SpectraMax® iD3 Multi-Mode Microplate Reader

User Guide

MOLECULAR DEVICES

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

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

Product manufactured by Molecular Devices, LLC.
1311 Orleans Drive, Sunnyvale, California, United States of America 94089.
Molecular Devices, LLC is ISO 9001 registered.

Contents

Safety Information

Information about the safe use of the SpectraMax® iD3 Multi-Mode Microplate Reader instrument 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.

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

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

Warnings, Cautions, Notes, and Tips

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

When warnings and cautions appear in this guide, be careful to follow the specific safety information related to them.

The following user-attention statements can display in the text of Molecular Devices® user documentation. Each statement implies a particular amount of observation or recommended procedure as described:

WARNING! A warning indicates a situation or operation that could cause personal injury if precautions are not followed. Some warnings can have a different symbol on the left, such as electric shock or biohazard warnings. The definition of the symbol is included in the text of the warning.

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. The following table lists the alert symbols on the instrument.

Table 3-1: Instrument Label Alert Symbols

Symbol	Indication
	This symbol indicates that you must consult the product documentation.
	This symbol indicates a potential lifting hazard. To prevent injury, use a minimum of two people to lift the instrument. For information about the weight of the instrument, see Physical Specifications on page 129.
	This symbol indicates a potential pinch hazard.
	This symbol indicates a potential biohazard.
	This symbol indicates a potential heat hazard.
	This symbol indicates an electrostatic sensitive device (ESD). Observe precautions for handling electrostatic sensitive devices.

Note: 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.

The following sticker appears on the back of your instrument.

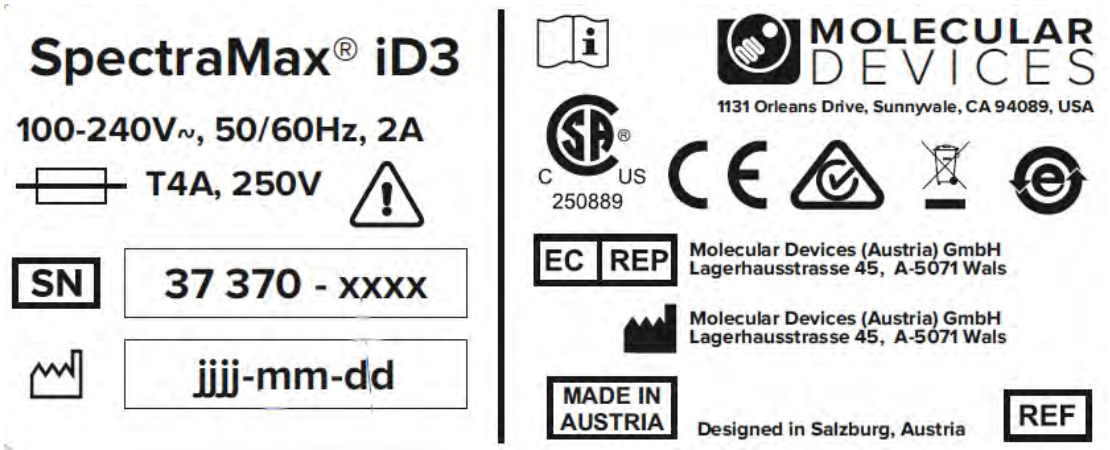
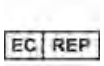


Table 3-2: Instrument Label Symbols

Symbol	Indication
	This symbol indicates the location of a fuse.
	This symbol indicates that you must consult the product documentation.
SN	This symbol indicates the instrument serial number.
	This symbol indicates the instrument manufacture date.
	This symbol indicates you should consult the instructions for use.
C US 250889	This symbol indicates CSA certification.
CE	This symbol signifies European technology conformity.
	This symbol signifies that the instrument complies with Australian radio communication requirements.

Table 3-2: Instrument Label Symbols (continued)

Symbol	Indication
	This symbol on the product 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.
	This symbol indicates the environmental friendly use period.
	This symbol indicates there is an authorized representative in the European community.
	This symbol indicates the instrument manufacturer.
	This symbol indicates the manufacturer catalog number.

Before 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.

Electrical Safety

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

WARNING! HIGH VOLTAGE. Within the instrument is the potential of an electrical shock hazard existing from a high-voltage source. All safety instructions must be read and understood before proceeding with the installation, maintenance, and servicing of all modules.

Do not remove the instrument covers. To prevent electrical shock, use the supplied power cords only and connect to a properly grounded wall outlet. Use only multi-plug power strips that are provided by the manufacturer.

To ensure sufficient ventilation and 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.

Turn the instrument power off when the instrument is not in use.

In the event that you need to change the instrument fuses, see Replace Fuses on page 104.

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. Therefore, 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.
- Observe the applicable cautionary procedures as defined by your safety officer when using flammable solvents in or near a powered-up instrument.
- Observe the applicable cautionary procedures as defined by your safety officer when using toxic, pathological, or radioactive materials.

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

CAUTION! Use of organic solvents can cause harm to the optics in the instrument. Extreme caution is recommended when using organic solvents. Always use a plate lid and do not place a plate containing these materials in the microplate chamber for prolonged periods of time. Damage caused by the use of incompatible or aggressive solvents is NOT covered by the instrument warranty.

CAUTION! When you use aggressive or corrosive reagents, you should turn the Auto Eject Plate setting on the Maintenance page Reader Settings tab to the On position. See Reader Settings Maintenance on page 64.

WARNING! BIOHAZARD. Depending on your usage, the injectors can have biohazardous material in and on them. Always use the personal protective equipment (PPE) prescribed by your laboratory.

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.

To prevent injury due to moving parts, observe the following:

- Never try to exchange labware, reagents, or tools while the instrument is operating.
- Never try to physically restrict the moving components of the instrument.
- Keep the instrument work area clear to prevent obstruction of the movement. Provide clearance in front of the instrument of 18 cm (7.1 in.) for the microplate drawer.
- The instrument has adjustable optics to define the read height, or z-height. In a top read, the read height is the gap between the lens and the top of the microplate, or the top of the lid if the microplate is lidded.

CAUTION! To prevent damage to the instrument, the height of the microplate must not exceed 22 mm, including the lid if the microplate is lidded.

Transport locks are placed on the transport slide and on the microplate drawer to protect the instrument from damage during shipment. You must remove the transport locks before you power on the instrument. See Remove Transport Locks on page 23.

To move the microplate drawer into or out of the instrument, always use the icon on the touchscreen or the controls in the software. See Load and Unload Microplates, see page 68.

When your instrument is equipped with the SpectraMax® Injector System with SmartInject™, the injectors in the instrument enable you to deliver a reagent to the wells of a microplate. Follow the instructions in this guide for injector use and maintenance. See Injectors on page 14.

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

Note: Observe all warnings and cautions listed for all external devices attached to or in use during the operation of the instrument. See the applicable user guide for the operating and safety procedures of that device.

Chapter 1: Introduction

The SpectraMax iD3 Multi-Mode Microplate Reader from Molecular Devices is a monochromator-based, multi-mode plate reader. The touchscreen interface provides integrated instrument control, data display, and the ability to export results for statistical data analysis.

The instrument supports the following read modes:

- UV and Visible Absorbance (ABS), see Absorbance Read Mode on page 90.
- Fluorescence Intensity (FL), see Fluorescence Intensity Read Mode on page 94.
- Luminescence (LUM), see Luminescence Read Mode on page 98.

For each read mode, you can setup and run the following read types:

- Endpoint Read Type on page 89
- Kinetic Read Type on page 89
- Spectrum Read Type on page 90

CAUTION! To prevent damage to the instrument, the height of the microplate must not exceed 22 mm, including the lid if the microplate is lidded.

Computer Integration

All software required to run basic non-injector reads is installed in the instrument and is accessible from the touchscreen. You must use a computer running SoftMax® Pro Software to operate the instrument for advanced acquisition settings and for protocols that use the SpectraMax Injector System.

Optional integration of the instrument with a computer enables you to export data over your intranet or to a USB flash drive in an Excel format for further analysis.

You can use SoftMax Pro Software to have the instrument collect data from one or more microplates and store the data in a single file, using the same or different instrument settings for different microplates. Assays that require a read in two or more read modes or read types can be combined in a single experiment and run with a single command in the SoftMax Pro Software, by defining separate microplate reads and enabling Auto Read. For information on the acquisition and analysis capabilities of the SoftMax Pro Software, see the *SoftMax Pro User Guide*.

Note: When you use a computer running the SoftMax Pro Software to operate the instrument, the instrument touchscreen is locked.

When you use SoftMax® Pro GxP Software to operate the SpectraMax iD3 Multi-Mode Microplate Reader and you log on as a user with the Sign Statements permission, two additional icons appear on the GxP tab.

- Users with the Sign Statements permission click **GxP Mode On**  to lock the instrument touchscreen and operate the instrument from the computer running the SoftMax Pro Software in GxP mode. This locks the instrument touchscreen for all users and the instrument must be operated from a computer running SoftMax Pro GxP Software.
- Users with the Sign Statements permission click **GxP Mode Off**  to release the lock from the instrument touchscreen and allow users to perform reads from the instrument.

Note: The instrument remains locked until the Sign Statements user clicks **GxP Mode Off**  to stop the GxP mode. You cannot use the Instrument Connection dialog to disconnect from an SpectraMax iD3 instrument that is locked in GxP mode.

The SpectraMax iD3 software version 1.0 is compatible with SoftMax Pro Software version 7.0.1.

The SpectraMax iD3 software version 1.1 is compatible with SoftMax Pro Software version 7.0.2.

To update the SpectraMax iD3 software, please contact Technical Support.

Applications

The high sensitivity and flexibility of the instrument make it useful for applications in the fields of biochemistry, cell biology, immunology, molecular biology, and microbiology.

Typical applications include ELISA, nucleic acid, protein, enzymatic type homogeneous and heterogeneous assays, microbial growth, endotoxin testing, and pipettor calibration.

Application notes with specific application protocol suggestions can be found in the Information Center and the Knowledge Base on the Molecular Devices web site at www.moleculardevices.com.

Optics

The 2x2 monochromators permit individual optimization of wavelengths for both excitation and emission in fluorescence readings. Mirrored optics shape the light, and a height-adjustable objective lens focuses the beam into the sample volume. PMT Gain can be set to automatic, high, medium, or low.

Dynamic Range

The dynamic range of detection is approximately from 10^{-6} to 10^{-12} molar fluorescein. Variations in measured fluorescence values are virtually eliminated by internal compensation for detector sensitivity, photomultiplier tube voltage and sensitivity, and excitation intensity. The photometric range is 0.000 to 4.000 ODs with a resolution of 0.001 OD.

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

CAUTION! Use of organic solvents can cause harm to the optics in the instrument. Extreme caution is recommended when using organic solvents. Always use a plate lid and do not place a plate containing these materials in the microplate chamber for prolonged periods of time. Damage caused by the use of incompatible or aggressive solvents is NOT covered by the instrument warranty.

Microplate Controls

Microplates up to a height of 22 mm can be placed in the microplate drawer of the instrument. A camera detects the height of a microplate in the microplate drawer and confirms that the height is consistent with the microplate type you select and that the plate is positioned properly on the microplate drawer.

Depending on the application, the instrument can read 6, 12, 24, 48, 96, and 384-well microplates. For micro-volume measurements, the instrument supports SpectraDrop 24-well Micro-Volume Microplates and SpectraDrop 64-well Micro-Volume Microplates. See Set Plate Format and Plate Type on page 72.

CAUTION! To prevent damage to the instrument, the height of the microplate must not exceed 22 mm, including the lid if the microplate is lidded.

Shake

A shake feature can be operated independently from a protocol to permit the contents of the wells in a microplate to be mixed outside of the microplate chamber for visual inspection. This makes it possible to do kinetic analysis of solid-phase, enzyme-mediated reactions. See External Shake Settings on page 46.

You also define shake settings as part of each protocol. The process related to the shake setting depends on the read mode you select. See Set Shake on page 81.

Temperature Regulation

The temperature inside the microplate chamber can be maintained at 5°C (9°F) above ambient to 65°C (149°F). See Microplate Chamber Temperature Settings on page 48.

The temperature sensors detect the temperature of the air inside the chamber, not the temperature of the samples in the microplate. If you use the instrument to warm the samples, use a seal or lid on the microplate to prevent evaporation of the sample. The seal or lid also helps to maintain a uniform temperature. It can take an hour or more for a prepared sample to equilibrate inside the microplate chamber. You can speed up equilibration by prewarming the sample and the assay reagents to the desired temperature before you place the microplate in the chamber. Validate the data quality to determine whether the seal or lid can stay on the microplate for the read.

Injectors

When your instrument is equipped with the SpectraMax Injector System, the injectors in the instrument enable you to deliver a reagent to the wells of a microplate. You use the injectors to deliver a specific volume of reagent to initiate a reaction that occurs rapidly and results in a luminescent or fluorescent signal that must be detected quickly. The injector system is method independent which means you can run injector protocols for all read modes: Absorbance, Luminescence (all wavelength), Luminescence Monochromator, Fluorescence Intensity top, and Fluorescence Intensity bottom.

Note: You must use a computer running SoftMax Pro Software to operate the instrument for advanced acquisition or injector protocols.

Before you use the injectors or do maintenance operations, make sure that you are familiar with the safety information in this guide. See Safety Information on page 5.

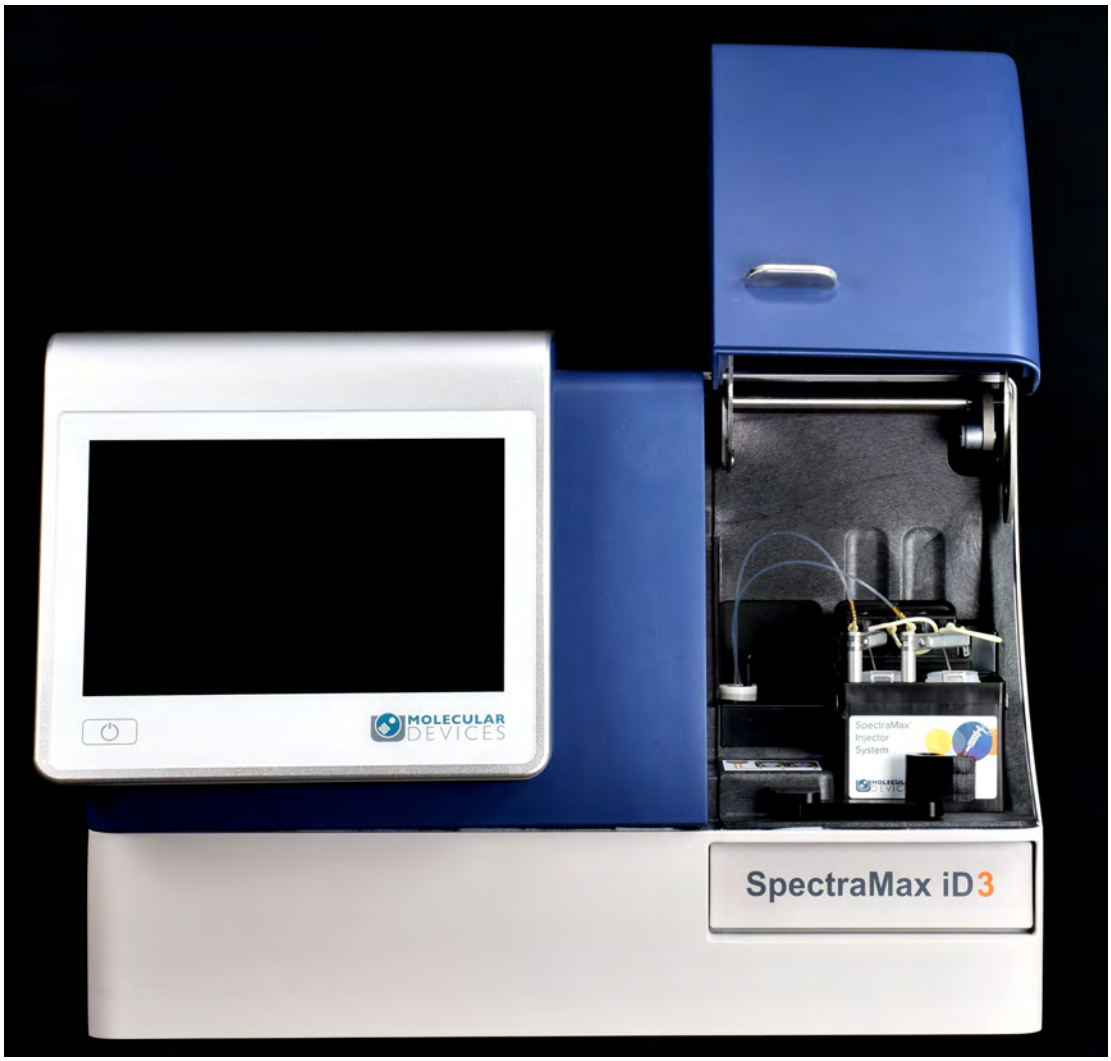
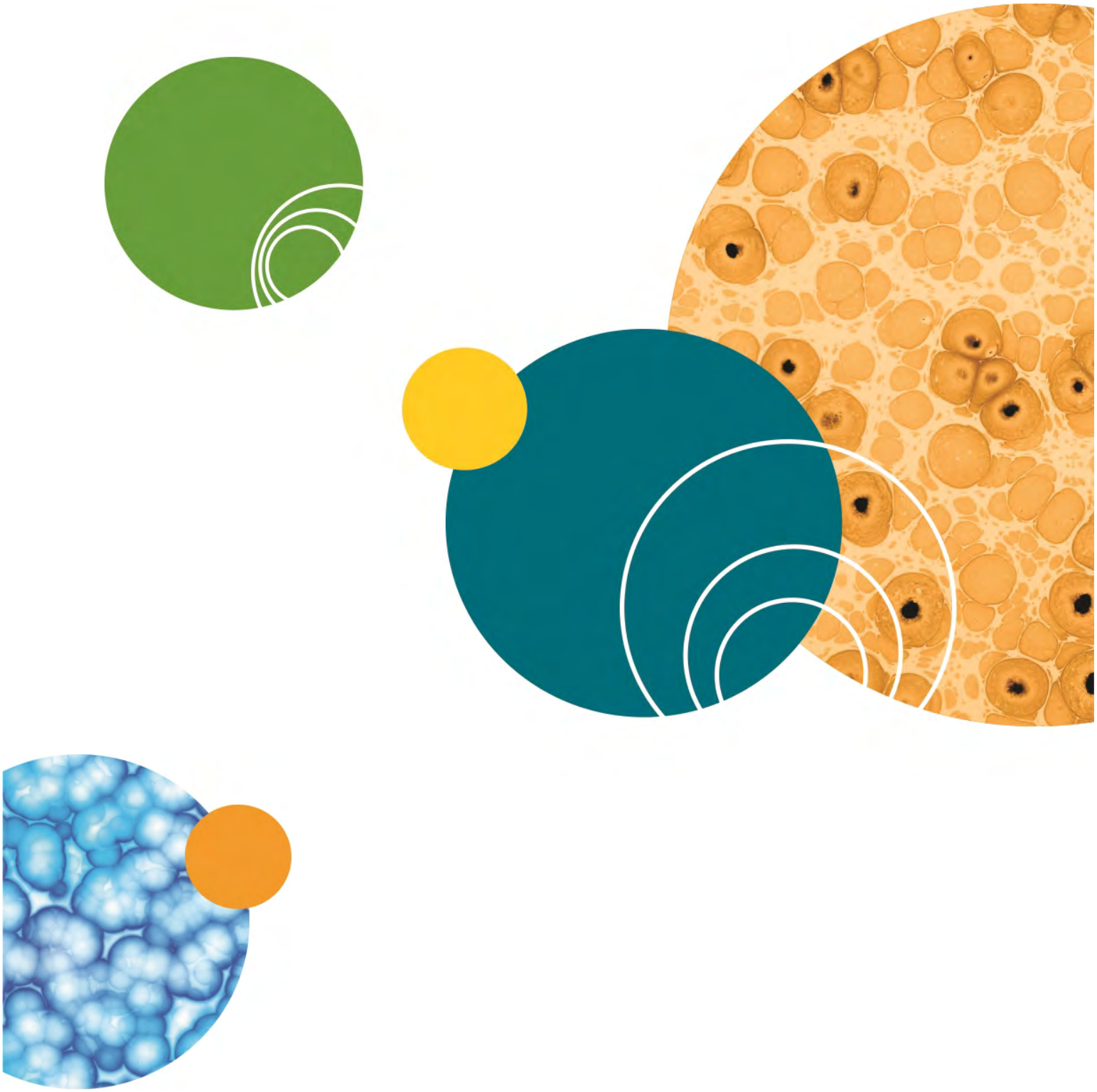


Figure 1-1: Hood open to display injectors

The SoftMax Pro Software can set up the instrument to inject and read well by well to reduce signal loss. To define the settings for a read with injection, you must use the Acquisition View on the Settings dialog. See the SoftMax Pro Software application help for details.

The Injector Maintenance page enables you to wash and prime the injectors. See Injector Maintenance - Wash and Prime on page 49.

WARNING! BIOHAZARD. Depending on your usage, the injectors can have biohazardous material in and on them. Always use the personal protective equipment (PPE) prescribed by your laboratory.



Chapter 2: Unpack and Setup the Instrument

Before you unpack and setup the SpectraMax® iD3 Multi-Mode Plate Reader instrument, prepare a dry, flat work area that has sufficient space for the instrument and required cables. See Instrument Specifications on page 125

All software required to run basic non-injector reads is installed in the instrument and is accessible from the touchscreen. You must use a computer running SoftMax Pro Software to operate the instrument for advanced acquisition settings and for protocols that use the SpectraMax Injector System.

You can export data to a computer over your intranet, through a direct Ethernet cable, or to a USB flash drive in an Excel format for further analysis.

- Unpack the instrument and save the original packaging. See Unpack the Instrument on page 20.
- Remove the transport locks from the transport slide and microplate drawer. See Remove Transport Locks on page 23.
- Connect the instrument cables. See Connect Instrument Cables on page 29.
- Turn on the instrument power. See Turn the Instrument On and Off on page 31.
- Get the instrument onto your network. See Get the Instrument On Your Network on page 32.
- Optionally, install the SoftMax® Pro Software on a computer to operate the instrument. You must use the SoftMax Pro Software to run injector reads. See the *SoftMax Pro User Guide*. The SoftMax Pro Software installation automatically installs the QuickSync® tool.
- You can choose to install only the QuickSync tool. See Install the QuickSync Tool on page 35.

Note: When you use a computer running the SoftMax Pro Software to operate the instrument, the instrument touchscreen is locked.

Contents of the Package

The package contains the instrument plus a box that contains the tools and accessories required to install the instrument.

Table 2-1: Package Contents

Illustration	Part Number	Description
	5054744	Installation guide
	YW 000 006	Hex key, 2.0 mm
	YW 000 012	Holex HEXAGON ballhead bolt driver 3 mm
	5052189	CAT6 Ethernet cable, 2 meter (6.56 foot)
	4400-0002 or 4400-0036	Power cord, 115 V or Power cord, 230 V
	5056338	Near Field Communication (NFC) sticker (NTAG 213, 180 byte) Stick sticker to an item to use as your swipe tool. Not the NFC key fob.
	5056339	Near Field Communication (NFC) key fob (NTAG 213, 180 byte). Do not put an NFC sticker on the key fob.
	5048887	SoftMax Pro Software with Product Key and QuickSync tool

When your instrument has the SpectraMax Injector System the package also contains the following.

Table 2-2: Package Contents

Illustration	Part Number	Description
	5055247	Injector Nozzle
	5044164	Tubing
	5055251	Bottle Holder
	5044165	Bottle Adapters
	5044163	Waste Plate
	Cannot order from Molecular Devices Wide-neck bottle, HDPE 50 mL capacity 36 mm square by 68 mm high 24 mm diameter inside neck Recommended supplier: VWR (215-0440)	Bottles
	Cannot order from Molecular Devices Strip wells, polystyrene 1x8, clear, flat-bottomed Recommended supplier: Greiner Bio-One (762001)	Strip well

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

Unpack the Instrument

The packaging is designed to protect the instrument during transportation.

Transport locks are placed on the transport slide and the microplate drawer to protect the instrument from damage during shipment. You must remove the transport locks before you power on the instrument.

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

Note: Retain the shipping box and all packaging materials for future transport needs. Do not use tools that can damage the packaging or the instrument.

CAUTION! When transporting the instrument, warranty claims are void if damage during transport is caused by improper packing.

To unpack the instrument:

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

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

2. With the box facing up as indicated on the packaging, use a box cutter to carefully cut open the side of the box labeled **Open Here**.

Figure 2-1: Open the box

3. Grasp the handle on the cardboard and slide the instrument out of the box.

Tip: It might be easier if a second person holds the box in place while the instrument is slid out on the cardboard.

