SpectraMax® Plus^{384}
SpectraMax 190
SpectraMax 340PC^{384}
VersaMax

Microplate Spectrophotometer
Operator's Manual

Molecular Devices

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Molecular Devices Corporation

SpectraMax® Plus^{384}, SpectraMax 190, SpectraMax 340PC^{384}, and VersaMax Operator's Manual

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1. Description

1.1. Introduction

SpectraMax and VersaMax microplate spectrophotometers provide rapid and sensitive measurements of a variety of analytes across a wide range of concentrations. They measure the optical density (OD) of samples at selected wavelengths in a number of reading modes, depending on the instrument model:

- **Endpoint:** at a single point in time.
- **Kinetic:** over a specified period of time.
- **Spectral scan:** over a specified wavelength range.

The VersaMax and SpectraMax 190 read 96-well microplates. The SpectraMax 340PC^{384} and SpectraMax Plus^{384} read both 96-and 384-well microplates.

The contents of the wells in a microplate can be mixed automatically by shaking before each read cycle, which makes it possible to perform kinetic analysis of solid-phase, enzyme-mediated reactions (mixing is not critical for liquid-phase reactions).

The temperature of the microplate chamber can be regulated, if desired from 4°C above ambient to 45°C.

Detailed specifications of each model reader are reported in Section 7, “Specifications”.

1.2. Components

The main components of SpectraMax and VersaMax readers described in this manual are:

- Control panel
- Microplate drawer
- Cuvette chamber (SpectraMax Plus^{384} only)
- Back panel (connections and power switch)

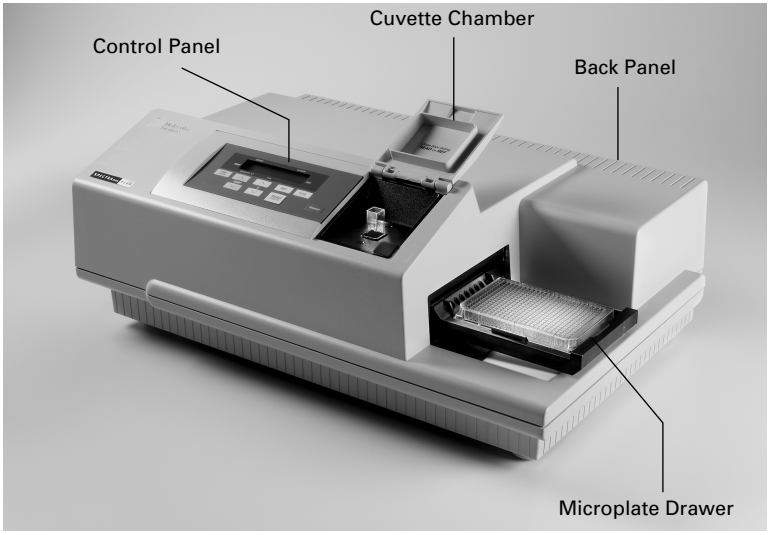


Figure 1.1: SpectraMax Plus^{384} components.

1.2.1. The Control Panel

The control panel consists of a 2-x-20-character LCD and eleven pressure-sensitive membrane keys that can be used to control some functions of the instrument. When you press a control panel key, the instrument performs the associated action.

TEMP on/off

The TEMP on/off key enables and disables the incubator.

- When the incubator is on, the set temperature and actual temperature are shown on the front panel LCD display.
- When the instrument is performing a kinetic or spectral scan, the temperature keys on the front panel are disabled.

TEMP

The TEMP keys allow you to enter a set point at which to regulate the microplate chamber temperature.

Pressing this key scrolls the temperature up or down, starting at the previous temperature setting (or the default of 37.0°C, if no setting had been made):

- Pressing the up (▲) or down (▼) arrow once increments or decrements the displayed temperature by 0.1°C.

- Pressing and holding either arrow increments or decrements the displayed temperature by 1°C until it is released.

λ (not available on VersaMax)

Selects the wavelength to be used for reading the microplate manually.

Pressing the up or down arrow key scrolls up or down through the available wavelengths, starting at the previous setting:

- Pressing the up (▲) or down (▼) arrow once increments or decrements the displayed wavelength by 1 nm.
- Pressing and holding either arrow increments or decrements the displayed wavelength by 10 nm until it is released.

96/384 (SpectraMax 340PC^{384} only)

Selects either 96-well or 384-well plate for a stand-alone read.

REF (SpectraMax Plus^{384} only)

A reading of buffer, water, or air taken in the cuvette that is used as I_0 to calculate Absorbance or % Transmittance. If no reference reading is taken, the instrument uses the I_0 values stored in the NVRAM (non-volatile memory) of the instrument.

READ CUVETTE (SpectraMax Plus^{384} only)

Initiates the sample reading of the cuvette.

READ (SpectraMax 340PC^{384} and SpectraMax 190 only)

Initiates reading of the microplate in stand-alone mode. Data is sent to the printer port.

%T / A (SpectraMax Plus^{384} only)

A toggle switch used to display cuvette data as percent transmission or absorbance.

DRAWER

The DRAWER key opens and closes the microplate drawer.

1.2.2. The Microplate Drawer

The microplate drawer is located on the right side of the SpectraMax and VersaMax readers, slides in and out of the microplate chamber. A small plastic pusher, located

in the front left corner of the drawer, holds the plate securely in place when the drawer is closed.

The drawer remains in the reading chamber during read cycles.

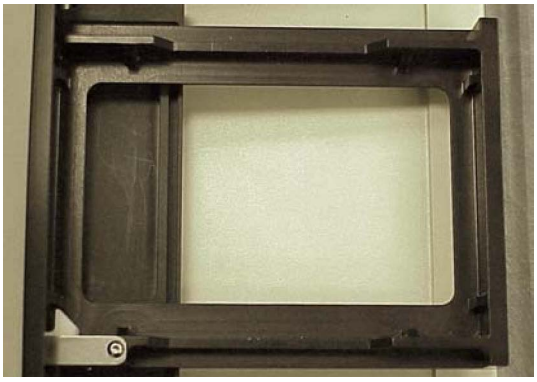


Figure 1.2: The microplate drawer.

Microplate drawer operation varies, depending on the incubator setting:

- If the incubator is off, the drawer remains open.
- If the incubator is on, the drawer closes after approximately 10 seconds to assist in maintaining temperature control within the microplate chamber.

Do not obstruct the movement of the drawer. If you must retrieve a plate after an error condition or power outage and the drawer does not open, it is possible to open it manually (see Section 6, “Troubleshooting”).

1.2.3. Microplates

SpectraMax 190 and VersaMax readers can accommodate standard 96-well and strip-well microplates.

SpectraMax Plus^{384} and 340PC^{384} readers can accommodate standard 96-well, 384-well, and strip-well microplates. When reading at wavelengths below 340 nm, special UV-transparent, disposable or quartz microplates allowing transmission of the deep UV spectra must be used.

Not all manufacturers' microplates are the same with regard to design, materials, or configuration. Temperature uniformity within the microplate may vary depending on the type of microplate used.

1.2.4. The Cuvette Chamber *(SpectraMax Plus^{384} only)*



Figure 1.3: The cuvette chamber.

Located at the right front of the SpectraMax Plus^{384}, the cuvette chamber has a lid that lifts up, allowing you to insert or remove a cuvette. The chamber contains springs that automatically position the cuvette in the proper alignment for a reading. The cuvette door must be closed before initiating a reading.

Cuvettes

The SpectraMax Plus^{384} can accommodate standard-height (45 mm), 1-cm cuvettes and 12 x 75 mm test tubes when used with the test tube cover (Figure 1.4).

Not all manufacturers' cuvettes are the same with regard to design, materials, or configuration. Temperature uniformity within the cuvette may vary depending on the type of cuvette used.

Be sure to handle cuvettes on the frosted sides only. Place the cuvette into the chamber so that the "reading" (clear) sides face left and right.

The SpectraMax Plus^{384} does not accept the short (25 mm high) microcuvettes.



Figure 1.4: Test tube cover.

1.2.5. The Back Panel

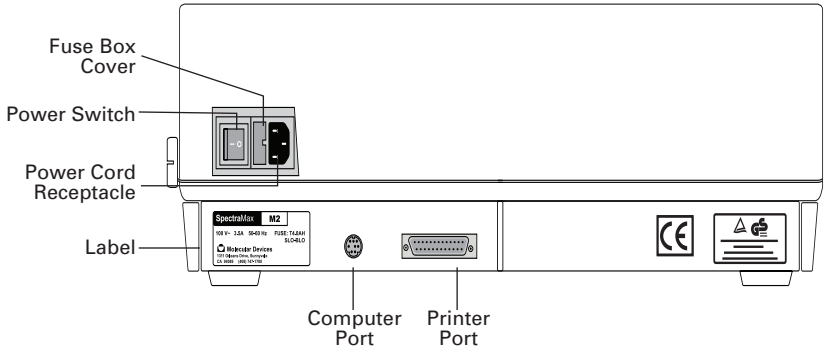


Figure 1.5: Schematic of the back panel of a reader.

