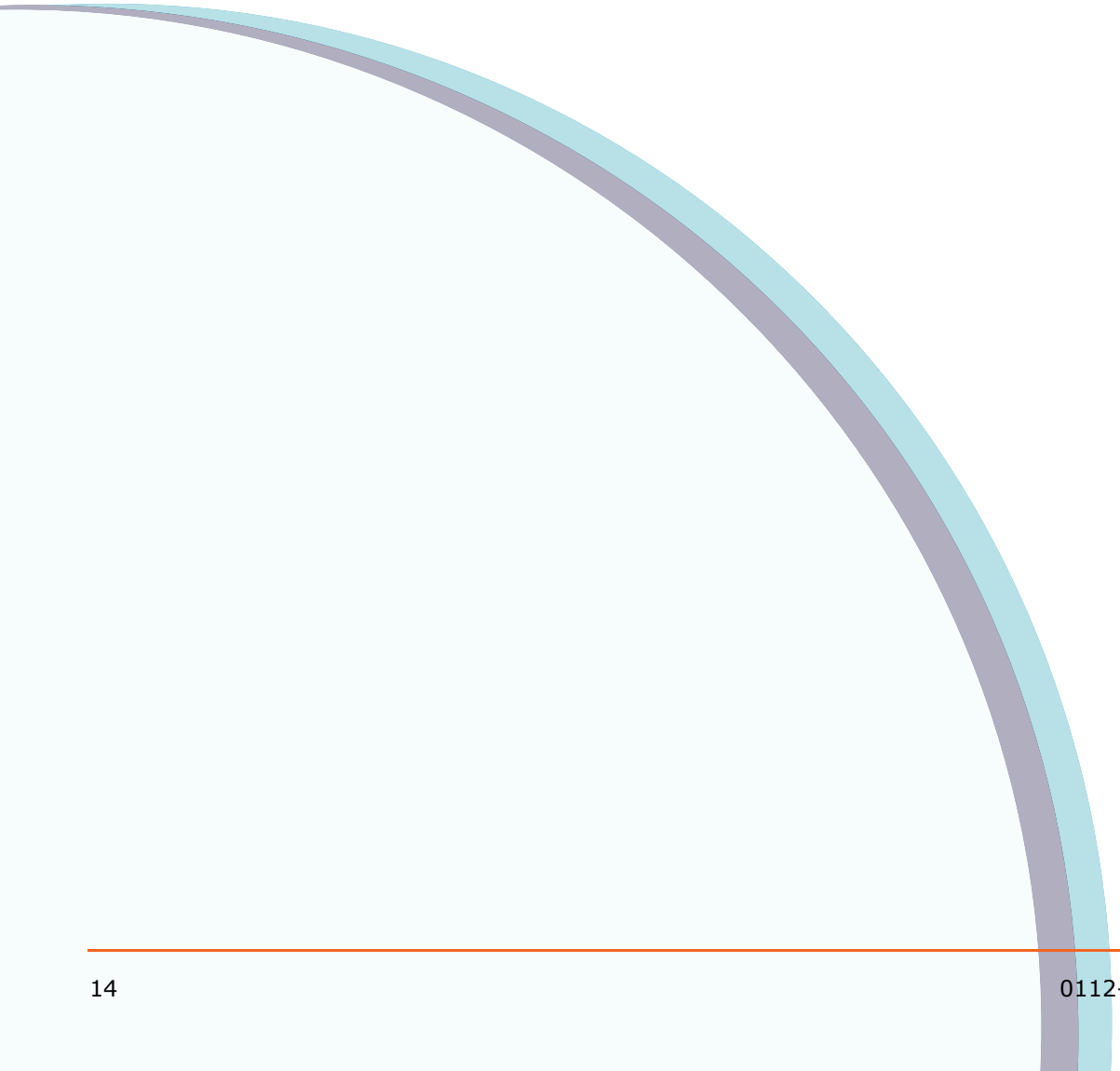
Instrument Specifications

Table 1-5 Instrument Specifications

Performance	Base Configuration	Multiple Detectors
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	Same as base configuration

Table 1-5 Instrument Specifications

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 microplates 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	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 or 12
Volume	10 to 100 µL in 1 µL increments	Same as base configuration
Accuracy	±1 µL all deliveries of requested volume	±1 µL ± 2 %
Precision	±1 µL all deliveries of requested volume	±1 µL ± 2 %
Physical	Base Configuration	Multiple Detectors
Size	8.75 inches (22 cm) high by 16.5 inches (42 cm) wide by 17 inches (43 cm) deep	• 22.5 inches wide for two (2) PMTs with injectors • 28.5 inches wide for six (6) PMTs with injectors
Weight	36 lb (16.4 kg)	• 40 lb (18.2 kg) for two (2) PMTs with injectors • 45 lb (20.4 kg) for six (6) PMTs with injectors
Line voltage and frequency	Input Adapter: 100−240VAC, 2A, 50/60Hz; Output: 24VDC, 6.25A	Additional input adapters for external injector modules: Output: 24VDC, 1.5 A



Installation

2

The SpectraMax® L Luminescence Microplate Reader should be unpacked and in position on a level surface before performing any of the installation procedures. Perform the procedures in the following order:

- Unpacking the instrument
- Installing the Wash Bottles, Reagent Containers, and Waste Bottle
- Installing the Software
- Preparing the Instrument for Use

Unpacking the instrument

The SpectraMax L Luminescence Microplate Reader is packed in a specially designed shipping container. Retain the container and the packing materials. If the instrument requires repair, you must return the instrument to Molecular Devices in the original packaging. If the container has been damaged in transit, you must retain it for inspection by the carrier.

WARNING! Shock Hazard. In an emergency, users must be able to safely disconnect the mains power cable without moving the instrument. Locate the instrument so that either the wall power outlet or the instrument's appliance connector is accessible.

WARNING! The SpectraMax L Luminescence Microplate Reader with external injector modules weighs over 40 pounds (18.2 kg). To avoid potential injury, a minimum of two people are needed to lift the instrument.

WARNING! Biohazardous Material. Do not operate the instrument in an environment where potentially damaging liquids or gases are present, or in a room with a temperature below 15° C.

To unpack the instrument

1. Open the shipping container and remove any foam blocks used to prevent movement of the assemblies during shipping.

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.

2. Place the instrument on a level surface, away from direct sunlight, dust, drafts, vibration, and moisture.

CAUTION! Leave at least three inches between the back of the instrument and the nearest objects or surfaces to ensure proper ventilation and cooling.

3. Connect the USB cable to the USB Type B port on the back panel of the instrument. Connect the other end of the cable to the USB Type A port on the control computer.

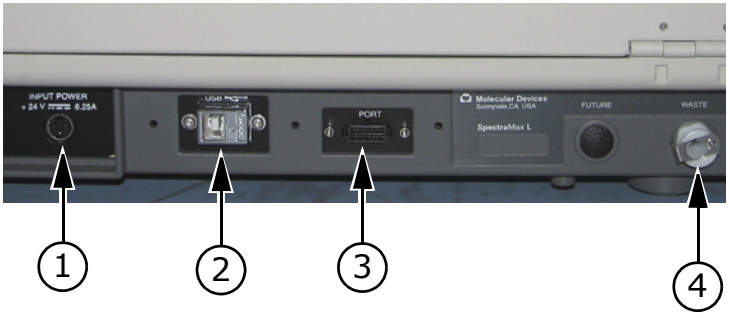


Figure 2-1 Back Panel

Item	Name
1	Main power cable connection
2	USB Type B port
3	External injector module communication port on instrument
4	Waste port

4. Plug the main power cable into the connection on the back panel of the instrument.
5. Connect the mains power cable to the AC adapter, and then connect the other end of the power cable into the lab's power outlet.

   Molecular Devices recommends you use a surge protector between the power cable and the grounded power outlet.
6. If you have purchased one or both external injector modules, plug the module's main power cable into the connection on the back of the module, connect the power cable to the AC adapter, and then plug the other end of the power cable into the lab's power outlet.

   Molecular Devices recommends you use a surge protector between the power cable and the grounded power outlet.
7. If you have purchased one or both external injector modules, you must connect each module's communication cable.

Condition	Connection
Single external injector	Connect the communication cable from the external injector module communication port on the back panel of the instrument to the COMM IN port on the external injector module. See Figure 2-2.
Dual external injectors	Connect the first communication cable from the external injector module communication port on the back panel of the instrument to the COMM IN port on the first injector module. Connect the second communication cable from the COMM OUT port on the first injector module to the COMM IN port on the second external injector module. See Figure 2-3.

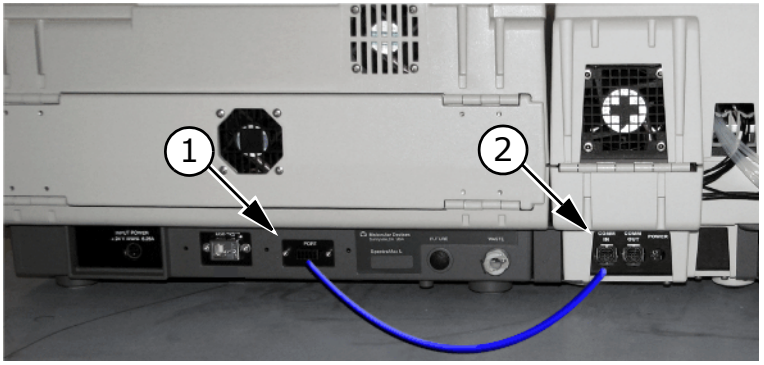


Figure 2-2 Communication cable connection for optional single external injector module

Item	Name
1	External injector module communication port on instrument
2	COMM IN port on external injector module

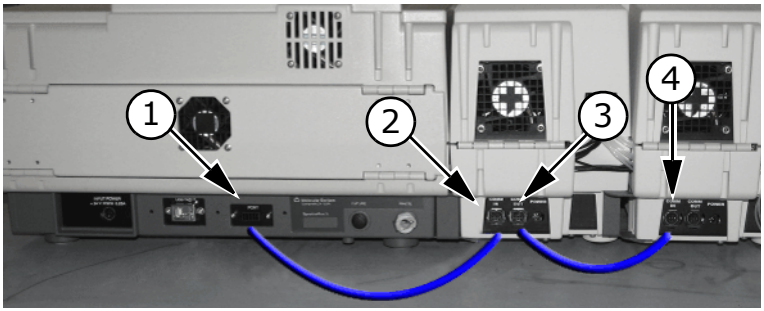


Figure 2-3 Communication cable connection for optional dual external injector modules

Item	Name
1	External injector module communication port on instrument
2	COMM IN port on first external injector module
3	COMM OUT port on first external injector module
4	COMM IN port on second external injector module

Installing the Wash Bottles, Reagent Containers, and Waste Bottle

Connect the wash bottles and reagent containers to the injector ports on the left side of the instrument (base configuration instrument). The SpectraMax L Luminescence Microplate Reader is shipped with two wash bottles; the reagent containers are customer-supplied vials, conical centrifuge tubes, or bottles.

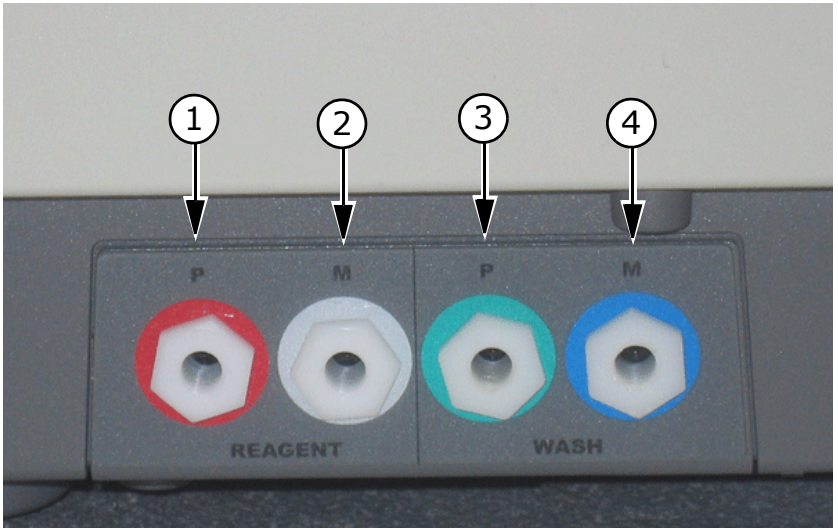


Figure 2-4 Injector ports – base configuration instrument

Figure 2-5

Item	Name	Description
1	P–Reagent injector	Reagent connected here is controlled by P–injector commands. If the SoftMax® Pro Software plate settings include both P and M–injections, the P–injection occurs before the M–injection.
2	M–Reagent injector	Reagent connected here is controlled by M–injector commands.
3	P–Wash injector	Wash solution connected here washes the P–injector from injector port through injector tip. Controlled by the P–Wash commands in the SoftMax Pro Software, and automatically when the instrument is turned off.
4	M–Wash injector	Wash solution connected here washes the M–injector from injector port through injector tip. Controlled by the M–Wash commands in the SoftMax Pro Software, and automatically when the instrument is turned off.