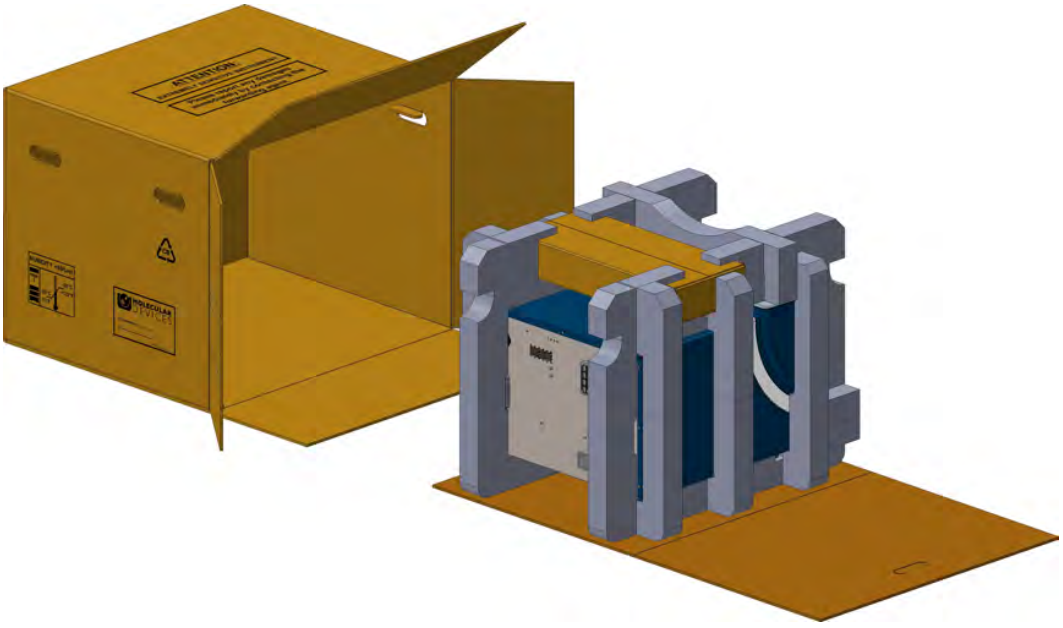


Figure 2-2: Slide the instrument out of the box

4. Remove the accessories tool box.

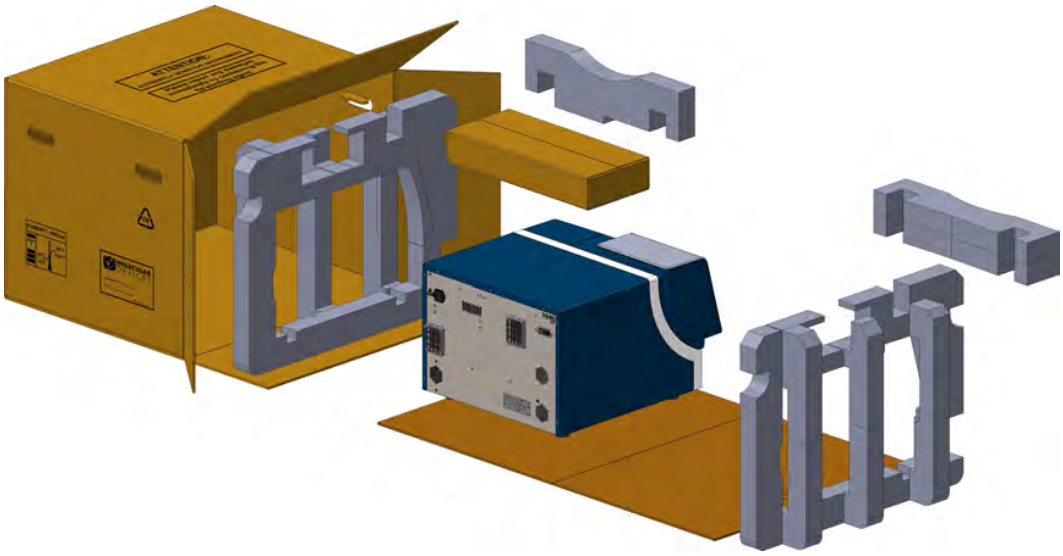


Figure 2-3: Remove the accessory tool box and foam packing

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

5. Lift one end of the instrument slightly and remove the foam packaging from that end.
6. Gently return the instrument to the ground.
7. Lift the other end of the instrument slightly and remove the foam packaging from that end.
8. Gently return the instrument to the ground.
9. Remove the large plastic bag from the instrument. You might need to slightly lift the instrument to get the bag over the feet.
10. With one person on each end, lift the instrument and gently place the instrument on a dry, flat area. You will need to access the back of the instrument to remove the transport lock and to connect the instrument cables. For information about the weight of the instrument, see Instrument Specifications on page 125.

Note: The feet are sticky and the instrument does not slide well. If you slide the instrument, it can mark the work surface.

Remove Transport Locks

CAUTION! The instrument can be damaged if the transport locks are not removed before the instrument is powered on.

Transport locks are placed on the transport slide and the microplate drawer to protect the instrument during shipment. You must remove the transport locks before you power on the instrument.

Note: If the rear of your instrument does not have the transport lock opening in the center, see Remove Transport Locks - Alternate on page 25.

This procedure requires the following tool:

Table 2-3: Required Tool

Illustration	Part Number	Description
	YW 000 006	Hex key, 2.0 mm
	YW 000 012	Holex HEXAGON ballhead bolt driver 3 mm

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 locks:

1. On the rear of the instrument, use the provided 3.0 mm Holex HEXAGON ballhead bolt driver to loosen the screw located inside the Transport Lock opening until you feel the spring release.

   Note: The screw remains inside the instrument. The screw is spring mounted and cannot get lost within the instrument. It could take ten full turns to loosen the screw until you feel the spring release. This unlocks the transport slide. If the instrument makes a grinding noise when you start a plate read, you have not released the transport slide.

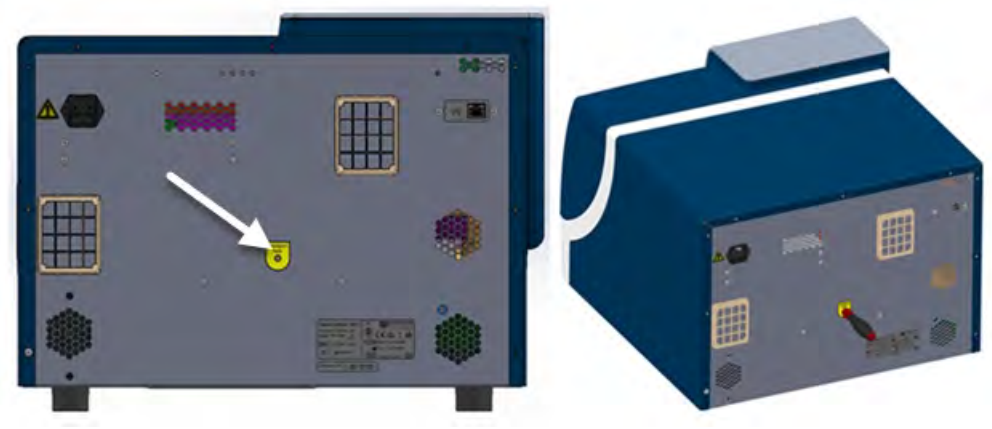


Figure 2-4: Transport lock opening

2. On the front of the instrument, gently pull the yellow tab protruding from the microplate chamber door to open the door. You must hold the microplate drawer door open while you remove the transport lock.

Note: Be careful not to tear the yellow tab. It must remain attached to the transport lock to make it easier to open the microplate chamber door.

3. Use the provided 2.0 mm hex key to loosen screw #1 in the upper-left corner of the transport lock (6) until the lock disconnects from the instrument frame. The screw has a retaining washer that prevents it from being removed from the lock.

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

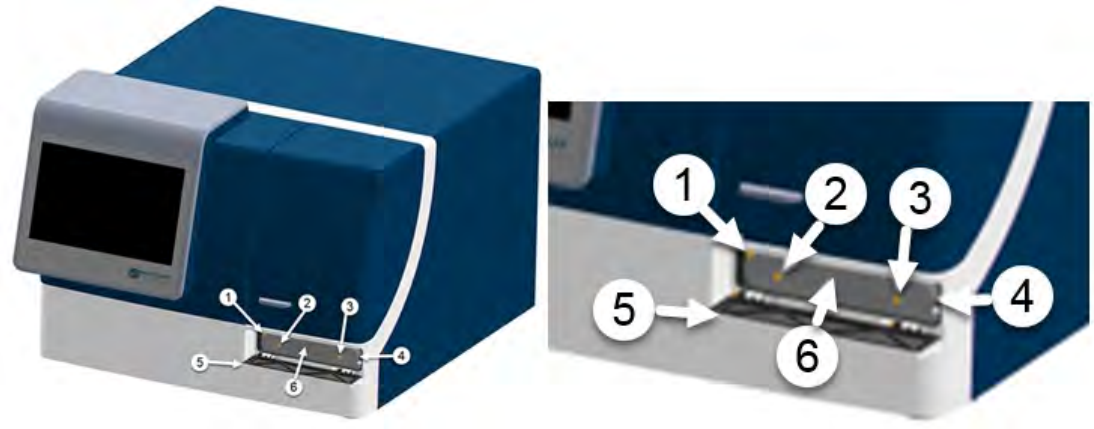


Figure 2-5: Microplate drawer transport lock

Item	Description
1	Screw #1 fastens the lock to the internal frame of the instrument
2	Screw #2 fastens the lock to the microplate drawer

Figure 2-5: Microplate drawer transport lock (continued)

Item	Description
3	Screw #3 fastens the lock to the microplate drawer
4	Microplate drawer
5	Microplate chamber door in open position
6	Microplate drawer transport lock

4. Loosen screws #2 and #3 until the lock comes free of the microplate drawer (4) and you can remove the lock from the instrument. The screws have retaining washers that prevent them from being removed from the lock. Store the transport lock in the accessories tool box included with the microplate reader.
5. Gently push the microplate drawer (4) back inside the instrument and close the chamber door (5).
6. Save the original carton, foam inserts, accessories tool box, and transport locks in case you must ship the instrument in the future.

Remove Transport Locks - Alternate

CAUTION! The instrument can be damaged if the transport lock is not removed before the instrument is powered on.

If the rear of your instrument does not have the transport lock opening in the center, perform the following steps to remove the transport lock. A transport lock is placed on the transport slide to protect the instrument during shipment. You must remove the transport locks before you power on the instrument.

If the rear of your instrument has a Transport Lock sticker in the center, see Remove Transport Locks on page 23.

This procedure requires the following tool:

Table 2-4: Required Tool

Illustration	Part Number	Description
	YW 000 044	Hex key, 4.0 mm

CAUTION! Do not touch or loosen screws or parts other than those designated in the instructions. Changes to other screws or parts can cause misalignment and possibly void the warranty.

To remove the transport lock:

1. Use the provided 4.0 mm hex key to loosen screw #1 in the upper-right corner on the back of the instrument. After a few turns, the touchscreen housing rises. You might hear a click when the housing is ready for the next step. Do not loosen the screw after the touchscreen is unlocked.

Figure 2-6: Instrument rear - release touchscreen housing

2. Walk to the front of the instrument and gently lift the touchscreen housing to access the transport lock face plate.

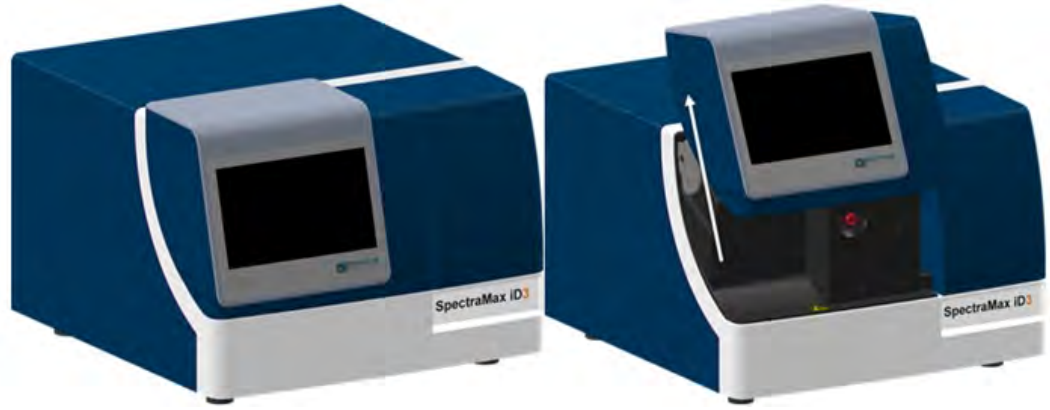


Figure 2-7: Instrument front - raise touchscreen housing

WARNING! You must hold the touchscreen housing in place for the following steps.

3. Loosen screw #2, by hand, to free the transport lock face plate. The screw is attached to the faceplate to prevent it from being misplaced.

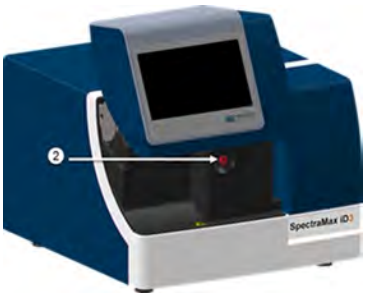


Figure 2-8: Transport lock face plate

4. Remove the transport lock face plate (with screw #2 attached) to access the transport locks.

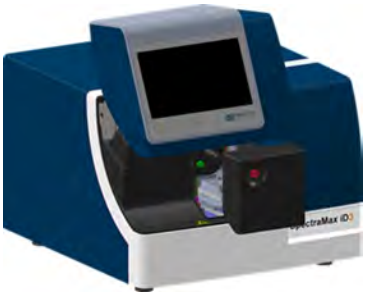


Figure 2-9: Remove transport lock face plate

5. By hand, remove the short transport lock #3 from the instrument case.

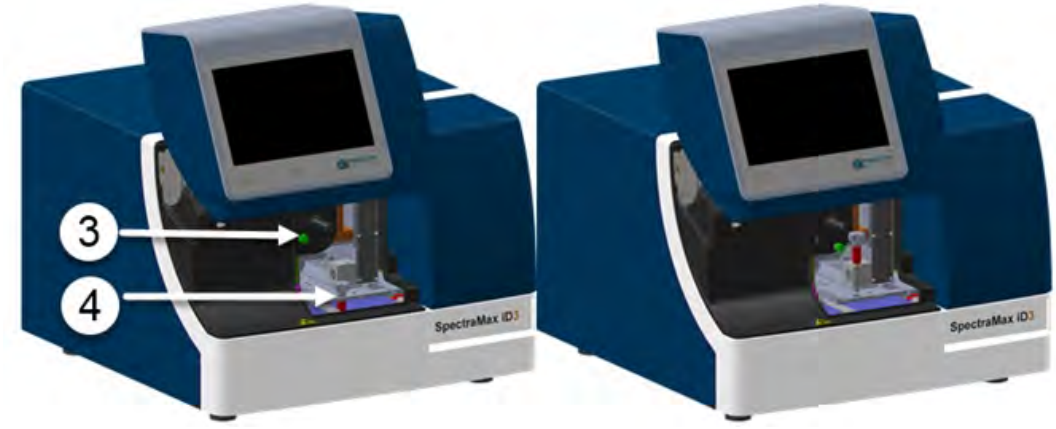


Figure 2-10: Remove transport locks

6. By hand, remove the long transport lock #4 from the transport slide.

7. Screw the long transport lock #4 into the slot on the instrument case from where you removed the short transport lock.

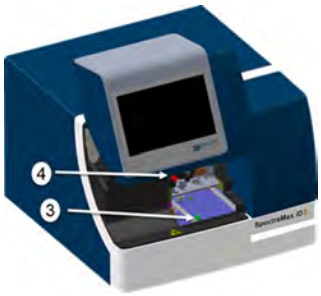


Figure 2-11: Switch transport locks

8. Move the transport slide toward the back of the instrument and screw the short transport lock #3 into the lower slot from where you removed the long transport lock.
9. Align the transport lock faceplate with screw #2 to its original position.

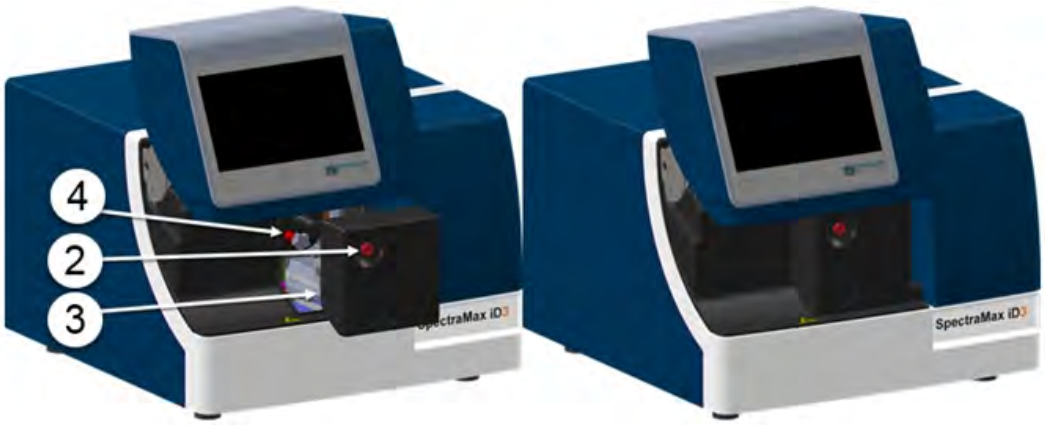


Figure 2-12: Replace transport lock face plate

10. Screw the transport lock faceplate back into place with screw #2 by hand.
11. Gently lower the touchscreen housing.

Note: Do not push the touchscreen housing down manually. It lowers automatically as you turn the hex key in the next step.

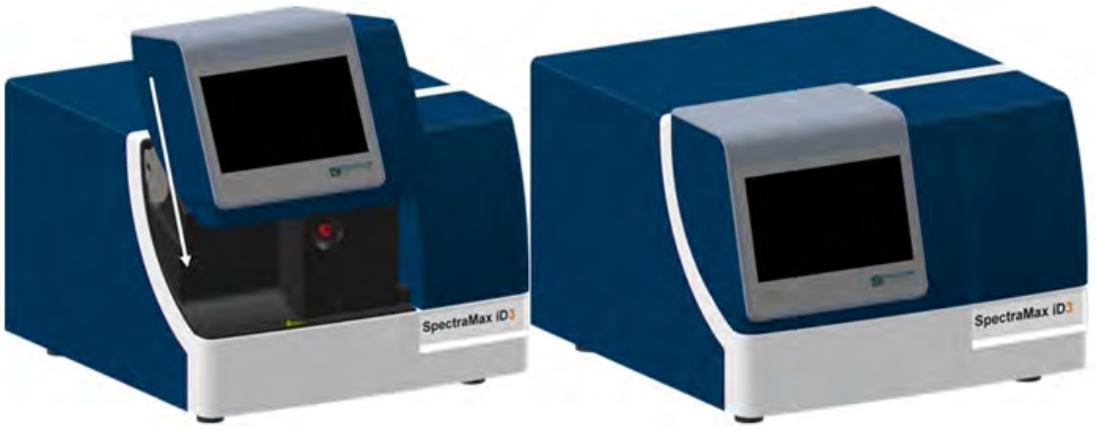


Figure 2-13: Lower touchscreen housing

12. Walk to the back of the instrument. Use the provided 4.0 mm hex key to tighten screw #1 in the upper-right corner on the back of the instrument until the touchscreen housing lowers and is locked in place. Do not over tighten the screw.

Figure 2-14: Lock touchscreen housing

13. Save the original carton, foam inserts, and accessories tool box in case the instrument must be shipped in the future.

Connect Instrument Cables

The power cord and Ethernet cable connect to the ports on the rear of the instrument. All software required to run non-injector reads is installed in the instrument and is accessible from the touchscreen.

Ethernet connection is optional. The Ethernet port enables you to connect the instrument to your intranet (similar to a printer) or to connect the instrument directly to a computer. When the instrument is connected to your intranet, you can synchronize any computer on the same intranet with the instrument, within security and firewall restrictions. You can synchronize multiple computers to an instrument and multiple instruments to a computer over your intranet.

You can use the SoftMax Pro Software on a connected computer to operate the instrument and you must use the SoftMax Pro Software to run injector reads. See the *SoftMax Pro User Guide*.

Table 2-5: Required Accessories

Illustration	Part Number	Description
	5052189	CAT6 Ethernet cable, 2 meter (6.56 foot)
	4400-0002 or 4400-0036	Power cord, 1 meter (3.3 foot)

To connect the cables to the instrument:

1. Make sure the instrument is placed on a dry, flat work area with sufficient space for the instrument and the required cables. To ensure ventilation and provide access to disconnect the 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.
2. Connect one end of the supplied Ethernet cable to the Ethernet port on the instrument and then connect the other end of the Ethernet cable to a network wall outlet.

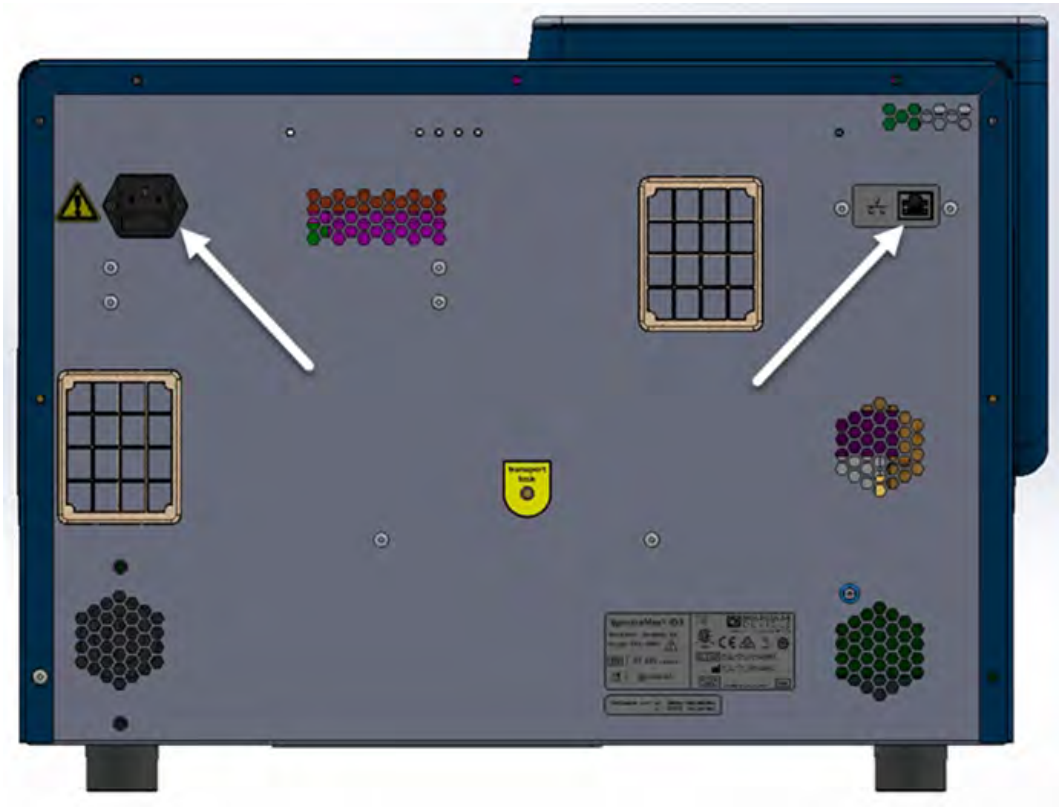


Figure 2-15: Instrument rear - power port and Ethernet port

3. Use the power cord supplied with the instrument to connect the instrument to a grounded electrical wall outlet.

You can optionally connect the instrument directly to a computer.

1. Turn on the power to the computer.
2. Connect one end of an Ethernet cable to the port on the rear of the instrument and then connect the other end of the Ethernet cable to the Ethernet port on the computer. If the computer to which you intend to directly connect the instrument does not have an available Ethernet port, you can use an Ethernet (instrument side) to USB (computer side) adapter. (Adapter not included.)
3. Install SoftMax Pro Software version 7.0.2 or newer on the computer. The SoftMax Pro Software installation automatically installs the QuickSync tool as well. See the *SoftMax Pro Software Installation Guide* and the *SoftMax Pro User Guide.* You can choose to install only the QuickSync tool. See Install the QuickSync Tool on page 35.

Note: When you use a computer running the SoftMax Pro Software to operate the instrument, the instrument touchscreen is locked.

Turn the Instrument On and Off

All software required to run basic non-injector reads is installed in the instrument and is accessible from the touchscreen. You must use a computer running SoftMax Pro Software to operate the instrument for advanced acquisition settings and for protocols that use the SpectraMax Injector System. Near Field Communication (NFC) tags enable you to easily save and view the protocols that matter to you.

The power button and NFC sensor are directly below the touchscreen on the front of the instrument.

CAUTION! You must remove the transport locks before you power on the instrument. See Remove Transport Locks on page 23.

