

SpectraMax Mini

Multi-Mode Microplate Reader
User Guide

SpectraMax Mini User Guide

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Safety Information

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

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

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

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



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

Warnings, Cautions, Notes, and Tips

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

The following user attention statements can display in the Molecular Devices user documentation. Each statement implies the amount of observation or a recommended procedure.



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



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








Note: A note calls attention to significant information.



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

Symbols on the Instrument












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

Symbol	Indication
	Consult the product documentation.
	Potential pinch hazard.
	Potential biohazard.
	Potential heat hazard.
	Electrostatic sensitive device (ESD). Observe precautions to handle electrostatic sensitive devices.

Near-field Communication

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at their own expense. Changes or modifications made to this equipment not expressly approved by the party responsible for compliance may void the FCC authorization to operate this equipment.

A sticker on the back of the instrument displays the following symbols:

Symbol	Indication
	The instrument serial number.
	The instrument manufacture date.
	You should consult the instructions for use.
	CSA certification.
	European technology conformity.
	United Kingdom technology conformity.
	The instrument complies with Australian radio communication requirements.
	This symbol is required in accordance with the Waste Electrical and Electronic Equipment (WEEE) Directive of the European Union. It indicates that you must not discard this electrical or electronic product or its components in domestic household waste or in the municipal waste collection system. For products under the requirement of the WEEE directive, contact your dealer or local Molecular Devices office for the procedures to facilitate the proper collection, treatment, recovery, recycling, and safe disposal of the device.
	Indicates the environmental friendly use period.
	The instrument manufacturer.
	California proposition 65 requires businesses to provide warnings to Californians about significant exposures to chemicals that cause cancer, birth defects, or other reproductive harm.

Chemical and Biological Safety

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

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



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

Electrical Safety

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

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

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

Power off the instrument when not in use.

Moving Parts Safety

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



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

To prevent injury:

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



The SpectraMax® Mini Multi-Mode Microplate Reader is a multi-detection instrument with a 6-well to 384-well plate read capability.

The instrument uses a paired monochromator and xenon flash lamp for absorbance reads that allow for optimization of wavelengths between 200 nm and 1000 nm. The Fluorescence mode is powered by a second xenon flash lamp and highly selective filter cubes. The flash lamp offers broad spectrum light that can be used with the available filter cubes to deliver optimal excitation and emission light to and from the sample. The optics focus the light into the sample volume, and cutoff filters reduce stray light and minimize background interference. Luminescence readings are also possible with the luminescence cube. You can vary the integration time of the read to optimize the sensitivity or read speed of your assays.

The instrument dynamic range for fluorescence detection is from 10^{-6} to 10^{-12} molar fluorescein. Internal compensation for detector sensitivity, photomultiplier tube voltage and sensitivity, as well as excitation intensity, provides a wide dynamic range without PMT gain adjustment. The photometric range for absorbance detection is 0–4 ODs with a resolution of 0.001 OD.

The flexibility and high sensitivity make this instrument appropriate for applications in the fields of biochemistry, cell biology, immunology, molecular biology, and microbiology. Typical applications include ELISA, nucleic acid and protein quantitation, enzyme assays, microbial growth, and pipettor calibration.

Read modes include:

- [Absorbance Read Mode on page 27](#)
- [Fluorescence Intensity Read Mode on page 30](#)
- [Luminescence Read Mode on page 32](#)

The instrument ships with one or more filter cubes, and your filter cube configuration determines which combination of read modes are available.

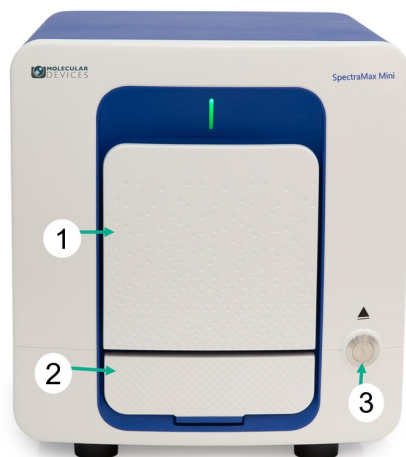
- Absorbance and Luminescence
- Absorbance and Fluorescence
- Absorbance, Luminescence, and Fluorescence

Read types include:

- Endpoint
- Kinetic
- Spectrum (Absorbance reads only)
- Well Scan

See [Read Types on page 33](#).

The following are the main components of the instrument.



Item	Description
1	Filter Chamber
2	Plate Drawer
3	Plate Drawer Open/Close Button

Computer Integration

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

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

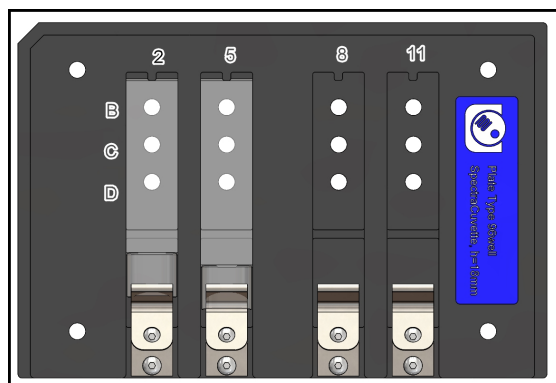
For information about the computer specifications required to run the software, the software installation and licensing instructions, and the directions to create the software connection between the computer and the instrument, see the *SoftMax Pro Data Acquisition and Analysis Software - Standard Edition and MiniMax Imaging Edition - Installation Guide*.



Note: The SoftMax Pro Software - GxP edition does not support this instrument.

Supported Plates

Depending on the application, the instrument can read 6, 12, 24, 48, 96, and 384-well plates. For the absorbance read mode you can use plates up to a height of 22 mm. For the fluorescence read mode and the luminescence read mode you can use plates up to a height of 20 mm and the instrument confirms that the height is consistent with the plate type you select. To read cuvettes, the instrument supports the use of the SpectraCuvette adapter that has a blue label.



This is the only cuvette adapter that will result in accurate readings from this instrument. Do not use other cuvette adapters. See [Filters and Accessories on page 47](#).

The plate list in the software includes half area and low-volume plates. The software allows you to define a new plate type based on the manufacturer's specifications for well size, spacing, and distance from the plate edge.

- Use polystyrene plates for absorbance wavelengths above 340 nm.
- To allow transmission of the deep UV spectra when you do a read at wavelengths below 340 nm, you must use special UV-transparent plates or quartz plates.
- Endpoint, Kinetic, and Well-Scan read types are available with any plate model.
- For low volume measurements, the instrument supports the SpectraDrop Micro-Volume Microplate. See [Filters and Accessories on page 47](#).



Note: Although not recommended, you can also use strip-well plates in the instrument. When you use strip-wells, use high quality support frames or holders. To avoid warping, you should fill the frame or holder with empty strips.

Temperature Regulation

The temperature in the plate chamber is isothermal, both at ambient and when you turn on the incubator. When you turn on the incubator, you can set the temperature from 4°C above ambient to 45°C. The ambient temperature must be at least 20°C to reach the 45°C operating temperature. The operating humidity must be non-condensing and can range from 15% to 75%.

Filters

You can insert one filter cube into the instrument filter chamber for fluorescence reads or luminescence reads. Each filter cube contains an NFC tag that the instrument uses to determine which filter cube you insert. The Instrument Information dialog in the SoftMax Pro Software displays the information related to the filter cube you insert in the instrument.

On each fluorescence filter cube, the excitation filters pass only the wavelength of light required for excitation and the emission filters separate fluorescence generated by the sample from background light and for some luminescence measurements.

Use the Luminescence filter cube for luminescence reads.