To install the wash bottles, reagent containers, and waste bottle

1. Clean the wash bottles with an appropriate solvent before installation.
2. Install the tubing and cap on the P–Wash bottle, and then connect the tubing to the P–Wash injector port on the instrument.
3. Install the tubing and cap on the M–wash bottle, and then connect the tubing to the M–Wash injector port on the instrument.
4. Install the tubing and caps on the reagent containers in accordance with the manufacturer's instructions, and then connect the tubing to the P and M–Reagent injector ports on the instrument.
5. Connect the waste line to the Waste Port on the back panel. Place the other end of the line in a suitable waste container.

   Make sure the waste container is in a secure location and will not spill.

Tip! Instruments purchased with the optional external injector modules will have all reagent and wash port lines pre-installed before leaving the Molecular Devices facility.

Installing the Spinner Flask

If you intend to inject cells, install the curved reagent and wash tube assemblies in the spinner flasks as described in this section.

Note: These assay methods are also described in the literature, e.g., in Boie et al., Eur. J. Pharmacol., 340(2-3):227-241 (1997), and in United States Patent 6,872,536 and European Patent 1,145,002. Users interested in the patented methods may wish to consult legal counsel in evaluating these patents.

To install the spinner flasks

1. Shut down the instrument. See Shutting Down the Instrument on page 30.
2. Remove the straight tube from the reagent or wash assembly, if it has been installed.

3. Pull the clear straight tube off the reagent or wash assembly. The tube fits into the assembly with a compression fitting.

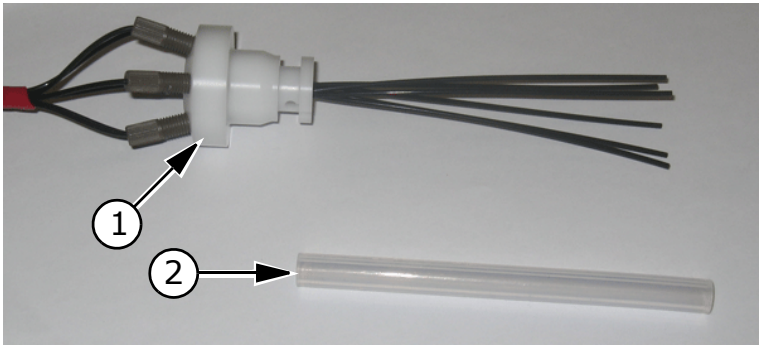


Figure 2-6 Clear straight tube removed

Item	Name
1	Reagent or wash assembly
2	Clear straight tube

4. Fit the clear curved tube over the thin black reagent or wash lines attached to the assembly, and then push the tube into the assembly until it is secure.

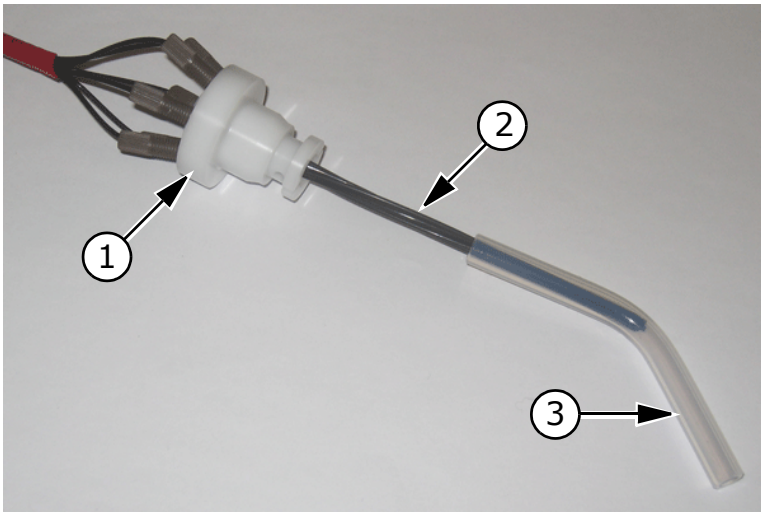


Figure 2-7 Fitting the clear curved tube

Item	Name
1	Reagent or wash assembly
2	Individual thin black reagent or wash lines
3	Clear curved tube

5. Position the metal clip over the assembly, and then insert and tighten the fastening ferrule through the metal clip into the assembly.

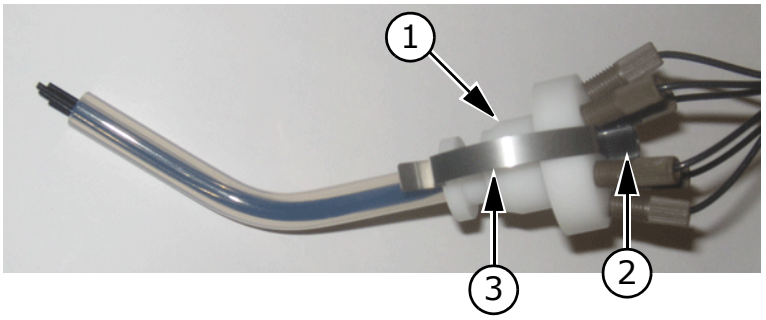


Figure 2-8 Positioning the metal clip

Item	Name
1	Reagent or wash assembly
2	Fastening ferrule
3	Metal clip

6. Insert the reagent or wash assembly into the spinner flask, and then lock the ends of the clip over the neck of the spinner flask.

   You may require a small screwdriver to pry the clip over the edges of the spinner flask.

Installing the Software

Before operating the instrument, you must install the SoftMax® Pro Software on a Windows- or Macintosh-based control computer.

To install the software

1. Locate the SoftMax Pro Software CD.

Note: To operate the SpectraMax L Luminescence Microplate Reader, the SoftMax Pro installer must install the hardware device driver. For more information, refer to the SpectraMax L *Read Me* file on the SoftMax Pro Software CD.

2. Insert the SoftMax Pro Software CD into the computer. Follow the on-screen instructions to install the SoftMax Pro Software.

Preparing the Instrument for Use

Before operating the instrument, you must:

- Turn on the instrument and start the SoftMax Pro Software
- Configure the instrument settings

To turn on the instrument

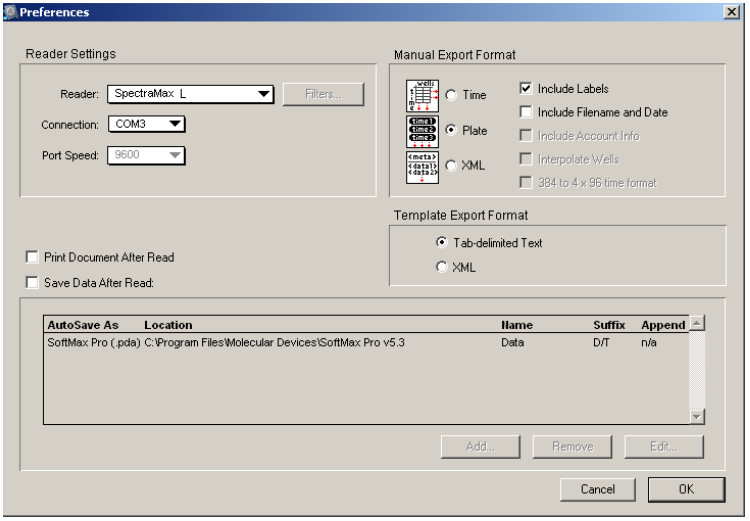
1. Turn on the instrument power switch, located on the front panel of the instrument.
2. Turn on the control computer.
3. Click **Start > Programs > Molecular Devices > SoftMax Pro.**

To configure the instrument settings

Once the instrument is turned on and the control computer is on, the SoftMax Pro Software will detect the COM port and configure the instrument settings. If successful the Connected icon appears in the top left corner of the SoftMax Pro window. See Figure 2-9 on page 26. If the preferred COM port is already used by another hardware device, you must manually configure the COM port. To do so, perform the following procedure:

1. In the SoftMax Pro Software, on the **Edit** menu, click **Preferences**.

   The Preferences dialog box appears.



2. In the **Reader** list, make sure SpectraMax L is selected.
3. In the **Connection** list, select the appropriate COM port.

   The Connected icon appears in the top left corner of the SoftMax Pro window.

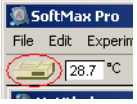


Figure 2-9 Connected icon

4. Click **OK**

Installing Filters

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

Required tools:

- 2 mm Allen wrench
- Phillips screwdriver
- Slotted screwdriver
- Powder-free latex or nitrile gloves

To install the filters

1. Shut down the instrument. See Shutting Down the Instrument on page 30.
2. Loosen the captive screws on the front of the instrument, and then raise the top cover up and over the instrument. See Figure 4-1 on page 57.
3. Remove the two 2 mm Allen screws that secure each detector to the instrument chassis.

The screws are located inside the access holes in the detector adapter.

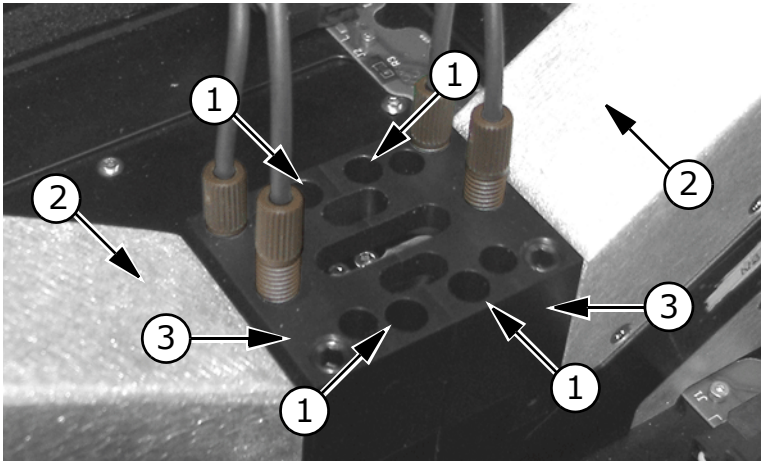


Figure 2-10 Detector attached to instrument chassis

Item	Name
1	Access holes for Allen screws
2	Detector
3	Detector adapter

CAUTION! The PMT must be kept away from any direct light source. Light can damage the PMT causing temporarily high background reads.

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 captured Phillips screws that secure the filter cover plate to the detector.

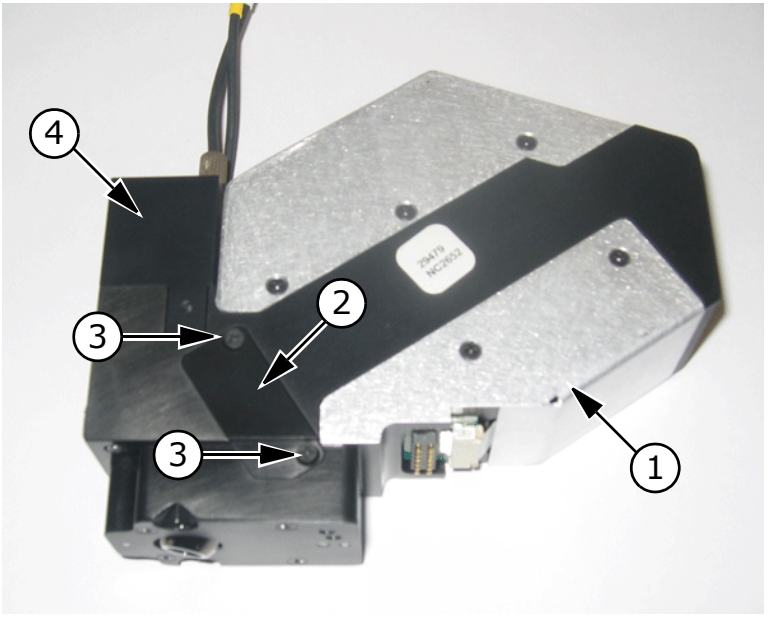


Figure 2-11 Detector with filter cover plate attached

Item	Name
1	Detector
2	Filter cover plate
3	Captured screw
4	Detector adapter

6. Lift the filter cover plate off the detector, and then remove the filter 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 Allen 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.