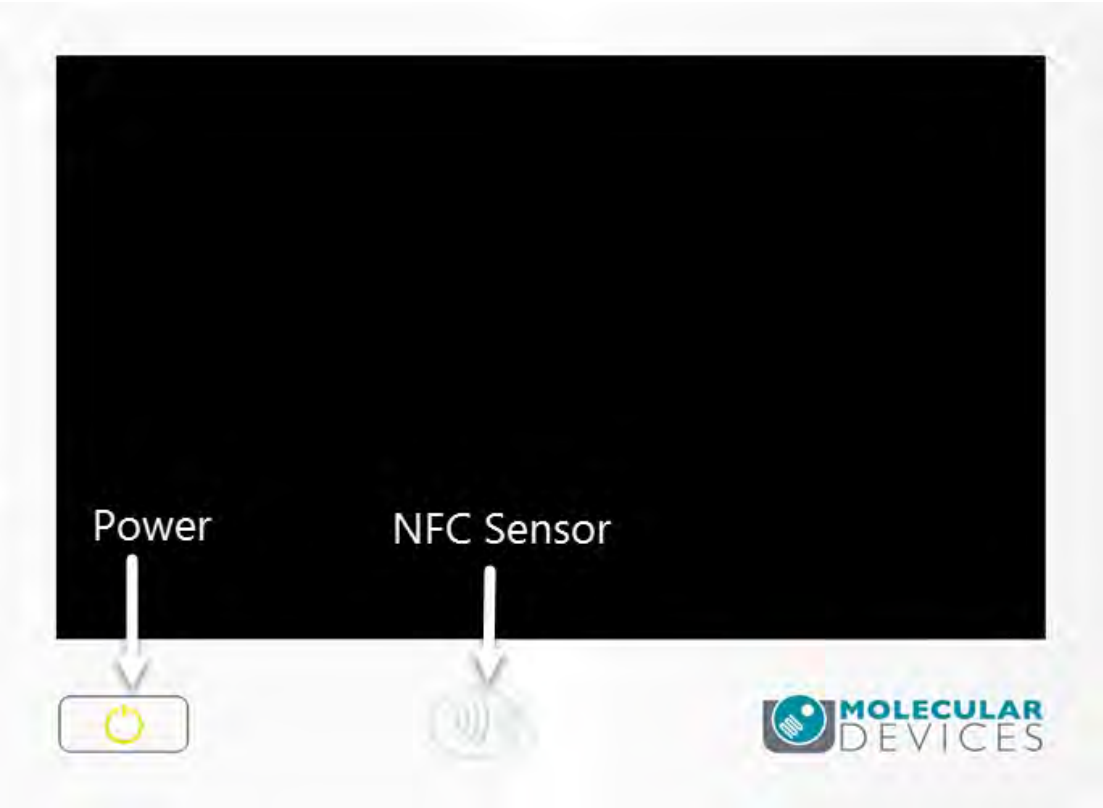


Figure 2-16: Touchscreen, power button, NFC sensor

1. To turn on the instrument, press the power button below the touchscreen.
2. Wait until the Home page displays and the  disappears. See Home Page on page 43.
3. If you use an NFC tag, after the Home page displays on the touchscreen, swipe your NFC tag over the NFC sensor below the touchscreen.

Note: The first time you power on the instrument, there is only one user - **Public**. You cannot edit or delete the **Public** user and **Public** has no associated NFC tag. To add users and to associate a user with an NFC tag, see Users Maintenance on page 62.

Get the Instrument On Your Network

The instrument is configured to automatically obtain an IP address. After you connect the Ethernet cable from the instrument to a networked wall outlet and after you power on the instrument, the instrument should automatically assign itself an IP address.

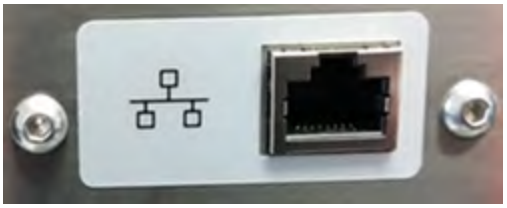


Figure 2-17: Ethernet port - instrument rear

Tip: You might have to work with your IT department to make sure your network will accept the addition of the instrument.

CAUTION! You must remove the transport locks before you power on the instrument.

Figure 2-18: Maintenance page - Instrument tab

The Instrument tab on the Maintenance page enables you to view the instrument IP address.

1. From the buttons on the left, touch  to display the Maintenance page.
2. Touch **Instrument** to display the Instrument tab.
3. The **Assigned IP** field displays the instrument IP address. If you disconnect the Ethernet cable and then reconnect the Ethernet cable or if you power off the instrument, the instrument IP address can change. Touch **Refresh** to update the display of the Assigned IP address.

Note: If you plan to use a computer running the SoftMax Pro Software to operate the instrument, write down the IP address. You may need the IP address to connect the computer to the instrument.

4. Touch **Reader Settings** to display the Reader Settings tab.
5. If you plan to have the instrument export data through an Ethernet cable to a computer that is on the same company network or is attached to the instrument, touch the **Export Excel** OFF to display ON and then install the QuickSync tool on the computer. See Install the QuickSync Tool on page 35.

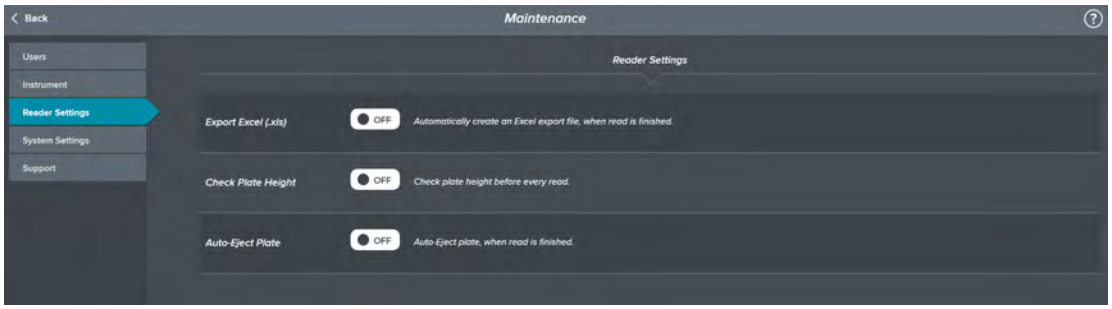


Figure 2-19: Maintenance page - Reader Settings tab - Export Excel setting

6. Touch **System Settings** to display the System Settings tab.
7. Touch in the **Date/Time** field to display a calendar. Use the calendar to change the system date then touch the clock and use the scroll bars to change the time. Touch **Set** to save the date/time changes.

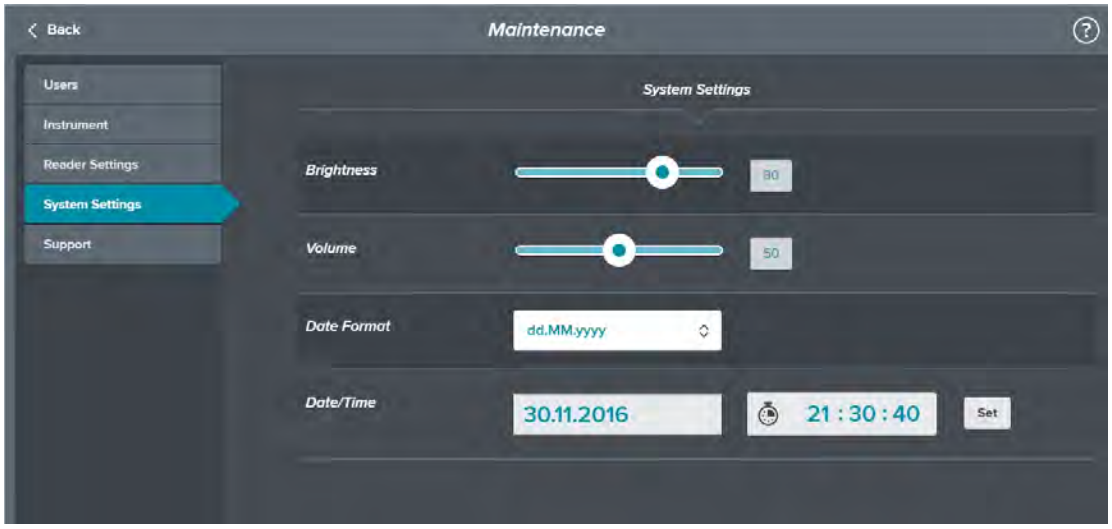


Figure 2-20: Maintenance page - System Settings tab - Date/Time setting

Install the QuickSync Tool

The QuickSync tool enables a computer to receive the raw data that the instrument exports, within the security and firewall restrictions of your network. When you install the SoftMax Pro Software on a computer, the SoftMax Pro Software installation process automatically installs the QuickSync tool.

You can install the QuickSync tool on any computer. The computer must be able to communicate with the instrument over your intranet or is directly connected to the instrument. See Use the QuickSync Tool on page 88.

The instrument acquires an IP address when you connect the instrument to your intranet. After you synchronize the computer with the instrument, the instrument exports result data to the computer for further analysis.

Tip: You can synchronize multiple computers to an instrument and multiple instruments to a computer.

The QuickSync tool is optional. You can insert a USB flash drive into the USB port located below the front of the touchscreen and export raw data to a flash drive. See Export Protocol Data on page 87.

Table 2-6: Required Accessory

Illustration	Part Number	Description
	5048887	SoftMax Pro Software with Product Key and QuickSync tool

Do the following on the computer to which you want to export data.

1. Insert the supplied USB flash drive or CD into your computer.
2. Navigate the file hierarchy to locate the **QuickSync<x.x>.exe** file.
3. Double-click **QuickSync<x.x>.exe** to start the QuickSync tool installation wizard.
4. Select the **I accept the terms of the license agreement** check box and click **Next**.
5. Wait for the installation to complete and the success message appears.
6. Click **Finish**.
7. The  QuickSync icon appears in your computer taskbar area, with the other programs running on your computer, at the bottom of the screen. You may have to look in the Show Hidden Icons area.
8. Click the big  in the taskbar to display a smaller version of the  in the computer tray near the clock at the bottom of the computer screen with the message "Scanning For Devices". Wait for the computer to find the instrument.

9. Right-click in the tray near the clock to display a menu and select **Available Services** to display the list of SpectraMax iD3 Multi-Mode Microplate Reader instruments on your intranet and/or the instrument to which you connect the computer through an Ethernet cable.

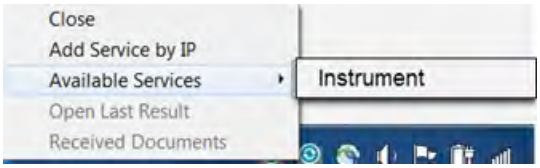


Figure 2-21: Connect your computer to the instrument

10. Click the name of instruments to which to synchronize the computer. A check mark appears next to each instrument name to which the computer is synchronized.

Add Service by IP Address

If the name of the instrument does not appear in the list of available services, do the following:

1. Right-click in the tray near the clock and select **Add Service by IP** to display the following.

2. Enter the IP address of the instrument to which to connect. See Get the Instrument On Your Network on page 32.
3. Click **Add Connect**.

Tip: If the computer still cannot find the instrument, contact your IT help desk to make sure that your company network setup and company intranet security allow the communication between the computer and the instrument.

Injector Assembly and Maintenance

This topic describes how to assemble the injectors when you receive a new instrument that has the SpectraMax Injector System. The steps in this topic also describe how to replace the bottle holder and the injector tubing.

Before you use the injector or do maintenance operations, make sure that you are familiar with the safety information in this guide. See Safety Information on page 5.

WARNING! BIOHAZARD. Depending on your usage, the injector can have biohazardous material in and on it. Always use the personal protective equipment (PPE) prescribed by your laboratory.

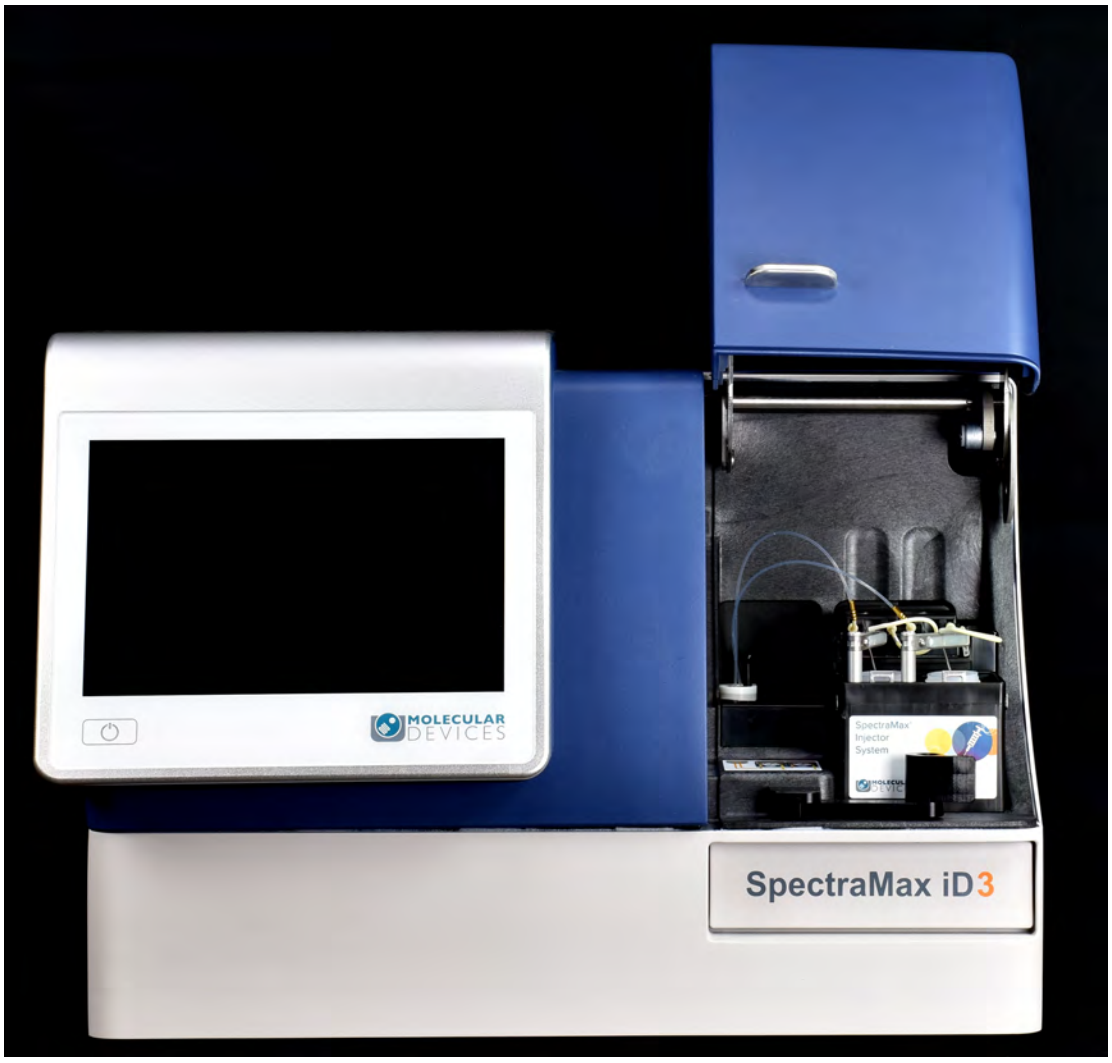


Figure 2-22: Injectors

The two injectors are located under the hood on the right of the instrument. Injector 1 is on the left and injector 2 is on the right as you face the instrument.

Bottle Holder

The bottle holder is mounted on two knobs for ease of installation, cleaning, and replacement.

Figure 2-23: Bottle holder

To install the bottle holder:

1. Align the two slots on the bottle holder with the two pegs on the rail.
2. Lightly press the bottle holder into position.
3. Insert the bottles in the bottle holder.

Injector Tubing

Each injector has an injector tube. The tubing line connects to an injector tip on one end and a snorkel on the other end. From the tip, the tube passes around the injector pump to the snorkel. The tubing around the pump is held in position by two rubber bumpers and a stabilizer flap.

The tips slide into the nozzle. The nozzle fits into the injector arm for maintenance, wash and prime and fits into an opening located in the back left of the injector space within the instrument for injector protocols.

The snorkels are held in the bottles by snorkel clamps.

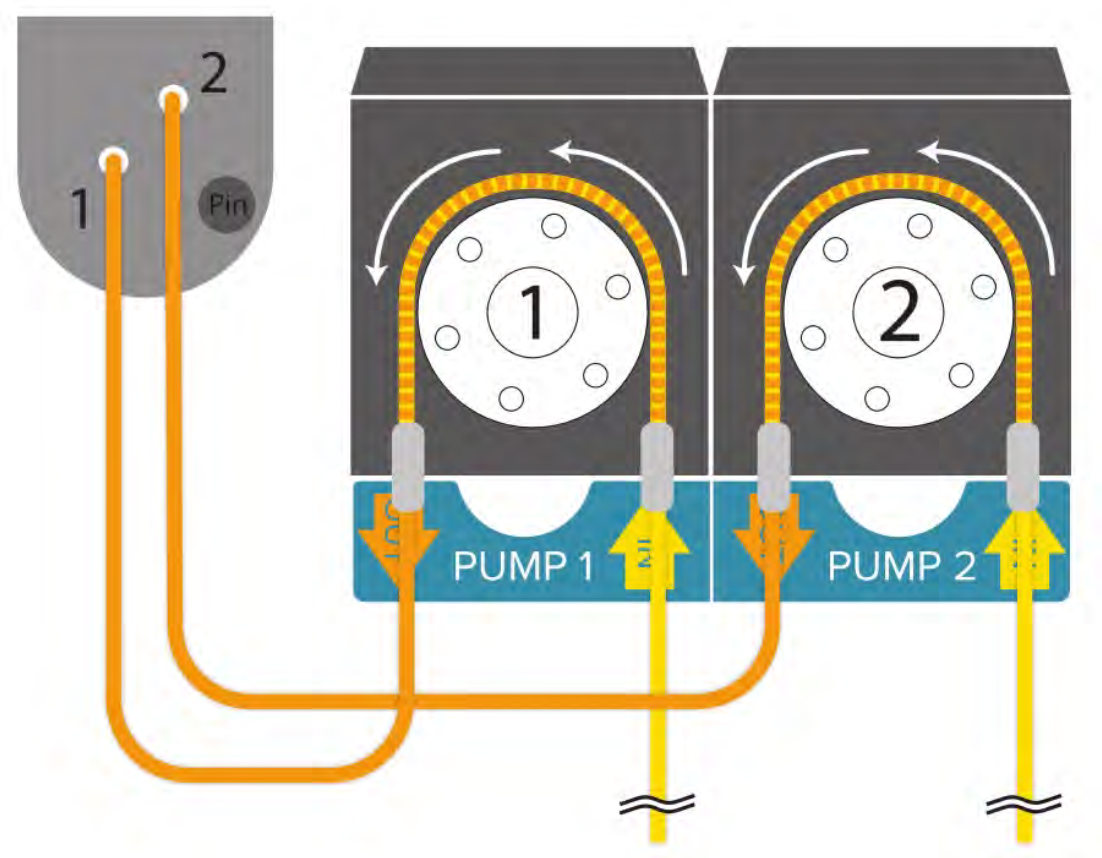


Figure 2-24: Injector tubing

To install or replace the injector tubing:

1. Move the injector arm away from the instrument and insert the nozzle into the injector arm.

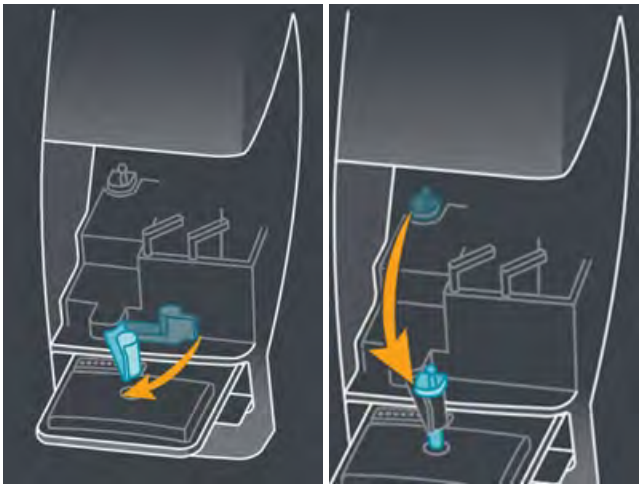


Figure 2-25: Move injector arm and insert nozzle

Perform each of the following steps for both injectors.

2. Insert the snorkel into the bottle and then insert the snorkel into the snorkel clamp. Clamp for injector 1 is on the left and clamp for injector 2 is on the right.

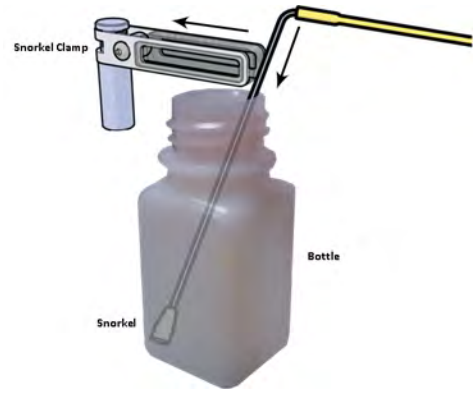


Figure 2-26: Install snorkel

3. Pump 1 is on the left and pump 2 is on the right. Lift the stabilizer lid over the pump, press the bumper into the bumper slot on the input (right) side of the injector pump , gently pull the tubing around the injector pump, and then seat the bumper on the output (left) side of the injector pump.

Tip: When the injector is not in use and the tube is empty, the stabilizer lid over the injector pump should be kept open to extend the tubing lifetime.

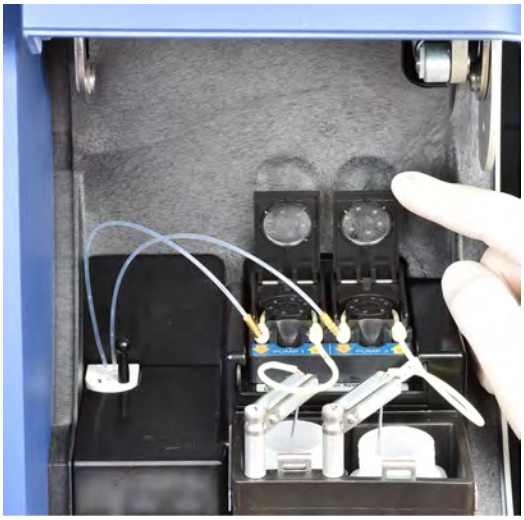


Figure 2-27: Install tubes

4. Slide the tip into the nozzle, the slots are labeled 1 and 2 for injector 1 and injector 2.

Figure 2-28: Insert tips in nozzle

5. Use the black pin to move the nozzle back to the rear of the injector space. Align the nozzle with the opening and press straight down until you feel it snap into place.

Figure 2-29: Insert nozzle into injector port

6. Move the injector arm to its original position.

Chapter 3: Home Page

3

All software required to run basic non-injector reads is installed in the instrument and is accessible from the touchscreen. You must use a computer running SoftMax Pro Software to operate the instrument for advanced acquisition settings and for protocols that use the SpectraMax Injector System. If you choose to use the SoftMax Pro Software on a computer to operate the instrument, the touchscreen is disabled.

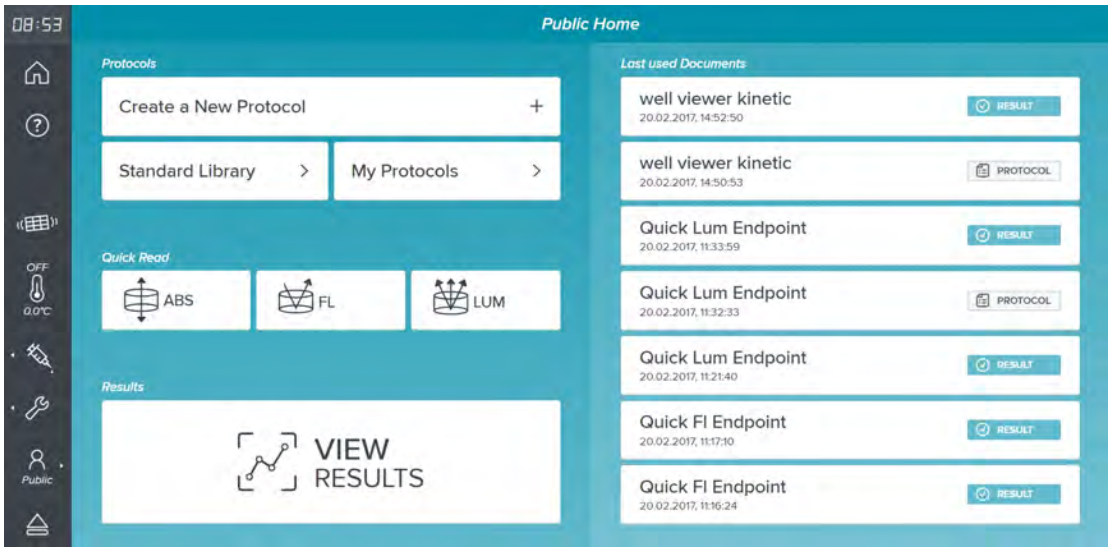
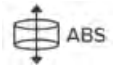


Figure 3-1: Touchscreen Home page

The left side of the page provides a set of buttons that display in most workflows.

-  - Touch to return to the Home page.
-  - Touch to access page-specific application help.
-  - Touch to shake a plate outside of a defined protocol. See External Shake Settings on page 46.
-  - Touch to change the temperature of the microplate chamber. See Microplate Chamber Temperature Settings on page 48.
-  - Touch to access injector maintenance settings to wash and prime the injectors. See Injector Maintenance - Wash and Prime on page 49.
-  - Touch to access instrument maintenance settings. See Maintenance Page on page 61.
-  - Touch to change the user. See Change User on page 67.
-  - Touch to open or close the microplate drawer. See Load and Unload Microplates on page 68.

The Home page provides the following controls.