The instrument ships with an accessories case that holds up to four filter cubes. Additional filter cubes arrive in a separate shipping box. See [Filters and Accessories on page 47](#).

Before you unpack and set up the instrument, prepare a dry, flat work area that is away from direct sunlight, dust, drafts, vibration, and moisture. The work area must have sufficient space for the instrument, host computer, and required cables. To provide access for disconnecting power from the instrument, maintain a 20 cm to 30 cm (7.9 in. to 11.8 in.) gap between the rear of the instrument and the wall. To ensure sufficient ventilation, do not block the ventilation grids on the rear of the instrument.

The package contains the instrument and the following accessories:

- SoftMax Pro Software, product key, and instructions to install the SoftMax Pro Software patch for the features that are specific to the SpectraMax Mini.
- Instrument installation guide
- USB computer connection cable
- Power supply
- Power cord
- Accessory case that contains:
 - Hex wrench for transport lock removal
 - Up to four filter cubes depending on how many you purchased. (Additional filter cubes are shipped in a separate box.)

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



Note: You must install SoftMax Pro Software version 7.1.2 or higher. Version 7.1.2 requires that you install the SpectraMax Mini software patch. See the *SoftMax Pro Data Acquisition and Analysis Software - Standard Edition and MiniMax Imaging Edition - Installation Guide*. SoftMax Pro Software version 7.2 and higher does not require the additional software patch.

Unpacking the Instrument

The package is designed to protect the instrument during shipment.



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

The instrument is packed in a specially designed carton. Before you unpack the instrument, check the box for any damage that might have occurred during transportation. If any damage is noted, inform the supplier immediately and keep the damaged packaging.



Note: Retain the shipping box and all packing materials for future transport needs.

To unpack the instrument:

1. Open the side of the box.



2. Grasp the cardboard handles below the top layer of foam packing material and slide the accessories package out of the carton. This contains the power supply, power cord, USB cable, and a case that contains the filter cubes and other accessories.



3. Remove the foam packing material that was above the accessories.



4. Grasp the cardboard handles below the instrument and slide the instrument out of the carton.



5. Lift the foam packing material above the instrument upward to reveal the top of the instrument.



6. Slide the five vertical pieces of foam packing material that surround the instrument upward to clear the sides of the instrument.



7. Lift the instrument from the bottom foam packing material and place the instrument on a level surface.



8. Save the carton, cardboard slides, and foam packing for future shipments.



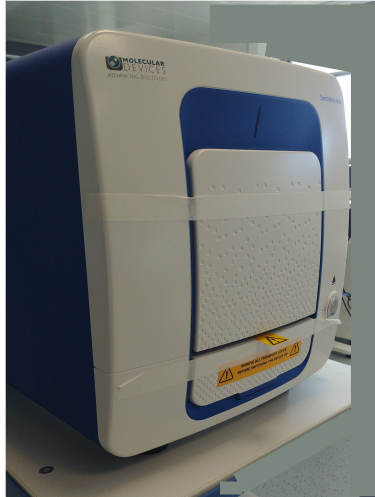
Removing the Transport Lock



CAUTION! The instrument can be damaged if you do not remove the transport lock before you power on the instrument.



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

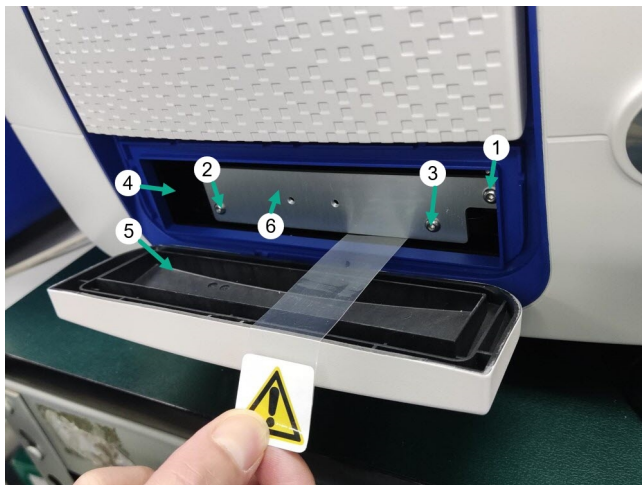


To remove the transport lock:

1. Remove the tape that holds the filter chamber door shut and remove the tape that holds the plate chamber door shut.
2. Gently pull the yellow tab that protrudes from the plate chamber door to open the door. Hold the door open while you remove the transport lock.




Note: Do not tear the yellow tab. It remains attached to the transport lock to make it easier to open the plate chamber door.



Item	Description
1	Screw #1 fastens the lock to the internal frame of the instrument (remove first)
2	Screw #2 fastens the lock to the plate drawer
3	Screw #3 fastens the lock to the plate drawer
4	Plate drawer
5	Plate chamber door in open position
6	Plate drawer transport lock

3. Use the hex key (included in the accessory case) to loosen screw #1 in the upper-right corner of the transport lock until the lock disconnects from the instrument frame. The screw has a retaining washer to prevent removal from the lock.

 **Tip:** After you loosen screw #1, pull the plate drawer slightly out of the instrument to hold the chamber door open.


4. Loosen screws #2 and #3 until the lock comes free of the plate drawer and you can remove the lock from the instrument. The screws have retaining washers that prevent removal from the lock.

 **Note:** Store the transport lock in slot within the accessory case.

5. Push the plate drawer back inside the instrument and close the chamber door.

Connecting Instrument Cables

The power cord and USB cable connect to the ports on the rear of the instrument.

 **Note:** Before you connect or disconnect the power cord, make sure that the power switch that is on the rear of the instrument is in the Off position.

To connect the instrument cables:

1. Turn the instrument around so that the rear of the instrument faces you.



2. Make sure that the power switch is in the Off position.
3. Connect the appropriate end of the supplied USB cable to the USB port, and then connect the other end to a USB port on the computer.
4. Connect the supplied power adapter to the power cord port, and then connect the other end to a grounded electrical wall outlet.
5. Turn the instrument around so that the front of the instrument faces you.

 **Note:** Ensure no cables run beneath the instrument.

Installing Filter Cubes

Filter cubes ship with the instrument in a specialized case. Each case can contain up to four filter cubes. Store filters and filter cubes in a protected, dust-free area, preferably in the supplied case.



CAUTION! Handle filter cubes by the stem only. Do not touch filter surfaces with fingers. Fingerprints left on filters negatively affect measurement results.



You insert the filter cube into the filter chamber on the front of the instrument. Each filter cube has an NFC tag that the instrument reads. The NFC tag contains the information the instrument uses for reads. Filter information displays in the Instrument Information dialog in the software.

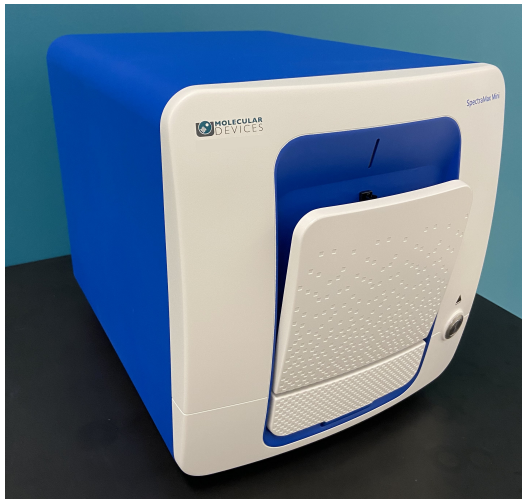
To install filter cubes:

1. Turn on the instrument power switch located on the rear of the instrument. Wait for the instrument to complete its diagnostic check and the indicator light to turn green.