The following components are located on the back panel of the SpectraMax and VersaMax readers:

- **Power switch:** a rocker switch, labeled I/O (for on and off, respectively).
- **Power cord receptacle:** plug the power cord in here.
- **Fuse box cover:** cannot be opened while the power cord is plugged in. When opened, it provides access to the fuse box containing two fuses that are required for operation.
- **Computer port** (double-shielded 8-pin RS-232 serial, for use with an external computer): plug one end of an 8-pin DIN serial cable into this port; the other end attaches to the serial (modem) port of the computer.
- **Printer port:** (double-shielded, 25-pin parallel, for use in stand-alone operation). Plug the 25-pin end of the cable into this port; the other end attaches to the port on the printer.

- **Label:** provides information about the reader, such as line voltage rating, cautionary information, serial number, etc. Record the serial number shown on this label for use when contacting Molecular Devices Technical Support.

2. Principles of Operation

2.1. Absorbance

Absorbance is the amount of light absorbed by a solution. To measure absorbance accurately, it is necessary to eliminate light scatter. In the absence of turbidity, absorbance = optical density.

$$A = log(I_0/I)$$

where I_0 is incident light, and I is transmitted light.

In this manual, we use the terms absorbance and optical density interchangeably.

2.2. Optical Density

Optical density is the amount of light passing through a sample to a detector relative to the total amount of light available. Optical density includes absorbance of the sample plus light scatter from turbidity.

2.3. Transmittance

Transmittance is the ratio of transmitted light to the incident light.

$$T = (I_0/I)$$
$$\%T = 100T$$

where I_0 is incident light, and I is transmitted light.

2.4. PathCheck™ (not VersaMax)

The Beer-Lambert law states that absorbance is proportional to the distance that light travels through the sample:

$$A = \varepsilon bc$$

where A is the absorbance, ε is the molar absorbtivity of the sample, b is the pathlength and c is the concentration of the sample. In short, the longer the pathlength, the higher the absorbance.

Microplate readers use a vertical light path so the distance of the light through the sample depends on the volume. This variable pathlength makes it difficult to perform extinction-based assays and also makes it confusing to compare results between microplate readers and spectrophotometers.

The standard pathlength of a cuvette is the conventional basis for quantifying the unique absorbtivity properties of compounds in solution. Quantitative analyses can

be performed on the basis of extinction coefficients, without standard curves (e.g. NADH-based enzyme assays). When using a cuvette, the pathlength is known and is independent of sample volume, so absorbance is proportional to concentration.

In a microplate, pathlength is dependent on the liquid volume, so absorbance is proportional to both the concentration and the pathlength of the sample. Standard curves are often used to determine analyte concentrations in vertical-beam photometry of unknowns, yet errors can still arise from pipetting the samples and standards. The PathCheck feature automatically determines the pathlength of aqueous samples in the microplate and normalizes the absorbance in each well to a pathlength of 1 cm. This novel approach to correcting the microwell absorbance values is accurate to within 2.5% of the values obtained directly in a 1-cm cuvette.

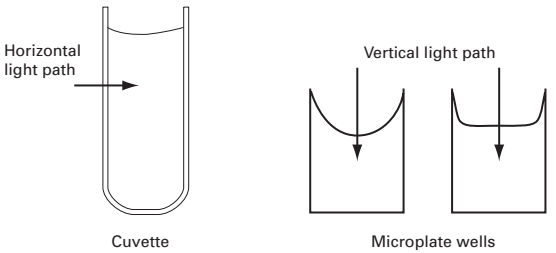


Figure 2.1: Cuvette and microwell light paths.

Reference measurements made by reading the cuvette (Cuvette Reference, SpectraMax Plus^{384} only) or using factory-stored values derived from deionized water (Water Constant) can be used to normalize the optical density data for microplate wells.

PathCheck pathlength correction is accomplished only when using the SoftMax Pro software.

The SpectraMax Plus and SpectraMax Plus^{384} offer both the Cuvette Reference and the Water Constant methods. The SpectraMax 190 and SpectraMax 340PC^{384} use the Water Constant only.

The actual pathlength, *d*, of a solvent is found from the following equation:

$$d(cm) = \frac{Sample(OD_{1000} - OD_{900})}{k}$$

When a Cuvette Reference is used for pathlength correction, the value of *k* is obtained by taking optical density measurements on the fluid in the cuvette at two wavelengths, 1000 and 900 nm:

$$k = Cuvette(OD_{1000} - OD_{900})$$

When the Water Constant is used for pathlength correction, the value of *k* is obtained from the instrument. This constant is saved in the instrument in the factory and may differ slightly from instrument to instrument.

Once the pathlength d is found, the following equation is used for the pathlength correction:

$$\frac{OD}{cm} = \frac{OD_{Sample}}{d(cm)}$$

PathCheck is applicable to almost all biological/pharmaceutical molecules in aqueous solution because they have little or no absorbance between 900 nm and 1000 nm at concentrations normally used. PathCheck can also be used with samples containing small amounts of organics or high buffer concentrations by using the Cuvette Reference (below).

2.4.1. Water Constant or Cuvette Reference Option (SpectraMax Plus^{384} Only)

The PathCheck measurement is based on the absorbance of water in the near infrared region (between 900 nm and 1000 nm). If the sample is completely aqueous, has no turbidity and has a low salt concentration (less than 0.5 M), the Water Constant is adequate. The Water Constant is determined during manufacture and is stored in the instrument.

If the sample contains an organic solvent such as ethanol or methanol, we recommend using the cuvette reference. It is important that the solvent does not absorb in the 900 nm to 1000 nm range (to determine whether or not a given solvent would interfere, see the discussion of interfering substances below). When a noninterfering solvent is added to the aqueous sample, the water absorbance decreases proportionally to the percentage of organic solvent present. For example, 5% ethanol decreases the water absorbance by 5% and results in a 5% underestimation of the pathlength. You can avoid the error by putting the same water/solvent mixture in a cuvette and using the Cuvette Reference.

To use the Cuvette Reference, place into the cuvette port a standard 1 cm cuvette containing the aqueous/solvent mixture that is used for the samples in the microplate. The cuvette must be in place when you read the microplate. When you click the Read

button in SoftMax Pro, the instrument first makes the 900 nm and 1000 nm measurements in the cuvette, and then makes the designated measurements in the microplate. The cuvette values are stored temporarily and used in the PathCheck calculations for the microplate samples.

Use of Cuvette Reference with PathCheck is different from a reference reading of a cuvette in a CuvetteSet section (by clicking the Ref button in the CuvetteSet section tool bar in SoftMax Pro). The cuvette reference used for PathCheck calculations (measurements at 900 nm and 1000 nm) does not produce data that can be viewed in a CuvetteSet section and is used only with data in microplates, not cuvettes.

2.4.2. Background Constant Subtraction and Blanking Considerations

Raw optical density measurements of microplate samples include both pathlength-dependent components (sample and solvent) and a pathlength-independent component (OD of microplate material). The latter must be eliminated from the PathCheck calculation in order to obtain valid PathCheck-normalized results. There are three ways to accomplish this — plate blanks, plate background constants, and plate pre-reads — all of which are described in the PathCheck section of the SoftMax Pro manual.

2.4.3. Pathcheck and Interfering Substances

Any material that absorbs in the 900 nm to 1000 nm spectral region could interfere with PathCheck measurements. Fortunately, there are few materials that do interfere at the concentrations typically used.

Turbidity is the most common interference: if you can detect any turbidity in your sample, you should not use PathCheck. Turbidity elevates the 900 nm measurement more than the 1000 nm measurement and causes an erroneously low estimate of pathlength. Using a Cuvette Reference does not reliably correct for turbidity.

Samples that are highly colored in the upper visible spectrum may have absorbance extending into the near infrared (NIR) and can interfere with PathCheck. Examples include Lowry assays, molybdate-based assays and samples containing hemoglobins or porphyrins. In general, if the sample is distinctly red or purple, you should check for interference before using PathCheck.

To determine possible color interference, do the following:

- Measure the optical density at 900 nm and 1000 nm (both measured with air reference).
- Subtract the 900 nm value from the 1000 nm value.

- Do the same for pure water.