- **Create a New Protocol** - Touch to create a protocol. The Create New Protocol dialog appears.
- **Standard Library** - Touch to select from a list of pre-defined protocols. See Protocol Libraries on page 44.
- **My Protocols** - Touch to select from the protocols you save for future use. See Protocol Libraries on page 44.
- **Quick Read** - See Quick Read on page 46.
  - 

 - Touch to run an absorbance quick read. See Absorbance Read Mode on page 90.
  - 

 - Touch to run a fluorescence quick read. See Fluorescence Intensity Read Mode on page 94.
  - 

 - Touch to run a luminance quick read. See Luminescence Read Mode on page 98.
- **View Results** - Touch to view the results of the read. See View Protocol Results on page 82.

The **Last Used Documents** section displays the list of your most recently used documents and protocols with the following details.

- **Document/Protocol Name** - Displays in bold large font.
- **Date and Time** - Displays the date and time the document/protocol was created or run last.
- **Protocol** - The document is a protocol without read results. Touch to display the protocol settings. See View Protocol Settings on page 69.
- **Result** - The protocol has been run and contains the results of the last read. Touch to display the read results. See View Protocol Results on page 82.

Protocol Libraries

Protocol libraries enable you to access pre-defined protocols. The Home page provides access to a Standard Library, to your My Protocols list, and to the protocols and documents that you used most recently. See Protocols on page 69.

The Last Used Documents list and the contents of the My Protocols list change based on the user you select from the Change User page. See Change User on page 67.

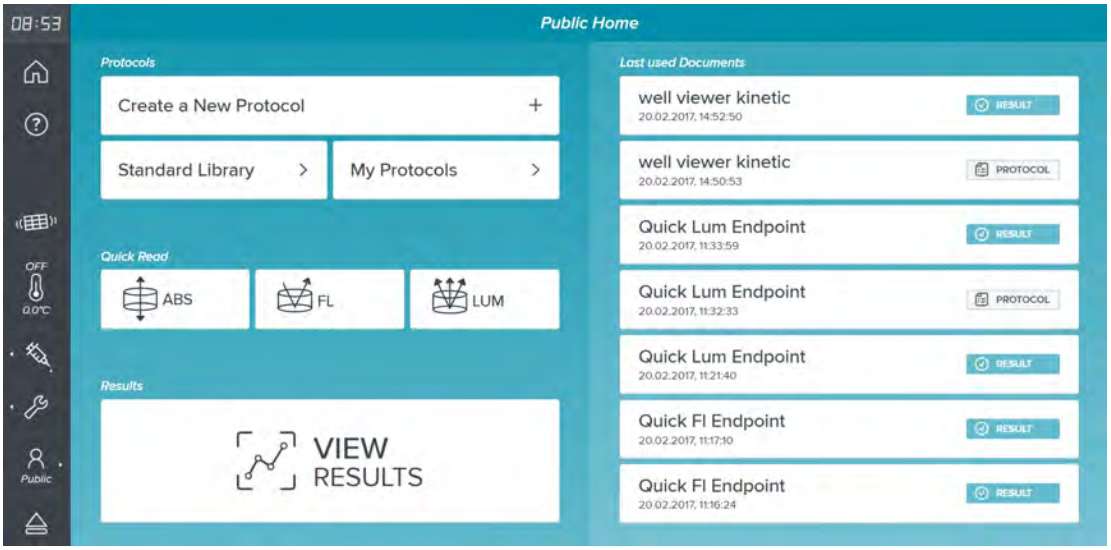


Figure 3-2: Protocol libraries on the Home page

From the Last Used Documents list, touch a protocol to display the protocol settings (see View Protocol Settings on page 69) or touch a result to display the read results (see View Protocol Results on page 82).

The Standard Library and the My Protocols list share a similar workflow. After you touch either of these buttons, you can navigate between both protocol lists.

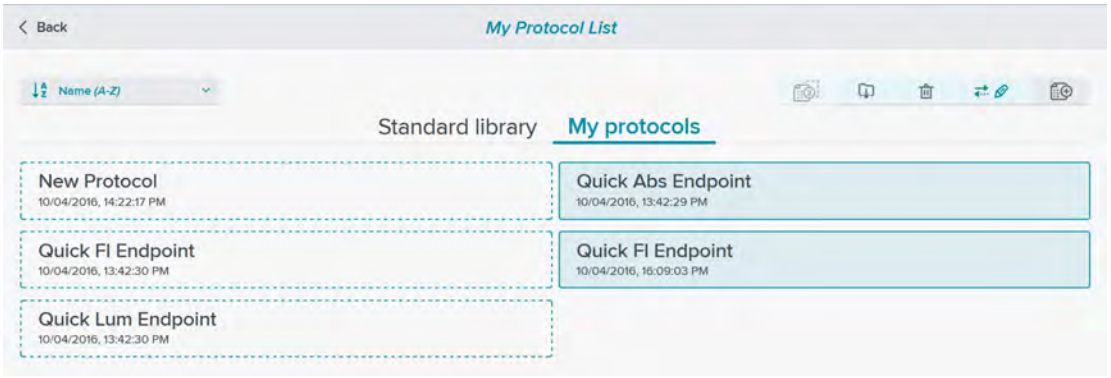


Figure 3-3: Protocol libraries - My Protocols List - edit mode

The protocols in the Standard Library are read only. These protocols enable you to create your own copy of a similar protocol. To use or edit a Standard Library protocol, touch the protocol to display its settings then touch **Save as** to save the protocol with a different name. The copy of the Standard Library protocol then appears in your My Protocols list.

1. From the Home page, touch **Standard Library** to display the Standard Protocol List or touch **My Protocols** to display the My Protocol List.

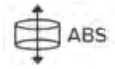
2. To toggle between the two lists, touch either **Standard Library** or **My Protocols** above the protocol list.
   - Touch 

to sort the protocols in either list alphabetically or by date.
   - In the My Protocols list, touch  to add a protocol to your My Protocols list. The Create New Protocol dialog appears.
   - Touch  to manage your My Protocols list.
     - Select a protocol in the list and touch  to make a copy of the protocol.
     - Select one or more protocols in the list and touch  to export the protocol.
     - Select one or more protocols in the list and touch  to delete the protocols you select.
3. To display the protocol settings, touch a protocol. See View Protocol Settings on page 69.

Quick Read

The Home page provides Quick Read buttons to enable you to quickly run an Absorbance, Fluorescence Intensity, and Luminescence read using default settings.

You can run the quick read protocols as defined or you can modify the protocol settings and save the updated settings in your protocol library for future use. See View Protocol Settings on page 69.

- 

- See Absorbance Read Mode on page 90.
- 

- See Fluorescence Intensity Read Mode on page 94.
- 

- See Luminescence Read Mode on page 98.

External Shake Settings

The external shake feature enables you to shake a plate outside of the instrument. This shake process is independent of a protocol.

When you create a protocol, a separate workflow enables you to define how to shake the microplate as part of the protocol. See Define Protocol Settings on page 71.

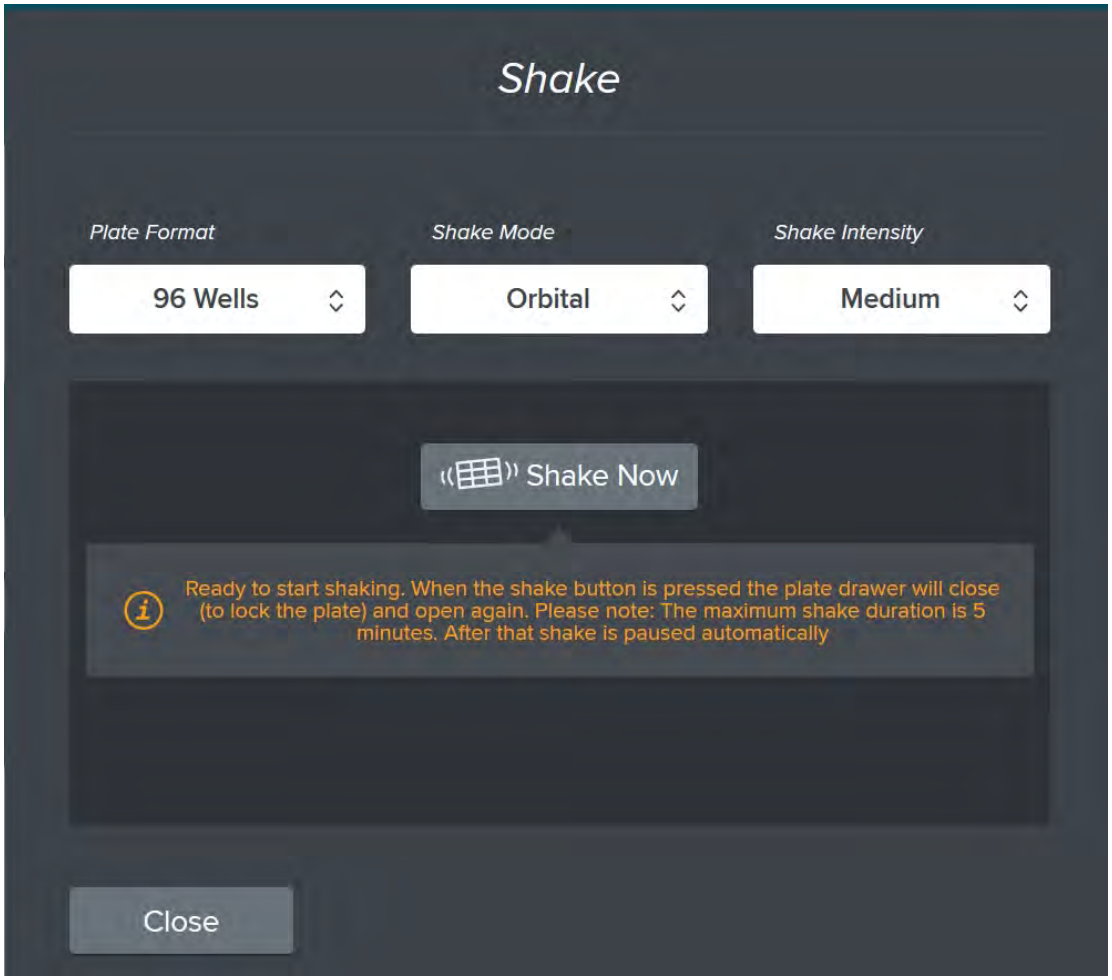


Figure 3-4: External Shake dialog

To shake a microplate outside the instrument, independently from a protocol:

1. From the buttons on the left, touch  to open the microplate drawer and insert the microplate to shake.

   Note: Leave the microplate drawer open.

2. From the buttons on the left, touch  to display the Shake dialog.
3. Touch the **Plate Format** drop-down and select the number of wells the plate contains.
4. Touch the **Shake Mode** drop-down and select **Orbital** or **Double Orbital**.
5. Touch the **Shake Intensity** drop-down and select **Low**, **Medium**, or **High**.
6. Touch **Shake Now**. The Shake Now button changes to Pause.
   - The plate drawer closes to lock the plate and then opens again.
   - The plate shakes until you touch Pause or for five minutes. After five minutes the shake process stops. Touch Resume for a shake duration longer than five minutes.
7. To stop the shake, touch **Pause**. The Pause button changes to Resume.

8. Touch one of the following:
   - Touch **Resume** to start the shake again.
   - Touch **Close Drawer** to close the microplate drawer to start a read.
   - Touch **Remove Plate** to remove the plate from the microplate drawer.

   Note: The microplate drawer closes to release the lock then reopens to enable you to remove the plate.

Microplate Chamber Temperature Settings

The temperature inside the microplate chamber can be maintained at 5°C (9°F) above ambient to 65°C (149°F). The temperature sensors detect the temperature of the air inside the chamber, not the temperature of the samples in the microplate. If you use the instrument to warm, the samples, use a seal or lid on the microplate to prevent evaporation of the sample. The seal or lid also helps to maintain a uniform temperature. It can take an hour or more for a prepared sample to equilibrate inside the microplate chamber. You can speed up equilibration by pre-warming the sample and the assay reagents to the desired temperature before you place the microplate in the chamber. Validate the data quality to determine whether the seal or lid can stay on the microplate for the read.

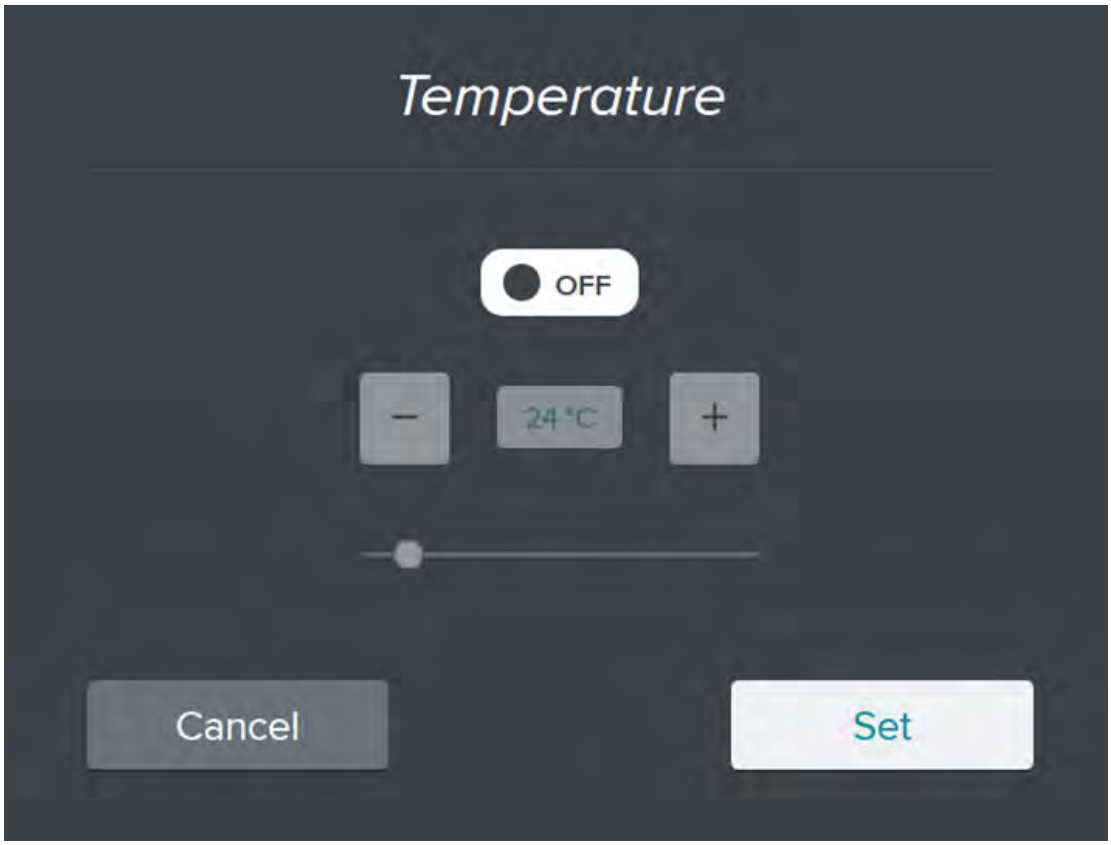


Figure 3-5: Temperature Settings dialog

To set the temperature of the microplate chamber:

1. From the buttons on the left, touch  to display the Temperature dialog.
2. Touch  to display  . The controls on the page are activated.
3. Touch:
   - - To granularly decrease the target temperature
   -  - To granularly increase the target temperature
   - The slider to broadly set the temperature.
4. Touch **Set** to set the microplate chamber temperature.

Injector Maintenance - Wash and Prime

The Preparation tab on the Injector Maintenance page enables you to wash and prime the injectors. The Status tab enables you to determine when to replace the injector tubing. See Injector Maintenance - Status on page 59.

To ensure optimal operation of the injector, it is important to wash the injector tubing after every use. The Manual settings on the Injector Maintenance page enable you to set the amount of liquid to dispense. You should wash the injector tubing before you switch the reagents you use for your experiments. Molecular Devices recommends that you wash the injector tubing with deionized water. For a list of compatible solutions, see List of Compatible Solutions on page 132.

Note: You must use a computer running SoftMax Pro Software to operate the instrument for advanced acquisition or injector protocols.

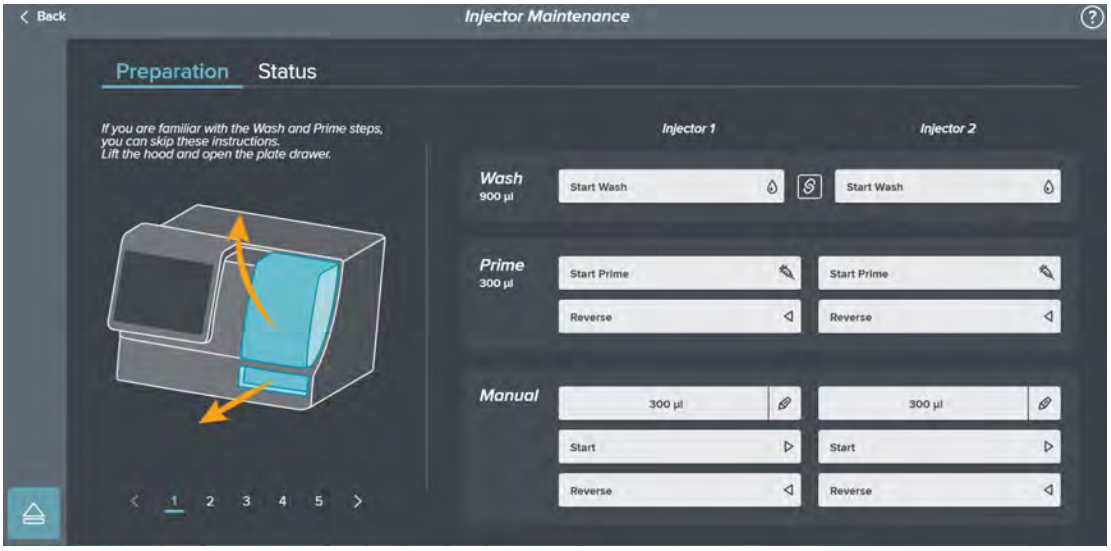


Figure 3-6: Injector Maintenance page - Preparation tab - Instructions page one

From the buttons on the left, touch  to display the Preparations tab on the Injector Maintenance page. The Preparation tab instructions have several pages. You do not need to scroll through the instruction pages to perform the wash and prime steps when you are familiar with the wash and prime procedures.

- Page 1: Lift the hood on the right and touch  to open the microplate drawer.
- Page 2: Insert an empty waste plate and empty strip wells in the microplate drawer. See Injector Waste Plate and Strip Wells on page 55
- Page 3: Move the injector arm so that it is over the waste plate.

Note: The injector arm does not line up with the hole in the waste plate. The instrument moves the waste plate to the proper position when you touch Wash or Prime.

- Page 4: Follow the injector tube to the nozzle in the back left. Use the black pin on the nozzle to pull straight up and free the nozzle. Insert the nozzle into the injector arm.
- Page 5: Insert the bottles that contain the liquid to dispense. See Injector Bottles on page 57.
- Page 6: Insert the snorkels in the bottles. Perform the wash or prime function. See Wash Injector Tubing on page 51 and Prime Injector Tubing on page 53

Note: To wash both injectors at the same time, touch  .

- Page 7: Remove the waste plate and strip wells and return the nozzle to the back left.

Touch the **Status** tab on the Injector Maintenance page to determine when to replace the injector tubing. See Injector Maintenance - Status on page 59.

Touch **Back** to return to the Home page. See Home Page on page 43.

Overfill Detection

An overfill detection sensor helps reduce the chance of spillage from dispensing too much liquid into a microplate well. To avoid overfill errors, make sure that the dispense volume you define in the Manual section or the SoftMax Pro Software is less than the volume of the well minus the volume of the sample in the well. See the SoftMax Pro Software application help or user guide.

If an overfill detection error occurs, do the following:

- Clean the bottom of the injector. See Clean Injector and Accessories on page 103.
- Make sure the dispense volume you enter is less than the volume of the well minus the volume of the sample in the well.
- Make sure you specify the correct microplate type and the plate definition is accurate.

Note: You must use a computer running SoftMax Pro Software to operate the instrument for advanced acquisition or injector protocols.

Wash Injector Tubing

To ensure optimal operation of the injector, periodically wash the injector tubing. Molecular Devices recommends that you wash the injector tubing with deionized or distilled water for rinse cycles and 70% alcohol for a disinfectant cycle. You can configure the wash operation to dispense up to three solutions. For a list of compatible solutions, see List of Compatible Solutions on page 132.

You can choose to use the predefined wash process that dispenses 900 µl or you can use the Manual section to define how much solution to dispense, to do reverse wash, and to do air aspiration steps.

During a wash or a read the nozzle that contains the injector tips lowers to 0.5 mm above the opening of the waste plate or the top of the microplate to inject the reagent.

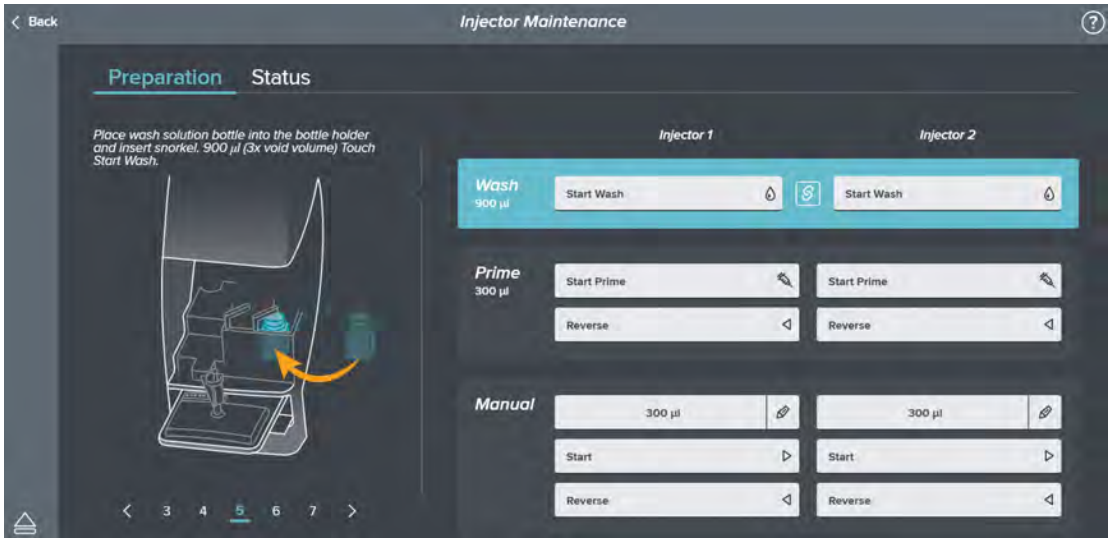


Figure 3-7: Injector Maintenance - Wash steps

The Injector Maintenance page enables you to perform the wash and prime steps for the injectors.

Tip: Instead of switching the bottles in and out of the bottle holder between the solutions, you can insert two different solutions in the bottle holder and then switch the snorkels between the bottles for each step of the wash operation.

1. To view the Injector Maintenance page - Preparation tab, touch  .
2. Lift the instrument's right hood to display the injectors.
3. If reagent is still in the tubing, run a **Reverse** operation from the Prime settings or the Manual settings. See Prime Injector Tubing on page 53.

The Manual settings enable you control the amount of liquid to move through the injector tubing for a wash or prime.

4. Touch  to open the microplate drawer.
5. Insert an empty waste plate and empty strip wells in the microplate drawer. See Injector Waste Plate and Strip Wells on page 55.
6. Position the injector arm over the waste plate.

Note: The injector arm does not line up with the hole in the waste plate. The instrument moves the waste plate to the proper position when you touch Wash or Prime.

7. Use the black pin to move the nozzle from the rear of the injector space to the injector arm. Pull straight up until the nozzle is free from the instrument.
8. Fill a bottle with enough solution for each injector tubing to wash and place the filled bottle in the left side, #1 injector, bottle holder. See Injector Bottles on page 57.
9. Fill another bottle with enough solution for each injector tubing to wash and place the filled bottle in the right side, #2 injector, bottle holder.

   If you use a third solution for the wash operation, fill a third bottle with enough solution for each injector tubing to wash and place the bottle to the side until the third wash step.
10. Place the snorkel for the injector #1 tubing to wash into the #1 bottle on the left.

    To wash both injector tubing place both snorkels in the #1 bottle.
11. Touch:
    -  to simultaneously run both injectors for the wash.
    - **Start Wash** to dispense 900 µl for the wash.
    - **Manual**  to use a keypad to define the amount of liquid to dispense then touch **Start**.
12. After the first wash step completes, move the snorkel or snorkels to the #2 bottle on the right.
13. Touch:
    -  to simultaneously run both injectors for the wash.
    - **Start Wash** to dispense 900 µl for the wash.
    - **Manual**  to use a keypad to define the amount of liquid to dispense then touch **Start**.
14. After the second wash step completes, move the snorkel or snorkels to the bottle that contains the third solution.

    If you use a third solution for the Wash operation, then remove the #1 bottle on the left and replace it with the bottle that contains the third solution before you move the snorkels.
15. Touch:

-  to simultaneously run both injectors for the wash.
- **Start Wash** to dispense 900 µl for the wash.
- **Manual**  to use a keypad to define the amount of liquid to dispense then touch **Start**.