Note: You can install a filter cube with the instrument powered off, but the instrument must be powered on and connected to the computer in order for the SoftMax Pro Software to recognize the filter cube you install.

2. Press the top of the filter chamber cover to release the latch. The door opens slightly.

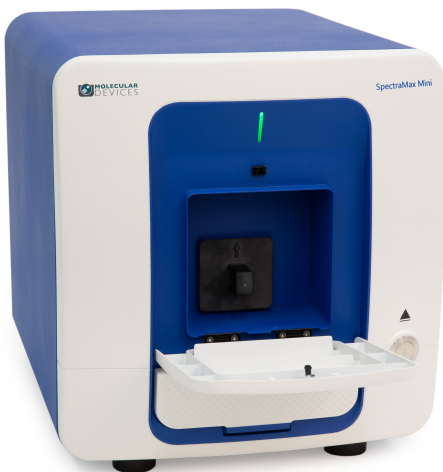


3. From the top of the door, gently pull down to open the filter chamber.

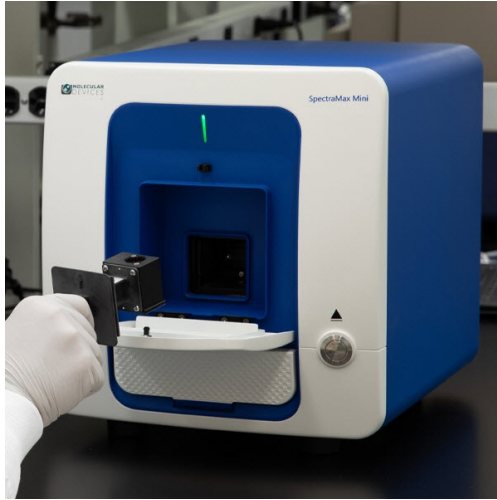



Note: The filter chamber should open with no resistance. Do not use excessive force to open the chamber.

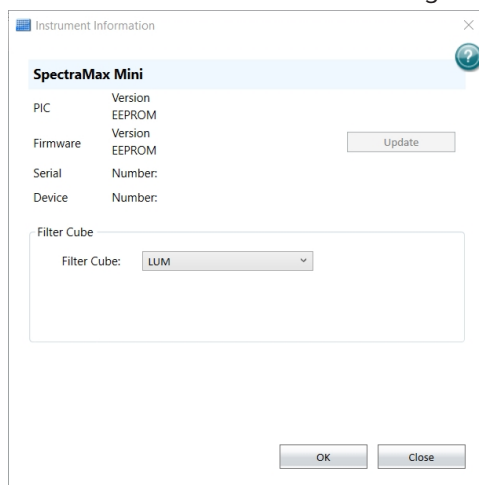
4. If there is a filter cube in the instrument, grab the filter cube stem and pull the cube out of the chamber. A magnet holds the cube in place. You must remove the existing filter cube before you insert a different filter cube.



5. Grasp the filter cube by the stem and with the arrow pointing up, insert the filter cube into the opening at the rear of the filter chamber. A magnet draws the filter cube into place and holds the filter cube in the correct position within the instrument.



6. Close the filter chamber cover.
7. In the SoftMax Pro Software, on the **Operations** tab in the Ribbon, click  **Info** to display the Instrument Information dialog to confirm successful filter cube installation. Click **OK** to exit the Instrument Information dialog.





Chapter 3: Getting Started

3

Now that you have installed the SoftMax Pro Software on the computer, connected the cables, and installed a filter cube, it is time to get started.

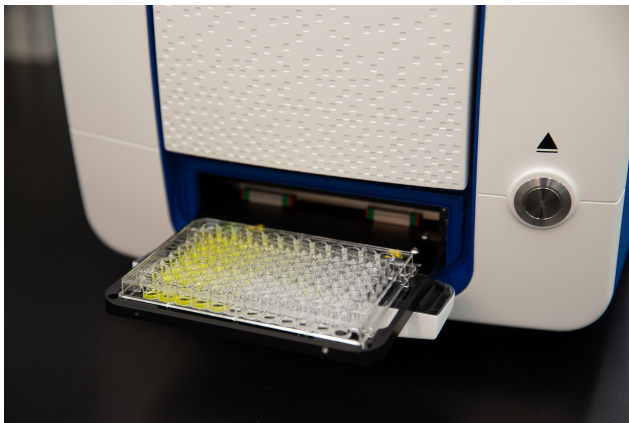
Turn on the instrument power switch located on the rear of the instrument. After a few seconds the indicator light displays red while the instrument completes its diagnostic check, and then the indicator light turns green.




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

Plate Drawer

The plate drawer is located on the front of the instrument and slides in and out of the plate chamber. You can use 6, 12, 24, 48, 96, and 384-well plates that conform to ANSI/SBS standard microplate footprint and dimensions in the instrument. See [Supported Plates on page 11](#).



Push the button on the front of the instrument or use the software to open and close the plate drawer. In the software, on the **Home** tab in the Ribbon click  **Open/Close** to open and close the plate drawer.



CAUTION! Do not obstruct the movement of the plate drawer. If you must retrieve a plate after an error condition or power outage and the plate drawer does not open, you can open the drawer manually. See [Opening the Drawer Manually on page 26](#).



CAUTION! To prevent damage to the instrument:


For the absorbance read mode, the plate height must not exceed 22 mm including the lid if the plate is lidded.

For the fluorescence read mode and the luminescence read mode, the plate height must not exceed 20 mm including the lid if the plate is lidded.




Note: For detailed software instructions, see the *SoftMax Pro Data Acquisition and Analysis Software User Guide* or the application help.

Temperature Settings

In the software, on the Home tab in the Ribbon, click  **Temperature** to display the Temperature Control dialog. Use the Temperature Control dialog to turn the incubator on and off and to set the chamber temperature.

You cannot set a temperature beyond the upper (45°C) or lower (4°C above ambient) instrument limits.

Shake Settings

The software allows you to mix of the contents of the wells by shaking the plate. In the Ribbon on the Home tab, click  **Shake** to shake the plate. This shake process is independent of a protocol.

When you create a protocol, the Settings dialog allows you to define how to shake the plate as part of the read.

- For the Endpoint, Spectrum, and Well Scan read types, you can shake the plate for 0 to 999 seconds before the read at each wavelength you set.
- For the Kinetic read type, you can shake the plate for 0 to 999 seconds before the initial read, and/or before each subsequent read.

There are three shake speeds:


- Low
- Medium
- High

There are three shake modes:

- Orbital
- Linear
- Double Orbital

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


Wavelength Settings

For absorbance reads, you use the Settings dialog to set the wavelengths. In the software, select a Plate section in the Navigation Tree and then click  **Acquisition Settings** to display the Settings dialog.

For fluorescence reads and luminescence reads, insert the filter cube that meets the read requirements. You cannot adjust the wavelength from the Settings dialog. See [Installing Filter Cubes on page 19](#).




Read Plates

Before you place a plate in the drawer, the underside of the plate must be dry. If the plate has fluid on the underside, you must dry it with a lint-free cloth before you place it in the drawer.

1. On the rear of the instrument, turn on the power switch.
2. Power on the computer.
3. Start the SoftMax Pro Software and connect the software to the instrument.
4. Do one of the following in the software:
 - Open a document that contains the experiment settings for the read.
 - Create a new protocol file with the settings for the read.
5. In the software, select a Plate section in the Navigation Tree and then click  **Acquisition Settings** to display the Settings dialog where you define the acquisition settings for the Plate section.
6. Place the plate onto the plate drawer, matching well A1 with position A1 in the drawer. Make sure that the plate is flat against the drawer bottom.