If the delta OD for the sample differs significantly from the delta OD for water, then it is advisable not to use the PathCheck feature. Use of Cuvette Reference does not correct for the interference with the current calculation scheme in SoftMax Pro. Currently, Cuvette Reference involves a single (automated) read at 900 nm and 1000 nm and the automated calculations in SoftMax Pro do not compensate for color or solvent interference. However, you could correct for such interference by taking two cuvette measurements and using a different set of calculations. For further information, contact Molecular Devices Technical Support.

Organic solvents could interfere with PathCheck if they have absorbance in the region of the NIR water peak. Solvents such ethanol and methanol do not absorb in the NIR region, so they do not interfere, except for causing a decrease in the water absorbance to the extent of their presence in the solution. Their passive interference can be avoided by using the Cuvette Reference. If, however, the solvent absorbs between 900 and 1000 nm, the interference would be similar to the interference of highly colored samples described above. If you are considering adding an organic solvent other than ethanol or methanol, you are advised to run a spectral scan between 900 nm and 1000 nm to determine if the solvent would interfere with PathCheck.

2.4.4. Making Absorbance Measurements Normalized to a 1-cm Pathlength

SoftMax Pro automatically reports absorbance values normalized to a 1-cm pathlength. The table below shows results obtained with 75 µL to 300 µL yellow reagent.

Well Volume (µL)	Pathlength (cm)	Raw Absorbance	Absorbance/ cm	SD	CV%
75	0.231	0.090	0.390	0.006	1.6
100	0.300	0.116	0.387	0.005	1.2
150	0.446	0.172	0.385	0.003	0.8
200	0.596	0.228	0.383	0.002	0.4
250	0.735	0.283	0.384	0.002	0.5
300	0.874	0.336	0.384	0.001	0.3
Absorbance in 1-cm cuvette = 0.386					

Optical pathlengths and raw absorbance values were directly proportional to well columns. After normalization to a 1-cm pathlength, all absorbance values, regardless of the volume in the wells, were within 1% of the value obtained by measuring the same solution in a 1-cm cuvette.

2.5. Functional Description

The full power of a SpectraMax or VersaMax instrument can only be harnessed when the instrument is controlled using SoftMax Pro software running on a computer connected to the instrument. For a complete description of the modes of operation, how to choose instrument settings, etc., refer to the SoftMax Pro User's Manual.

However, some functionality is available directly on the instrument without having to use SoftMax Pro:

- Temperature control (all models)
- Wavelength control (not VersaMax)
- Fixed-point microplate readings (SpectraMax 190 and SpectraMax 340PC^{384})
- Fixed-point cuvette readings (SpectraMax Plus^{384})

2.5.1. Temperature Regulation

SpectraMax and VersaMax instruments have been designed to regulate the temperature of the microplate chamber from 4°C above ambient to 45°C. Upon power up, when the incubator is off, the temperature in the microplate chamber is ambient and isothermal. Turning on the incubator by pressing the TEMP on/off key causes the instrument to begin warming the microplate chamber. The temperature set point defaults to 37.0°C at start-up.

Accuracy of the temperature set point is guaranteed only if the set point is at least 4°C above ambient. If the temperature set point is lower than the ambient temperature, the chamber temperature remains at ambient. Temperature regulation is controlled by heaters only and, therefore, cannot cool the temperature to a setting lower than ambient. Additionally, the highest setting (45°C) can be achieved only if the ambient temperature is greater than 20°C.

Typically, the microplate chamber reaches 37.0°C in less than 30 minutes. The microplate chamber temperature is maintained at the set point until you press the incubator TEMP on/off key again, turning temperature regulation off.

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

Temperature regulation and control of the microplate chamber is achieved through electric heaters, a fan, efficient insulation, and temperature sensors. The heaters are located in the microplate chamber, which is insulated to maintain the temperature set point. The sensors are mounted inside the chamber and measure the air temperature.

The temperature feedback closed-loop control algorithms measure the chamber air temperature, compare it to the temperature set point, and use the difference to calculate the regulation of the heating cycles. This technique results in accurate, precise control of the chamber temperature with a temperature variation of the air inside the chamber of less than 1.0°C. The temperature uniformity within the microplate depends on its design and composition.

Note: *the temperature display on the SpectraMax Plus^{384} control panel shows the temperature of the cuvette chamber. The microplate chamber temperature can be seen only using SoftMax Pro.*

2.5.2. Read Types

SpectraMax and VersaMax readers can perform up to three types of read, depending on the instrument model: endpoint, kinetic and spectrum. Instrument setup parameters for each read type are discussed in the SoftMax Pro User's Manual.

Endpoint Read

In an endpoint read, a reading of each microplate well is taken at a single or multiple wavelengths.

Depending on the read type selected, values can be reported as optical density or %Transmittance.

Kinetic Read

In a kinetic read the data are collected over time with multiple readings taken at regular intervals. To achieve the shortest possible interval for kinetic readings, choose wavelengths in ascending order.

Kinetic analysis can be performed for up to 99 hours. The kinetic read interval depends upon the instrument setup parameters chosen in SoftMax Pro.

Kinetic analysis has many advantages when determining the relative activity of an enzyme in different types of microplate assays, including ELISAs and the purification and characterization of enzymes and enzyme conjugates. Kinetic analysis is capable of providing improved dynamic range, precision, and sensitivity relative to endpoint analysis.

Spectrum Read (not VersaMax)

Spectral analysis measures optical density or % Transmittance across a spectrum of wavelengths from 340 nm to 850 nm in the SpectraMax 340PC[384], 190 nm to 850 nm in the SpectraMax 190, and 190 nm to 1000 nm in the SpectraMax Plus[384]

All spectrum readings are made using the scanning monochromators of the instrument.

2.5.3. Automix

The Automix function permits automatic linear shaking along the long axis of the microplate at preset intervals, thereby mixing of the contents within each well. Automix must be selected before beginning a reading. The actions associated with the Automix setting depend on the read mode chosen:

- **Endpoint mode:** Automix shakes the plate for a definable number of seconds and then reads at all selected wavelengths.
- **Kinetic mode:** two types of Automix can be enabled; Automix can shake the plate for a definable number of seconds before the initial reading, and/or for a definable number of seconds before each subsequent reading.

Use of Automix is strongly recommended for ELISAs and other solid-phase, enzyme-mediated reactions to enhance accuracy.

2.5.4. Computer Control

SpectraMax and VersaMax readers are equipped with an 8-pin DIN RS-232 serial port through which the computer communicates with the instrument. (Different types of cables are available for connecting to different types of computers — see Appendix 8.1., “Cables”).

SoftMax Pro software, version 4.1 or greater, is required to control SpectraMax and VersaMax instruments.

3. Installation

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

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

⚠ **CAUTION:** Do not touch or loosen any screws or parts other than those specifically designated in the instructions. Doing so might cause misalignment and voids the instrument warranty.

3.1. Unpacking

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

⚠ **WARNING:** SpectraMax and VersaMax instruments weigh approximately 30 pounds (13.6 kg) and should be lifted with care. It is recommended that two persons lift the instrument together, taking the proper precautions to avoid injury.

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

3.2. Setting Up the Instrument

1. Place the instrument on a level surface, away from direct sunlight, dust, drafts, vibration, and moisture.
2. Turn the instrument around so that the back of the instrument is facing you.
3. Insert the serial cable into the RS-232 serial port receptacle on the back panel of the instrument. (A Keyspan USB adapter is necessary for a Macintosh computer or a Windows computer without a serial port; see Appendix 8.1., “Cables” for more information on adapter cables.) Attach the other end to your computer.

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

5 If you are using a printer for reading microplates in stand-alone mode, locate the printer port (25-pin parallel) on the rear panel. Connect one end of the cable to this port and connect the other end to the printer. Load paper into the printer according to the manufacturer's instructions and connect the printer's power cord.

6 Turn the instrument around so that the control panel now faces you. Ensure no cables run beneath the instrument. Leave at least three inches between the back of the instrument and the nearest objects or surfaces to ensure proper ventilation and cooling.

7 For the SpectraMax Plus^{384}, remove the tape from the cuvette door.

8 Turn on the power to the instrument, wait for the microplate drawer to open, and remove the tape and protective covering from the drawer subplate.

4. Operation

This chapter contains operating information for SpectraMax and VersaMax Spectrophotometers.

4.1. Quick Overview

If you are an experienced user of this instrument, the following steps provide a quick reminder of the basic operating procedures required to perform an assay using a SpectraMax or VersaMax instrument:

1. Turn on the power switch (located on the back panel). The microplate drawer opens automatically.
2. If you want to regulate the temperature inside the chamber, touch the TEMP on/off (incubator) key to turn the incubator on and bring the chamber to the default temperature of 37.0°C. The microplate drawer closes.
3. If the incubator is on, the LCD shows the current temperature along with the temperature set point. To change the set point (to any setting from ambient +4° to 45°C), press the up or down arrow keys.
4. Select the desired instrument settings (read mode, type of analysis, template, etc.) using SoftMax Pro software on the external computer.
5. If you are performing kinetic analysis, add substrate at this time.
6. Load the prepared microplate into the drawer, being sure to match well A1 with the upper left-hand corner of the drawer.
7. Using SoftMax Pro, start the reading.