16. After the third wash step completes, empty the bottles and optionally return them to the bottle holder.
17. Return the snorkel for injector 1 to the left side snorkel clamp and the snorkel for injector 2 to the right side snorkel clamp.
18. Use the black pin to move the nozzle back to the rear of the injector space. Align the nozzle with the opening and press the nozzle straight down until you feel it snap into place.
19. Move the injector arm to its original position.
20. Remove the waste plate from the microplate drawer and empty the contents to waste as prescribed by your laboratory procedures.
21. Touch  to close the microplate drawer.

Tip: When the injector is not in use and the tube is empty, the tube stabilizer lid over the injector pump should be kept open to extend the tubing lifetime.

Prime Injector Tubing

Before you run a read with the injectors, prime the injector tubing with the reagent that you use for the experiment.

You can choose to use the predefined prime process that dispenses 300 µl or you can use the Manual section to define how much solution to dispense, to do reverse prime, and to do air aspiration steps.

During a prime or a read the nozzle that contains the injector tips lowers to 0.5 mm above the opening of the waste plate or the top of the microplate to inject the reagent.

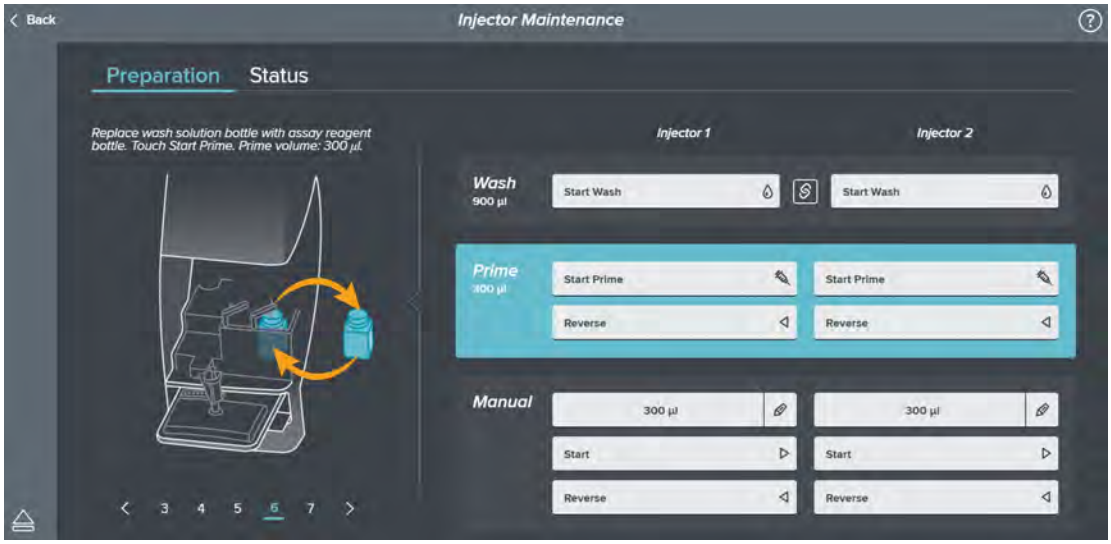


Figure 3-8: Injector Maintenance - Prime steps

The Injector Maintenance page enables you to perform the wash and prime steps for the injectors.

1. To view the Injector Maintenance page, touch  .

   The Manual settings enable you control the amount of liquid to move through the injector tubing for a wash or prime.
2. Lift the instrument's right hood to display the injectors.
3. If reagent is still in the tubing, run a **Reverse** operation from the Prime settings or the Manual settings.
4. Touch  to open the microplate drawer.
5. Insert the empty waste plate and the empty strip wells on the microplate drawer. See Injector Waste Plate and Strip Wells on page 55.
6. Position the injector arm over the waste plate.

   Note: The injector arm does not line up with the hole in the waste plate. The instrument moves the waste plate to the proper position when you touch Wash or Prime.
7. Use the black pin to move the nozzle from the rear of the injector space to the injector arm. Lift straight up until the nozzle is free from the instrument.
8. Fill the bottles with enough reagent for your experiment plus at least 2 mL to account for the prime operation and the quick-prime operation before the plate is read, and for the dead volume in the bottle and the tubing. Place the bottle for injector #1 on the left and the bottle for injector #2 on the right. See Injector Bottles on page 57.
9. Place the left side snorkel for injector 1 into bottle #1 and the right side snorkel for injector 2 into bottle #2.

10. Touch:
    - **Start Prime** for injector 1 to dispense 300 µl from bottle #1.

      If the protocol uses both bottles, touch **Start Prime** for injector 2 after the first prime operation completes.
    - **Manual** to use a keypad to define the amount of liquid to dispense then touch **Start**.
11. Use the black pin to move the nozzle back to the rear of the injector space. Align the nozzle with the opening and press the nozzle straight down until you feel it snap into place.
12. Move the injector arm to its original position.
13. Remove the waste plate from the microplate drawer and replace it with the prepared microplate.
14. Place the microplate for your experiment onto the microplate drawer.
15. Touch to close the microplate drawer.

After you finish a read that uses the injectors, do a reverse prime to clear the reagent from the injector tubing and return it to the bottle. This can save valuable reagents from going to waste.

1. To view the Injector Maintenance page, touch .
2. Touch to open the microplate drawer.
3. Remove the microplate from the microplate drawer, if applicable.
4. Insert the empty waste plate on the microplate drawer. See Injector Waste Plate and Strip Wells on page 55.
5. Touch **Reverse** for each injector that has reagent in its tubing.
6. After you clear the injector tubes, you can remove the bottles from the instrument.

Tip: When the injector is not in use and the tube is empty, the tube stabilizer lid over the injector pump should be kept open to extend the tubing lifetime.

Injector Waste Plate and Strip Wells

The waste plate captures excess liquid during the wash and prime operations. You use the strip wells during the quick-prime of the injectors that occurs when you start a read with injectors.

Note: Make sure that the waste plate and strip wells are empty before you insert them.

Touch to open the microplate drawer.

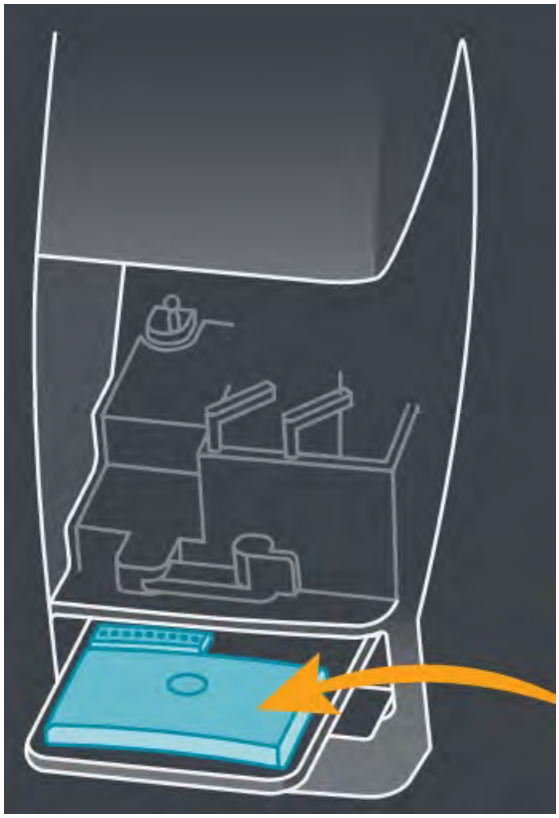


Figure 3-9: Waste plate and strip wells

- Insert the empty waste plate in the same location as a microplate.
- Insert the empty strip wells in the smaller slot next to the microplate.

When you are ready to run an experiment, replace the waste plate with your prepared microplate. The empty strip wells remain in the microplate drawer for use during the 10 µL quick-prime of the injectors when you start a read with injectors.

Note: You must use a computer running SoftMax Pro Software to operate the instrument for advanced acquisition or injector protocols.

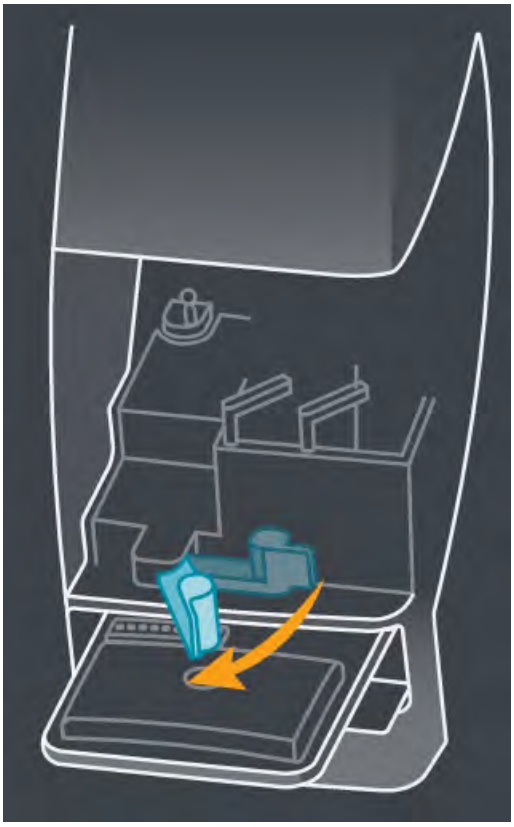


Figure 3-10: Injector arm in position over waste plate

Note: The injector arm does not line up with the hole in the waste plate. The instrument moves the waste plate to the proper position when you touch Wash or Prime.

Injector Bottles

The bottle holder holds two bottles that correspond with the two injectors. Fill the bottles with enough reagent for your experiment plus at least 2 mL to account for the prime operation and the quick-prime operation that occur before the plate is read, and for the dead volume in the bottle and the tubing.

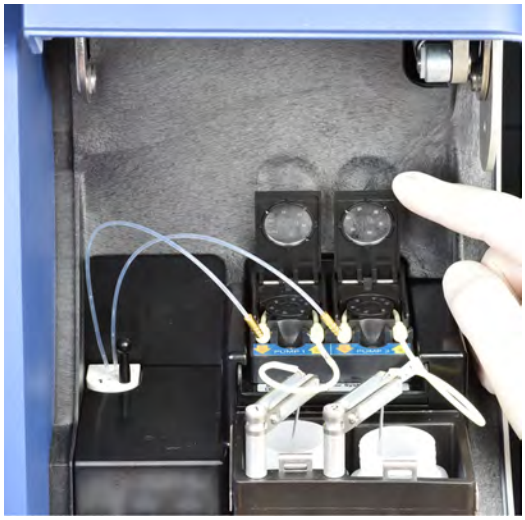


Figure 3-11: Injector bottle holders

Place bottle #1 on the left and bottle #2 on the right. The tubes from the snorkel holders to the injector pumps should not cross.

The injector comes with adapters that you can insert in the bottle holder to accommodate smaller labware. Each adapter has several hole positions, one for 1 mL tubes and others for larger vessels. Insert the adapters in the bottle holder before you insert the alternate labware. After you install the labware, insert the snorkels into the labware and secure the snorkels in the snorkel clamps.

1. Lift the right instrument hood.
2. Slide the snorkel tube out of the open side of the snorkel clamp and then slide it upward out of the bottle.

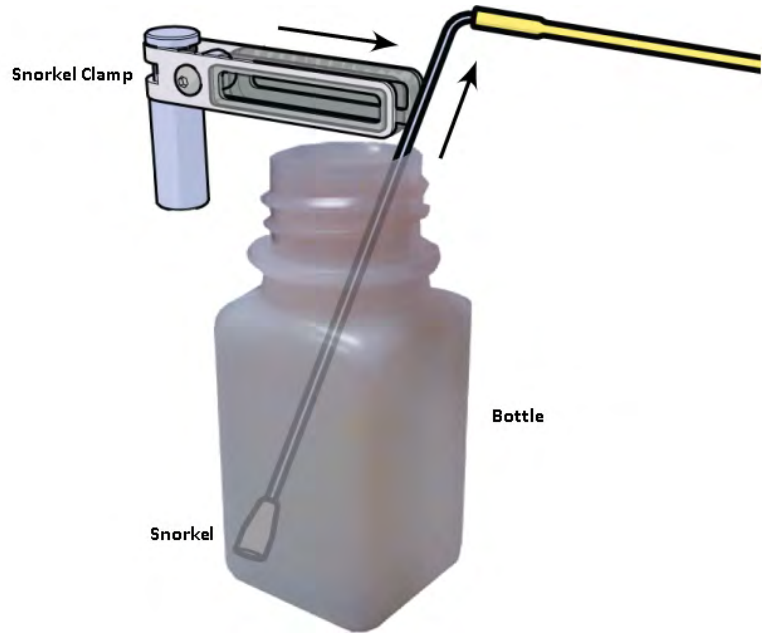


Figure 3-12: Remove snorkel

3. Twist the snorkel clamp to clear the position where the bottle is to be placed.
4. Remove the old bottle, if present, and then place the new bottle into its position.
5. Move the snorkel clamp back into position over the bottle.
6. Slide the snorkel all the way down into the bottle and then slide the snorkel tube into the open end of the snorkel clamp.

   The bottle holder is slightly tilted toward one corner. To extract the maximum amount of liquid from the bottle, place the end of the snorkel in the lowest point that is located in the corner of the bottle closest to the closed end of the snorkel clamp.

Figure 3-13: Lowest points of the bottle holder

7. Follow the previous steps to insert a second bottle into the right side bottle position for injector 2, if needed.

Injector Maintenance - Status

The Status tab on the Injector Maintenance page display how many milliliters (ml) of liquid have been dispensed through the tubing. The lifetime of the tubing is limited and you must replace the tubing when worn.

After you replace the tubing, touch **Reset Volume** to reset the counters to zero.

Tip: When the injector is not in use and the tube is empty, open the tube stabilizer lid over the injector pump to extend the tubing lifetime.

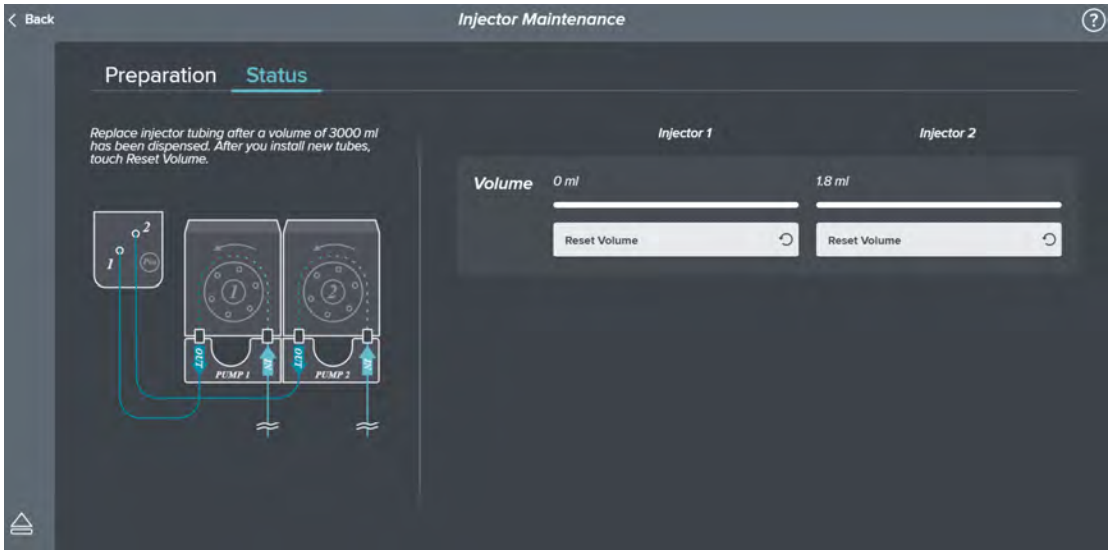


Figure 3-14: Injector Maintenance page - Status tab

You must use a computer running SoftMax Pro Software to operate the instrument for advanced acquisition or injector protocols. The SoftMax Pro Software displays messages as the dispensed volume reaches the following milestones:

- After 2000 ml have been dispensed through the tubing, a message appears to remind you that the tubing needs to be changed soon.
- After 3000 ml have been dispensed through the tubing, you must change the tubing before you can use the injectors.

For instructions how to install new tubing, see Injector Assembly and Maintenance on page 37.

Maintenance Page

The Maintenance page enables you to manage users, view instrument information, manage reader settings, manage system settings, and to do support tasks such as view instructional videos, export support log files, and to set the transport slide in a position to accept the physical transport lock for shipment and storage.

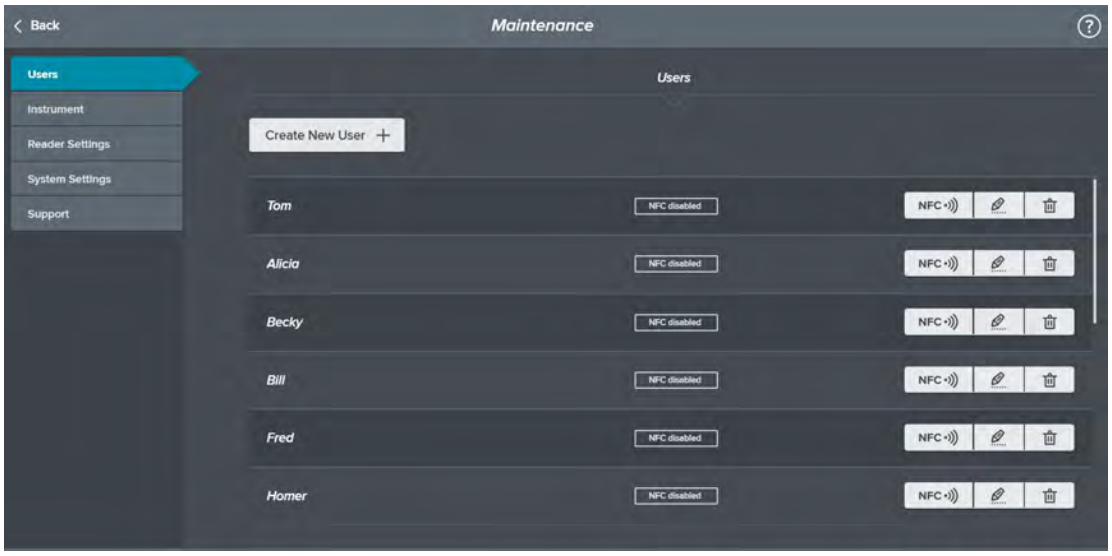


Figure 3-15: Maintenance page - Users tab

From the buttons on the left, touch  to display the Maintenance page. The Maintenance page has five tabs on the left. The Users tab appears by default the first time you access the page.

- Touch **Users** to maintain the list of users. The Users tab enables you to add, rename, and delete users and enables you to associate a user with an NFC tag. See Users Maintenance on page 62.
- Touch **Instrument** to view the instrument computer name, firmware version, PIC version, serial number, device number, assigned IP address, and software version. See Instrument Maintenance on page 63.
- Touch **Reader Settings** to set preference for protocol reads such as result export, check plate height, and auto plate eject. See Reader Settings Maintenance on page 64.
- Touch **System Settings** to adjust the brightness, date format, and volume. See System Settings Maintenance on page 65.
- Touch **Support** to view how-to videos, access the log files that are useful for support purposes, and to set the transport slide in a position to accept the transport lock for shipment and storage. See Support on page 66.

Touch **Back** to return to the Home page. See Home Page on page 43.

Users Maintenance

The Users tab appears selected by default when you first access the Maintenance page.

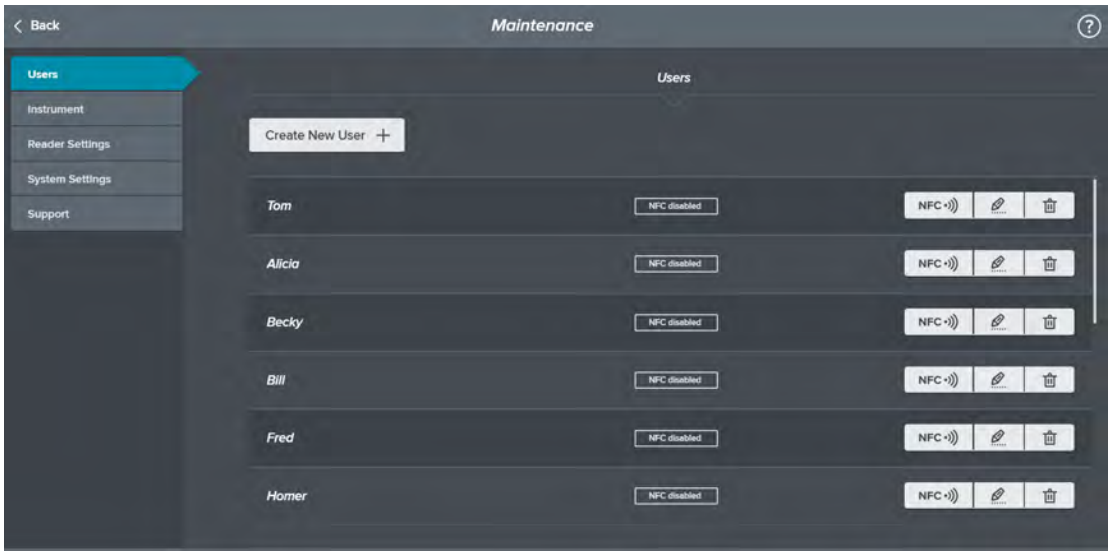


Figure 3-16: Maintenance page - Users tab

The first time you power on the instrument, there is only one user - **Public**. You cannot edit or delete the **Public** user and **Public** has no associated NFC tag.

- From the buttons on the left, touch  to display the Maintenance page.
- Touch **Users** to display the Users tab, if needed.
- Touch **Create New User** to display the New User dialog where you add a user.
- Touch  to display the NFC Pairing dialog where you assign a user an NFC tag.
- Touch  to display the Rename User dialog where you rename the user.
- Touch  to delete a user.

Note: The  in the buttons on the left of the Home page enables you to change users and to add users. If you use an NFC tag, swipe the tag over the NFC sensor below the touchscreen to change users. See Change User on page 67.

Touch **Back** to return to the Home Page on page 43.

Instrument Maintenance

The Instrument tab on the Maintenance page enables you to view instrument information.

Figure 3-17: Maintenance page - Instrument tab

If you power off the instrument or if you disconnect the Ethernet cable and then reconnect the Ethernet cable, the instrument IP address can change. Touch **Refresh** to update the display of the Assigned IP address.

- From the buttons on the left, touch  to display the Maintenance page.
- Touch **Instrument** to display the Instrument tab and to view the following information.
  - Computer Name
  - Firmware Version
  - PIC Version
  - Serial Number
  - Device Number
  - Assigned IP
  - Software Version

Touch **Back** to return to the Home Page on page 43.

Reader Settings Maintenance

The Reader Settings tab on the Maintenance page enables you to define preferences for protocol reads.

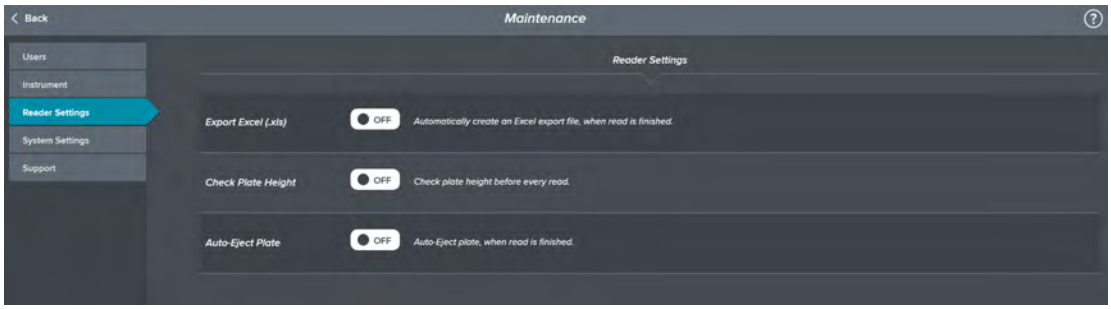


Figure 3-18: Maintenance page - Reader Settings tab

- From the buttons on the left, touch  to display the Maintenance page.
- Touch **Reader Settings** to display the Reader Settings tab.
- Touch the **Export Excel (.xls)**  to display  to export read results to an Excel format for further analysis. See Export Protocol Data on page 87. You must insert a USB flash drive into the slot below the touchscreen or install the QuickSync tool on the computers to which you export data. See Install the QuickSync Tool on page 35.
- Touch the **Check Plate Height**  to display  to have the instrument confirm that the actual plate height matches the plate definition of the plate you select for the protocol.
- Touch the **Auto Eject Plate**  to display  to have the plate drawer open automatically after the read is complete.

Touch **Back** to return to the Home Page on page 43.

System Settings Maintenance

The System Settings tab on the Maintenance page enables you to adjust instrument settings.