Note: Make sure that the filter cube chamber door is fully closed. If the door is open, data quality may be compromised.

7. If you change the filter cube between reads, do one of the following to confirm that the software recognizes the correct filter cube:
 - In the software, select a Plate section in the Navigation Tree and then click  **Acquisition Settings** to display the Settings dialog. Select the Wavelength category, if needed to confirm the wavelength and click **OK** to exit the Settings dialog.
 - In the Ribbon, select the **Operations** tab and click  **Info** to display the Instrument Information dialog to confirm the filter is correct. Click **OK** to exit the Instrument Information dialog.
8. Click  **Read** to read the plate. The indicator light flashes green until the read is complete. After the read is complete, the plate drawer opens, and you can remove the plate.

Opening the Drawer Manually

Do the following if an error occurs while the drawer is closed, and you need to remove a plate.

- Press the open/close plate drawer button on the front of the instrument.
- If the drawer does not open, then turn off the power to the instrument and then on again.
- If the drawer remains closed after you cycle the power, and the incubator is on, then turn off the incubator.
- If the drawer remains closed, power off the instrument, unplug the power cord and then at the groove in the upper left side wall of the drawer, try to use a blunt, flat object, such as a spatula, to open the door. With your index finger, pull the plate drawer out of the instrument (do not force the drawer) and remove the plate. This action will not harm the instrument but should only be taken if the first two options fail to open the drawer.

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

Absorbance Read Mode

The instrument uses the Absorbance (ABS) read mode to measure the Optical Density (OD) of the sample solutions.

Absorbance is the quantity of light absorbed by a solution. To measure absorbance accurately, it is necessary to eliminate light scatter. If there is no turbidity, then absorbance = optical density.

$$A = \log_{10}(I_0 / I) = -\log_{10}(I/I_0)$$

where I_0 is intensity of the incident light before it enters the sample divided by the light after it passes through the sample, and A is the measured absorbance.

The instrument allows you to choose whether to display absorbance data as Optical Density (OD) or %Transmittance (%T).

Optical Density

Optical density (OD) is the quantity of light passing through a sample to a detector relative to the total quantity of light available. Optical Density includes absorbance of the sample plus light scatter from turbidity and background. You can compensate for background using blanks.

A blank well contains everything used with the sample wells except the chromophore and sample-specific compounds. Do not use an empty well for a blank.

Some applications are designed for turbid samples, such as algae or other micro-organisms in suspension. The reported OD values for turbid samples are likely to be different when read by different instruments.

For optimal results, you should run replicates for all blanks, controls, and samples. In this case, the blank value that will be subtracted is the average value of all blanks.

% Transmittance

%Transmittance is the ratio of transmitted light to the incident light for absorbance reads.

$$T = I/I_0$$

$$\%T = 100T$$

where I is the intensity of light after it passes through the sample and I_0 is incident light before it enters the sample.

Optical Density and %Transmittance are related by the following formulas:

$$\%T = 10^{2-OD}$$

$$OD = 2 - \log_{10}(\%T)$$

The factor of two comes from the fact that %T is expressed as a percent of the transmitted light and $\log_{10}(100) = 2$.

When in %Transmittance analysis mode, the instrument converts the raw OD values reported by the instrument to %Transmittance using the above formula. All subsequent calculations are done on the converted numbers.

Applications of Absorbance

Absorbance-based detection is commonly used to evaluate changes in color or turbidity, permitting widespread use including ELISAs, protein quantitation, endotoxin assays, and cytotoxicity assays.

Optimizing Absorbance Read Mode

You can adjust the wavelength of the transmitted light in 1-nm increments between 200 nm and 1000 nm. You can also use the instrument for reading up to six wavelengths per plate, which allows for reference wavelength readings such as A260 and A280 for nucleic determination.

For an assay blank, you should use appropriate plate blanks or group blanks in a template that you define in the software. You can also use the PathCheck technology feature to normalize the data to a 1 cm pathlength.

PathCheck Technology

The temperature-independent PathCheck technology normalizes absorbance values to a 1 cm path length based on the near-infrared absorbance of water.

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

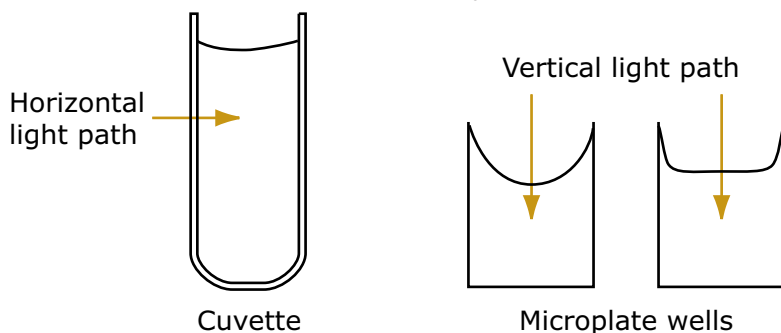
$$A = \epsilon cL$$

where A is the absorbance, ϵ is the molar absorptivity of the sample, c is the concentration of the sample, and L is the pathlength. 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 do extinction-based assays and makes it confusing to compare results between microplate readers and spectrophotometers.

The standard pathlength of a 1 cm cuvette is the conventional basis to quantify the unique absorptivity properties of compounds in solution. Quantitative analysis can be done on the basis of extinction coefficients, without standard curves (for example, NADH-based enzyme assays). When you use a cuvette, the pathlength is known and is independent of sample volume, so absorbance is directly proportional to concentration when there is no background interference.

In a plate, 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 occur from pipetting the samples and standards. The PathCheck technology determines the pathlength of aqueous samples in the plate and normalizes the absorbance in each well to a pathlength of 1 cm. This way of correcting the microwell absorbance values can be accurate to within $\pm 4\%$ of the values obtained directly in a 1 cm cuvette.



PathCheck technology normalizes the data acquired from an absorbance read mode endpoint read type to a 1 cm pathlength, correcting the OD for each well to the value expected if the sample were read in a 1 cm cuvette. The instrument uses the factory installed water constant to obtain the 1 cm values.



Note: You must select the PathCheck check box before a read because you cannot apply the PathCheck technology after the read. After you read a plate with PathCheck technology turned on, the software stores PathCheck information permanently within the document.

Water Constant

The PathCheck technology is based on the absorbance of water in the near infrared spectral 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 correction method is sufficient. The water constant is determined for each instrument during manufacture and is stored in the instrument.

Eliminating the Pathlength-Independent Component

Raw OD measurements of plate samples include both pathlength-dependent components (sample and solvent) and a pathlength-independent component (OD of plate material). The pathlength-independent component must be eliminated from the calculation to get valid results that have been normalized by the PathCheck technology. You can do this using a plate blank or using a plate background constant.

Using Plate Blanks

You can use this method if all samples in the plate are the same volume, and the read does not depend on the PathCheck technology to correct for variability in volumes.

To use a plate blank:

1. Designate a minimum of one well (preferably several) as Plate Blank.
2. Pipette buffer (for example, your sample matrix) into those wells and read along with the samples. Do not use an empty well for a blank.
The instrument automatically subtracts the average of the blank wells from each of the samples. The OD of the plate material is subtracted as part of the blank.
3. Select the Use Plate Blank check box in the Data Reduction dialog.

Using Plate Background OD

If your sample volumes are not identical or if you choose not to use a Plate Blank, then you must use a Plate Background OD. Omitting a Plate Background OD results in artificially high values after being normalized by the PathCheck technology.