4.2. Preparing for a Reading

4.2.1. Turn the Instrument and Computer On

The power switch is located on the back panel. Press the rocker switch to the ON position.

The instrument automatically performs diagnostic checks to ensure that it is functioning correctly. Turn the computer on at this time also and start the SoftMax Pro software program.

4.2.2. Set the Temperature (Optional)

To set the temperature within the microplate chamber, you should turn on the incubator first, allowing enough time for the temperature to reach the set point before performing a reading. When you first turn the instrument on, up to 60 minutes may be required for the temperature within the chamber to reach the set point. Turning on the incubator and choosing a temperature set point can be done using the software or the front panel of the instrument (described here).

Temperature cannot be regulated at a set point that is lower than 4°C above the ambient temperature.

To enable the incubator:

- Press the incubator TEMP on/off key.
- The LCD display indicates that temperature control is on and shows the set point and current temperature of the microplate chamber.

To change the temperature set point:

- Press the up or down arrow keys until the desired temperature set point is shown in the display.

The microplate chamber temperature is maintained at the set point until you disable temperature control by touching the incubator key again. When the incubator is off, the temperature within the microplate chamber gradually returns to ambient.

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

4.3. Read the Microplate

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

- Insert the filled microplate into the drawer, matching well A1 with the upper left hand position of the drawer. Make sure the microplate is flat against the drawer bottom.
- You must have SoftMax Pro software running on a computer connected to the instrument. Press the READ button in SoftMax Pro to start the plate read.
- When reading is complete, the drawer of the instrument opens, allowing you to remove the microplate. If the incubator is on, the drawer closes again after approximately 10 seconds.

- If you return to the instrument and find the drawer closed after a reading has finished, press the DRAWER key. When the drawer opens, you can remove the microplate.

For more information about plate reading, please consult the SoftMax Pro User's Manual.

4.4. Standalone Endpoint Cuvette Reading (SpectraMax Plus^{384} Only)

When operating the SpectraMax Plus^{384} as a stand-alone system, you can obtain a fixed-wavelength reading of the cuvette:

- Choose the wavelength by pressing the up or down arrows beneath the display labeled λ.
- Set the temperature of the cuvette/microplate chamber using the keys on the left side of the control panel.

To read the cuvette:

- Press the REF key for a reference reading.
- Press the READ CUVETTE key for a sample reading.

The resulting data can be displayed as absorbance or percent transmission by toggling the %T/A key.

The data is sent to the printer port of the instrument. A new cuvette can be loaded into the SpectraMax Plus^{384} while the results from the first reading are being printed.

Note: due to the continuous changes in printer technology leading to printer obsolescence, and the ability of SoftMax Pro software to print on PC-compatible and Macintosh-compatible printers, Molecular Devices does not recommend relying on printing in standalone mode.

Keep the cuvette door closed when reading a cuvette.

See Appendix 8.3., “Cuvettes in SpectraMax Plus384” for cuvette types that have been tested with the SpectraMax Plus^{384}.

4.5. Standalone Endpoint Microplate Reading (SpectraMax 190 and SpectraMax 340PC^{384} Only

When operating the SpectraMax 190 and SpectraMax 340PC^{384} as stand-alone systems, you can obtain a fixed-wavelength reading of the microplate:

- Choose the wavelength by pressing the up or down arrows beneath the display labeled λ.
- Set the temperature of the microplate chamber using the keys on the left side of the control panel.

To read the microplate:

- Press the READ key.

The resulting data is sent to the printer port of the instrument.

Note: due to the continuous changes in printer technology leading to printer obsolescence, and the ability of SoftMax Pro software to print on PC-compatible and Macintosh-compatible printers, Molecular Devices does not recommend relying on printing in standalone mode.

5. Maintenance

5.1. Technical Support

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

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

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

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

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



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

⚠ **WARNING:** Never perform any operation on the instrument in an environment where liquids or potentially damaging gases are present.

⚠ **WARNING:** Risk of electrical shock. Refer servicing to qualified personnel.

⚠ **CAUTION:** Use of organic solvents (such as dichloromethane) may cause harm to the optics in the instrument. Extreme caution is advised when using organic solvents. Always use a plate lid and avoid placing a plate containing these materials in the reading chamber for prolonged periods of time. Damage caused by the use of incompatible or aggressive solvents is NOT covered by the instrument warranty.

⚠ **CAUTION:** Never touch any of the optic mirrors, filters, or cables or their housing, or manifold. The optics are extremely delicate, and critical to use of the instrument.

⚠ **CAUTION:** Do not touch or loosen any screws or parts other than those specifically designated in the instructions. Doing so could cause misalignment and possibly void the warranty.

5.2. Moving the VersaMax or SpectraMax

If you need to relocate a VersaMax or SpectraMax, follow these steps.

⚠ **WARNING:** SpectraMax and VersaMax instruments weigh approximately 35 pounds (16 kilograms). To avoid injury, it is recommended that two people lift the instrument together, using proper lifting techniques.

1. Remove any microplate from the drawer and then close the drawer.
2. Turn off the power switch and unplug the power cord from the source and from the receptacle on the back of the instrument.
3. Depending on the distance that you are moving the instrument, you may want to repackage the instrument in its original shipping carton. Otherwise, carry the instrument or place it on a rolling cart to transport it.
4. Ensure that the new location meets the proper specifications as described in Section 3.2., “Setting Up the Instrument”.

5.3. General Maintenance

Keep the drawer closed when the instrument is not in use. The drawer can be opened by pressing the DRAWER button. Always close the drawer immediately prior to switching the instrument off.

5.4. Cleaning

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

⚠ **WARNING:** Never clean the inside of the instrument.

Periodically, you should clean the outside surfaces of the instrument using a cloth or sponge that has been dampened with water:

- Do not use abrasive cleaners.
- If required, clean the surfaces using a mild soap solution diluted with water or a glass cleaner and then wipe with a damp cloth or sponge to remove any residue.

- Do not spray cleaner directly onto the instrument.

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

Should fluids spill in the drawer area (when the drawer is out), they are directed to a tray at the bottom of the instrument, from which they exit to the bench or counter beneath the instrument. Wipe up any spills immediately.

Do not allow excess water or other fluids to drip inside the instrument.

5.5. Cleaning the Fan Filter

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

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

5.6. Changing the Fuses

Fuses burn out occasionally and must be replaced.

If the instrument does not seem to be getting power after switching it on (the LCD shows no display):

- Check to see whether the power cord is securely plugged in to a functioning power outlet and to the receptacle at the rear of the SpectraMax or VersaMax.

If power failed while the instrument was already on:

- Check that the power cord is not loose or disconnected and that power to the power outlet is functioning properly.

If these checks fail to remedy the loss of power, follow the steps listed below to replace the fuses. Spare fuses (two U.S. and two metric) are shipped with the instrument. The

U.S. and metric fuses are identical except for physical size. They may be taped to the back of the instrument.

If you no longer have spare fuses, you may obtain new ones from Molecular Devices (part numbers: 4601-0013 for U.S., 4601-0014 for metric) or from a local hardware store. Make sure fuses are rated SLOWBLOW (U.S.: 4-amp time-delay; metric: 4-amp, 5 x 20 mm, time-delay).

To change fuses:

1. Switch power to the instrument off and then remove the power cord from the outlet and from the instrument power cord receptacle.
2. Remove the printer cable and computer cable (if connected) from the back of the instrument.
3. Turn the instrument around for easy access to the rear panel.
4. On the left-hand side of the rear panel (viewed from the back) is the power switch, fuse box, and power cord receptacle. As shown in the figures below, press to the left of the black plastic cover of the fuse box to release it. Pull the fuse box cover away from the instrument. The fuse box will begin to slide forward.

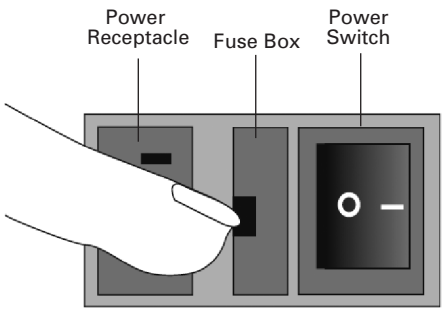


Figure 5.1: Power receptacle, fuse box, and power switch.