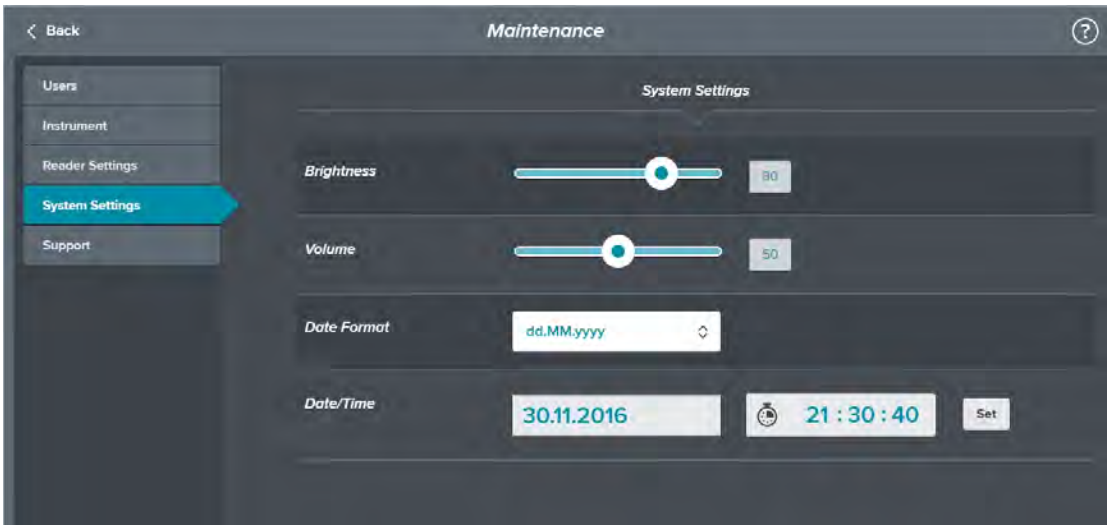


Figure 3-19: Maintenance page - System Settings tab

- From the buttons on the left, touch  to display the Maintenance page.
- Touch **System Settings** to display the System Settings tab.
- Use the **Brightness** slider to adjust the brightness of the touchscreen.
- Use the **Volume** slider to change the volume of the instrument speakers that play the how-to videos. How-to videos are on the Support tab. See Support on page 66.
- Touch the **Date Format** drop-down and select a format for date and time display.
- Touch in the **Date/Time** field to display a calendar. Use the calendar to change the system date then touch the clock and use the scroll bars to change the time. Touch **Set** to save the date/time changes.

Touch **Back** to return to the Home Page on page 43.

Support

The Support tab on the Maintenance page enables you to view how-to videos, to export log files to a location from where you can send the log file to a support engineer, and to move the transport slide into a position where it can accept the physical transport lock in preparation for instrument shipment or storage. See Install Transport Lock Alternate on page 110.

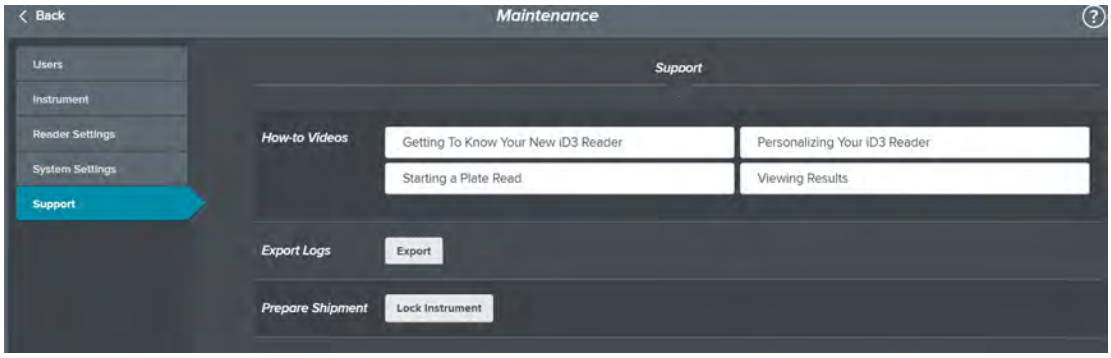


Figure 3-20: Maintenance page - Support tab

- From the buttons on the left, touch  to display the Maintenance page.
- Touch **Support** to display the Support tab.
- Touch a how-to video for instrument instructions.
- Touch **Export** to export a log file to assist with technical support. See Obtain Support on page 124.
- Touch **Lock Instrument** to lock the instrument for shipment. This moves the transport slide into a position where it can accept the transport lock and powers off the instrument. See Before You Move the Instrument on page 106.

Touch **Back** to return to the Home Page on page 43.

Change User

The Change a User page enables you to define which protocols display in the Last Used Documents section and in the My Protocols list. See Protocol Libraries on page 44.

If you use NFC tags, you swipe your NFC tag over the sensor below the touchscreen to change users. You do not need to do the following steps.

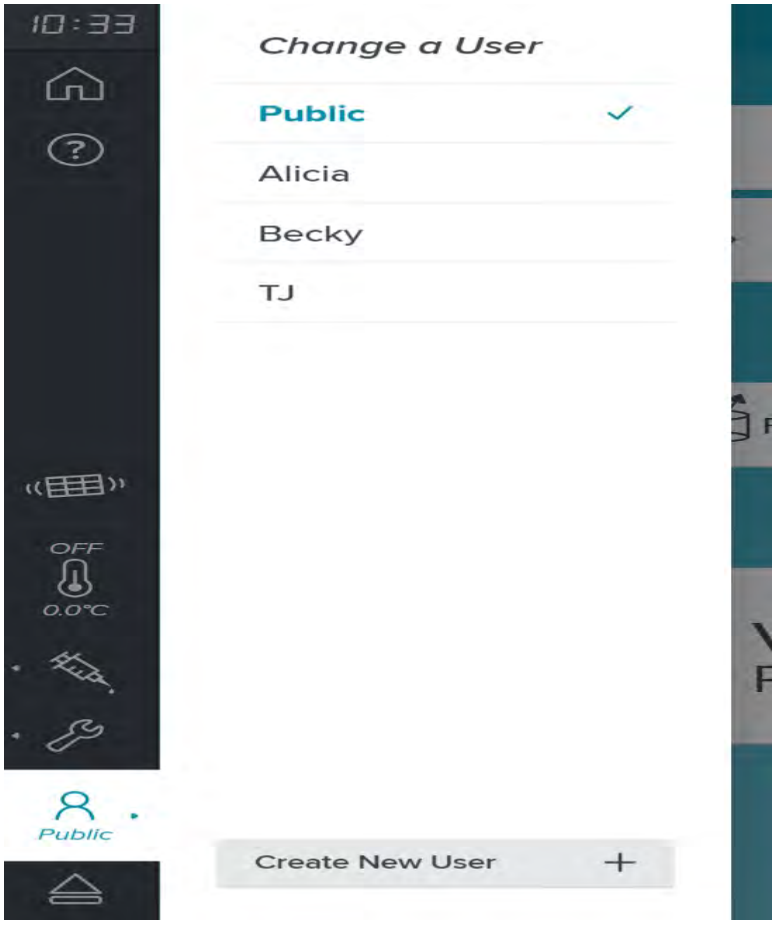


Figure 3-21: Change User page

If you do not use NFC tags, do the following to change the user and to display their user-specific protocols and documents.

- From the buttons on the left, touch  to display the Change a User page.
- Touch the user name whose protocol list and whose last used documents you want to display on the Home page.
- Touch **Create New User** to display the New User dialog.

Touch  to return to the Home page. See Home Page on page 43.

Load and Unload Microplates

The buttons on the left include  to enable you to load or unload a microplate.

1. Touch  to move the microplate drawer outside of the instrument.
2. Place the microplate onto the drawer or remove the microplate from the drawer.

Note: Place the microplate on the microplate drawer in landscape orientation with well A1 in the left corner closest to the instrument.

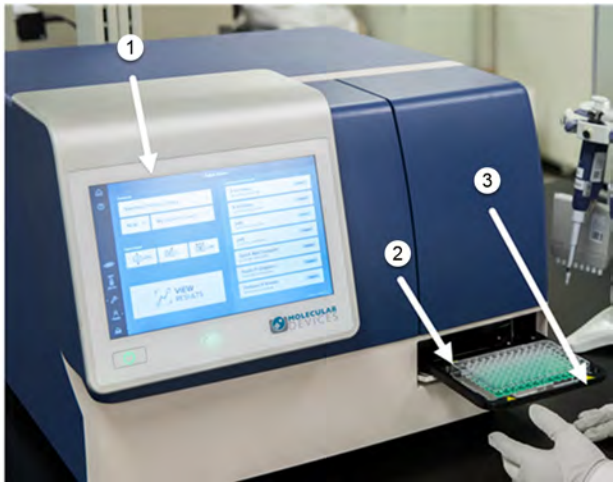


Figure 3-22: Microplate drawer with microplate loaded

Item	Description
1	Touchscreen
2	Well A1 on the microplate
3	Microplate drawer

3. Touch  to move the microplate drawer inside the instrument.

CAUTION! To prevent damage to the instrument, the height of the microplate must not exceed 22 mm, including the lid if the microplate is lidded.

Chapter 4: Protocols

Protocols are experiment files that contain microplate well layout assignments, reader configuration information, and reduction parameters, but no data. Protocol files are useful if you repeat a particular type of experiment frequently.

You can use a quick read protocol, create a protocol from scratch, or you can copy an existing protocol and save it with a new name. For a description of the supported read modes and read types, see Read Modes and Read Types on page 89.

On the Home page, touch **ABS**, **FL**, or **LUM** to do a quick absorbance, quick fluorescence, or quick luminescence read using default settings. The Endpoint read type is the default setting for quick read protocols. You can use the quick read protocols as defined or you can modify the protocol settings and save the updated settings in your protocol library for future use. See Quick Read on page 46.

Touch **Standard Library**, **My Protocols**, or **Last Used Documents** to select protocols stored in the file system. See Protocol Libraries on page 44.

For instruction about how to use the instrument to define protocol-specific settings, see View Protocol Settings on page 69.

Application notes with specific application protocol suggestions can be found in the Information Center and the Knowledge Base on the Molecular Devices web site at www.moleculardevices.com.

View Protocol Settings

You can use a quick read protocol, create a protocol from scratch, or you can copy an existing protocol and save it with a new name. Protocol settings for the plate you select display on the right.

Note: You must use a computer running SoftMax Pro Software to operate the instrument for advanced acquisition or injector protocols.

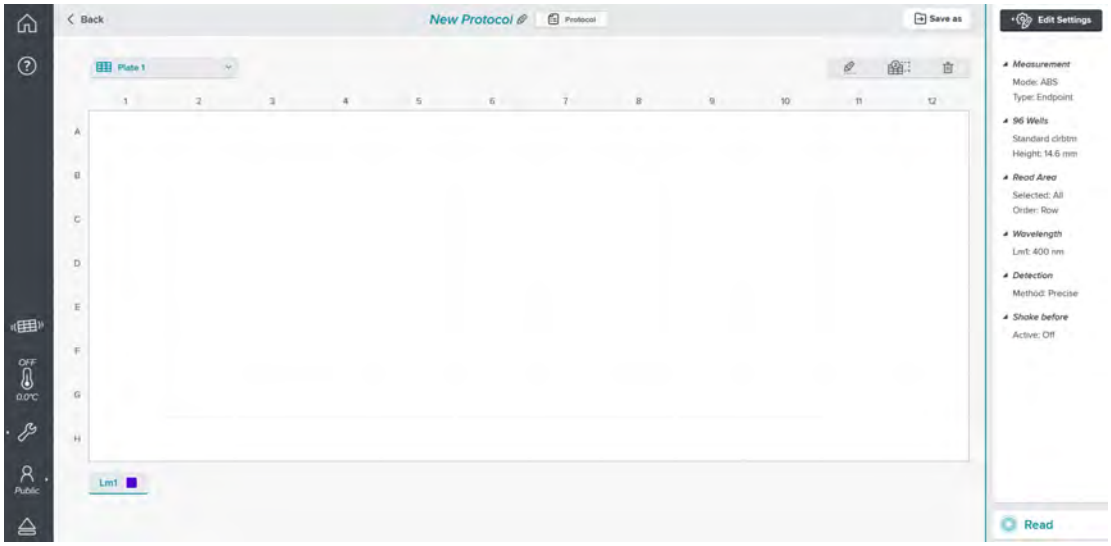


Figure 4-1: Plate-specific protocol information

To view and manage protocol settings:

1. On the Home page, touch **Create a New Protocol** or select an existing protocol from the **Standard Library**, **My Protocols**, or the list of **Last Used Documents**.
2. To name the protocol:
   - For a new protocol, the Create New Protocol dialog appears.
   - From an existing protocol, touch **<protocol name>** to rename the protocol. The Rename Protocol dialog appears.
   - Touch Save as to create a copy of the protocol. The Save as New Protocol dialog appears.

   Note: Protocols in the Standard Library are read only. You can touch Save as or Edit Settings to rename the protocol before you run or edit a protocol from the Standard Library. The copy of the Standard Library protocol is saved to your My Protocols list.
3. The plate name (**<plate name>**) appears above the plate layout diagram. You define the plate format and type along with the read area etc. as part of the plate-specific protocol settings see Define Protocol Settings on page 71.
   - Touch **<plate name>** and select a plate to view and manage the settings for that plate.
   - Touch to change the name of the plate you select. The Rename Plate dialog appears.
   - Touch to add a plate based on the plate you select. The Copy Plate dialog

appears.

- Touch to delete the plate you select.

4. Settings for the plate you select appear on the right. Touch Edit Settings to edit the plate-specific protocol settings. See Define Protocol Settings on page 71.
5. Touch **Read** to read the microplate.
6. Touch **<plate name>** and select an additional plate to read, view, or manage the settings.

Define Protocol Settings

The plate-specific settings page enables you to define the protocol settings for each plate to be read. Settings vary depending upon the read mode and read type you select. For a description of the supported read modes and read types, see Read Modes and Read Types on page 89.

Note: You must use a computer running SoftMax Pro Software to operate the instrument for advanced acquisition or injector protocols.

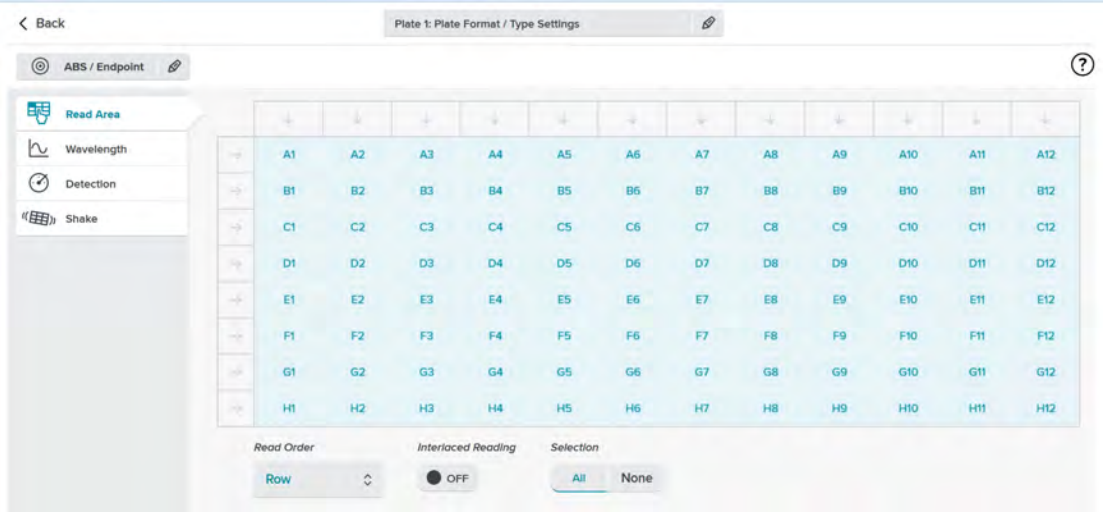


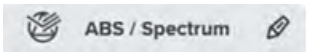
Figure 4-2: Define plate-specific protocol settings

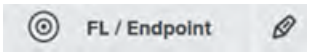
Do the following to define the plate format/plate type, read mode/read type, and other settings for how to read each plate in a protocol.

1. From the protocol settings page, touch the next to **<plate name>** and select the plate to define.
2. Touch Edit Settings to define the plate settings and read settings for each plate in the protocol.

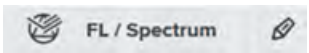
3. The plate name appears at the top center of the page. Touch **<plate name> Plate Format/Type Settings** to display the Plate Settings page where you can select the plate format and plate type. See Set Plate Format and Plate Type on page 72.
4. The read mode and read type appear on the upper left side of the page. Touch **<read mode/read type>** to change the read mode and read type. The Read Mode/Read Type dialog appears. See Set Read Mode and Read Type on page 74.
   - 

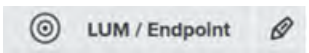
- Absorbance read mode - Endpoint read type.
   - 

- Absorbance read mode - Kinetic read type.
   - 

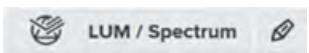
- Absorbance read mode - Spectrum read type.
   - 

- Fluorescence Intensity read mode - Endpoint read type.
   - 

- Fluorescence Intensity read mode - Kinetic read type.
   - 

- Fluorescence Intensity read mode - Spectrum read type.
   - 

- Luminescence read mode - Endpoint read type.
   - 

- Luminescence read mode - Kinetic read type.
   - 

- Luminescence read mode - Spectrum read type.
5. The **Read Area** tab appears for all read mode/read type combinations. Touch to select the wells to read. See Set Read Area on page 75.
6. The **Wavelength** tab appears for all read mode/read type combinations. Touch to define the wavelengths. See Set Wavelength on page 77.
7. The **Detection** tab appears for all read mode/read type combinations. Touch to define detection settings. See Set Detection on page 79.
8. The **Shake** tab appears for all read mode/read type combinations. Touch to define shake settings. See Set Shake on page 81
9. The **Timing** tab appears for Kinetic read types. Touch to define the timing settings. See Set Timing on page 82.

Set Plate Format and Plate Type

Depending on the application, the instrument can read 6, 12, 24, 48, 96, and 384-well microplates and strip wells. For micro-volume measurements, the instrument supports SpectraDrop 24-well Micro-Volume Microplates and SpectraDrop 64-well Micro-Volume Microplates.

To read optical density at wavelengths below 340 nm, special UV-transparent, disposable, or quartz microplates that permit transmission of the far UV spectra must be used.

CAUTION! To prevent damage to the instrument, the height of the microplate must not exceed 22 mm, including the lid if the microplate is lidded.

The Plate Settings dialog enables you to select the plate format and to select the plate type. Changes you make here affect the other protocol settings. See Define Protocol Settings on page 71.

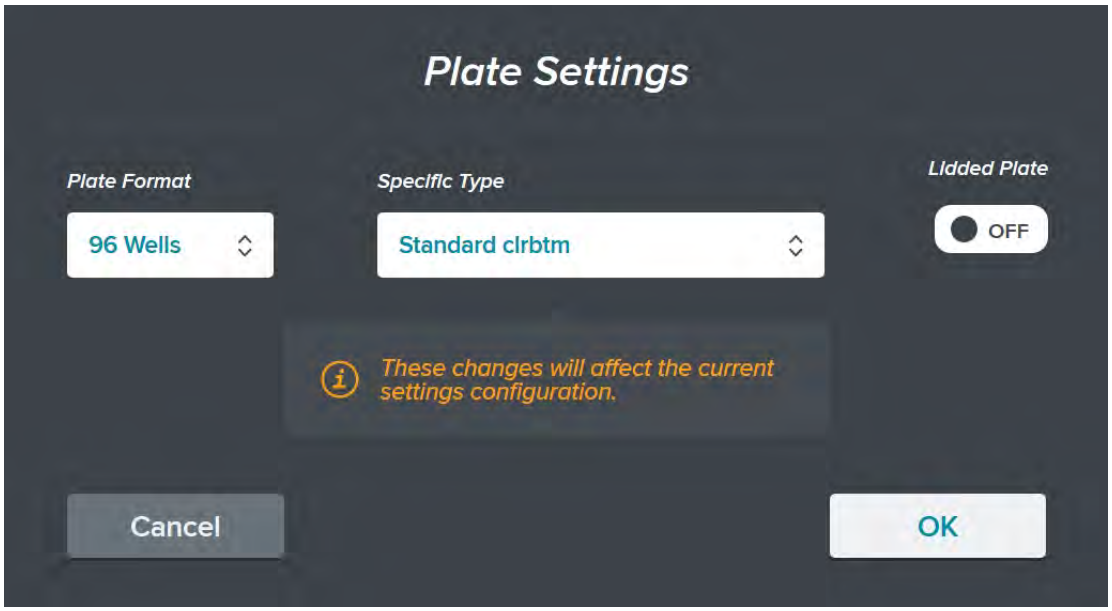


Figure 4-3: Plate Settings dialog

To define the plate settings:

1. On the Protocol Settings page, touch **<plate name>** to display the Plate Settings dialog.
2. Touch the **Plate Format** drop-down and select the number of wells in the plate.
3. Touch the **Specific Type** drop-down and select the plate type.
4. Touch the **Lidded Plate** OFF to display ON if the plate has a lid.
5. Touch **OK** to save the microplate settings.

The type of microplate and the way it is handled can have an effect on the measurement performance of the instrument. Select a microplate type with properties suited for the application.

- Never touch the clear well bottom of microplates.
- Visually inspect the bottom and the rim of the microplate before use to make sure that it is free of dirt and contaminants.
- Keep unused microplates clean and dry.
- Make sure that the strips on strip plates are inserted correctly and level with the frame.
- Do not use V-bottom microplates unless the performance has been tested and validated with this instrument. Irregular plastic density in the tip of the well can cause inaccurate measurements.

Set Read Mode and Read Type

The Read Mode/Type dialog enables you to set the protocol read mode and protocol read type. After you select the read mode and read type on this dialog, the read mode/type displays at the top left on the protocol settings page. See View Protocol Settings on page 69.

Changes you make here affect other Protocol Settings. See Define Protocol Settings on page 71.

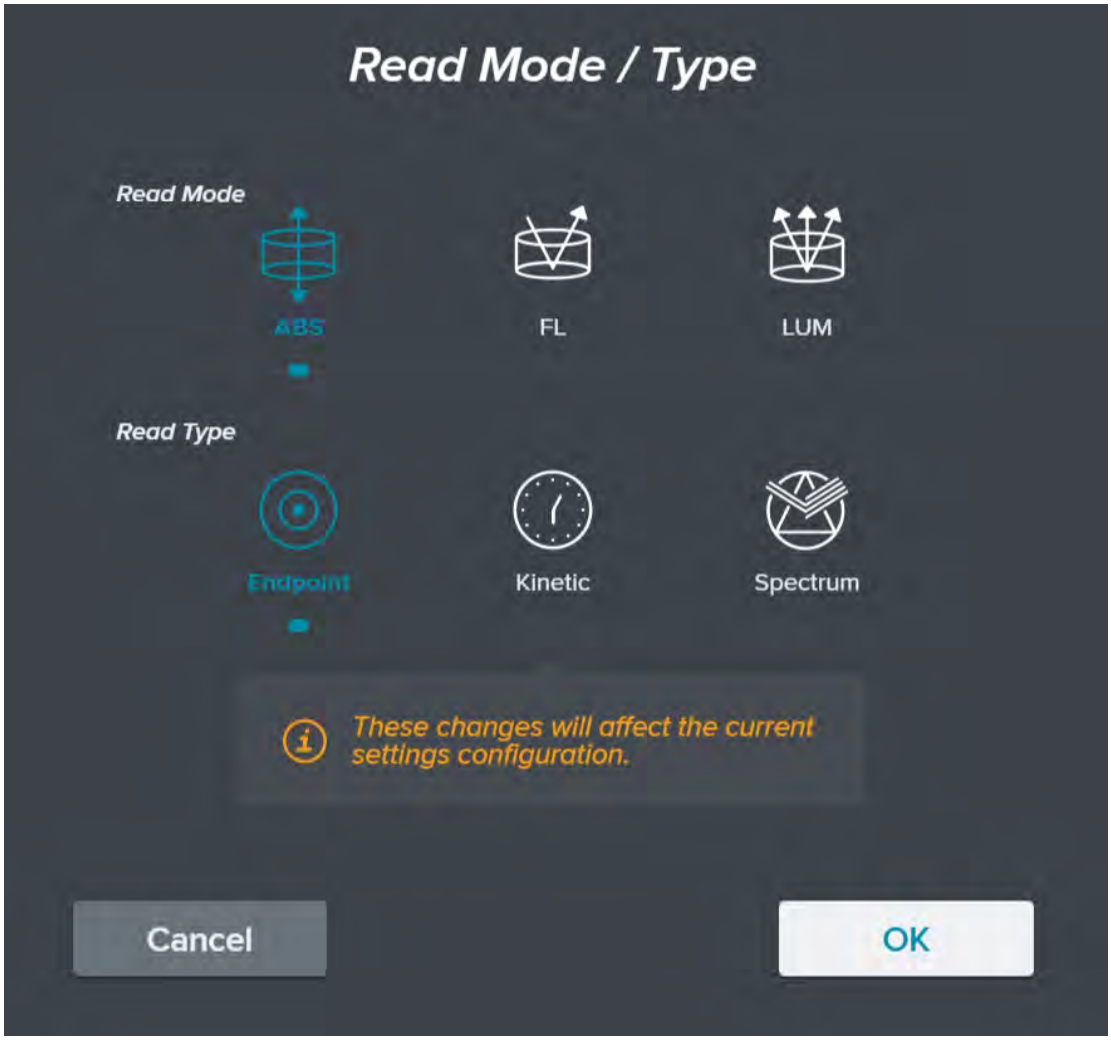
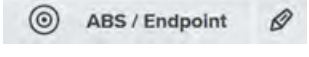


Figure 4-4: Read Mode/Type dialog

For a description of the supported read modes and read types, see Read Modes and Read Types on page 89.

To set the protocol read mode and read type:

1. From the plate-specific settings page, touch 

to display the Read Mode/Type dialog.

Note: The icon changes depending upon the read mode and read type. This example is for an Absorbance read mode, Endpoint read type.