Shutting Down the Instrument

Proper shut down of the instrument makes sure the pumps and fluid lines are clear, and the instrument is ready for future use.

To shut down the instrument

1. Reverse the injectors. See To reverse the injectors on page 62.
2. Disconnect the tubing from the reagent bottles and place the tubing in a container of deionized water.
3. Wash the injectors as recommended by the assay vendor. See To wash the injectors on page 60. Injectors may also be washed through the reagent lines by checking this option in the Wash Injectors dialog box.
4. Turn off the instrument power switch.

   The instrument goes through the shutdown sequence, automatically performing the following:

   - 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, flushing wash fluid through the injectors and fluid lines.
   - The microplate drawer is placed in the lock position. This prevents movement of the drawer if the instrument is relocated.

Configuring Operating Parameters

3

You must configure the instrument operating parameters for the SpectraMax® L Luminescence Microplate Reader before reading any microplates. You can configure the instrument parameters for the instrument connected to the control computer, or you can configure and save the parameters for a remote instrument used elsewhere in the lab, or in a lab in another facility.

Read Types

The SpectraMax L Luminescence Microplate Reader can operate in four read types: Endpoint, Dual Read, Kinetic, and Fast Kinetic. Each read type has several groups of settings and parameters which can be configured. See Table 3-2 on page 32.

Table 3-1 Read types

Read type	Description
Endpoint	Each user-selected microplate well receives up to two injections, and then is read once. You can set a delay time after each injection, from 0 to 3,600 seconds for the M-injection, and from 0.1 to 3,600 seconds for the P-injection.
Dual Read	Each user-selected microplate well receives two injections and two reads. 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 after each injection, from 0 to 3,600 seconds for the M-injection, and from 0.1 to 3,600 seconds for the P-injection.
Kinetic	Data is collected over time with readings taken at regular intervals. Each user-selected microplate well can receive multiple injections and reads. You can specify single injections, followed by single reads, for multiple timepoints. The minimum integration interval is dependent on the number of wells selected, the integration time selected, and the number of injections selected.

Table 3-1 Read types

Read type	Description
Fast Kinetic	Fast Kinetic performs repeated readings of one or more wells of a 96 or 384-well microplate up to a 200 point maximum integration. All readings of a single well are made before the next well is read. Pre-reading a microplate is not possible in Fast Kinetic type. Each user-selected microplate 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.

Read Types, Settings, and Parameters.

Table 3-2 Read types and settings available in each type

Setting	Read Type			
	Endpoint	Dual Read	Kinetic	Fast Kinetic
Integration Time	X	X		
Integration and Timing			X	
Integration/Point Count				X
Sensitivity	X	X	X	X
Automix	X	X	X	X
Injection and Delay	X	X	X	X
Assay Plate Type	X	X	X	X
Wells to Read	X	X	X	X
Injection Wells	X	X	X	X
Dark Adept	X	X	X	X
AutoRead	X	X	X	X

Table 3-3 Settings

Setting	Description
Integration Time	The amount of time photons from the microplate well are measured. In Dual Read, you can specify separate integration times for the P and M–injections.
Integration and Timing	The amount of time each data point on the kinetic curve is measured. Run time is the total time for the Kinetic measurement. Interval is the amount of time between reads.
Integration/Point Count	The amount of time each data point on the Fast Kinetic curve is measured. Point count is the number of data points read per well.
Sensitivity: Four options are available	AutoRange: Provides greater than nine orders of dynamic range by simultaneously measuring in photon counting and analog mode. An algorithm automatically chooses which measurement to report.
	Analog Only: This setting turns down the PMT voltage so that very bright signals can be detected in a linear fashion over two orders of magnitude. The RLU (relative luminescence unit) values from reads using the Analog Only setting are different compared to Photon Counting and AutoRange. In Analog Only mode, it is common for an empty, dark-adapted white plate to have RLU values as high as 1,000,000.
	Photon Counting: Provides six orders of dynamic range with excellent sensitivity.
	MaxRange: Combines AutoRange and Analog Only modes for the widest dynamic range with high sensitivity.
Automix	Automatic microplate shaking before and/or during the read cycle. Four methods are available: Classic, Single, Dual, and Orbital.
Injection and Delay	Specify injection parameters such as the volume, speed, and the amount of time between the injection and the read.
Assay Plate Type	Select 96- or 384-well microplates.
Wells to Read	Specify which microplate wells are read.
Injection Wells	Specify which wells are to receive P– and M–injections.
Dark Adapt	Moves microplate into instrument and delays beginning of read.
AutoRead	Automatically re-reads the plate using the settings in the next plate section in the protocol.

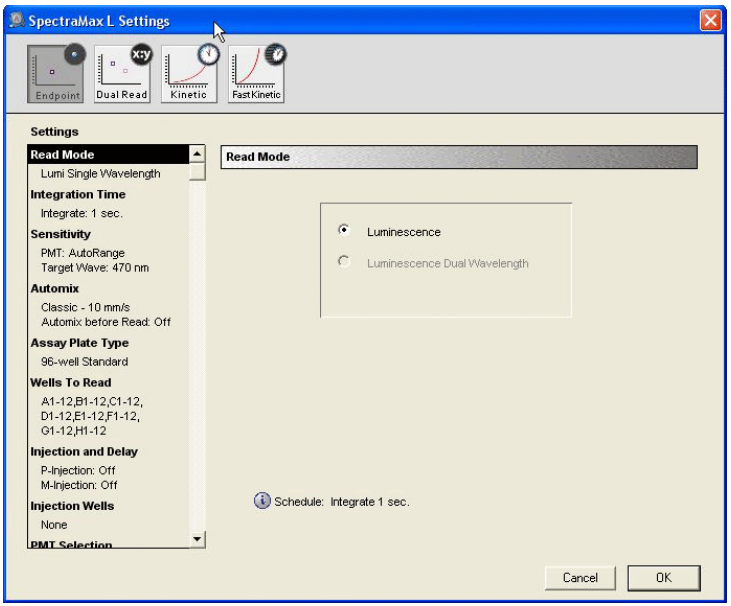
Configuring Operating Parameters for Each Read Type

Follow the procedures below to configure the operating parameters for each read type, for an instrument connected to the local control computer. To configure operating parameters for a remote instrument, see To configure the parameters for a remote instrument on page 50.

To configure the parameters for Endpoint type

1. In the SoftMax® Pro Software, in the experiment window, click **Settings**.

   The SpectraMax L Settings window appears.



2. Click **Endpoint**.

   The settings and parameters specific to the Endpoint read type are displayed in the left pane of the window.

3. Click **Integration Time**, and then configure the following.

Parameter	Description
Integration Time (sec.)	Type the amount of time required for well measurement.

4. Click **Sensitivity**.
   - Select one of the options from the **PMT Setting** list.

PMT Setting	Description
AutoRange	Select for assays requiring high sensitivity with a wide dynamic range.
Analog Only	Select for very bright assays.
Photon Counting	If selected you must also select a Correction Option: No Correction or Custom Calibration. No Correction: Select to maintain linearity in the low range of detection, below 10,000,000 cps. Custom Calibration: Select to choose one of two user-defined custom calibrations that can be applied. See Creating Custom Calibrations on page 55.
MaxRange	Select for assays with massive dynamic range.

   - In the **Target Calibration Wavelength** group, select the option closest to the wavelength of the assay to read. Available in AutoRange, Analog Only, and Max Range types.
5. Click **Automix**, if applicable, and then select a **Mix Type**.

Mix Type	Description
Classic	Select to shake the microplate along the X–axis, alternating between fast and slow shaking.
Single	Select to shake the microplate along the X–axis at a constant rate.
Dual	Select to shake the microplate in an 'L' pattern.
Orbital	Select to shake the microplate in a circular pattern.

In the **AutoMix Settings** group, configure the following.

Parameter	Description
Mix Duration	Type the amount of time the microplate will be shaken.
Mix Speed	Type the mixing speed.
Size	Type the mix size. Available in Single, Dual, and Orbital mix types.
Enable Automix before Read	Select to shake the microplate between the injection and the read.

6. Click **Injection and Delay,** and then configure the following parameters for either or both **P**–**Injection** (pre-injection) and **M**–**Injection** (measurement injection).

Parameter	Description
Injector volume (µL)	Type the volume of fluid to be injected.
Post injection delay (sec)	Set the delay time between the injection and the first read. The delay time for the P–Injection is the time between the start of the P–Injection and start of M–Injection. The delay time for the M–Injection is the time between start of M–Injection and start of the read.
Injection speed (µL/s)	The recommended injection speed for reagent injections is 320 µL/s. Applications involving the injections of cells may benefit from slower injection speeds to reduce damage to cells.
Shake after injection (sec)	Type the amount of time to shake after injection and prior to measurement.
Number of baseline reads	Type the number of reads before the injection.

7. Click **Assay Plate Type**, and then select either a 96-well or 384-well microplate.
8. Click **Wells to Read**, and then select each microplate well to be read.

   Click **Clear** to clear all of the selected wells. Click **Ctrl**–**Clear** to clear individual wells.

9. Click **Injection Wells**, and then select the wells to receive a P–injection or an M–injection, or both.
   - Click **P Injector**, and then select the wells to receive a P–injection. Click the well again to clear it.
   - Click **M Injector,** and then select the wells to receive a M–injection. Click the well again to clear it.
10. Click **PMT Selection** (multi-detector instrument), and then configure the following:

Parameter	Description
Default PMT Selection	Select to use the factory configured PMTs.
Manual PMT Selection	Select to specify which PMTs perform the read.

11. Click **Dark Adapt**, and then configure the following:

Parameter	Description
Dark Adapt	Select to delay the start of reading. This allows the microplate autophosphorescence to subside.
Delay (min)	Type the amount of time to delay.

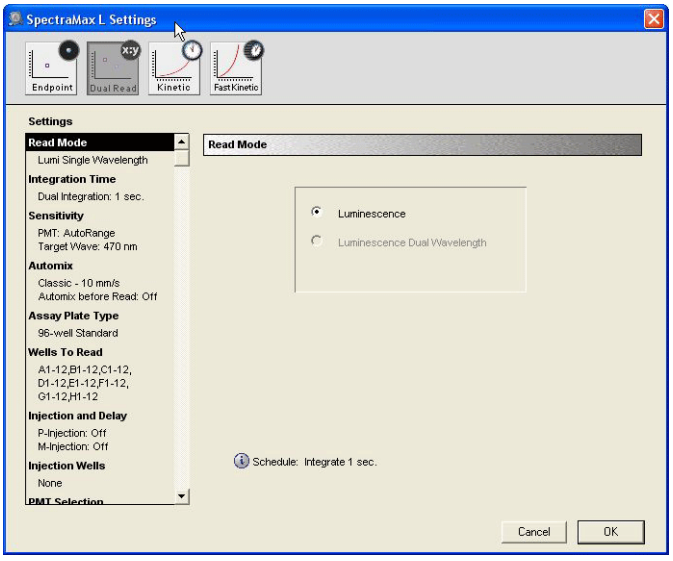
12. Click **AutoRead**, and then configure the following:

Parameter	Description
AutoRead	Select to automatically re-read the plate according to the next plate section in the protocol.
Delay (sec)	Type the amount of time between the end of the current plate being read and beginning of the read according to the next plate section.

To configure the parameters for Dual Read type

1. In the SoftMax Pro Software, in the plate section window, click **Settings**.

   The SpectraMax L Settings window appears.



2. Click **Dual Read**.

   The settings and parameters specific to the Dual Read type are displayed in the left pane of the window.