To determine the Plate Background OD:

1. Fill a clean plate with water.
2. Read at the wavelengths you will use for the samples.

The average OD value is the Plate Background OD. If you intend to read your samples at more than one wavelength, there should be a corresponding number of Plate Background OD values for each wavelength.



Note: It is important that you put water in the wells and do not read a dry plate for the Plate Background OD. A dry plate has a slightly higher OD value than a water filled plate because of differences in refractive indices. Use of a dry plate results in PathCheck technology normalized values that are lower than 1 cm cuvette values.

Interfering Substances

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

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

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

To determine possible color interference:

- Measure the OD 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 you should not use the PathCheck technology.

Organic solvents could interfere with the PathCheck technology if the solvents have absorbance in the region of the NIR water peak. Solvents such as ethanol and methanol do not absorb in the NIR region, so the solvents do not interfere, except to cause a decrease in the water absorbance to the extent of their presence in the solution. If the solvent absorbs between 900 nm and 1000 nm, the interference would be similar to the interference of highly colored samples. If you add an organic solvent other than ethanol or methanol, you should run a Spectrum scan between 900 nm and 1000 nm to determine if the solvent would interfere with the PathCheck technology.

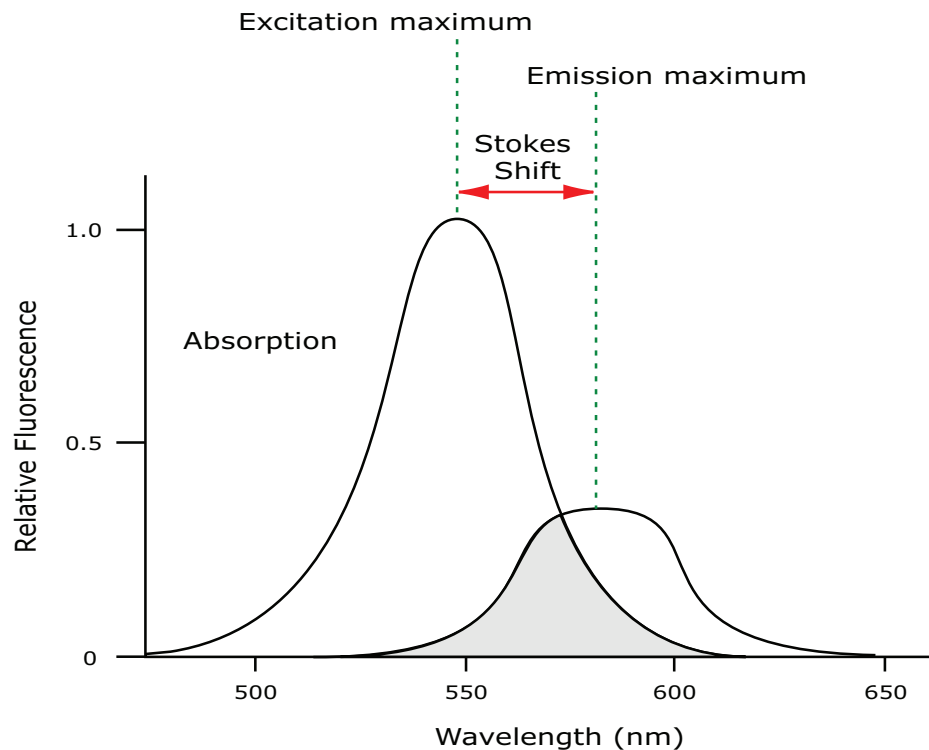
Fluorescence Intensity Read Mode

Fluorescence occurs when absorbed light is re-radiated at a longer wavelength. In the Fluorescence Intensity read mode, the instrument measures the intensity of the re-radiated light and expresses the result in Relative Fluorescence Units (RFU).

The governing equation for fluorescence is:

$$\text{Fluorescence} = \text{extinction coefficient} \times \text{concentration} \times \text{quantum yield} \times \text{excitation intensity} \\ \times \text{pathlength} \times \text{emission collection efficiency}$$

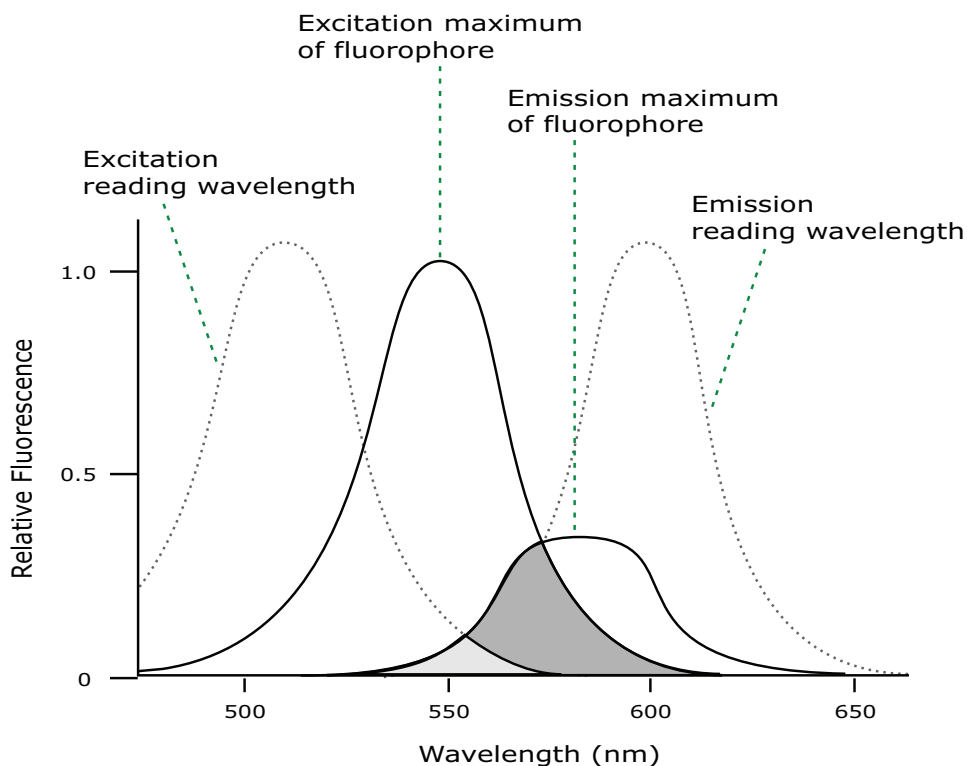
Fluorescent materials absorb light energy of a characteristic wavelength (excitation), undergo an electronic state change, and instantaneously emit light of a longer wavelength (emission). Most common fluorescent materials have well-characterized excitation and emission spectra. The following figure shows an example of excitation and emission spectra for a fluorophore. The excitation and emission bands are each fairly broad with half-bandwidths of approximately 40 nm, and the difference between the wavelengths of the excitation and emission maxima (the Stokes shift) is generally fairly small, about 30 nm. There is considerable overlap between the excitation and emission spectra (gray area) when a small Stokes shift is present.



Excitation and Emission Spectra

Because the intensity of the excitation light is usually many tens of thousands of times greater than that of the emitted light, you must have sufficient spectral separation to reduce the interference of the excitation light with detection of the emitted light.

*** Tip:** If the Stokes shift is small, you should choose an excitation wavelength that is as far away from the emission maximum as possible while still able to excite the fluorophore so that less of the excited light overlaps the emission spectrum, which permits better selection and quantitation of the emitted light.



Fluorescence intensity data is dependent on several variables.

Applications of Fluorescence Intensity

Fluorescence intensity is used widely in applications such as fluorescent ELISAs, protein assays, nucleic acid quantitation, reporter gene assays, cell viability, cell proliferation, and cytotoxicity. One more major application is to study the kinetics of ion release.