2. Touch a read mode.
   -  - Absorbance
   -  - Fluorescence
   -  - Luminescence
3. Touch a read type.
   -  - Endpoint
   -  - Kinetic
   -  - Spectrum
4. Touch **OK** to return to the plate-specific settings page. See Define Protocol Settings on page 71.

Set Read Area

The plate-specific settings page enables you to define the read area. All read mode read type combinations have a Read Area tab on the left to enable you to define which wells on the plate to read. See Define Protocol Settings on page 71.

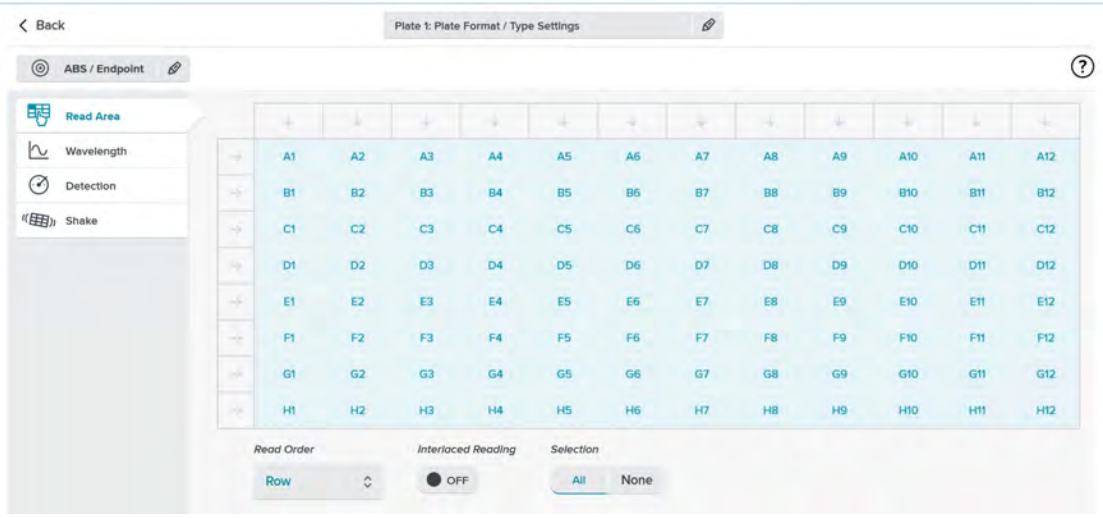


Figure 4-5: Plate-Specific settings page - Read Area tab (96 well plate)

Your plate format selection adjusts the display of the Read Area tab. See Set Plate Format and Plate Type on page 72.

You can choose to read an entire microplate or a subset of wells. Columns do not need to start with column one. Wells in the read area that display a shaded background and the well number will be read.

-

- Well read
- - Well not read

To define which wells on the microplate to read:

1. Start at the bottom of the tab. Select a **Read Order**.
   - Select **Row** to read each row in sequence.
   - Select **Column** to read each column in sequence.
   - Select **Well** to read each well individually with all wavelengths and intervals defined for the read before the next well is read.
2. Touch the **Interlaced Reading** 

to display 

to use interlaced reading. See below for information about interlaced reading.
3. Select a **Selection** option.
   - Select **All** to read all wells, then do the following to de-select the wells to not read.
   - Select **None** to read only well A1, then do the following to select additional wells to read.
4. Touch the following to select/de-select wells.
   - - Touch to select all wells in a row. If all wells in the row are selected, this de-selects the entire row.
   - - Touch to select all wells in a column. If all wells in the column are selected, this de-selects the entire column.
   - Touch individual wells to select/de-select the well.
   - To select a section of a plate:
     - 

Long touch (touch and hold) the well in the corner of the area to select until the well turns dark blue.
     - Touch the well in the opposite corner. All wells in between appear selected/de-selected.

Interlaced Reading Explained

When the instrument reads a sample with a small signal, an interference can occur from the afterglow of a very strong emitting adjacent sample that was measured just before. Such cross talk can occur through the wall of a white 384 well microplate. To prevent such interference, select the Interlaced Reading option to read every other well in a checkerboard pattern in the first run and then read the previously omitted wells in a second run. Molecular Devices recommends that you use blank or assay controls for background correction. The background can be effectively measured using blank replicates. You should use replicates for all blanks, controls, and samples.

Set Wavelength

The plate-specific settings page enables you to define the wavelength. All read mode read type combinations have a Wavelength tab on the left to enable you to define which wavelengths to use for the plate read. See Define Protocol Settings on page 71.

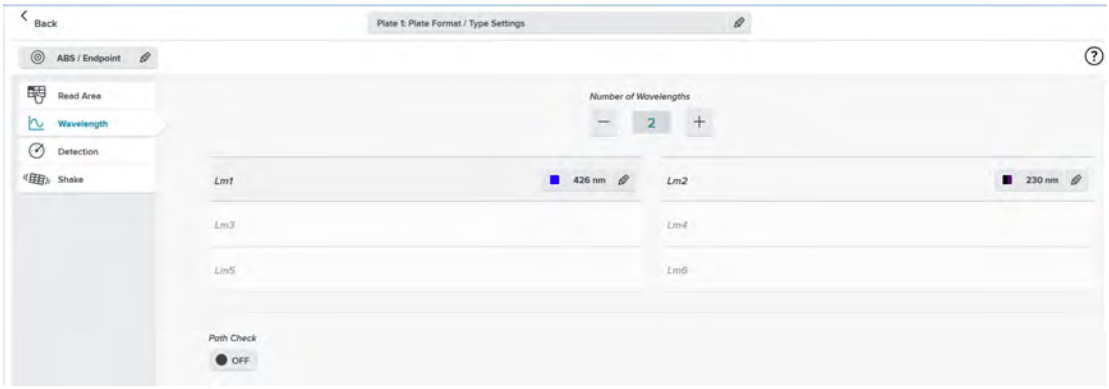


Figure 4-6: Plate-specific settings page - Wavelength tab (Absorbance mode Endpoint type)

The read mode and read type setting determines which wavelength settings are applicable.

Absorbance Mode Wavelength Settings

For the Absorbance read mode with the Endpoint and Kinetic read types, the following wavelength settings are available.

1. Touch the **Number of Wavelengths** − or + to define up to six wavelengths.
2. For each wavelength, touch ✎ to display a numeric keypad.
3. Use the keypad to enter the wavelength value then touch anywhere outside the keypad to save your setting. The wavelength range can be set from 230 - 1000 nm.

4. For Absorbance mode Endpoint type reads, touch the **Path Check** 

to display 

to use PathCheck® technology. The temperature-independent PathCheck Pathlength Measurement Technology normalizes your absorbance values to a 1 cm path length based on the near-infrared absorbance of water. See PathCheck Pathlength Measurement Technology on page 91.

For the Absorbance read mode with the Spectrum read type, the following wavelength settings are available.

1. Touch the **Start** to display a numeric keypad.
2. Use the keypad to enter the wavelength value then touch outside the keypad to save your setting.
3. Touch the **Step** to display a numeric keypad.
4. Use the keypad to enter the wavelength value then touch outside the keypad to save your setting.
5. Touch the **Stop** to display a numeric keypad.
6. Use the keypad to enter the wavelength value then touch outside the keypad to save your setting.

Fluorescence Mode Wavelength Settings

For the Fluorescence read mode with the Endpoint and Kinetic read types, the following wavelength settings are available.

1. Touch the **Number of Wavelength Pairs** − or + to define up to four wavelength pairs.
2. For each wavelength pair, touch the **Excitation** to display a numeric keypad.
3. Use the keypad to enter the wavelength value then touch outside the keypad to save your setting.
4. Touch the **Emission** to display a numeric keypad.
5. Use the keypad to enter the wavelength value then touch outside the keypad to save your setting.

For the Fluorescence read mode with the Spectrum read type, the following wavelength settings are available.

1. Touch  to change between an Excitation Scan and an Emission Scan.
2. Touch each wavelength **Start** to display a numeric keypad.
3. Use the keypad to enter the wavelength value then touch outside the keypad to save your setting.
4. Touch each wavelength **Stop** to display a numeric keypad.
5. Use the keypad to enter the wavelength value then touch outside the keypad to save your setting.
6. Touch each wavelength **Step** to display a numeric keypad.

7. Use the keypad to enter the wavelength value then touch outside the keypad to save your setting.

Luminescence Mode Wavelength Settings

For the Luminescence read mode with the Endpoint and Kinetic read types, the following wavelength settings are available.

Touch the **All Wavelength** OFF to display ON and use all wavelengths.

OR

1. Touch the **Number of Wavelengths** − or + to define up to four wavelengths.
2. For each wavelength, touch to display a numeric keypad.
3. Use the keypad to enter the wavelength value then touch anywhere outside the keypad to save your setting.

For the Luminescence read mode with the Spectrum read type, the following wavelength settings are available.

1. Touch the **Emission Start** to display a numeric keypad.
2. Use the keypad to enter the wavelength value then touch outside the keypad to save your setting.
3. Touch the **Emission Stop** to display a numeric keypad.
4. Use the keypad to enter the wavelength value then touch outside the keypad to save your setting.
5. Touch the **Emission Step** to display a numeric keypad.
6. Use the keypad to enter the wavelength value then touch outside the keypad to save your setting.

Set Detection

The plate-specific settings page enables you to define the detection speed. All read mode read type combinations have a Detection tab on the left. See Define Protocol Settings on page 71.

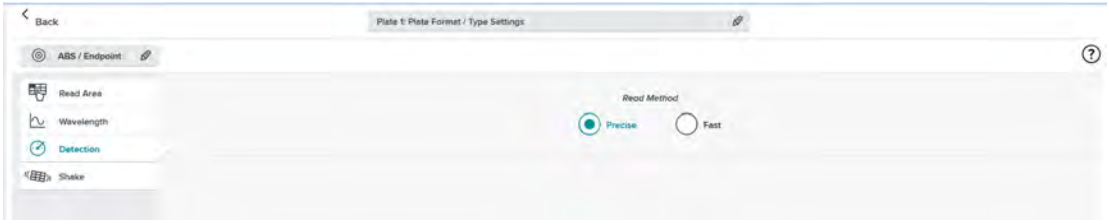


Figure 4-7: Plate-specific settings page - Detection tab (Absorbance mode Endpoint type)

The read mode and read type setting determines which detection settings are applicable.

Absorbance Mode Detection Settings

For the Absorbance read mode, there are two microplate detection speeds.

- **Precise** - The instrument stops above each selected well and does the read. This provides more precise results than the Fast mode for demanding assays.
- **Fast** - The instrument continually moves the plate and the read is timed to occur when the plate reaches the read position.

The following table compares the read time for different plate types in each detection speed. These read times do not include the time needed for the microplate drawer to move the plate into the instrument and start the read, and then move the plate out of the instrument, which can add approximately 25 seconds to the overall read time.

Table 4-1: Plate Read Times (± 5 seconds)

Mode	96-well	384-well
Precise - Optimized for performance	28 seconds	45 seconds
Fast - Optimized for speed	20 seconds	33 seconds

1. Select a **Read Method**:
   - Select **Precise** to use a slightly slower more precise detection method.
   - Select **Fast** to use a faster less precise detection method.

Fluorescence Mode Detection Settings

For the Fluorescence read mode, the following detection settings are available.

1. Touch the **PMT Gain** drop-down:
   - Select **Auto** to have the instrument adjust the PMT voltage automatically for varying concentrations of samples in the microplate.
   - Select **High** for samples that have lower concentration (dim samples).
   - Select **Medium** for samples that have average concentration.
   - Select **Low** for samples that have higher concentration (bright samples).
2. Touch the **Read From Bottom** 

to display 

to read the plate up from below rather than down from above.
3. If you set the Read From Bottom setting to Off, touch the **Read Height**  to display a numeric keypad.
4. Use the keypad to enter the distance between the objective lens and the microplate in millimeters, then touch outside the keypad to save your setting.
5. Touch the **Integration Time**  to display a numeric keypad.

Note: Integration time is the interval to allow the instrument to acquire information per each flash.

6. Use the keypad to enter the integration time in milliseconds then touch outside the keypad to save your setting.

Luminescence Mode Detection Settings

For the Luminescence read mode, the following detection settings are available.

1. Touch the **Read Height** to display a numeric keypad.
2. Use the keypad to enter the read height from the plate then touch anywhere outside the keypad to save your setting.
3. Touch the **Integration Time** to display a numeric keypad.
4. Use the keypad to enter the integration time in milliseconds then touch outside the keypad to save your setting.

Set Shake

The plate-specific settings page enables you to define the plate shake settings. All read mode read type combinations have a Shake tab on the left. See Define Protocol Settings on page 71.

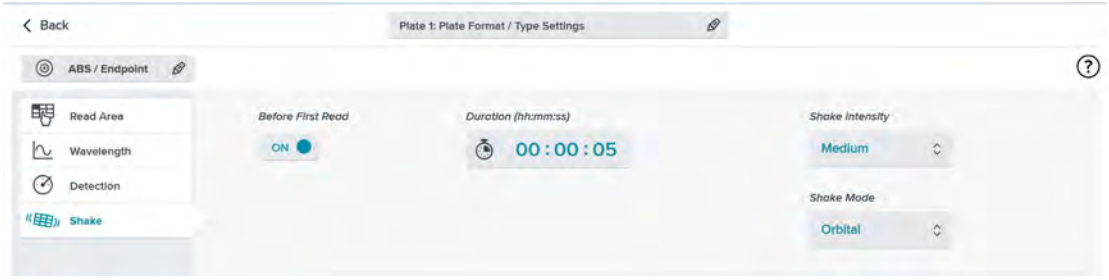


Figure 4-8: Plate-specific settings page - Shake tab (Absorbance mode Endpoint type)

To define how to shake the plate:

1. Touch the **Before First Read** OFF to display ON to shake the plate before the first read. The following settings are then activated.
2. Touch the **Duration** field and then use the scroll bars to set the number of minutes and seconds to shake the plate.
3. Touch the **Shake Intensity** drop-down and select **Low**, **Medium**, or **High**.
4. Touch the **Shake Mode** drop-down and select **Orbital** or **Double Orbital**.
5. For Kinetic read types, touch the **Between Reads** OFF to display ON to define the shake duration between subsequent reads. The following settings are then activated.
6. Touch the **Duration** field and then use the scroll bars to set the number of minutes and seconds to shake the plate.
7. Touch the **Shake Intensity** drop-down and select **Low**, **Medium**, or **High**.
8. Touch the **Shake Mode** drop-down and select **Orbital** or **Double Orbital**.

Set Timing

For Kinetic read types, the plate-specific settings page enables you to define timing settings. All read mode Kinetic read types have a Timing tab on the left. See Define Protocol Settings on page 71.

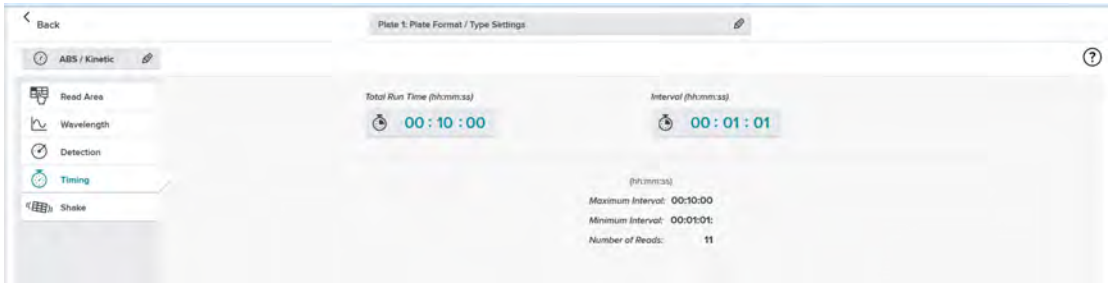


Figure 4-9: Plate-specific settings page - Timing tab (Kinetic read types only)

The number of reads is calculated based on the value you enter for the Total Run Time and the Interval. To define the total run time and interval for Kinetic read types.

1. Touch the **Total Run Time** field and then use the scroll bars to set the total run time hours, minutes, and seconds.
2. Touch the **Interval** field and then use the scroll bars to set the interval hours, minutes, and seconds.

View Protocol Results

The Result List page displays the results for your reads.

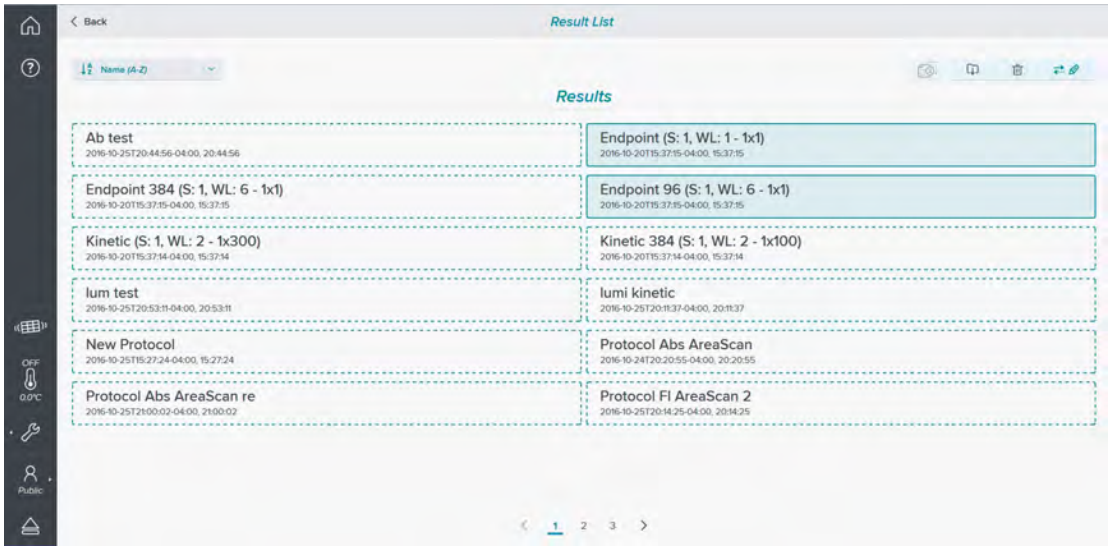


Figure 4-10: Results List page

To manage the list of results:

- From the Home page, touch **View Results** to display the Result List page.
- Touch  to sort the results alphabetically or by date.

- Touch  to manage your results list.
  - Select a result in the list and touch  to make a copy of the result.
  - Select one or more results in the list and touch  to export the protocol to a USB or to a computer over your intranet. See Export Protocol Data on page 87.
  - Select one or more results in the list and touch  to delete the results you select.
- Touch a result to display the result details. See Manage Results on page 84.

Endpoint Read Type Results

Depending on the read mode, endpoint read type raw absorbance, fluorescence, or luminescence data values are reported as optical density (OD), % Transmittance (%T), relative fluorescence units (RFU), or relative luminescence units (RLU). See Endpoint Read Type on page 89.

Kinetic Read Type Results

Kinetic results provide improved dynamic range, precision, and sensitivity relative to endpoint analysis. Raw data displays the change in optical density (OD), relative fluorescence units (RFU), or relative luminescence units (RLU) over time, displayed as a plot. The SoftMax Pro 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. See Kinetic Read Type on page 89.

Spectrum Read Type Results

Depending on the read mode, a spectrum read type raw absorbance, fluorescence, or luminescence data displays optical density (OD), % Transmittance (%T), relative fluorescence units (RFU), or relative luminescence units (RLU) across a spectrum of wavelengths that display as a plot. See Spectrum Read Type on page 90.

Manage Results

From the Result List page or from your Last Used Documents list, touch a result to display the raw data results of a read. The protocol read results appear as follows.

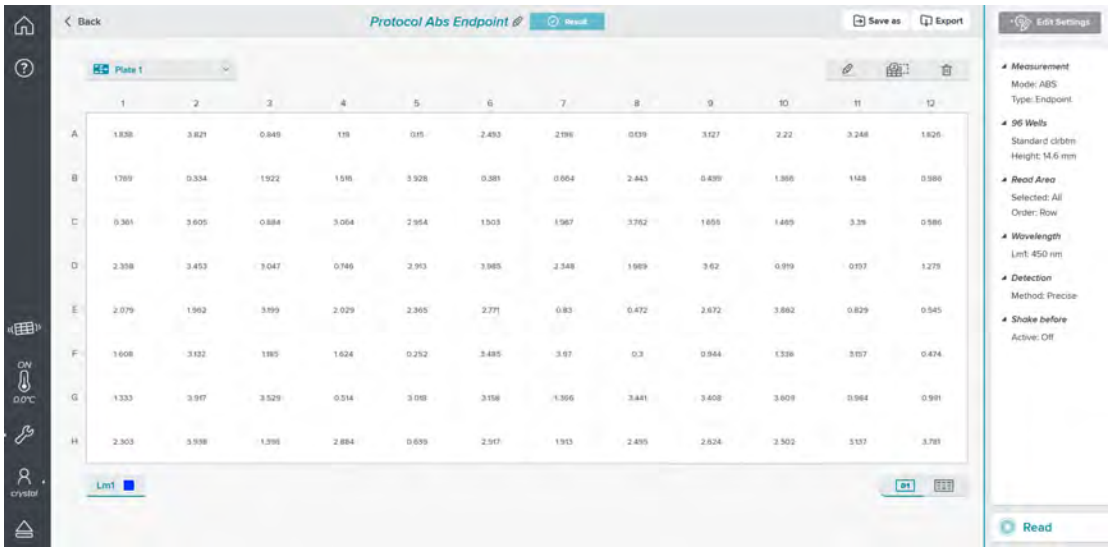


Figure 4-11: Protocol results (Absorbance mode Endpoint type)

To view the read results of a protocol:

- Touch **<result name>** to rename the result. The Rename Result dialog appears.
- Touch Save as to make a copy of the result. The Save as New Protocol dialog appears.
- Touch Export to export raw protocol data over your network or to a USB flash drive for further analysis.
- Touch **<plate name>** and select a plate to view the results for each plate in the protocol.
  - Touch to change the plate name. The Rename Plate dialog appears.
  - Touch to make a copy of the plate. The Copy Plate dialog appears.
  - Touch to delete the plate.
- Pinch the screen to zoom in or zoom out and swipe left or right as needed.
- Touch Lm1 Lm2 Lm3 to view the results for each wavelength.
- Touch 01 to view numeric results.
- Touch to view heat map results.

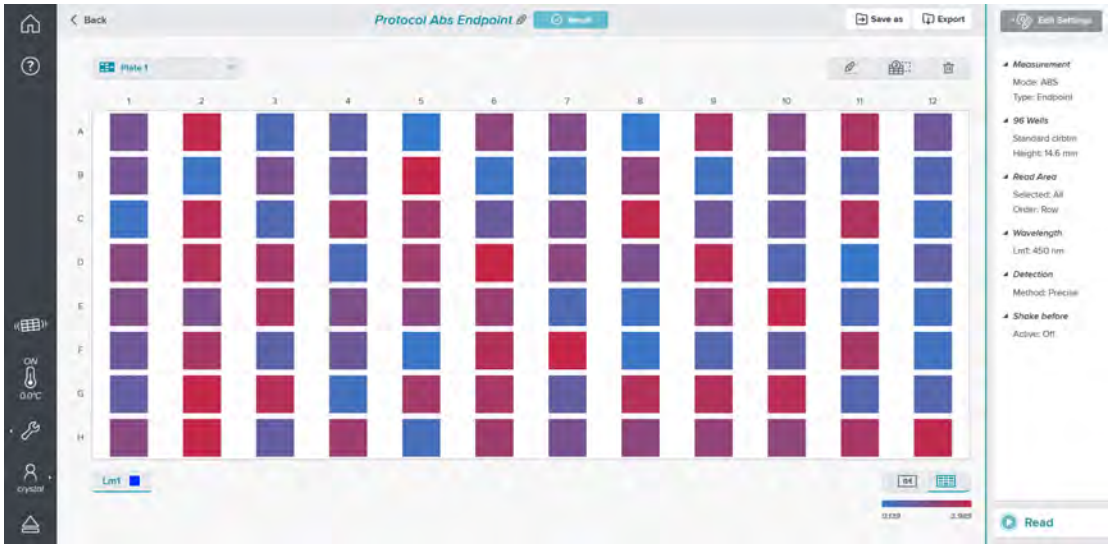


Figure 4-12: Protocol results - heat map

Compare Wells

For results that enable you to compare data in the wells, do the following to compare data.