3. Click **Integration Time**, and then configure the following:

Parameter	Description
Normal	Select if the integration times for the read following both the P– and the M–injection are the same.
Special	Select if the integration times for the read following the P– and the M–injection are different.
Dual Integration Time (sec)	Type the amount of time required for well measurement.

4. Click **Sensitivity**.
   - Select one of the options from the **PMT Setting** list:

PMT Setting	Description
AutoRange	Select for assays requiring high sensitivity with a wide dynamic range.
Analog Only	Select for very bright assays.
Photon Counting	If selected you must also select a Correction Option: No Correction or Custom Calibration. No Correction: Select to maintain linearity in the low range of detection, below 10,000,000 cps. Custom Calibration: Select to chose one of two user-defined custom calibrations that can be applied. See Creating Custom Calibrations on page 55.
MaxRange	Select for assays with massive dynamic range.

   - In the **Target Calibration Wavelength** group, select the option closest to the wavelength of the assay to read. Available in AutoRange, Analog Only, and Max Range types.
5. Click **Automix**, if applicable, and then select a **Mix Type**.

Mix Type	Description
Classic	Select to shake the microplate along the X–axis, alternating between fast and slow shaking.
Single	Select to shake the microplate along the X–axis at a constant rate.
Dual	Select to shake the microplate in an 'L' pattern.
Orbital	Select to shake the microplate in a circular pattern.

- In the **AutoMix Settings** group, configure the following:

Parameter	Description
Mix Duration	Type the amount of time the microplate will be shaken.
Mix Speed	Type the mixing speed.
Size	Type the mix size. Available in Single, Dual, and Orbital mix types.
Enable Automix before Read	Select to shake the microplate between the injection and the read.

6. Click **Injection and Delay,** and then configure the following parameters for either or both **P**–**Injection** (pre-injection) and **M**–**Injection** (measurement injection).

Parameter	Description
Injector volume (µL)	Type the volume of fluid to be injected.
Post injection delay (sec)	Set the delay time between the start of the injection and the start of the read.
Injection speed (µL/s)	The recommended injection speed for reagent injections is 320 µL/s. Applications involving the injections of cells may benefit from slower injection speeds to reduce damage to cells.
Shake after injection (sec)	Type the amount of time to shake after injection and prior to measurement.
Number of baseline reads	Type the number of reads before the injection.

7. Click **Assay Plate Type** and then select either a 96- or 384-well microplate.
8. Click **Wells to Read**, and then select each microplate well to be read.

   Click **Clear** to clear all of the selected wells. Click **Ctrl**–**Clear** to clear individual wells.
9. Click **Injection Wells**, and then select the wells to receive a P–injection or an M–injection, or both.
   - Click **P Injector**, and then select the wells to receive a P–injection. Click the well again to clear it.
   - Click **M Injector**, and then select the wells to receive an M–injection. Click the well again to clear it.

10. Click **PMT Selection**, and then configure the following:

Parameter	Description
Default PMT Selection	Select to use the factory configured PMTs.
Manual PMT Selection	Select to specify which PMTs perform the read.

11. Click **Dark Adapt**, and then configure the following:

Parameter	Description
Dark Adapt	Select to delay the start of reading. This allows microplate autophosphorescence to subside.
Delay (min)	Type the amount of time to delay.

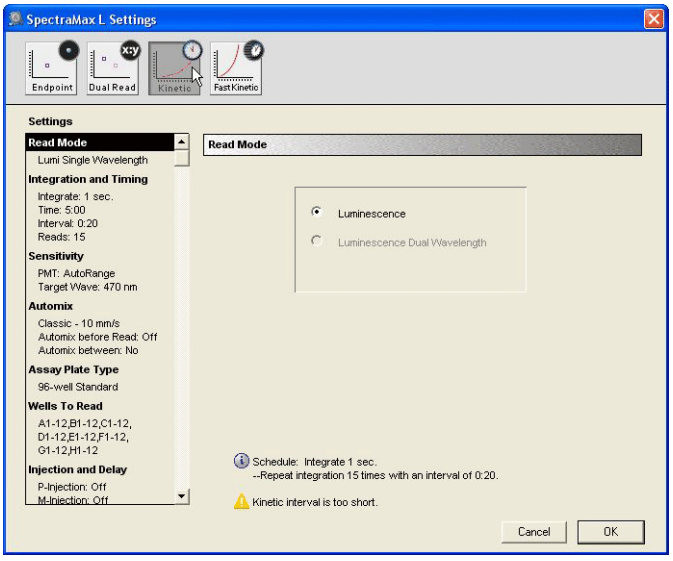
12. Click **AutoRead**, and then configure the following:

Parameter	Description
AutoRead	Select to automatically re-read the plate according to the next plate section in the protocol.
Delay (sec)	Type the amount of time between the end of the current plate being read and beginning of the read according to the next plate section.

To configure the parameters for Kinetic read type

1. In the SoftMax Pro Software, in the experiment window, click **Settings**.

   The SpectraMax L Settings window appears.



2. Click **Kinetic**.

   The settings and parameters specific to the Kinetic read type are displayed in the left pane of the window.

3. Click **Integration and Timing**, and then configure the following:

Parameter	Description
Integration Time (sec.)	Type the amount of time required for well measurement.
Run Time	Type the total time to read the plate.
Interval	Type the time between each reading of the microplate.
Number of Reads	Non-editable field.
Minimum Interval	Non-editable field.

4. Click **Sensitivity**.
   - Select one of the options from the **PMT Setting** list:

PMT Setting	Description
AutoRange	Select for assays requiring high sensitivity with a wide dynamic range.
Analog Only	Select for very bright assays.
Photon Counting	If selected you must also select a Correction Option: No Correction or Custom Calibration. No Correction: Select to maintain linearity in the low range of detection, below 10,000,000 cps. Custom Calibration: Select to chose one of two user-defined custom calibrations that can be applied. See Creating Custom Calibrations on page 55.
MaxRange	Select for assays with massive dynamic range.

   - In the **Target Calibration Wavelength** group, select the option closest to the wavelength of the assay to read. Available in AutoRange, Analog Only, and Max Range types.
5. Click **Automix**, and then select a **Mix Type**.

Mix Type	Description
Classic	Select to shake the microplate along the X–axis, alternating between fast and slow shaking.
Single	Select to shake the microplate along the X–axis at a constant rate.
Dual	Select to shake the microplate in an 'L' pattern.
Orbital	Select to shake the microplate in a circular pattern.

- In the **AutoMix Settings** group, configure the following:

Parameter	Description
Mix Duration	Type the amount of time the microplate will be shaken.
Mix Speed	Type the mixing speed.
Size	Type the mix size. Available in Single, Dual, and Orbital mix types.
Enable Automix before Read	Select to shake the microplate between the injection and the read.
Enable Automix between Kinetic Reads	Select to shake the microplate between reads.

6. Click **Injection and Delay,** and then configure the following parameters for either or both **P**–**Injection** (pre-injection) and **M**–**Injection** (measurement injection).

Parameter	Description
Injector volume (µL)	Type the volume of fluid to be injected.
Post injection delay (sec)	Set the delay time between the start of the injection and the start of the next read.
Injection speed (µL/s)	Type the injection speed.
Shake after injection (sec)	Type the amount of time to shake after injection and prior to measurement.
Number of baseline reads	Type the number of reads before the injection.

7. Click **Assay Plate Type** and then select either a 96- or 384-well microplate.
8. Click **Wells to Read**, and then select each microplate well to be read.

   Click **Clear** to clear all of the selected wells. Click **Ctrl**–**Clear** to clear individual wells.
9. Click **Injection Wells**, and then select the wells to receive a P–injection or an M–injection, or both.
   - Click **P Injector**, and then select the wells to receive a P–injection. Click the well again to clear it.
   - Click **M Injector**, and then select the wells to receive an M–injection. Click the well again to clear it.

10. Click **PMT Selection**, and then configure the following:

Parameter	Description
Default PMT Selection	Select to use the factory configured PMTs.
Manual PMT Selection	Select to specify which PMTs perform the read.

11. Click **Dark Adapt**, and then configure the following:

Parameter	Description
Dark Adapt	Select to delay the start of reading. This allows microplate autophosphorescence to subside.
Delay (min)	Type the amount of time to delay.

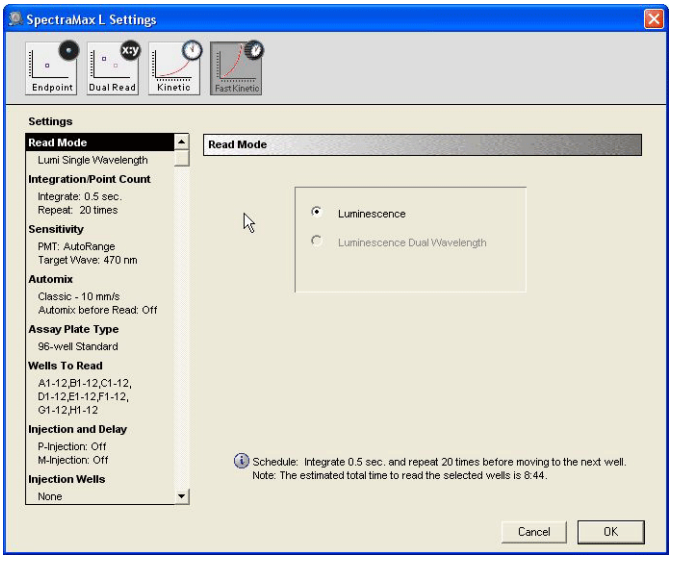
12. Click **AutoRead**, and then configure the following:

Parameter	Description
AutoRead	Select to automatically re-read the plate according to the next plate section in the protocol.
Delay (sec)	Type the amount of time between the end of the current plate being read and beginning of the read according to the next plate section.

To configure the parameters for Fast Kinetic read type

1. In the SoftMax Pro Software, in the experiment window, click **Settings**.

   The SpectraMax L Settings window appears.



2. Click **Fast Kinetic**.

   The settings and parameters specific to the Fast Kinetic read type are displayed in the left pane of the window.

3. Click **Integration/Point Count**, and then configure the following:

Parameter	Description
Integration Time (sec)	Type the amount of time required for well measurement.
Number of Reads per Well	Type the number of reads in each well.

4. Click **Sensitivity**.
   - Select one of the options from the **PMT Setting** list:

PMT Setting	Description
AutoRange	Select for assays requiring high sensitivity with a wide dynamic range.
Analog Only	Select for very bright assays.
Photon Counting	If selected you must also select a Correction Option: No Correction or Custom Calibration. No Correction: Select to maintain linearity in the low range of detection, below 10,000,000 cps. Custom Calibration: Select to chose one of two user-defined custom calibrations that can be applied. See Creating Custom Calibrations on page 55.
MaxRange	Select for assays with massive dynamic range.

   - In the **Target Calibration Wavelength** group, select the option closest to the wavelength of the assay to read. Available in AutoRange, Analog Only, and Max Range types.
5. Click **Automix**, and then select a **Mix Type**.

Mix Type	Description
Classic	Select to shake the microplate along the X–axis, alternating between fast and slow shaking.
Single	Select to shake the microplate along the X–axis at a constant rate.
Dual	Select to shake the microplate in an 'L' pattern.
Orbital	Select to shake the microplate in a circular pattern.

In the **AutoMix Settings** group, configure the following:

Parameter	Description
Mix Duration	Type the amount of time the microplate will be shaken.
Mix Speed	Type the mixing speed.
Size	Type the mix size. Available in Single, Dual, and Orbital mix types.
Enable Automix before Read	Select to shake the microplate between the injection and the read.

6. Click **Injection and Delay,** and then configure the following parameters for either or both **P**–**Injection** (pre-injection) and **M**–**Injection** (measurement injection).

Parameter	Description
Injector volume (µL)	Type the volume of fluid to be injected.
Post injection delay (sec)	Set the delay time between the last injection and the read. The delay time for the P–injection is the time between the start of the injection and the start of the read. The delay time for the M–injection is automatically calculated by the SoftMax Pro Software.
Injection speed (µL/s)	Type the injection speed.
Shake after injection (sec)	Type the amount of time to shake after injection and prior to measurement.
Number of baseline reads	Type the number of reads before the injection.