Some assays use a fluorescent label to selectively attach to certain compounds. The quantity or concentration of the compound can then be quantified by measuring the fluorescence intensity of the label, which is attached to the compound. Such methods are often used to quantify low concentrations of DNA or RNA, for example.

Luminescence Read Mode

With the luminescence filter cube, the instrument allows you to do top luminescence reads. You should use solid white plates or white plates with clear bottoms.

Luminescence is the emission of light by processes that derive energy from essentially non-thermal changes, the motion of subatomic particles, or the excitation of an atomic system by radiation. Luminescence detection relies on the production of light from a chemical reaction in a sample.

In luminescence (LUM) read mode, no excitation is necessary as the measured species emit light naturally. For this reason, the lamp does not flash, so no background excitation interference occurs.

For the luminescence read mode, the instrument provides measurements in Relative Light Units (RLUs).

To help eliminate background luminescence from a plate that has been exposed to light, you should dark adapt the plate by placing the sample-loaded plate inside the instrument for several minutes before you start the read.

Concentrations or qualitative results are derived from raw data with a standard curve or by comparison with reference controls.

Luminescence read times are not designated by multiple reads per well, but rather by the total integration time you enter (between 1 ms and 1500 ms). Typical luminescence assays require between 500 ms and 1000 ms integration.

If wells are incubated for a long period of time, you should use the Shake setting to mix the plate before the read.

Applications of Luminescence

Chemiluminescent or bioluminescent reactions can be induced to measure the quantity of a particular compound in a sample. Examples of luminescent assays include the following:

- Reporter gene assays (the measurement of luciferase gene expression)
- Quantitation of adenosine triphosphate (ATP) as an indication of cell counts with cell-proliferation, cytotoxicity, and biomass assays
- Enzyme measurements with luminescent substrates, such as immunoassays

Read Types

The instrument support the following read types:

Endpoint

In an endpoint read type, a reading of each plate well is taken in the center of each well, at a single wavelength or at multiple wavelengths. Raw data values are reported as optical density (OD), % transmittance (%T), relative fluorescence units (RFU), or relative light units (RLU).

Kinetic

In a kinetic read type, the instrument collects data over time with multiple reads taken in the center of each well at regular intervals. To achieve the shortest possible interval for kinetic reads, choose wavelengths in ascending order.

The software can do the following calculations based on raw data: VMax, VMax per Sec, Time to VMax, and Onset Time. Kinetic readings can be single wavelength or multiple wavelength readings.

The kinetic read type can collect data points in time intervals of seconds, minutes, or hours (up to 99 hours).

Kinetic analysis has many advantages to determine the relative activity of an enzyme in different types of plate 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

For the absorbance read mode, the Spectrum read type measures optical density (OD) or %Transmittance across a specified wavelength range, with allowed values from 200 nm to 1000 nm.

Well Scan

The Well Scan read type takes reads at more than one location on an evenly spaced, user defined grid or other pattern inside of each well at single or multiple wavelengths. The fill pattern is either round or square to match the well shape. The number of points that are available depend on the well size of the plate you select.

The Read Order you select in the More Settings category determines the available options.

For the Row and Well read order, select:



For a Column read order, select:



Drag the **Density** slider and the **Point Spacing** slider to define the well scan specifications. The values for Density and Point Spacing are dependent on each other and on the plate you select.

Perform only the maintenance tasks described in this guide. Contact a Molecular Devices service engineer to inspect and perform a preventive maintenance service on the instrument each year. See [Obtaining Support on page 48](#).

Before you operate the instrument or perform maintenance operations, make sure you are familiar with the safety information in this guide. See [Safety Information on page 4](#).



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

Troubleshooting



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



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

Observe the cleaning procedures outlined in this guide for the instrument.

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

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

Do only the maintenance described in this guide. Maintenance procedures other than those specified in this guide must be done by qualified Molecular Devices personnel only. See [Obtaining Support on page 48](#).

To clean the instrument, use disinfectant wipes according to the supplier instructions. Disinfect the entire instrument outer surface with an emphasis on the following areas you will handle when packing, unpacking and servicing the instrument:

- Plate Carrier
- Instrument Top
- Cover Edges
- Underneath Between Instrument Feet
- Rear Edges (do not damage the warranty seal)

Preventive Maintenance

To ensure optimal operation of the instrument, do the following preventive maintenance procedures as required:

- Wipe off visible dust from exterior surfaces with a lint-free cloth to avoid dust build up on the instrument.
- Wipe up all spills immediately.
- Follow applicable decontamination procedures as instructed by your laboratory safety officer.
- Respond as required to all error messages the software displays.

You should power off the instrument when not in use.

Cleaning the Instrument



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



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

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

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

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

Storing or Shipping the Instrument

Before you move the instrument, make sure that the new location is a dry, flat work area that has sufficient space for the instrument, host computer, and required cables.



Tip: Although you can always carry the instrument, depending on the distance that you are moving it, you might use a rolling cart instead.

If you must store the instrument, then store it in a dry, dust-free, environmentally controlled area. The storage temperature can range from -20°C to 65°C.

To minimize the possibility of damage during storage or shipment, you should pack the instrument in the original packaging materials. Correctly repacking the instrument includes following applicable decontamination procedures and packing instructions.



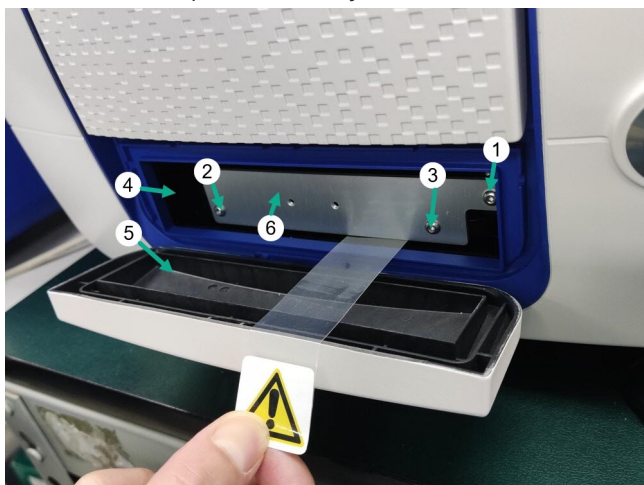
CAUTION! When transporting the instrument, warranty claims are void if improper packing results in damage to the instrument.

Packing the Instrument

The original packaging is designed to protect the instrument during shipment and storage. You must always pack the instrument before you ship it or store it.

To pack the instrument:

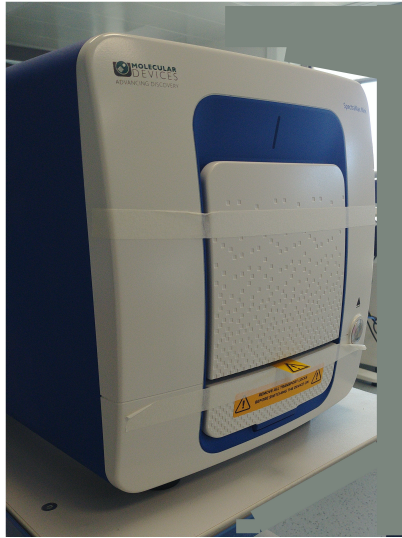
1. Make sure the plate drawer chamber and filter cube chamber are empty.
2. Power off the instrument.
3. Disconnect the power cord and USB cable from the rear of the instrument.
4. Locate the transport lock that you stored in the accessories case.