5. Continue gently pulling the fuse box forward until it is free of the instrument.

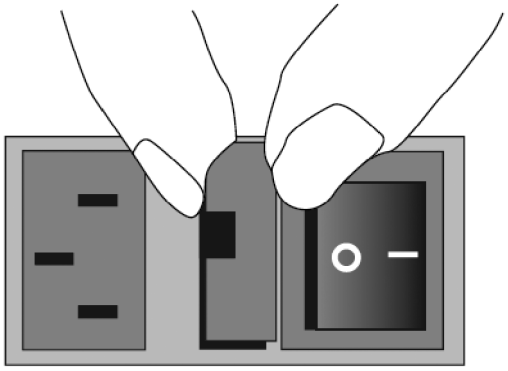


Figure 5.2: Removing the fuse box.

6 When removed, the fuse assembly will appear as shown in Figure 5.3. The holder inside contains two fuses.

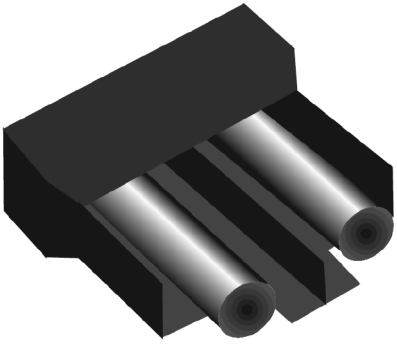


Figure 5.3: The fuse box and holder (with fuses), removed from instrument.

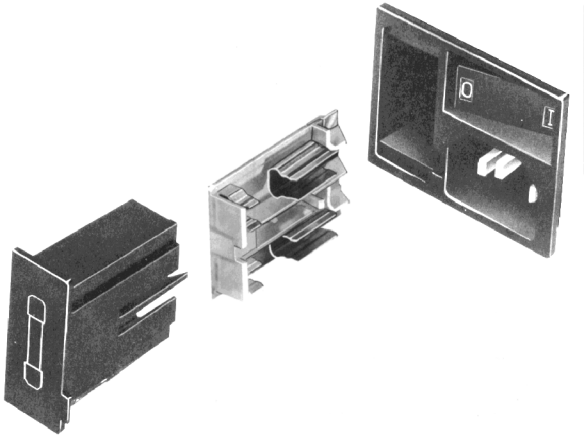


Figure 5.4: The fuse holder (with fuses), removed from the fuse box.

7 It is possible that only one of the fuses may have blown. However, Molecular Devices recommends that you replace both fuses to ensure continued proper operation. Pull both fuses out of the holder and discard them.

8 Insert new SLOWBLOW-rated fuses into the fuse holder. Either end of the fuse may be forward.

9 Insert the fuse box into the opening in the instrument, making sure that the fuses are on the left side (toward the power receptacle). Press the fuse box into place, making sure the cover snaps closed.

10 Reconnect the power cord to the instrument and to the wall outlet and reconnect other cables previously disconnected.

6. Troubleshooting

This chapter lists error codes that may occur while using the instrument, followed by their most likely causes and remedies.

Maintenance procedures are described in Chapter 5.

For problems with the SpectraMax or VersaMax that are not listed here, in the U.S., contact Molecular Devices Technical Services group at 1-800-635-5577; elsewhere, call your local representative.

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

6.1. Error Codes and Resolutions

The LCD displays Fatal Error codes when a situation arises that requires attention. Any reading in progress will stop.

Warning messages do not stop a reading but are logged in the error buffer. Warning messages indicate a situation that requires attention but is not sufficient to stop or prevent a reading. Examples of situations that might cause warning messages are low memory, entries being out of range, or operations that could result in loss of data. These messages are generally self-explanatory.

For assistance regarding warning messages, contact your local Molecular Devices representative.

6.1.1. Error Code Classifications

Not all error messages are listed in this manual. The errors are grouped in relationship to possible causes as follows:

Table 6.1: SpectraMax and VersaMax error code ranges.

ERROR CODE NUMBERS	POSSIBLE CAUSES
100–199	Errors possibly caused by unrecognized commands being sent from the computer to the instrument.
200–299	Errors probably due to a main board failure or an error in the firmware code. Most of these errors require the assistance of Technical Support.
300–399	Instrument errors due to either a main board failure or other system failure. Most of these errors require the assistance of Technical Support.
400–499	Errors caused by a motor motion failure. Most of these errors require the assistance of Technical Support.
500–599	Errors due to failure or improper initialization of the instruments non-volatile memory (NVRAM). All of these errors require the assistance of Technical Support.

Some errors (shown in boldface in the following table) are considered fatal in that if they are detected during power up, the instrument aborts the power up sequence and displays "FATAL ERROR" on the LCD panel.

Check the following list to see if there is something that you can do to change the condition of the instrument to prevent the fatal error. (e.g., closing the cuvette door during the power up sequence prevents errors 111, 219, 302, and 310).

After correcting the problem, leave the instrument on for about five minutes, turn it off and then back on.

If you continue to get the fatal error message on power up, record the error message number and contact Molecular Devices Technical Support or your local representative for assistance.

If the instrument is functioning normally when using SoftMax Pro, no errors should be in the buffer (except error number 100).

Table 6.2: Error codes, error messages, and notes about the errors.

ERROR CODE	ERROR MESSAGE	NOTES
100–199: UNRECOGNIZED COMMAND ERRORS SENT FROM THE COMPUTER		
100	command not found	Command string not recognized.
101	invalid argument	Command argument not recognized.
102	too many arguments	Too many arguments after command.
103	not enough arguments	Missing arguments.
104	input line too long	Too many characters in the input line.
105	command invalid, system busy	Instrument could not perform the give command because it was busy doing another task.
106	command invalid, measurement in progress	Instrument could not perform command because a measurement was in progress.
107	no data to transfer	Inputting transfer when there's no data in the buffer.
108	data buffer full	Too many data sets in the buffer. Can be caused by setting up a long kinetic and disconnecting computer or SoftMax Pro is preempted by another application.
109	error buffer overflow	More than 65 errors in the buffer, clear the buffer.
110	stray light cuvette, door open?	Cuvette door open while doing a read.
111	invalid read settings	
200–299: FIRMWARE ERRORS		
200	assert failed	Firmware error.
201	bad error number	Firmware error.
202	receive queue overflow	Caused by external device sending too much data over serial port and ignoring flow control.

ERROR CODE	ERROR MESSAGE	NOTES
203	serial port parity error	Parity bit error detected with incoming serial data.
204	serial port overrun error	Caused by host computer sending too much data and ignoring the flow control signal.
205	serial port framing error	
206	cmd generated too much output	Firmware error.
207	fatal trap	Instrument error. Instrument locks up.
208	RTOS error	Firmware error.
209	stack overflow	Firmware error.
210	unknown interrupt	Firmware error.
300–399: HARDWARE ERRORS		
300	thermistor faulty	Unable to read a reasonable thermistor value. Thermistor faulty or disconnected, Main board problem, or ambient temperature out of range.
301	safe temperature limit exceeded	A temperature of over 50°C detected on one or more of the 4 thermistors. Temperature will be shut off and remain off until a successful completion of power-up reset.
302	low light	Not enough light detected to make an accurate measurement. If doing a cuvette read, the cuvette door may be open.
303	**unable to cal dark current**	**Too much stray light detected on power-up, faulty or disconnected pre-amp boards**.
304	signal level saturation	During a cuvette read, could be due to cuvette door being open.
305	reference level saturation	During a cuvette read, could be due to cuvette door being open.
306	plate air cal fail, low light	Minimum signal/reference ratio not met during air calibration.

ERROR CODE	ERROR MESSAGE	NOTES
307	cuv air ref fail	
308	stray light	Light leak in reading chamber or cuvette door open. Could also be a faulty pre-amp board.
309	front panel not responding	LCD front panel bad or disconnected.
312	**gain calibration failed**	**Power-up calibration and check of signal path gain is out of tolerance. Could be due to bad or disconnected pre-amp or excessive stray light.**
313	**reference gain check fail**	**Power-up check of the Reference amplifier's gain out of tolerance. Could be due to bad or disconnected pre-amp board or excessive stray light.**
314	**low lamp level warning**	
315	**can't find zero order**	**On power-up, grating motor could not find zero-order home position.**
316	**grating motor driver faulty**	**Grating motor didn't move to where it was commanded to in a reasonable time.**
317	**monitor ADC faulty**	
400–499: MOTION ERRORS		
400	**carriage motion error**	**Carriage did not move to either of its photo interrupts in a reasonable time, or can't find its photo interrupt.**
401	**filter wheel error**	**Filter wheel did not move to its photo interrupt in a reasonable time, or can't find its photo interrupt.**
402	**grating error**	**Grating did not move to its photo interrupt in a reasonable time, or can't find its photo interrupt.**
403	**stage error**	**Stage did not move to its photo interrupt in a reasonable time, or can't find its photo interrupt.**

ERROR CODE	ERROR MESSAGE	NOTES
500–599: NVRAM ERRORS		
500	NVRAM CRC corrupt	The CRC for the NVRAM data is corrupt.
501	NVRAM Grating cal data bad	Grating calibration data is unreasonable.
502	NVRAM Cuvette air cal data error	Cuvette air calibration data is unreasonable.
503	NVRAM Plate air cal data error	Plate air calibration data is unreasonable.
504	NVRAM Carriage offset error	Carriage offset data is unreasonable.
505	NVRAM Stage offset error	Stage offset data is unreasonable.
506	NVRAM Battery	Time to replace the NVRAM battery (U3).