7. Click **Assay Plate Type** and then select either a 96- or 384-well microplate.
8. Click **Wells to Read**, and then select each microplate well to be read.

   Click **Clear** to clear all of the selected wells. Click **Ctrl**–**Clear** to clear individual wells.

9. Click **Injection Wells**, and then select each well to receive a P-injection or an M-injection, or both.
   - Click **P Injector**, and then select the wells to receive a P-injection. Click the well again to clear it.
   - Click **M Injector**, and then select the wells to receive an M-injection. Click the well again to clear it.
10. Click **PMT Selection**, and then configure the following:

Parameter	Description
Default PMT Selection	Select to use the factory configured PMTs.
Manual PMT Selection	Select to specify which PMTs perform the read.

11. Click **Dark Adapt**, and then configure the following:

Parameter	Description
Dark Adapt	Select to delay the start of reading. This allows microplate autophosphorescence to subside.
Delay (min)	Type the amount of time to delay.

12. Click **AutoRead**, and then configure the following:

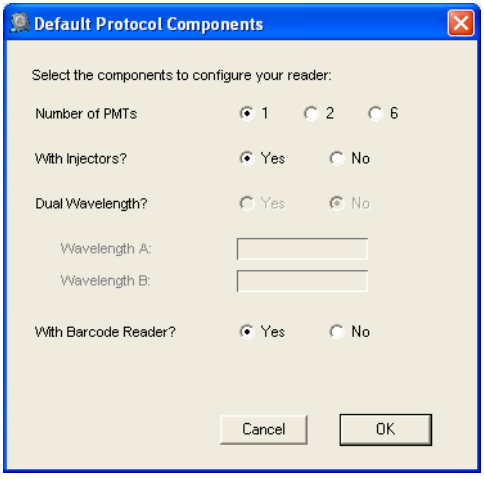
Parameter	Description
AutoRead	Select to automatically re-read the plate according to the next plate section in the protocol.
Delay (sec)	Type the amount of time between the end of the current plate being read and beginning of the read according to the next plate section.

Configuring Operating Parameters for a Remote Instrument

Follow this procedure to configure the operating parameters for a remote instrument.

To configure the parameters for a remote instrument

1. In the SoftMax Pro Software, on the **Edit** menu, click **Preferences**.
   The Preferences dialog box appears.
2. In the **Reader** list, select **Spectramax L.**
3. In the **Connection** list, select the COM port named "None".
4. Click **Default Protocol Components**.
   The Default Protocol Components dialog box appears.

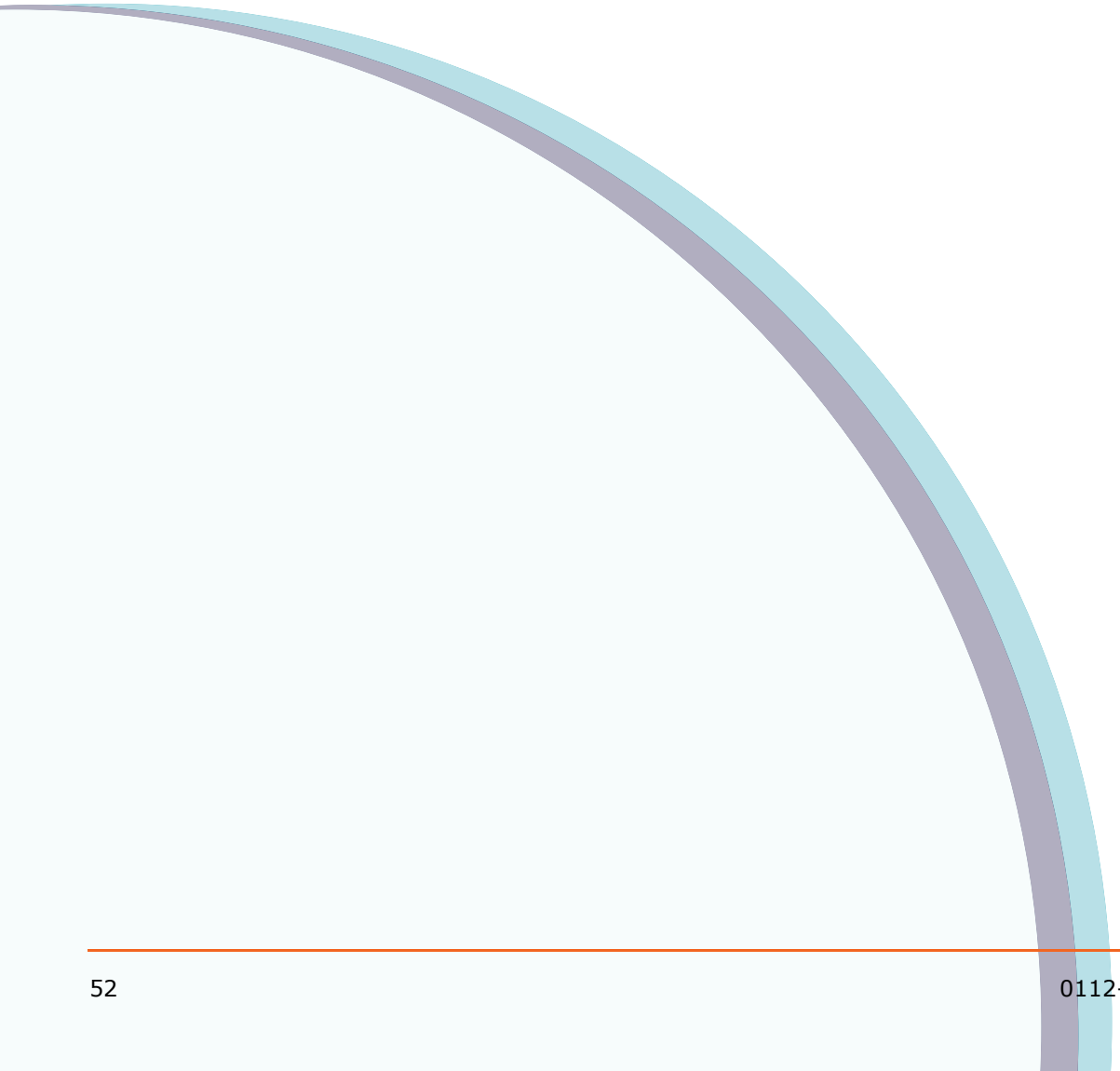


5. Configure the following options:

Parameter	Description
Number of PMTs	Select the number of PMTs installed in the remote instrument.
With Injectors	Select **Yes** if the remote instrument includes the injector option.
Dual Wavelengths	Select **Yes** if the remote instrument includes the wavelength option, and then type the values for **Wavelength A** and **Wavelength B**.
With Barcode Reader	Select **Yes** if the remote instrument includes the barcode reader.

6. Click **OK**.
7. Configure the parameters for Endpoint type. See To configure the parameters for Endpoint type on page 34.
8. Configure the parameters for Dual Read type. See To configure the parameters for Dual Read type on page 38.
9. Configure the parameters for Kinetic type. See To configure the parameters for Kinetic read type on page 42.
10. Configure the parameters for Fast Kinetic type. See To configure the parameters for Fast Kinetic read type on page 46.
11. Click **File** > **Save As** to save the protocol as a .ppr file.
12. Close the SoftMax Pro Software.
13. Connect the USB cable to the local instrument and the control computer.
14. Turn on the local instrument, and then start the SoftMax Pro Software.

    If the SoftMax Pro Software does not recognize the local instrument, ensure the correct COM port is selected in the Preferences dialog box.



Control Menu Functions

4

The functions accessed from the Control menu are intended to make sure the instrument is performing at an optimum level. Perform the following procedures at the intervals listed in the table below.

Table 4-1 Routine procedure frequency

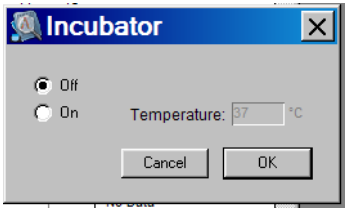
Procedure	Recommended Frequency
Setting the reading chamber temperature	When running assays requiring temperatures above ambient
Calibrating the instrument	Once a year
Creating custom calibrations	Before using the instrument for the first time
Changing the aperture	When switching between 96- and 384-well microplates
Priming the injectors	Before each assay that requires injection
Washing the injectors	After running each assay
Reversing the injectors	After running each assay
Cleaning the instrument	Once a month

Setting the Reading Chamber Temperature

If your assays require specific temperatures, you can regulate the temperature of the microplate chamber from 5° C to 45° C above the ambient room temperature.

1. In the SoftMax® Pro Software, on the **Control** menu, click **Incubator**.

   The Incubator dialog box appears.



2. Select **On** to turn on the incubator, and then type the appropriate **Temperature**.
3. Click **OK**.

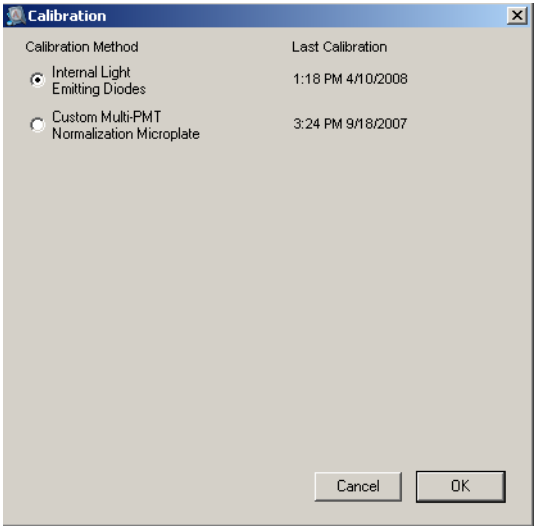
Calibrating the Instrument

Calibrate the instrument once a year for maximum performance.

To calibrate the instrument

1. Remove any microplate from the microplate drawer.
2. In the SoftMax Pro Software, on the **Control** menu, click **Calibration**.

   The Calibration dialog box appears.



3. Select **Internal Light Emitting Diodes**.
4. Click **OK**.

Creating Custom Calibrations

Create your own custom calibrations if you prefer to use the light spectrum from your assays, rather than the light spectrum from the factory configured LEDs.

You can create two custom calibrations.

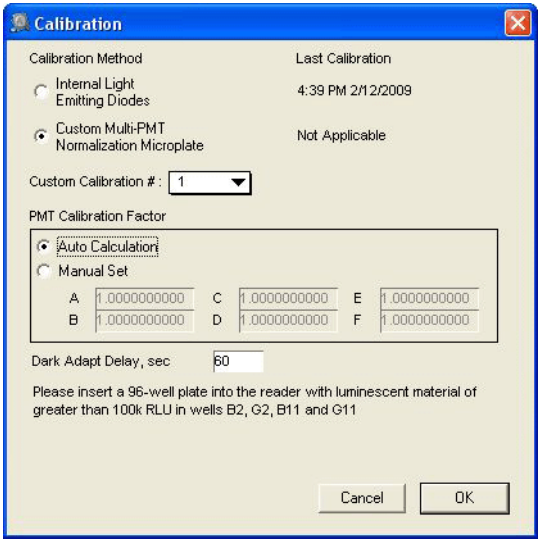
To create custom calibrations

1. In the SoftMax Pro Software, on the **Control** menu, click **Calibration**.

   The Calibration dialog box appears.

2. Click **Custom Multi-PMT Normalization Microplate.**

   A number of additional fields appear in the dialog box.



Note: Make sure there is a microplate in the reading chamber.

3. Select a **Custom Calibration #** from the list.

4. Select either **Auto Calculation** or **Manual Set**.

PMT Calibration Factor	Description
Auto Calculation	Click to accept the standard calibration configured in the SoftMax® Pro Software, and then complete step 5 below.
Manual Set	Click to specify your own calibration, and then complete step 6 below.

5. If you selected **Auto Calculation**, type a value for **Dark Adapt Delay, sec.**
6. If you selected **Manual Set**, type a value for each of the selected PMTs (A,B,C,D,E, or F)**.**
7. Click **OK**.