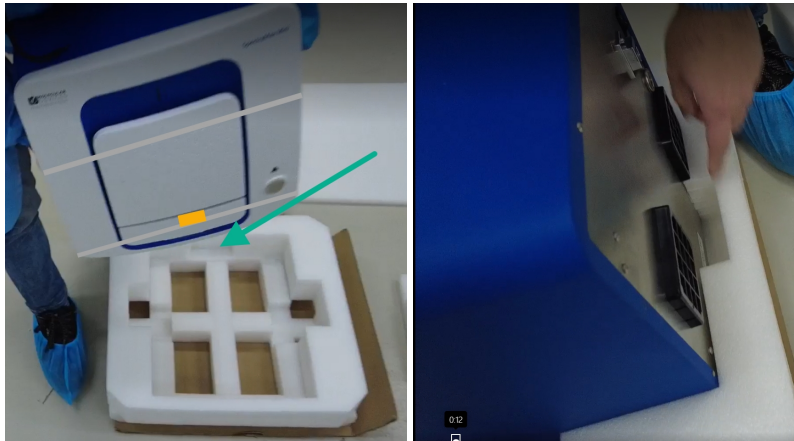
Item	Description
1	Screw #1 fastens the transport lock to the internal frame of the instrument (install last)
2	Screw #2 fastens the transport lock to the plate drawer
3	Screw #3 fastens the transport lock to the plate drawer
4	Plate drawer
5	Plate chamber door in open position
6	Plate drawer transport lock

5. Manually open the plate chamber door, gently slide the plate drawer out of the instrument, and place the transport lock on the end of the of the plate drawer.
6. Use the hex key (included in the accessory case) to tighten screws #2 and #3 until the transport lock is attached to the plate drawer.
7. Gently push the plate drawer into the instrument so that screw #1 aligns with the hole in the instrument frame. Hold the plate drawer open until you complete the next steps.
8. Tighten screw #1 in the upper-right corner of the transport lock to secure the plate drawer to the instrument frame.
9. Route the yellow tab connected to the transport lock so that it passes over the top of the plate chamber door when the door is closed.
10. Gently close the plate chamber door.

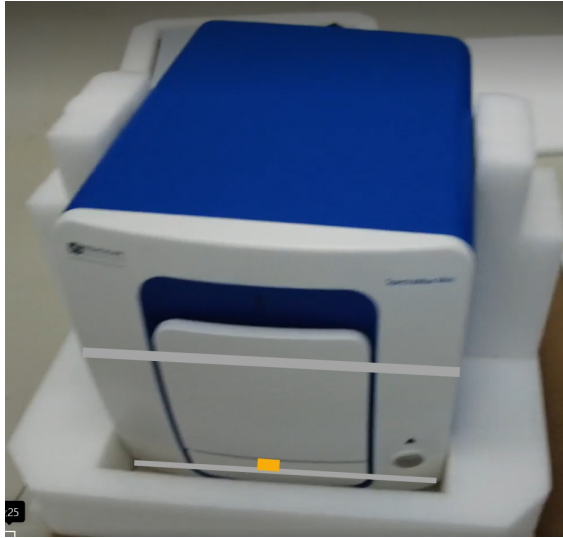
11. Use a strip of minimally adhesive tape to hold the filter chamber door shut and a strip of tape to hold the plate chamber door shut.



12. With the instrument's original cardboard shipping carton open on the side, place the bottom cardboard slide at the opening with the handles oriented away from the carton.
13. Place the bottom foam packing material onto the cardboard slide with the medium insert slot oriented towards the cardboard carton (see following images).
14. Place the instrument onto the bottom foam packaging material with the rear of the instrument aligned with the medium insert slot (toward the cardboard carton side).



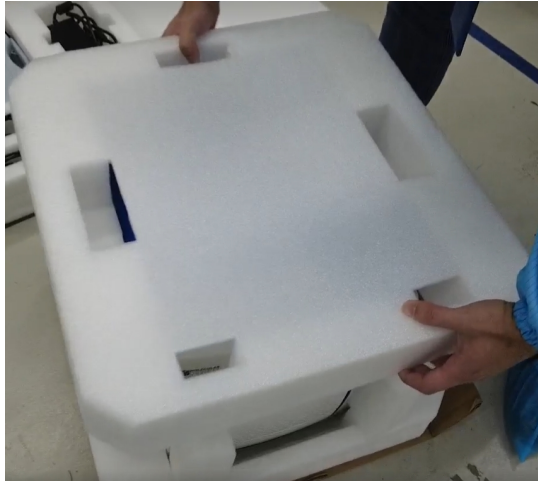
15. Place the two larger vertical foam packing materials on the sides of the instrument and the medium vertical foam packing material at the rear of the instrument.



16. Place the small vertical foam packing material, with the slot, in front of the instrument power button and the other small vertical foam packing material on the other side of the filter door in the front of the instrument.



17. Align the holes in the top foam packing material and place it over the vertical foam packing material.



18. Fold the handles on the cardboard slide upward and use the cardboard to slide the instrument into the carton.



19. Place the accessories into the upper foam packaging material.



20. Place the accessories foam packaging material onto the second cardboard slide, fold the handles upward, and slide it into the carton above the instrument.



21. Slide the top foam packing material into the carton above the accessories.



22. If needed, turn the box upright to ensure that all items are settled inside the carton.



23. Seal the carton with packing tape.



Appendix A: Specifications



Thermal specifications for plates used in the instrument apply to flat-bottom plates with isolated wells. All other plate specifications apply to standard 96-well polystyrene flat-bottom plates.



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

Read Times

Read Mode	96 Wells	384 Wells	Settings
Absorbance	0:57 min after time	2:54	Regular
Absorbance	0:27 min after time	1:21	Speed read
Fluorescence Intensity	0:28 min after time	1:27	100 ms integration time, PMT gain fixed
Luminescence	0:30 min after time	1:30	100 ms integration time

Absorbance Photometric Performance

Item	Description
Wavelength Range	200-1000 nm
Wavelength Selection	Monochromator, tunable in 1 nm increments
Wavelength Bandwidth	≤5.0 nm full width half maximum
Wavelength Accuracy	2 nm across wavelength range
Wavelength Repeatability	±0.2 nm
Dynamic Range	0 to 4 OD
Photometric Resolution	0.001 OD
Photometric Accuracy Linearity	< ± 0.006 OD ± 1.0%, 0-3 OD
Photometric Precision	< ± 0.003 OD ± 1.0%, 0-3 OD
Stray Light	≤0.05% at 230 nm
Light Source	Xenon flash lamp (5 Watts)
Lamp Lifetime	1 billion flashes or 2 years normal operation

Fluorescence Intensity Performance

Item	Description
Filter Based	Top Read
Excitation Wavelength and Emission Wavelength Range	For fluorescence filter cube wavelengths, see Filters and Accessories on page 47 .
Wavelength Range	250-850 nm
Dynamic Range	> 6 Decades
Sensitivity: Fluorescein	1 pM in 96-well plates
Light Source	Xenon flash lamp (5 Watts)
Lamp Lifetime	1 billion flashes or 2 years normal operation
Adjustable Read Height	Z range from upper plate @ 1 mm to max height 15.98 mm