For all other error messages (codes not listed here), please contact your local Molecular Devices representative for assistance.

6.2. Opening the Drawer Manually

- If an error occurs while the drawer is closed and you need to remove a microplate, press the DRAWER key.
- If the drawer does not open, turn power to the instrument off and then on again.
- If the drawer remains closed, turn the incubator off (if it was on) by pressing the TEMP on/off key.
- If the drawer still remains closed, try using a blunt, flat object (such as a spatula) to open the door. With your index finger, pull the microplate drawer out of the instrument (do not force the drawer) and remove the microplate. This action will not harm the instrument, but should only be taken if the first two options have failed to open the drawer.

If you are still unable to open the drawer, contact your local Molecular Devices representative.

7. Specifications

7.1. SpectraMax Plus384 Specifications

Thermal specifications for microplates used in the SpectraMax Plus384 apply to flat-bottom microplates with isolated wells.

All other microplate specifications apply to standard 96-well polystyrene flat-bottom microplates.

Performance specifications for cuvette readings apply only to aqueous solutions having solute molal concentrations less than 0.4 M.

When pathlength compensation is applied to microplate absorbance measurements, agreement with cuvette absorbance measurements for the same solution requires that the solution volume in the microplate well is between 100 µL and 300 µL.

Technical specifications are subject to change without notice.

PHOTOMETRIC PERFORMANCE	
Wavelength range	190–1000 nm
Wavelength selection	Monochromator tunable in 1-nm increments
Wavelength bandwidth	≤ 2.0 nm full width half maximum
Wavelength accuracy	± 1.0 nm across wavelength range
Wavelength repeatability	± 0.2 nm
Photometric range	0 to 4.000 OD
Photometric resolution	0.001 OD
Photometric accuracy/linearity (microplate), 0–2.0 OD	190–1000 nm < ± 1.0% and ± 0.006 OD
Photometric accuracy/linearity (cuvette), 0–2.0 OD	190–1000 nm < ± 1.0% and ± 0.005 OD
Photometric precision (repeatability), 0–2.0 OD	190–1000 nm < ± 1.0% and ± 0.003 OD
Stray light	≤ 0.05% at 230 nm
Photometric stabilization	Instantaneous

Photometric drift	None — continuous referencing of monochromatic input
Calibration	Automatic before first kinetic read and before every endpoint reading
Optical alignment	None required
Light source	Xenon flash lamp (5 Watts)
Average lamp lifetime	1 billion flashes
Illumination	Top down (microplates); horizontal (cuvettes)
Photodetectors	Silicon photodiode
PHOTOMETRIC ANALYSIS MODES	
Standalone	• Single wavelength Absorbance or %Transmittance reading of the cuvette (or test tube)
Using SoftMax Pro	• Express data as Absorbance or %Transmittance • Single wavelength reading of microplate and/or cuvette • Multiple wavelength (up to six) reading of microplate or cuvette • Kinetic and kinetic graphics of microplate and/or cuvette • Spectral scan (190–1000 nm) of microplate and/or cuvette

MEASUREMENT TIME (CALIBRATION OFF)	
Microplate read time (endpoint) — standard read* *Measurement conditions: endpoint, column priority (for dual-wavelength measurements), calibrate off.	• 96 wells in 9 seconds (single wavelength) • 96 wells in 19 seconds (dual wavelength, 425 & 650 nm) • 384 wells in 29 seconds (single wavelength) • 384 wells in 59 seconds (dual wavelength, 425 & 650 nm)
Microplate read time (endpoint) — speed read* *Measurement conditions: endpoint, column priority (for dual-wavelength measurements), calibrate off.	• 96 wells in 5 seconds (single wavelength) • 96 wells in 12 seconds (dual wavelength, 425 & 650 nm) • 384 wells in 16 seconds (single wavelength) • 384 wells in 34 seconds (dual wavelength, 425 & 650 nm)
Microplate kinetic read intervals	• 96 wells, 9-second minimum interval between readings (single wavelength) • 1 column, 2-second minimum interval between readings (single wavelength) • 384 wells, 29-second minimum interval between readings (single wavelength)
Cuvette read time (endpoint)	• 1 second (single wavelength)
Cuvette kinetic read intervals	• 2-second minimum interval between readings (single wavelength)
SCAN SPEED	
Cuvette: normal scan	45*K nm/min (K = wavelength interval)
Cuvette: speed scan	130*K nm/min
Microplate: normal scan	33*K nm/min (8-well strip); 21*K nm/min (16-well strip)
Microplate: speed scan	135*K nm/min (8-well strip); 77*K nm/min (16-well strip)

TEMPERATURE REGULATION	
Reading chamber	Isothermal when temperature regulation is not enabled
Range	4°C above ambient to 45°C when temperature regulation enabled. The ambient temperature must be >20°C to achieve temperature regulation at 45°C.
Resolution	± 0.1°C
Accuracy	± 1.0°C for microplate and cuvette chamber
Temperature uniformity at equilibrium	± 0.5°C at 37°C
Chamber warm-up time	15–30 minutes (measured on air) after initiation of temperature regulation
Temperature regulation	4 sensors
Drift	± 0.2°C (regulated)
Temperature regulation diagnostics	Temperature regulation system is continuously monitored and updated
Evaporation	Plate lid required to minimize evaporative cooling
Recommended microplate	Flat-bottom microplates with isolated wells and lid
AUTOMIX WITH SOFTMAX PRO	
Plate mixing modes	Selectable: off, once prior to any reading, and once prior to and between kinetic readings
Plate mixing duration	Selectable: 1 to 999 seconds (three-second default)

COMPATIBILITY	
Microplates	Standard and half-area 96-well flat-bottomed microplates (0.3 mL). 384-well flat-bottomed microplates. Polystyrene plates for wavelengths above 340 nm; UV transparent plates above 220 nm; quartz plates above 190 nm.
Cuvettes	Standard height (45 mm) cells with 10 mm pathlength (12.5 mm x 12.5 mm outside) with minimum inside width of 4 mm (typical for 3 mL volume cells). See Section 8.3., "Cuvettes in SpectraMax Plus384" for more information.
Test tubes	12 x 75 mm test tubes can be used in the cuvette chamber with the test tube cover.
GENERAL INSTRUMENT	
Display	2-x-20-character backlit LCD
Operating panel	8-key membrane keypad
Self-diagnosis	Continuous on-board diagnostics
Spill control	Drawer mechanism and reading chamber assembly protected from accidental spillage by drainage ports
Computer interface	8-pin DIN RS-232 serial (double shielding required)
Printer interface	Parallel 25-pin to Centronics (double shielding required)
Microplates supported	All 96-well and strip-well microplates, including lids

ENVIRONMENTAL	
Operating temperature	15°C to 40°C
Operating humidity	0 to 70%, non-condensing
Storage temperature	-20°C to 65°C
PHYSICAL	
Size (h x w x d)	8.6" (220 mm) x 22.8" (580 mm) x 15" (380 mm)
Weight	30 lb (13.6 kg)
Power consumption	< 250 W
Line voltage and frequency	90–250 VAC autoranging, 50/60 Hz

7.2. SpectraMax 340PC^{384} Specifications

Thermal specifications for microplates used in the SpectraMax 340PC^{384} apply to flat-bottom microplates with isolated wells.

All other microplate specifications apply to standard 96-well polystyrene flat-bottom microplates.

Technical specifications are subject to change without notice.