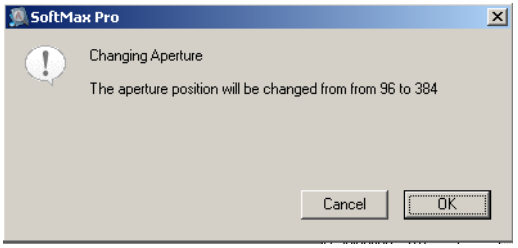
Manually Changing the Aperture

The SpectraMax® L Luminescence Microplate Reader is available with either manual or optional automatic switching between 96 and 384-well plates. If your instrument does not include the automatic switching option, you must use the Change Aperture command in the SoftMax Pro Software when switching between 96 and 384-well plates. The command instructs the reader to check whether a microplate is in the drawer. If no microplate is present, the plate carriage moves away from the read head to allow you to manually change the aperture.

To change the aperture

1. In the SoftMax Pro Software, on the **Control** menu, click **Change Aperture**.

   A message box appears informing you the aperture will be changed.



2. Remove any microplate from the microplate drawer.

3. Loosen the captive screws on the front of the instrument, and then 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.

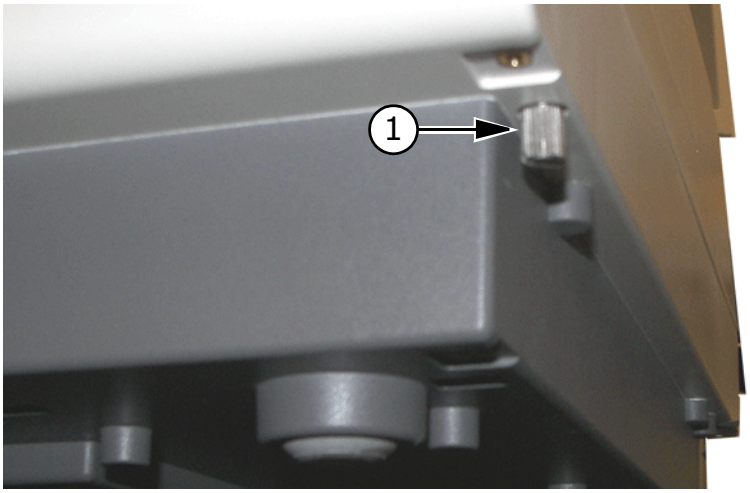


Figure 4-1 Captive screw below front of instrument

Item	Name
1	Captive screw

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.

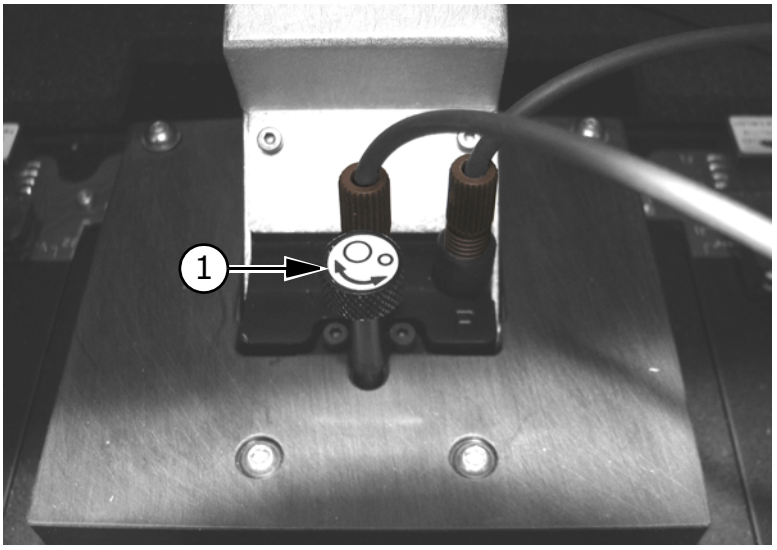


Figure 4-2 Aperture switch

Item	Name
1	Aperture switch dial

- To select the 384-well plate, push down on the dial and rotate counter-clockwise until the small circle is directly 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 directly in front of the M–injector tip assembly.

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

Priming the Injectors

Priming the injectors flushes the system with reagent solution to remove air bubbles and prepare the system for injection.

To prime the injectors

1. In the SoftMax Pro Software, on the **Control** menu, click **Prime Injectors**.
   The Prime Injectors dialog box appears.

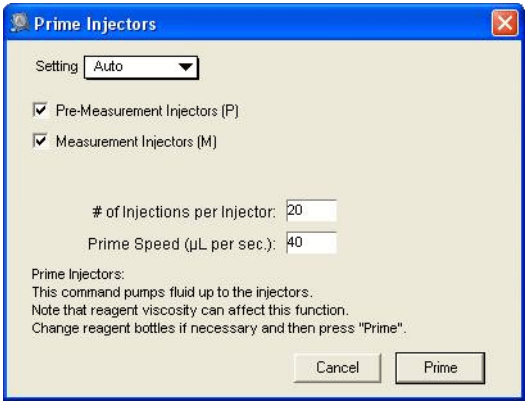


Figure 4-3 Prime Injectors – automatic

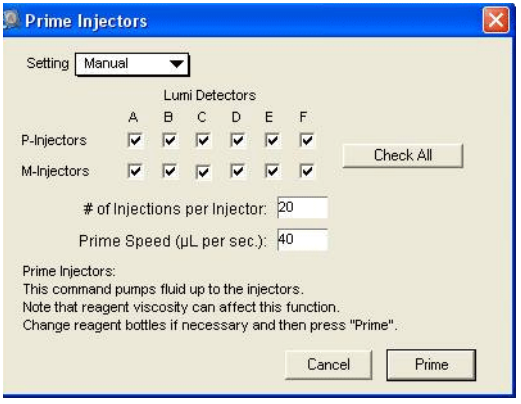


Figure 4-4 Prime Injectors – manual

2. In the **Setting** list, click **Auto** to prime all of the injectors, or click **Manual** to select individual injectors.
   - If you clicked **Auto**, select **Pre-Measurement Injectors (P)** to prime all of the P–injectors, and select **Measurement Injector (M)** to prime all of the M–injectors.
   - If you clicked **Manual**, select the individual injectors or click **Check All** to select all injectors.
3. In the **# of injections per injector** box, type a number or accept the default. Each injection draws 100 µL of fluid.
4. In the **Prime Speed (µL per sec.)** box, type a value.
5. Click **Prime**.

   Fluid is drawn from the source bottle and flushed through the fluid lines.

Washing the Injectors

Wash the injectors after running any assays.

To wash the injectors

1. In the SoftMax Pro Software, on the **Control** menu, click **Wash Injectors**.

   The Wash Injectors dialog box appears.

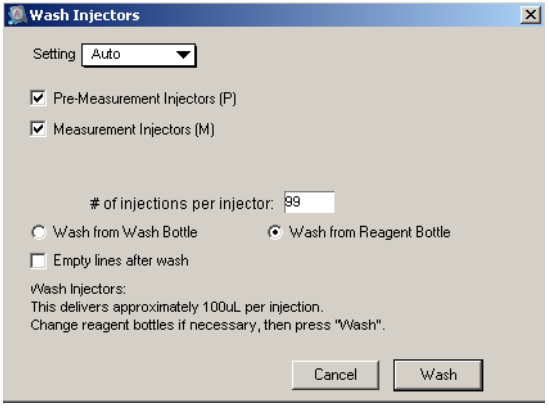


Figure 4-5 Wash injectors dialog box – automatic

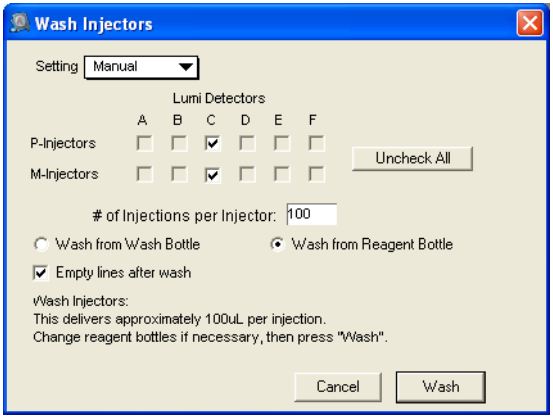


Figure 4-6 Wash injectors dialog box – manual

2. In the **Setting** list, click **Auto** to wash all of the injectors, or click **Manual** to select individual injectors.
   - If you clicked **Auto**, select **Pre-Measurement Injectors (P)** to wash all of the P–injectors, and select **Measurement Injector (M)** to wash all of the M–injectors.
   - If you clicked **Manual**, select the individual injectors or click **Check All** to select all injectors.
3. In the **# of injections per injector** box, type a number or accept the default. Each injection draws 100 µL of fluid.
4. Click either **Wash from Wash Bottle** or **Wash from Reagent Bottle**.
5. Select **Empty lines after wash** to drain the wash lines.
6. Click **Wash**.

   The reagent tubing and the injector tips are flushed with fluid, and then power to the instrument is shut off.

Reversing the Injectors

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 expensive reagent for future use.

To reverse the injectors

1. In the SoftMax Pro Software, on the **Control** menu, click **Reverse Injectors**.

   The Reverse Injectors dialog box appears.