Luminescence Performance

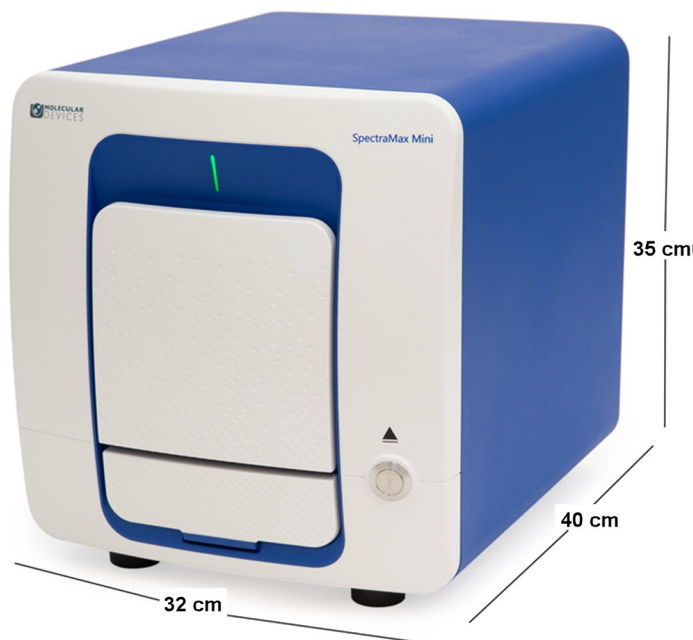
Item	Description
Filter Based	Top Read (For luminescence filter cube wavelengths, see Filters and Accessories on page 47 .)
Dynamic Range	> 7 Decades
Sensitivity	2 pM (ATP) in 96-well plates
Crosstalk	< 0.1%

Temperature Regulation

Item	Description
Reading chamber	Isothermal when temperature regulation is not enabled
Range	4°C above ambient to 45°C when temperature regulation enabled. Note: To achieve temperature regulation at 45°C, the ambient temperature must be > 20°C.
Resolution	Resolution \pm 0.1°C
Accuracy	\pm 2.0°C for plate chamber
Temperature uniformity at equilibrium	\pm 1°C at 37°C
Chamber warmup time	30-50 minutes (measured on air) after initiation of temperature regulation
Temperature regulation	2 sensors
Drift	\pm 0.2°C (regulated)
Temperature regulation diagnostics	Temperature regulation system is continuously monitored and updated
Evaporation	Plate lid required to minimize evaporative cooling

System Dimensions

In the following image, the dimensions are show in centimeters.



Physical Specifications

The following table lists the physical specifications of the instrument.

Physical Specifications

Item	Description
Environment	Indoor use only
Power requirements	External 24V DC power supply compatible with 100-240V AC @50-60 Hz 160W maximum consumption
Dimensions	32 cm W x 35 cm H x 40 cm D (12.6 in. W x 13.8 in. H x 15.7 in. D)
Rear clearance	20 cm to 30 cm (7.9 in. to 11.8 in.) between the rear of the instrument and the wall for ventilation and cable disconnects
Weight	18 kg (≤40 lbs)
Plate formats	6, 12, 24, 48, 96, 384-well plates ANSI/SLAS conformant Absorbance read mode: Maximum height 22 mm Fluorescence read mode and luminescence read mode: Maximum height 20 mm
Reading capability	Plates Cuvettes with SpectraCuvette Adapter part number 5301193 (see Supported Plates on page 11) Note: Do not use any other SpectraCuvette Adapter.

Physical Specifications (continued)

Item	Description
Shake	Orbital, double orbital, and linear
Absorbance Wavelength selection	1.0 nm Increments
Ambient operating temperature	15°C to 40°C (59°F to 104°F)
Ambient storage temperature	-5°C to 40°C (23°F to 104°F) continuous -20°C to 50°C (-4°F to 122°F) transient (up to 10 hours)
Humidity restrictions	15% to 75% (non-condensing) at 30°C (86°F)
Altitude restrictions	Up to 2000 m (6562 ft)
Air pressure restrictions	54 kPa to 106 kPa (7.8 PSI to 15.4 PSI)
Sound pressure level	Maximum sound pressure: 73 dBA Maximum sound pressure at one meter: 68 dBA
Installation category	II
Pollution degree	2
Data connection	One USB port
NFC antenna reader/writer	SANGOMA-MSMA 2V5 13.56 MHz Multi Standard - Multi Antenna Reader/Writer Contains FCC ID: 2AKHW-SANGMSMA4 Contains IC: 22202-SANGMSMA4 Changes or modifications made to this equipment not expressly approved by the party responsible for compliance may void the FCC authorization to operate this equipment.

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

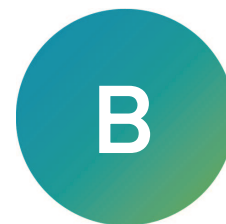
This ISM device complies with Canadian ICES-001.

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

ISM Equipment Classification (Group 1, Class A)

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

Appendix B: Filters and Accessories

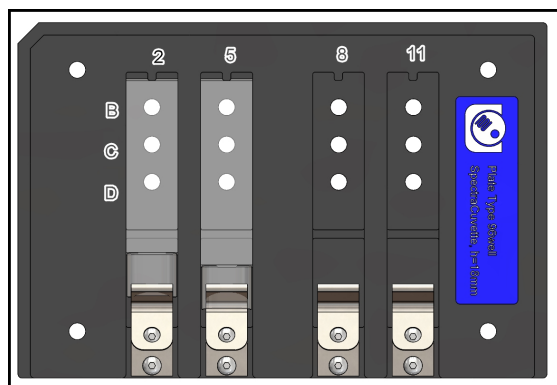


Filters

Item	Excitation		Dichroic	Emission		Part #
	Center WL (nm)	Bandwidth (nm)	Center WL (nm)	Center WL (nm)	Bandwidth (nm)	
Standard Fluorescence Filter Cubes						
Fluorescence Filter Cube FL - 465	360	35	405	465	35	5089096
Fluorescence Filter Cube FL - 535	485	20	508	535	25	5089097
Fluorescence Filter Cube FL - 595	535	25	560	595	35	5089098
Optional Fluorescence Filter Cubes						
Fluorescence Filter Cube FL - 360	280	20	310	360	35	5089099
Fluorescence Filter Cube FL - 565	465	35	508	565	30	5089100
Fluorescence Filter Cube FL - 635	590	20	612	635	20	5089102
Fluorescence Filter Cube FL - 680	625	35	658	680	20	5089103
Fluorescence Filter Cube FL - 810	710	50	765	810	40	5089104
Luminescence Filter Cube	Clean-up filter with turn over wavelength at 675 (nm)					5089334

SpectraCuvette Adapter

Part Number 5301193



SpectraDrop Plates

Part Number	Description	Details
0200-6262	SpectraDrop Starter Kit	Contains one microplate adapter, two 24-well low volume bottom slides, and one of each cover slide (2 μ L and 4 μ L)
0200-6267	SpectraDrop High-Throughput Screening (HTS) Kit	Contains one microplate adapter, five 24-well low volume slide, five 64-well low volume bottom slides, and five of each cover slide (2 μ L and 4 μ L)
0200-6263	SpectraDrop Micro-Volume Refills, 24-Well Bottom Slides	Three low volume 24-well bottom slides
0200-6264	SpectraDrop Micro-Volume Refills, 64-Well Bottom Slides	Three low volume 64-well bottom slides
0200-6265	SpectraDrop Micro-Volume Refills, 2 μ L Cover Slides	Three 2 μ L top slides (0.5 mm clear path length)
0200-6266	SpectraDrop Micro-Volume Refills, 4 μ L Cover Slides	Three 4 μ L top slides (1.0 mm clear path length)

Obtaining Support

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

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

You can contact your local representative or Molecular Devices Technical Support at 800-635-5577 x 1815 (North America only) or +1 408-747-1700.

In Europe call +44 (0) 118 944 8000.

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

Contact Us

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Web: moleculardevices.com
Email: info@moldev.com

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