PHOTOMETRIC PERFORMANCE	
Wavelength range	340–850 nm
Wavelength selection	Monochromator tunable in 1-nm increments
Wavelength bandwidth	≤ 2.0 nm full width half maximum
Wavelength accuracy	± 1.0 nm across wavelength range
Wavelength repeatability	± 0.2 nm across all optical channels
Photometric range	0 to 4.000 OD
Photometric resolution	0.001 OD
Photometric accuracy/linearity (microplate), 0–2.0 OD	340–850 nm < ± 1.0% and ± 0.006 OD

Photometric precision (repeatability), 0–2.0 OD	340–850 nm < ± 1.0% and ± 0.003 OD
Stray light	≤ 0.05% at 340 nm
Photometric stabilization	Instantaneous
Photometric drift	None — continuous referencing of monochromatic input
Calibration	Automatic before first kinetic read and before every endpoint reading
Optical alignment	None required
Light source	Xenon flash lamp (5 Watts)
Average lamp lifetime	1 billion flashes
Illumination	Top down
Photodetectors	Silicon photodiode
PHOTOMETRIC ANALYSIS MODES	
Standalone	• Single wavelength Absorbance reading of the microplate
Using SoftMax Pro	• Express data as Absorbance or %Transmittance • Single wavelength reading of microplate • Multiple wavelength (up to six) reading of microplate • Kinetic and kinetic graphics of microplate • Spectral scan (340–850 nm) of microplate

MEASUREMENT TIME (CALIBRATION OFF)	
Read time (endpoint) — standard read	• 96 wells in 9 seconds (single wavelength) • 96 wells in 19 seconds (dual wavelength, 425 & 650 nm) • 384 wells in 29 seconds (single wavelength) • 384 wells in 59 seconds (dual wavelength, 425 & 650 nm)
Kinetic read intervals	• 96 wells, 9-second minimum interval between readings (single wavelength) • 1 column, 2-second minimum interval between readings (single wavelength) • 384 wells, 29-second minimum interval between readings (single wavelength)
SCAN SPEED	
Microplate: normal scan	33*K nm/min (8-well strip); 21*K nm/min (16-well strip)
Microplate: speed scan	135*K nm/min (8-well strip); 77*K nm/min (16-well strip)
TEMPERATURE REGULATION	
Reading chamber	Isothermal when temperature regulation is not enabled
Range	4°C above ambient to 45°C when temperature regulation enabled. The ambient temperature must be >20°C to achieve temperature regulation at 45°C.
Resolution	± 0.1°C
Accuracy	± 1.0°C for microplate and cuvette chamber
Temperature uniformity at equilibrium	± 0.5°C at 37°C
Chamber warm-up time	15–30 minutes (measured on air) after initiation of temperature regulation

Temperature regulation	4 sensors
Drift	± 0.2°C (regulated)
Temperature regulation diagnostics	Temperature regulation system is continuously monitored and updated
Evaporation	Plate lid required to minimize evaporative cooling
Recommended microplate	Flat-bottom microplates with isolated wells and lid
AUTOMIX WITH SOFTMAX PRO	
Plate mixing modes	Selectable: off, once prior to any reading, and once prior to and between kinetic readings
Plate mixing duration	Selectable: 1 to 999 seconds (3-second default)
COMPATIBILITY	
Microplates	Standard and half-area 96-well flat-bottomed microplates (0.3 mL). 384-well flat-bottomed microplates. Polystyrene plates for wavelengths above 340 nm; UV transparent plates above 220 nm; quartz plates above 190 nm.
GENERAL INSTRUMENT	
Display	2-x-20-character backlit LCD
Operating panel	8-key membrane keypad
Self-diagnosis	Continuous on-board diagnostics
Spill control	Drawer mechanism and reading chamber assembly protected from accidental spillage by drainage ports
Computer interface	8-pin DIN RS-232 serial (double shielding required)
Printer interface	Parallel 25-pin to Centronics (double shielding required)
Microplates supported	All 96-well and strip-well microplates, including lids

ENVIRONMENTAL (FOR INDOOR USE ONLY)	
Operating temperature	5°C to 40°C
Operating altitude	< 2000 m
Installation category	II
Pollution degree	2
Operating humidity	< 80%
Storage temperature	-20°C to 65°C
PHYSICAL	
Size (h x w x d)	8.6" (220 mm) x 22.8" (580 mm) x 15" (380 mm)
Weight	30 lb (13.6 kg)
Power consumption	< 250 W
Line voltage and frequency	90–250 VAC autoranging, 50/60 Hz

7.3. SpectraMax 190 Specifications

Thermal specifications for microplates used in the SpectraMax 190 apply to flat-bottom microplates with isolated wells.

All other microplate specifications apply to standard 96-well polystyrene flat-bottom microplates.

Technical specifications are subject to change without notice.

PHOTOMETRIC PERFORMANCE	
Wavelength range	190–850 nm
Wavelength selection	Monochromator tunable in 1-nm increments
Wavelength bandwidth	≤ 2.0 nm full width half maximum
Wavelength accuracy	± 1.0 nm across wavelength range
Wavelength repeatability	± 0.2 nm across all optical channels
Photometric range	0 to 4.000 OD

Photometric resolution	0.001 OD
Photometric accuracy/linearity (microplate), 0–2.0 OD	190–850 nm < ± 1.0% and ± 0.006 OD
Photometric precision (repeatability), 0–2.0 OD	190–850 nm < ± 1.0% and ± 0.003 OD
Stray light	≤ 0.05% at 340 nm
Photometric stabilization	Instantaneous
Photometric drift	None — continuous referencing of monochromatic input
Calibration	Automatic before first kinetic read and before every endpoint reading
Optical alignment	None required
Light source	Xenon flash lamp (5 Watts)
Average lamp lifetime	1 billion flashes
Illumination	Top down
Photodetectors	Silicon photodiode
PHOTOMETRIC ANALYSIS MODES	
Standalone	• Single wavelength Absorbance reading of the microplate
Using SoftMax Pro	• Express data as Absorbance or %Transmittance • Single wavelength reading of microplate • Multiple wavelength (up to six) reading of microplate • Kinetic and kinetic graphics of microplate • Spectral scan (190–850 nm) of microplate

MEASUREMENT TIME (CALIBRATION OFF)	
Read time (endpoint) — standard read	• 96 wells in 9 seconds (single wavelength) • 96 wells in 19 seconds (dual wavelength, 425 & 650 nm)
Kinetic read intervals	• 96 wells, 9-second minimum interval between readings (single wavelength) • 1 column, 2-second minimum interval between readings (single wavelength)
SCAN SPEED	
Microplate: normal scan	33*K nm/min (8-well strip)
Microplate: speed scan	135*K nm/min (8-well strip)
TEMPERATURE REGULATION	
Reading chamber	Isothermal when temperature regulation is not enabled
Range	4°C above ambient to 45°C when temperature regulation enabled. Ambient temperature must be >20°C to achieve temperature regulation at 45°C.
Resolution	± 0.1°C
Accuracy	± 1.0°C for microplate and cuvette chamber
Temperature uniformity at equilibrium	± 0.5°C at 37°C
Chamber warm-up time	15–30 minutes (measured on air) after initiation of temperature regulation
Temperature regulation	4 sensors
Drift	± 0.2°C (regulated)
Temperature regulation diagnostics	Temperature regulation system is continuously monitored and updated
Evaporation	Plate lid required to minimize evaporative cooling

Recommended microplate	Flat-bottom microplates with isolated wells and lid
AUTOMIX WITH SOFTMAX PRO	
Plate mixing modes	Selectable: off, once prior to any reading, and once prior to and between kinetic readings
Plate mixing duration	Selectable: 1 to 999 seconds (three-second default)
COMPATIBILITY	
Microplates	Standard and half-area 96-well flat-bottomed microplates (0.3 mL).
GENERAL INSTRUMENT	
Display	2-x-20-character backlit LCD
Operating panel	8-key membrane keypad
Self-diagnosis	Continuous on-board diagnostics
Spill control	Drawer mechanism and reading chamber assembly protected from accidental spillage by drainage ports
Computer interface	8-pin DIN RS-232 serial (double shielding required)
Printer interface	Parallel 25-pin to Centronics (double shielding required)
Microplates supported	All 96-well and strip-well microplates, including lids
ENVIRONMENTAL (FOR INDOOR USE ONLY)	
Operating temperature	5°C to 40°C
Operating altitude	< 2000 m
Installation category	II
Pollution degree	2
Operating humidity	< 80%
Storage temperature	-20°C to 65°C

PHYSICAL	
Size (h x w x d)	8.6" (220 mm) x 22.8" (580 mm) x 15" (380 mm)
Weight	30 lb (13.6 kg)
Power consumption	< 250 W
Line voltage and frequency	90–250 VAC autoranging, 50/60 Hz

7.4. VersaMax Specifications

Thermal specifications for microplates used in the VersaMax apply to flat-bottom microplates with isolated wells.

All other microplate specifications apply to standard 96-well polystyrene flat-bottom microplates.

Technical specifications are subject to change without notice.