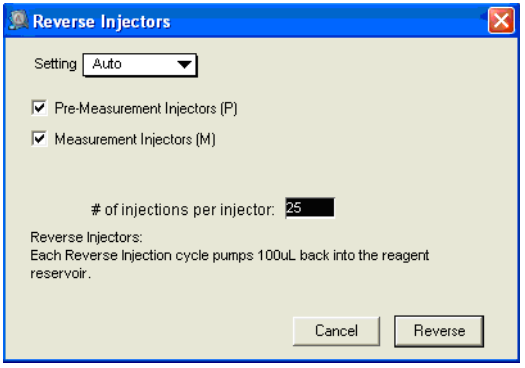


Figure 4-7 Reverse Injectors – automatic

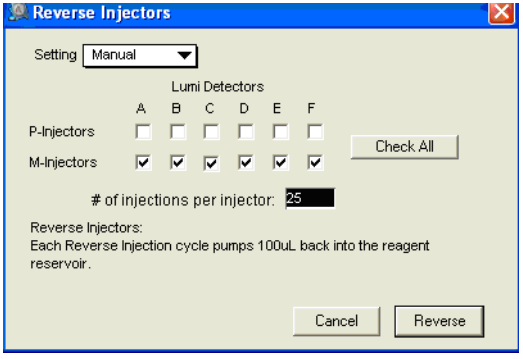


Figure 4-8 Reverse Injectors – manual

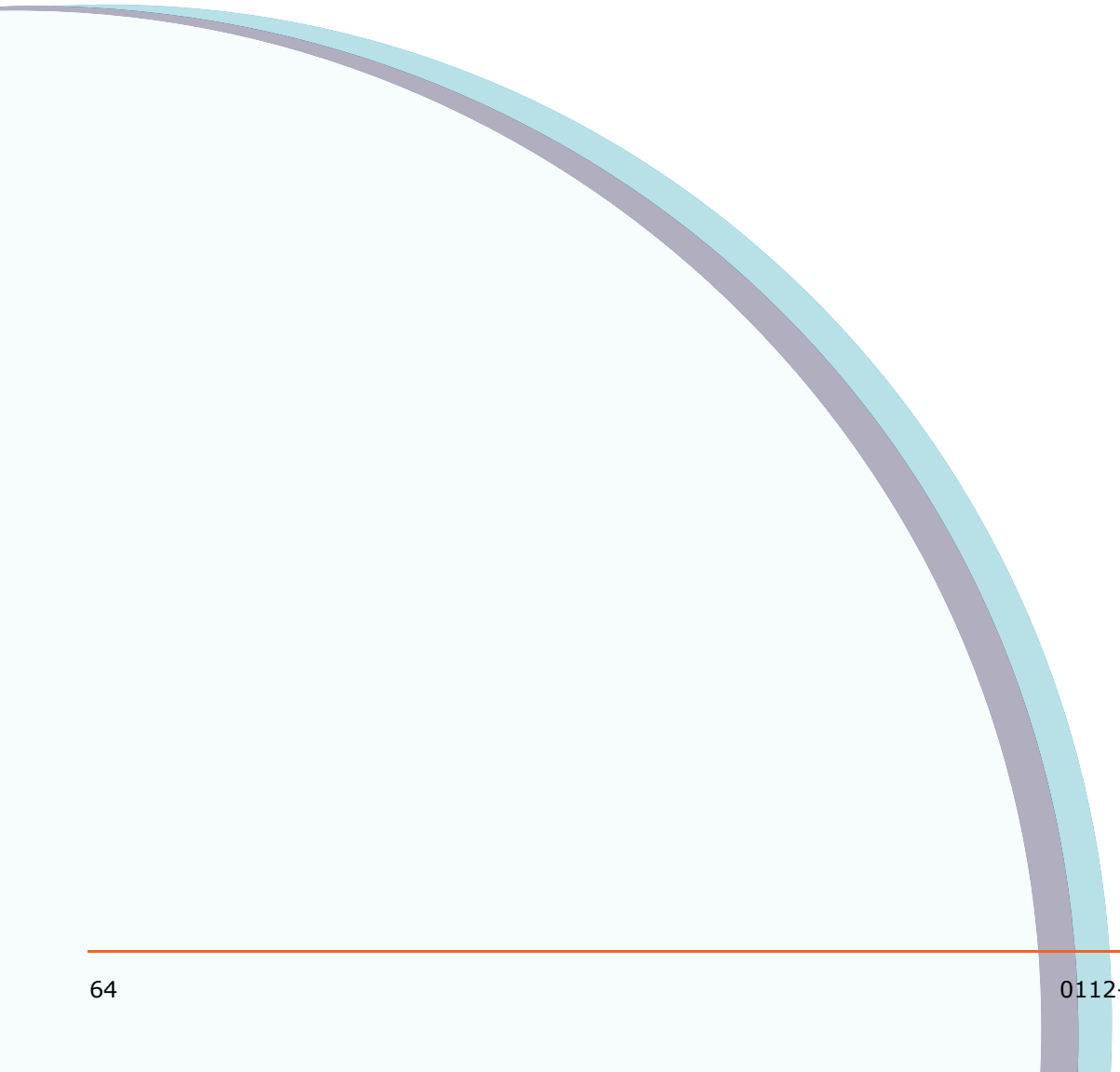
2. In the **Setting** list, click **Auto** to reverse all of the injectors, or click **Manual** to select individual injectors.
   - If you clicked **Auto**, select **Pre-Measurement Injectors (P)** to reverse all of the P–injectors, and select **Measurement Injector (M)** to reverse all of the M–injectors.
   - If you clicked **Manual**, select the individual injectors or click **Check All** to select all injectors.
3. In the **# of injections per injector** box, type a number or accept the default.
4. Click **Reverse**.
5. The injectors run in the opposite direction, removing reagent from the fluid lines and returning the fluid to the reagent containers.

Cleaning the Instrument

Clean the exterior of the instrument using mild soap and water, or a glass cleaning solution.

To clean the microplate drawer, dampen a lint-free cloth or sponge with water. Fluids spilled inside the microplate drawer are directed to a tray in the base of the instrument. Any overflow that has spilled onto the bench should be removed immediately.

CAUTION! Do not use abrasive cleansers, or spray any cleaning solution inside the instrument.



Maintenance and Troubleshooting

5

The SpectraMax® L Luminescence Microplate Reader requires very little maintenance. The procedures are limited to:

- Moving the instrument
- Manually opening the microplate drawer
- Replacing the injector tips
- Replacing the peristaltic tubing

WARNING! Biohazardous Material. It is your responsibility to decontaminate the instrument, as well as any accessories, before requesting service from Molecular Devices representatives, or before returning the instrument or any components to Molecular Devices.

WARNING! Biohazardous Material. Never perform any maintenance procedures on the instrument in an environment where potentially damaging gases or liquids are present.

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

WARNING! Shock Hazard. Do not remove any of the protective covers marked with the high-voltage warning symbol.

WARNING! Shock Hazard. Always turn the power switch off and disconnect the mains power cable before performing any maintenance procedures.

WARNING! Lifting Hazard. The SpectraMax L Luminescence Microplate Reader weighs over 40 pounds (18.2 kg). To avoid potential injury, a minimum of two people are needed to lift the instrument.

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

CAUTION! The use of organic solvents (such as dichloromethane) can damage the instrument optics. Use extreme caution when handling organic solvents. Damage caused by the use of incompatible or aggressive solvents is NOT covered by the instrument warranty.

Moving the Instrument

If the SpectraMax L Luminescence Microplate Reader must be moved outside the lab, or returned to Molecular Devices for service, the instrument must be prepared for transport.

If you do not have the original shipping container, contact Molecular Devices and a new container can be provided for an additional charge.

CAUTION! The SpectraMax® L Luminescence Microplate Reader with external injector modules weighs over 40 pounds (18.2 kg). Molecular Devices recommends that two people lift the instrument together, using proper lifting techniques to avoid injury.

To move the instrument

1. Remove any microplate from the microplate drawer.

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

2. Turn off the power switch on the instrument.

   During the shutdown sequence, the instrument carriage is locked automatically to prevent damage during transit.
3. Disconnect the mains power cable from the back of the instrument, and the wall outlet.
4. If the instrument is to be returned to Molecular Devices for service, pack the instrument in its original shipping container. Otherwise, have two people carry the instrument or place it on a rolling cart to transport it to the new location.
5. To install the instrument in a new location, follow the instructions as specified in To unpack the instrument on page 16.

Manually Opening the Microplate Drawer

If the microplate drawer fails to open either by pressing the DRAWER button, by using the Open Drawer command in the SoftMax® Pro Software, there are steps you can take to manually open the drawer.

Required tools:

- Carriage puller

To manually open the microplate drawer

1. Turn off the power switch on the instrument, wait five seconds, and then turn the power switch back on.

   If the microplate drawer does not open, complete steps 2 to 6.

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

2. Turn off the power switch on the instrument.

3. Loosen the captive screws on the front of the instrument, and then raise the top cover up and over the instrument. See Figure 4-1 on page 57.

Tip! If the captive screws are too tight to loosen by hand, use a slotted screwdriver.

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.

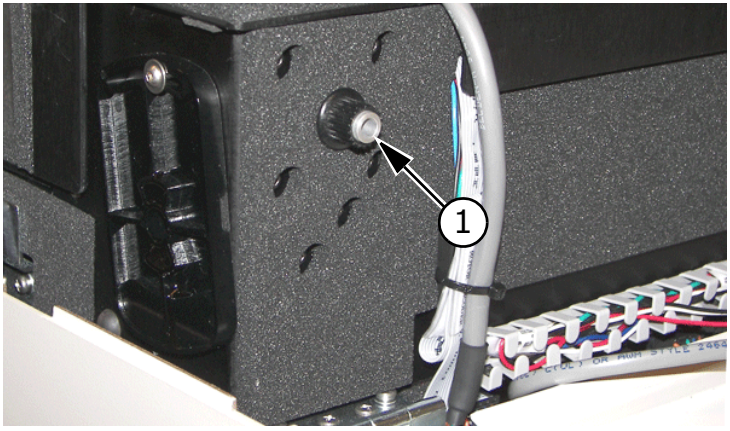


Figure 5-1 Plate carriage release knob

Item	Name
1	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.
6. Insert the end the carriage puller in the pull point on the left side of the microplate carriage, and then pull the carriage through the drawer opening.

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

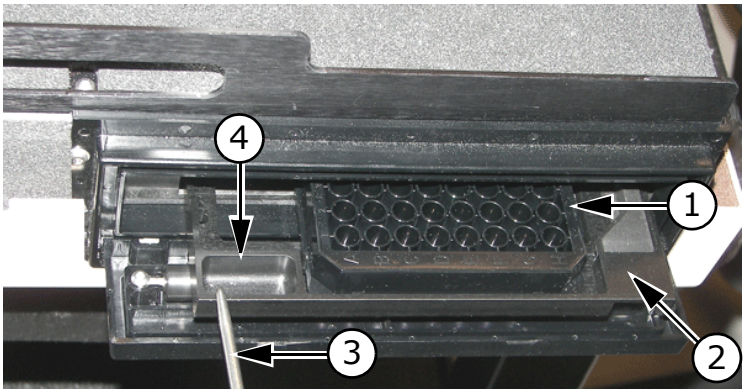


Figure 5-2 Pull point on microplate carriage

Item	Name
1	Microplate
2	Microplate drawer
3	Carriage puller
4	Pull point

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

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

Replacing the Injector Tips

Replace the injector tips if performance deteriorates or you suspect cross-contamination. The SpectraMax L Luminescence Microplate Reader can use either metal or plastic tips.

Required tools:

- Injector tool

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

1. Turn off the instrument power switch.
2. Loosen the captive screws on the front of the instrument, and then raise the top cover up and over the instrument. See Figure 4-1 on page 57.

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.

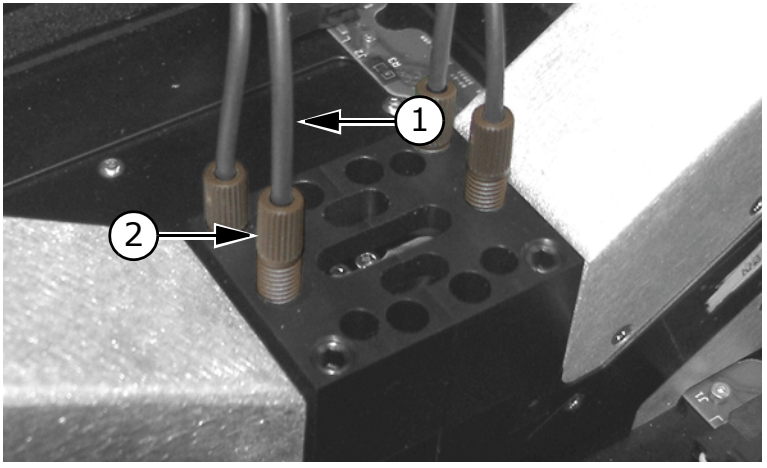


Figure 5-3 Injector and tubing assembly

Item	Name
1	Injector tubing
2	Injector fitting

4. Remove the injector tip as instructed in the table below.

Tip	Instruction
Metal	Insert the magnetic end of the injector tool into the injector port, and then pull the injector tip and attached adapter out of the port.
Plastic	Insert the pointed end of the injector tool into the injector port, and then pull the adapter out of the port. Insert the injector tool into the injector port a second time to remove the tip.

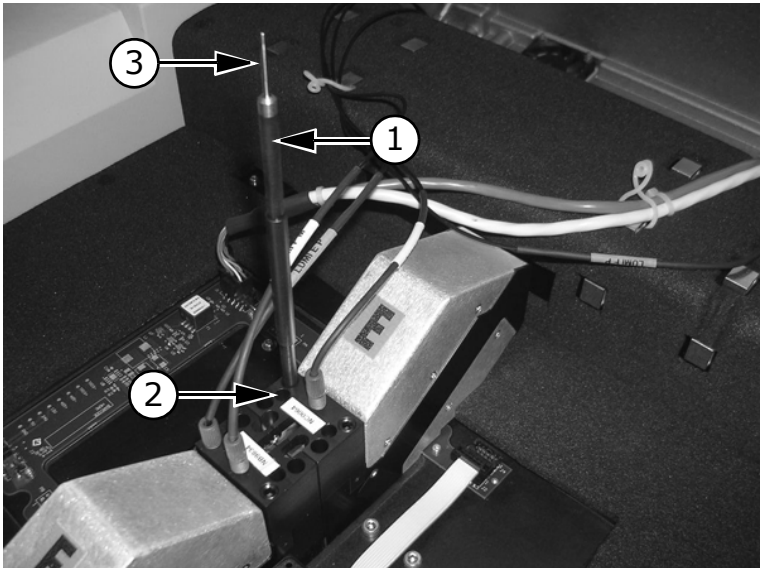


Figure 5-4 Injector tool

Item	Name
1	Injector tool – end for metal tip inserted in adapter
2	Injector port
3	Pointed end of injector tool for removing plastic tips.