PHOTOMETRIC PERFORMANCE	
Wavelength range	340–850 nm
Wavelength selection	Monochromator tunable in 1-nm increments
Wavelength bandwidth	≤ 2.0 nm full width half maximum
Wavelength accuracy	± 1.0 nm across wavelength range
Wavelength repeatability	± 0.2 nm across all optical channels
Photometric range	0 to 4.000 OD
Photometric resolution	0.001 OD
Photometric accuracy/linearity (microplate), 0–2.0 OD	340–850 nm < ± 1.0% and ± 0.006 OD
Photometric precision (repeatability), 0–2.0 OD	340–850 nm < ± 1.0% and ± 0.003 OD
Stray light	≤ 0.05% at 340 nm
Photometric stabilization	Instantaneous

Photometric drift	None — continuous referencing of monochromatic input
Calibration	Automatic before first kinetic read and before every endpoint reading
Optical alignment	None required
Light source	Xenon flash lamp (5 Watts)
Average lamp lifetime	1 billion flashes
Illumination	Top down
Photodetectors	Silicon photodiode
PHOTOMETRIC ANALYSIS MODES	
Using SoftMax Pro	• Express data as Absorbance or %Transmittance • Single wavelength reading of microplate • Dual wavelength reading of microplate • Kinetic and kinetic graphics of microplate
MEASUREMENT TIME (CALIBRATION OFF)	
Read time (endpoint) — standard read	• 96 wells in 9 seconds (single wavelength) • 96 wells in 19 seconds (dual wavelength, 425 & 650 nm)
Kinetic read intervals	• 96 wells, 9-second minimum interval between readings (single wavelength) • 1 column, 2-second minimum interval between readings (single wavelength)
TEMPERATURE REGULATION	
Reading chamber	Isothermal when temperature regulation is not enabled
Range	4°C above ambient to 45°C when temperature regulation enabled. The ambient temperature must be >20°C to achieve temperature regulation at 45°C.

Resolution	± 0.1°C
Accuracy	± 1.0°C for microplate chamber
Temperature uniformity at equilibrium	± 0.5°C at 37°C
Chamber warm-up time	15–30 minutes (measured on air) after initiation of temperature regulation
Temperature regulation	4 sensors
Drift	± 0.2°C (regulated)
Temperature regulation diagnostics	Temperature regulation system is continuously monitored and updated
Evaporation	Plate lid required to minimize evaporative cooling
Recommended microplate	Flat-bottom microplates with isolated wells and lid
AUTOMIX WITH SOFTMAX PRO	
Plate mixing modes	Selectable: off, once prior to any reading, and once prior to and between kinetic readings
Plate mixing duration	Selectable: 1 to 999 seconds (three-second default)
COMPATIBILITY	
Microplates	Standard and half-area 96-well flat-bottomed microplates (0.3 mL).
GENERAL INSTRUMENT	
Display	2-x-20-character backlit LCD
Operating panel	8-key membrane keypad
Self-diagnosis	Continuous on-board diagnostics
Spill control	Drawer mechanism and reading chamber assembly protected from accidental spillage by drainage ports
Computer interface	8-pin DIN RS-232 serial (double shielding required)

Printer interface	Parallel 25-pin to Centronics (double shielding required)
Microplates supported	All 96-well and strip-well microplates, including lids
ENVIRONMENTAL (FOR INDOOR USE ONLY)	
Operating temperature	5°C to 40°C
Operating altitude	< 2000 m
Installation category	II
Pollution degree	2
Operating humidity	< 80%
Storage temperature	-20°C to 65°C
PHYSICAL	
Size (h x w x d)	8.6" (220 mm) x 22.8" (580 mm) x 15" (380 mm)
Weight	30 lb (13.6 kg)
Power consumption	< 250 W
Line voltage and frequency	90–250 VAC autoranging, 50/60 Hz

8. Appendices

8.1. Cables

Molecular Devices recommends that you use high-quality, double-shielded cables to connect your SpectraMax or VersaMax reader to the computer. Choose cables that meet the following requirements:

Serial Interface Cable

The serial interface cable used to connect the instrument to the computer is a custom cable designed and built by Molecular Devices. Please use the cable supplied by Molecular Devices, or contact Molecular Devices for specific pin-out requirements:

Male DB8 to Female DB9 (custom cable made by Molecular Devices, PN 9000-0149)

USB Adapter

iMac, G4 and G5 Macintosh computers, and many newer Windows computers do not have a serial port. You can connect a serial cable between these computers and the instrument using a USB-to-serial adapter.

Molecular Devices has tested many third-party serial-to-USB adapter cables and has found the Keyspan USA-19HS to be the most reliable. It is the only one we recommend and support.



Figure 8.1: Molecular Devices' custom serial cable (left) and a serial-to-USB converter (right).

8.2. Accessories

Description	Part #
SpectraTest ABS1 Absorbance Validation Test Plate	0200-6117
Cuvette Absorbance Validation Kit	9000-0161
SpectraPlate-Quartz UV-transparent microplate	R8024
Cable, RS-232, 8-pin DIN to 8-pin DIN (instrument to pre-G3 Macintosh)	9000-0091
Cable, RS-232, 9-pin DIN to 8-pin DIN (instrument to PC serial port)	9000-0149
Power Cord (US, Canada, Japan, Mexico, India)	4400-0002
Power Cord, EC1 (Germany, France, Scandinavia, Italy, Korea)	4400-0036
Power Cord, EC2 (UK, Indonesia, Singapore, Malaysia)	4400-0037
Power Cord, AP1 (Australia, Hong Kong, China)	4400-0038
Fuse, 4-amp (5 x 20 mm) Time Delay	4601-0014
SpectraMax Mouse Pad	9000-0133
Adapter USB-Serial High-Speed (KeySpan adapter; instrument to USB-only instrument)	9000-0938
Test Tube Cover	2300-0277

8.3. Cuvettes in SpectraMax Plus^{384}

The guidelines for cuvette use in the SpectraMax Plus^{384} are the same as those that apply to any high-quality spectrophotometer. The user must ensure that the meniscus is comfortably above the light beam in standard cuvettes and that the sample chamber in a microcuvette is aligned properly with the beam. The light beam is 0.625 in (15.87 mm) above the cuvette bottom.

Below are some cuvettes that have been tested. All have an optical pathlength of 1 cm (10 mm) and standard external dimensions (12.5 cm x12.5 cm). Their fill volumes differ only because of their different internal width and chamber height dimensions.

Standard and Semi-micro Cuvettes

(Several brands available)[1]

Internal Width	Minimum Volume	Maximum Volume
10 mm	~ 1.8 mL	4.0 mL
4 mm	~ 0.75 mL	1.4 mL
2 mm	~ 0.40 mL	0.7 mL

Ultra-micro Cuvettes (Hellma)

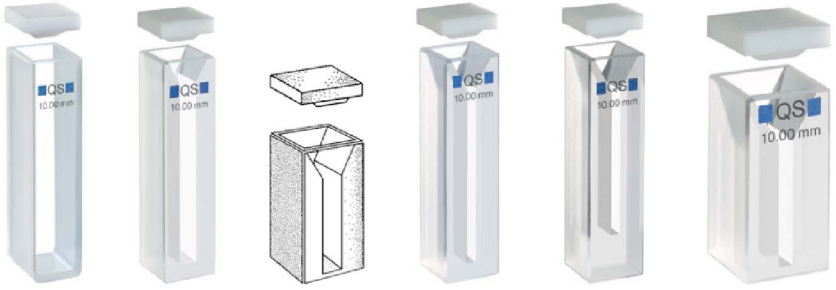
When ordering, specify the Z-dimension to be 15 mm.

Hellma Cat. No.	Window Size	Chamber Volume	Fill Volume
105.201-QS	2.0 x 5.0 mm	100 µL	120 µL
105.202-QS[a]	2.0 x 2.5 mm	50 µL	70 µL
105.210-QS[b]	0.8 mm diameter	5 µL	10 µL

a.You must put a riser (0.8–1 mm) on cuvette bottom to match the cuvette window to the beam.
b.You must put a riser (0.8–1 mm) on cuvette bottom to match the cuvette window to the beam. Gives good qualitative results (i.e. spectral scans), but quantitative results are impractical because the window is smaller than the beam.

1.Hellma: http://www.hellma-worldwide.com.

Standard, Semi-micro, and Microcuvettes (Hellma)



	Standard	Semi-micro		Micro		
Hellma Cat. No.	100	104	105.004	104.002	108.002	105
Internal Dimensions	10 x 10	4 x 10	4 x 10	2 x 10	2 x 10	2 x 10
Fill Volume	4 mL	1.4 mL	600 µL	700 µL	500 µL	300 µL

Ultra-micro Cuvettes (Hellma)



Hellma Cat. No.	105.200	105.201	105.202	105.210
Optical Pathlength	10 mm	10 mm	10 mm	10 mm
Fill Volume	180 µL	120 µL	70 µL	10 µL