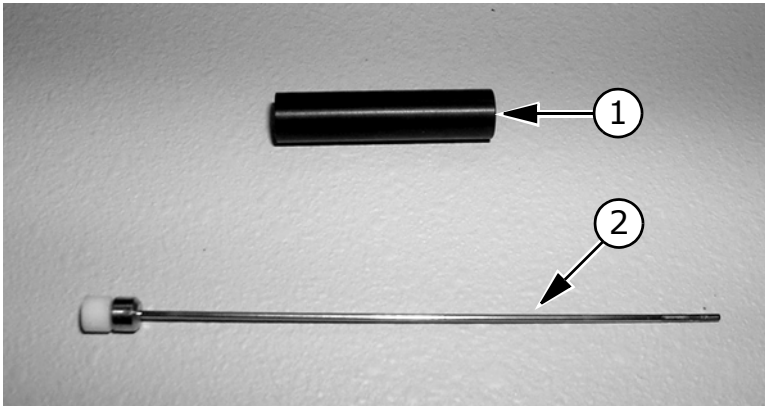


Figure 5-5 Metal injector tip and adapter

Item	Name
1	Injector adapter
2	Metal injector tip

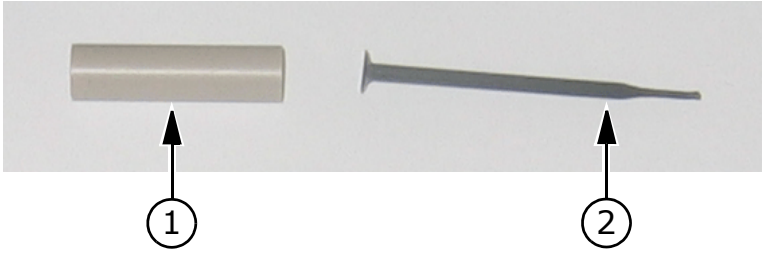


Figure 5-6 Plastic injector tip and adapter

Item	Name
1	Injector adapter
2	Plastic injector tip

5. Replace the injector tip as instructed in the table below.

Tip	Instruction
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.

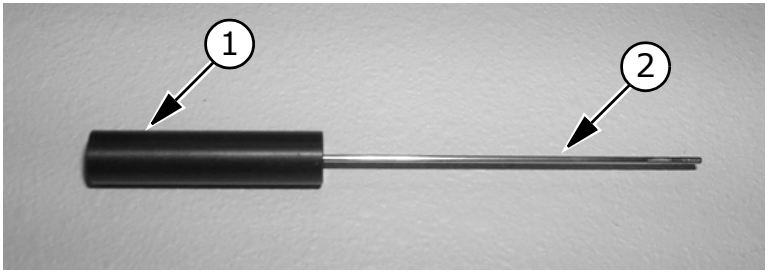


Figure 5-7 Metal injector tip inserted in adapter

Item	Name
1	Injector adapter
2	Metal injector tip

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 then tighten the captive screws.

Replacing the Peristaltic Tubing

The SpectraMax L Luminescence Microplate Reader records the volume injected by each injector. At 75,000,000 µL (approximately 3,800 96-well microplates) SoftMax® Pro Software issues a warning recommending replacement of the peristaltic tubing. Perform the following procedures to replace the old tubing and test the newly installed tubing.

- Replace the peristaltic tubing
- Prepare the instrument
- Perform an injector accuracy test

Required equipment:

- Precision electronic scale
- 96 and 384-well microplates
- Filtered de-ionized water
- Two clean laboratory bottles

To replace the peristaltic tubing

1. Loosen the captive screws on the front of the instrument, and then raise the top cover up and over the instrument. See Figure 4-1 on page 57.

Tip! If the captive screws are too tight to loosen by hand, use a slotted screwdriver.

2. Open the injector pump access door.

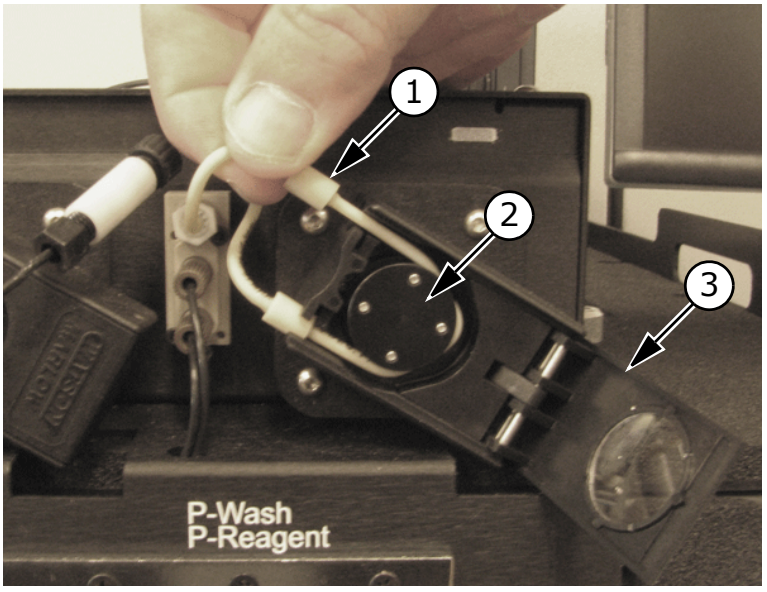


Figure 5-8 Injector pump with access door opened

Item	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.

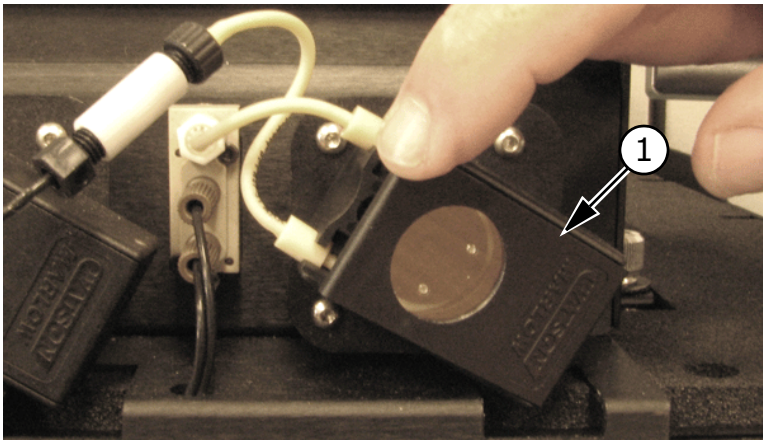


Figure 5-9 Injector pump with access door closed

Item	Name	Description
1	Injector pump access door	Closed position

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

To prepare the instrument

1. Connect the tubing for the P– and M–reagent bottles to the ports on the left side of the instrument. See Figure 2-4 on page 20.
2. Fill a bottle with filtered de-ionized 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's 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. Turn on the instrument power switch.
5. Turn on the control computer.
6. Click **Start > Programs > Molecular Devices > SoftMax Pro.**

7. Prime the injectors. See To prime the injectors on page 59.
8. Prime the injectors two more times while checking for leaks at the reagent ports.

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

1. Prime the injectors. See To prime the injectors on page 59.
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, in the experiment window, click **Settings**.

   The SpectraMax L Settings window appears.
6. Click **Endpoint**.

   The settings and parameters specific to the Endpoint read type are displayed in the left pane of the window.
7. Click **Integration Time**, and then type an Integration Time of 1 second.
8. Click **Sensitivity**, and then click **AutoRange** in the PMT Setting list.
   - In the **Target Calibration Wavelength** group, click **395 nm**.
9. Click **Injection and Delay**, and then set the following parameters for M–Injection:
   - Select **M–Injection** to configure the measurement injection parameters.

Parameter	Value
Injector volume (mL)	50
Injection speed (mL/s)	230
Number of baseline reads	0

10. Click **Assay Plate Type**, and then click **96-well Standard** microplate.

11. Click **Wells to Read**, and then select all of the microplate wells.
12. Click **OK**.
13. Click **Read** in the SoftMax Pro menu bar.

    The M–injections are initiated.
14. When the injections have finished, 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, perform the correction procedure. See To perform the correction procedure on page 79.
15. Repeat steps 3 to 14 but select P–injection in step 9.

    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, perform the correction procedure. See To perform the correction procedure on page 79.

To perform the correction procedure

1. In the SoftMax Pro Software, on the **Control** menu, click **Injector Pump Tubing**.

   The Injector Pump Tubing window appears.

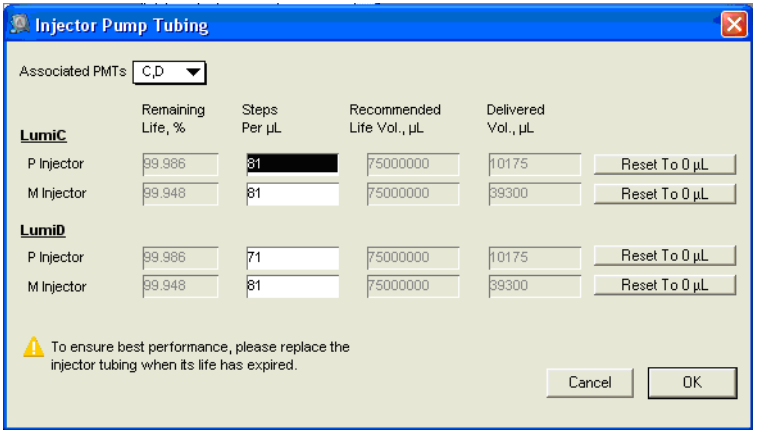


Table 5-1 Injector Pump Tubing dialog box field descriptions

Field	Description
Remaining Life, %	Life left before recommended tubing replacement
Steps per µL	Specific value for each peristaltic tube, calculated by completing step 3 below
Recommended LIfe Vol., µL	Fixed value determined by the SoftMax Pro Software
Delivered Vol., µL	Volume of fluid injected since the last reset
Reset to 0 µL	Click to reset the Delivered Vol to 0

2. In the **Associated PMTs** list, select the desired PMT.
3. Calculate the **Steps per µL** for each injector using the formula

   Steps per µL = 4.8 grams / actual weight (determined from step 14 and 15 in the procedure To perform an injector accuracy test on page 78.
4. Type the **Steps per µL** for either or both of the M–injectors and the P–injectors as calculated in step 3 above.
5. Click **Reset to 0 µL** for either or both of the M–injectors and the P–injectors.
6. Click **OK**.
7. Close the SoftMax Pro Software.
8. Turn off the power switch on the instrument, wait five seconds, and then turn the power switch back on.

Warranty and Service

A

Molecular Devices is committed to ensuring the highest quality of our products and customer service.

If you have any problems with your SpectraMax® L Luminescence Microplate Reader, contact our Technical Support group. In the US, contact us at 1-800-635-5577. For locations outside the U.S, please contact your local sales representative.

Standard Warranty

Molecular Devices warrants its non-consumable hardware products to be free from defects in materials and workmanship for 12 (twelve) months from date of invoice or date of purchased installation visit, whichever is later. The warranty covers the cost of parts and labor to repair the product.

Please keep the shipping container for future use. If you should need an additional container, one can be provided for an additional charge. Products returned to Molecular Devices for repair should be properly packaged with transportation charges prepaid. Molecular Devices will pay for the return shipping of the product to the customer. If the shipment is to a location outside the United States, the customer is responsible for all duties, taxes and freight clearance charges.

The warranty is valid when the product is used for its intended purpose and does not cover products which have been modified without approval from Molecular Devices, or which have been damaged by abuse, accident or connection to incompatible equipment.

This warranty is in lieu of all other warranties, expressed or implied.

Out-of-Warranty Repair Service

Out-of-warranty repair service is available. Contact Molecular Devices Technical Support for more information.

Optional Service Agreement

Purchasing an optional Service Agreement extends the coverage of the Standard Warranty. Contact the supplier for current rates.

Technical Support

In order to receive the best possible technical support, we encourage you to register on our website **www.moleculardevices.com**, especially if your sales transaction was conducted by a purchasing agent. Your name in our database ensures that we can contact you directly with important information about product upgrades and special promotional opportunities. Once you register your name, you can then register your instrument.

If you require advice on the use of your SpectraMax L Luminescence Microplate Reader, do not hesitate to contact Molecular Devices' Technical Support. Visit the website www.moleculardevices.com and complete the Technical Support Request form. You can also phone Molecular Devices' Technical Support at 800-635-5577. Follow the prompts for **SpectraMax Plate Readers and Liquid Handling Products Technical Support**.

At Molecular Devices, staying in touch with all our customers is a valuable part of our ongoing development process, ensuring the excellence of every product we offer.

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