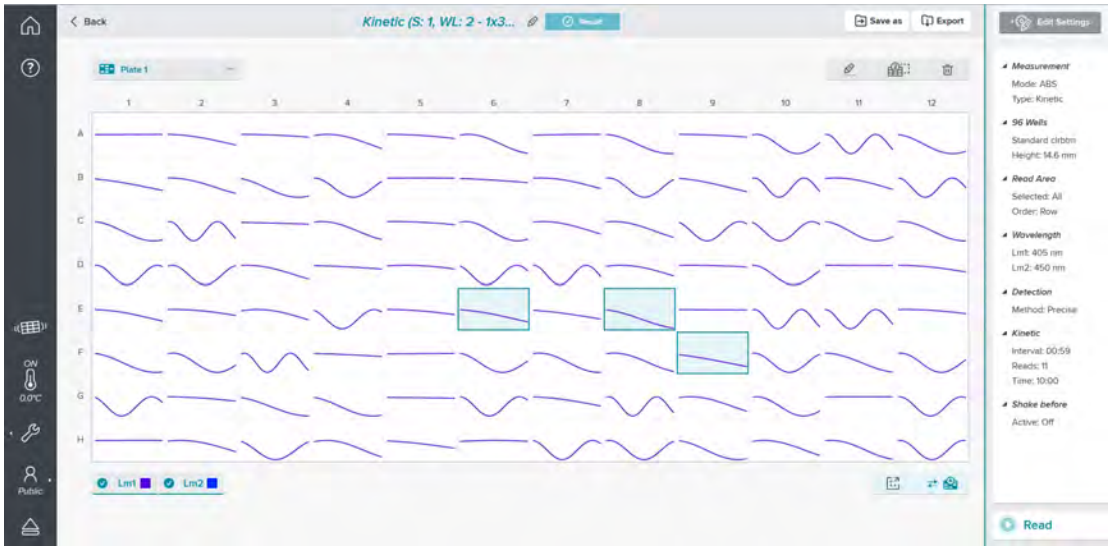


Figure 4-13: Compare data in multiple wells

1. Touch  to compare the results in several wells. The icon spins and an additional icon appears.
2. Touch up to four wells to compare. Each well you select displays a shaded background.
3. Touch  to display the well comparison.

Well Specific Linear Results

Touch a single well in the result or select to compare multiple wells to display a view of a single well with the one result or the compared results.

Figure 4-14: Well-specific results

The Navigator on the top right displays the selected well or the wells that are being compared.

Touch the arrows below the Navigator to navigate the plate to view results in other wells.

For linear graph results,

- Touch **Zoom Mode** and select:
  - **Auto** to use the Auto zoom mode
  - **Ratio** to use the Ratio zoom mode.
  - **Horizontal** to zoom horizontally.
  - **Vertical** to zoom vertically.
- Pinch the screen to zoom in and to zoom out.
- Touch Scale to scale the image.
- Touch 100% to zoom to the original depth that displays for all wells in the result.
- Touch Zoom to zoom to the depth where individual point become visible.

Export Protocol Data

If you operate the instrument from a computer running the SoftMax Pro Software, the touchscreen is disabled and all results appear in the SoftMax Pro Software.

You do not need to connect the instrument to your network or directly to a computer to export raw result data. When your instrument is not connected to your intranet or to a computer, leave the Maintenance - Reader Settings - Export Excel option to Off. See below.

- Insert a USB flash drive into the USB port located below the front of the touchscreen.
- Each read result provides an Export button on the top right of the screen. Touch to export raw data to the flash drive.

Tip: The file extension is .xml so you will need to open the Excel program on the computer and drag the file into an open Excel spreadsheet to view the data.

Figure 4-15: USB port below touchscreen

When you connect the instrument to your intranet or you connect the instrument directly to a computer with an Ethernet cable, you must install the QuickSync tool on the computer to which you want to export data. See Install the QuickSync Tool on page 35.

Set the Maintenance - Reader Settings - Export Excel option to On and the instrument automatically exports raw protocol data to computers on which you have installed the QuickSync tool. See Use the QuickSync Tool on page 88.

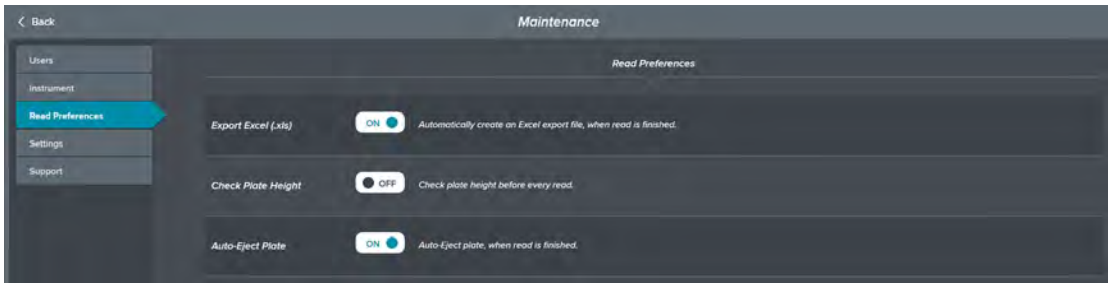


Figure 4-16: Maintenance page - Reader Settings tab - Export Excel setting

The Reader Settings tab on the Maintenance page provides an **Export Excel** option that enables the instrument to automatically export raw protocol data for further analysis.

1. From the buttons on the left, touch  to display the Maintenance page.
2. Touch the **Reader Settings** tab to view the Reader Settings tab.
3. Touch the **Export Excel (.xls)** 

to display 

to export read results to an Excel format for further analysis.

Note: If a well result is saturated, the touchscreen displays #SAT.

Use the QuickSync Tool

The QuickSync tool enables you to use the raw data the instrument exports for further analysis.

This is optional. The steps in this topic are done on a computer, not on the instrument touchscreen.

After you install the QuickSync tool (see Install the QuickSync Tool on page 35) and after you export read data (see Export Protocol Data on page 87), do the following steps to use the QuickSync tool.

The  QuickSync icon appears in your computer taskbar area, with the other programs running on your computer, at the bottom of the screen. You may have to look in the Show Hidden Icons area.

1. Click the big  in the taskbar to display a smaller version of the  in the computer tray near the clock at the bottom of the computer screen. If you set the Maintenance - Read Preference - Export Excel setting to On or after you touch the 

button for a result, the  icon in the computer tray near the clock displays a message that displays the result name.
2. Right-click  in the computer tray to display a menu and select **Open Last Result**.

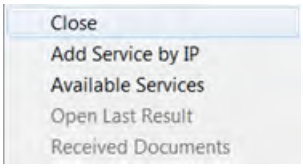


Figure 4-17: Open exported results

3. To copy single wavelength endpoint results from the QuickSync tool into the SoftMax Pro Software, you must have an entry in each well field. Enter **0** into any well that was not read.

Note: If a well result is saturated, the touchscreen displays #SAT.

Chapter 5: Read Modes and Read Types

5

The instrument can measure samples in Absorbance (ABS), Fluorescence Intensity (FL), and Luminescence (LUM) read modes. This chapter describes these read modes and their associated read types.

Application notes with specific application protocol suggestions can be found in the Information Center and the Knowledge Base on the Molecular Devices web site at www.moleculardevices.com.

For information on the supported read modes, see the following topics:

- Absorbance Read Mode on page 90
- Fluorescence Intensity Read Mode on page 94
- Luminescence Read Mode on page 98

The touchscreen enables you to define the settings to achieve the expected results for the read mode using the following read types:

- Endpoint Read Type on page 89
- Kinetic Read Type on page 89
- Spectrum Read Type on page 90

For instructions on steps to define protocols, see View Protocol Settings on page 69.

Endpoint Read Type

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

Kinetic Read Type

In a kinetic read type, the instrument collects data over time with multiple readings taken in the center of each well at regular intervals.

The SoftMax Pro 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.

Kinetic analysis has many advantages to determine 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 Type

Spectrum readings are made using the scanning monochromators of the instrument and can measure across the spectrum of absorbance wavelengths 230 nm to 1000 nm. Fluorescent intensity reads scan excitation wavelengths between 250 nm to 830 nm and emission wavelengths between 270 nm to 850 nm, where the emission wavelength must be a minimum of 20 nm greater than the excitation wavelength. Luminescence reads scan emission wavelengths between 300 nm to 850 nm.

Depending on the read mode selected, a spectrum read measures optical density (OD), %Transmittance (%T), relative fluorescence units (RFU), or relative luminescence units (RLU) across a spectrum of wavelengths.

Absorbance Read Mode

In the Absorbance (ABS) read mode, the instrument measures 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 incident light before it enters the sample, I is the intensity of light after it passes through the sample, and A is the measured absorbance.

For Absorbance reads, you can 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, Molecular Devices recommends that you run replicates for all blanks, controls, and samples. In this case, the blank value that can 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. With absorbance readers that are capable of measuring in the ultraviolet (UV) range, the concentration of nucleic acids (DNA and RNA) can be found using their molar extinction coefficients.

For micro-volume measurements, you can use SpectraDrop 24-well Micro-Volume Microplates and SpectraDrop 64-well Micro-Volume Microplates.

You can use the Protocol Libraries to quickly find and open a predefined protocol.

You can download additional protocols and updated protocols from the **Protocol Home Page** button on the Protocols tab. (www.softmaxpro.org).

PathCheck Pathlength Measurement Technology

The temperature-independent PathCheck® Pathlength Measurement Technology normalizes your 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:

$$a - b = c\ d\ e + f$$

where *a* is absorbance, *b* is blank, *c* is concentration, *d* is the depth of sample layer, *e* is extinction (coefficient of...), and *f* is further terms, e.g., non-linearity caused from turbidity.

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 also makes it confusing to compare results between microplate readers and spectrophotometers.

The standard pathlength of a 1 cm cuvette is the conventional basis for quantifying 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 using 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 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 occur from pipetting the samples and standards. The PathCheck technology automatically determines the pathlength of aqueous samples in the microplate and normalizes the absorbance in each well to a pathlength of 1 cm. This way of correcting the microwell absorbance values is accurate to within ±4% of the values obtained directly in a 1 cm cuvette.

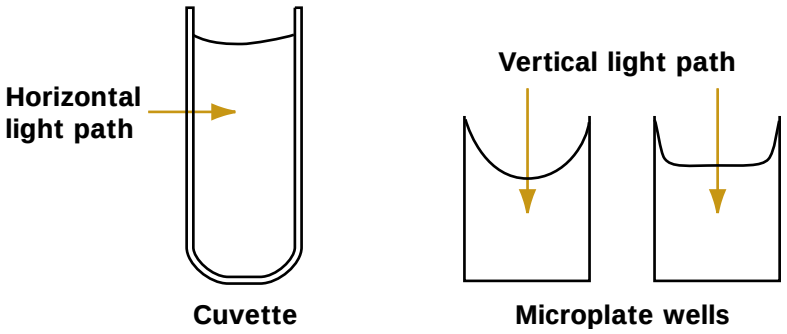


Figure 5-1: Cuvette and Microplate Well Light Paths

The 1 cm values can be obtained by using the factory installed *Water Constant*. PathCheck technology is used to normalize the data acquired from absorbance endpoint microplate readings 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.

Water Constant

The Water Constant correction method is supported for absorbance endpoint reads.

The PathCheck technology is based on the absorbance of water in the near infrared spectral region (between 900 nm to 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 sufficient. The Water Constant is determined for each instrument during manufacture and is stored in the instrument.

Note: After you read a plate with PathCheck technology turned on, PathCheck information is stored permanently in the data file. You have the option to apply, or not apply, PathCheck technology to the absorbance values. If you do not have PathCheck technology turned on during the plate read, you cannot apply the PathCheck Pathlength Measurement Technology feature after the read.

Eliminate the Pathlength Independent Component

Raw OD measurements of microplate samples include both pathlength-dependent components (sample and solvent) and a pathlength-independent component (OD of microplate 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.

Plate Blank

This method can be used if all samples in the microplate are the same volume and you do not depend on the PathCheck technology to correct for variability in volumes.

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 your 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 microplate material is subtracted as part of the blank.
3. In SoftMax Pro, make sure the **Use Plate Blank** check box is selected under **Other Options** on the Data Reduction dialog.

Plate Background Constant

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

1. Fill a clean microplate with water.
2. Read at the wavelengths that you which you plan to read your samples.

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

Note: It is important that you put water in the wells and not read a dry microplate for the Plate Background Constant. A dry microplate has a slightly higher OD value than a water-filled microplate because of differences in refractive indices. Use of a dry microplate 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. Using 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 it is recommended to not use the PathCheck technology.

Organic solvents could interfere with the PathCheck technology if they have absorbance in the region of the NIR water peak. Solvents such as 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. If, however, the solvent absorbs between 900 nm and 1000 nm, the interference would be similar to the interference of highly colored samples as previously described. If you plan to 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 (FL) 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:

Fluorescence = extinction coefficient × concentration × quantum yield × excitation intensity × pathlength × 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.

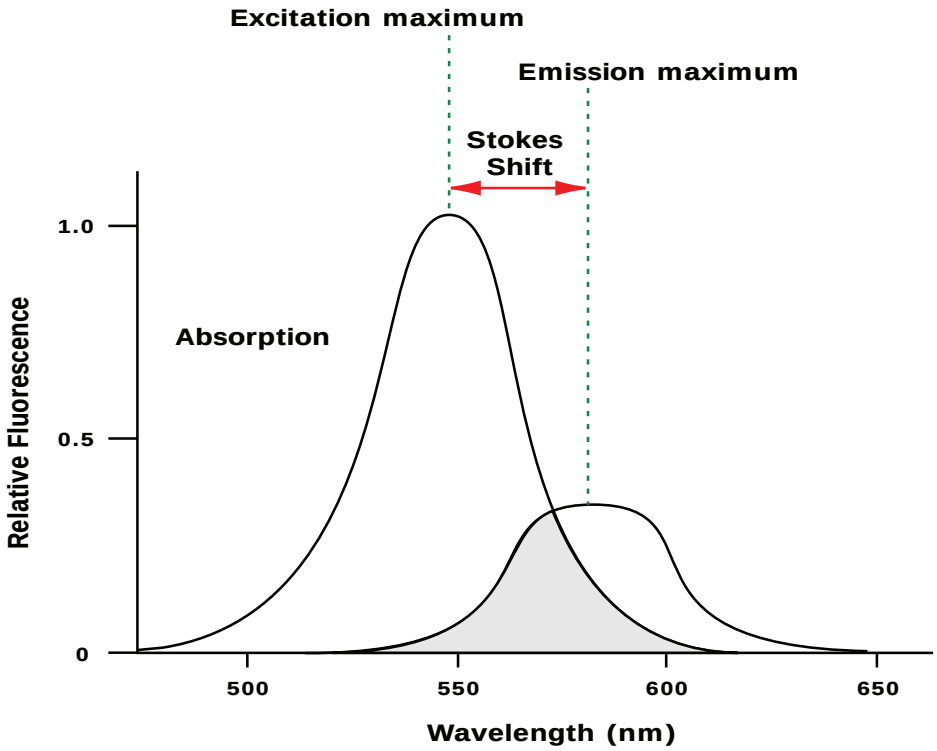


Figure 5-2: Excitation and Emission Spectra

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 being capable of stimulating the fluorophore so that less of the excited light overlaps the emission spectrum, which permits better selection and quantitation of the emitted light.

In the SoftMax Pro Software, the **Spectral Optimization Wizard** provides the best settings for maximizing the signal to background window, (S-B)/B, while minimizing the optimization time.

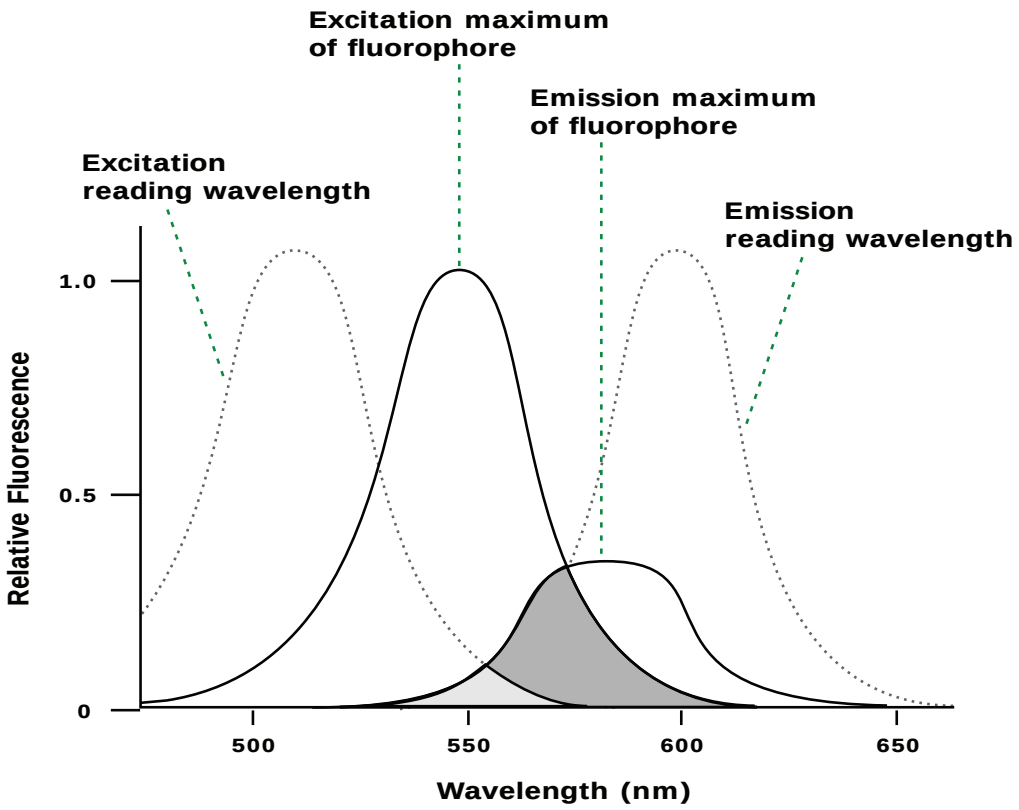


Figure 5-3: Optimized Excitation and Emission Reading Wavelengths

The previous figure shows that the best results are often obtained when the excitation and emission wavelengths used for reading are not the same as the peak wavelengths of the excitation and emission spectra of the fluorophore. When the reading wavelengths for excitation and emission are separated, a smaller quantity of excitation light passes through to the emission monochromator (gray area) and on to the PMT, resulting in a purer emission signal and more accurate data.

The instrument permits scanning of both excitation and emission wavelengths, using separate tunable dual monochromators. One benefit of scanning emission spectra is that you can determine more accurately whether the emission is, in fact, the expected fluorophore, or multiple fluorophores, and not one generated by a variety of background sources or by contaminants. One more benefit is that you can find excitation and emission wavelengths that prevent interference when interfering fluorescent species are present.

For this reason, it is desirable to scan emission for both an intermediate concentration of labeled sample, as well as the background of unlabeled sample. The optimal setting is where the ratio of the sample emission to background emission is at the maximum.

Fluorescence intensity data is dependent on a number of variables. See Analyze Fluorescence Intensity Data on page 97.

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 of this mode 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.

You can use the Protocol Libraries to quickly find and open a predefined protocol.

You can download additional protocols and updated protocols from the **Protocol Home Page** button on the Protocols tab. (www.softmaxpro.org).

Analyze Fluorescence Intensity Data

Fluorescence intensity data is dependent on a number of variables. Raw data is compared to a standard curve with known concentrations of a reference label.

A standard curve consists of, at a minimum, a blank sample and a reference standard sample of known concentration. The raw data can then be expressed in equivalent concentration of a reference label.

Analyzing and validating fluorescence intensity data generally consists of the following:

- Background Correction and Quantification on page 97
- Detection Limit on page 98
- Linearity and the Linear Dynamic Range on page 98

Background Correction and Quantification

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

The blank sample reveals the offset underlying each data sample. This offset does not carry information on the label, and is generally subtracted before data reduction is done.

Within the linear detection range, the blank-subtracted raw data are proportional to the quantity of label in a sample such that the label concentration is quantified by the following equation.

$$\text{conc}_{\text{label}} = \frac{(\text{sample} - \text{blank})}{\frac{\text{std} - \text{blank}}{\text{conc}_{\text{std}}}}$$

where $conc_{std}$ is the concentration of the *standard*, and *sample*, *blank*, and *standard* are average values of replicates for the sample, blank, and standard wells. In the general case where the standard curve covers a concentration range of more than a few linear logs, *(standard – blank) / $conc_{std}$* is equivalent to the slope of the standard curve, and so the concentration of the label is determined by *(sample – blank) / (slope of standard curve)*.

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

Detection Limit

The detection limit is the smallest sample concentration that can be measured reliably above the blank. Determining the detection limit requires taking a number of blank measurements and calculating an average value and standard deviation for the blanks. The detection threshold is defined as the average blank plus three standard deviations. If the average sample value measures above the threshold, the sample can be detected at a statistically significant level.

The detection limit can be described by the following equation:

$$\text{Det Limit} = \frac{3\ \text{Stdev}_{\text{blank}}}{\frac{\text{std} - \text{blank}}{\text{conc}_{\text{std}}}}$$

where $conc_{std}$ is the concentration of the standard, $StDev_{Blank}$ is the standard deviation of the blank replicates, and *blank* and *Std* are average values of the replicates for the blank and standard wells.

Determining the detection limit for an assay requires multiple blanks to calculate their standard deviation.

Linearity and the Linear Dynamic Range

Within a wide range at moderately high concentrations, blanked raw data is proportional to the quantity of label in a sample.

The linear dynamic range (LDR) is defined by:

$$\text{LDR} = \log_{10}\left(\frac{\text{max conc lin}}{\text{detection limit}}\right)$$

where *LDR* is expressed as a log, and *max conc lin* is the highest concentration in the linear range that can be quantified.

When the standard curve after blank reduction is not linear in concentration at the lower end, there might be an incorrect or contaminated blank.

When the standard curve levels are off at the highest concentrations, this can be addressed to the inner filter effect: excitation does not reach as deep into the sample for lower concentrations, without being more significantly attenuated (absorbance) layer by layer.

Luminescence Read Mode

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

In the Luminescence (LUM) read mode, the instrument provides measurements in Relative Luminescence Units (RLUs).

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.

To help eliminate background luminescence from a microplate that has been exposed to light, Molecular Devices recommends dark adaptation of the microplate by placing the sample-loaded microplate in the instrument for several minutes before starting the read.

The instrument bypasses the emission monochromator for luminescence reads that detect all wavelengths.

If wavelength selection is desired, you can choose the wavelength where peak emission is expected to occur. Also, multiple wavelength choices let species with multiple components be differentiated and measured easily.

Luminescence can be read from the top of a microplate. Solid white microplates are recommended for luminescence reads.

Concentrations or qualitative results are derived from raw data with a standard curve or by comparison with reference controls. See Analyze Luminescence Data on page 99.

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

You can use the Protocol Libraries to quickly find and open a predefined protocol.

Analyze Luminescence Data

The conversion rate of photons to counts is individual for each reader. Therefore, raw data from the same plate can seem significantly different from one instrument to the next. Also, the data format used by other manufacturers might not be counts per second and can be different by several orders of magnitude. It is important to know that the number of counts and the size of figures is in no way an indication of sensitivity. See Detection Limit on page 100.

Concentrations or qualitative results are derived from raw data with a standard curve or by comparison with reference controls. A standard curve consists of, at a minimum, a blank sample and a reference standard sample of known concentration. The raw data can then be expressed in equivalent concentration of a reference label. The raw data is normalized to counts per second by dividing the number of counts by the read time per well.

Analyzing and validating luminescence data generally consists of the following:

Background Correction

The light detected in a luminescent measurement generally has two components: specific light from the luminescent reaction and an approximately constant level of background light caused by various factors, including the plate material and impurities in the reagents. The background can be effectively measured using blank replicates. Blanks should include the luminescent substrate (chemical energy source) but not the luminescence agent (generally an enzymatic group which makes the substrate glow).

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

The blank sample reveals the offset underlying each data sample. This offset does not carry information on the label, and is generally subtracted before data reduction is done.

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

To help eliminate background luminescence from a microplate that has been exposed to light, Molecular Devices recommends dark adaptation of the microplate by placing the sample-loaded microplate in the instrument for several minutes before starting the read.

Detection Limit

The detection limit is the smallest sample concentration that can be measured reliably above the blank. Determining the detection limit requires taking a number of blank measurements and calculating an average value and standard deviation for the blanks. The detection threshold is defined as the average blank plus three standard deviations. If the average sample value measures above the threshold, the sample can be detected at a statistically significant level.

The detection limit can be described by the following equation:

$$\text{Det Limit} = \frac{3\ \text{Stdev}_{\text{blank}}}{\frac{\text{std} - \text{blank}}{\text{conc}_{\text{std}}}}$$

where $conc_{std}$ is the concentration of the standard, $StDev_{Blank}$ is the standard deviation of the blank replicates, and *blank* and *Std* are average values of the replicates for the blank and standard wells.

Determining the detection limit for an assay requires multiple blanks to calculate their standard deviation.

Sample Volumes and Concentration of Reactants

The concentration of the luminescent agent impacts the quantity of light output in a luminescent reaction. Light is emitted as a result of a reaction between two or more compounds. Therefore, the quantity of light output is proportional to the quantity of the limiting reagent in the sample.

For example, in an ATP/luciferin-luciferase system, when total volume is held constant and ATP is the limiting reagent, the blanked light output is proportional to the concentration of ATP in the sample, at very high concentrations of ATP. Substrate can be used up and become rate-limiting, providing it is the rate-limiting component. In this case, the non-linearity is an effect of the assay and not caused by the microplate reader.

Data Optimization

The measurement noise is dependent on the read time per sample (time per plate or time per well). In particular, the detection limit improves when the read time is increased. Therefore, it is important to specify the read time when comparing measurements.

All low-light-level detection devices have some measurement noise in common. To average out the measurement noise, optimization of the time per well involves accumulating as many counts as possible. Within some range, you can reduce noise (CVs, detection limit) by increasing the read time per well, as far as is acceptable from throughput and sample stability considerations.

Z´ is the standard statistical parameter in the high-throughput screening community for measuring the quality of a screening assay independent of test compounds. It is used as a measure of the signal separation between the positive controls and the negative controls in an assay.

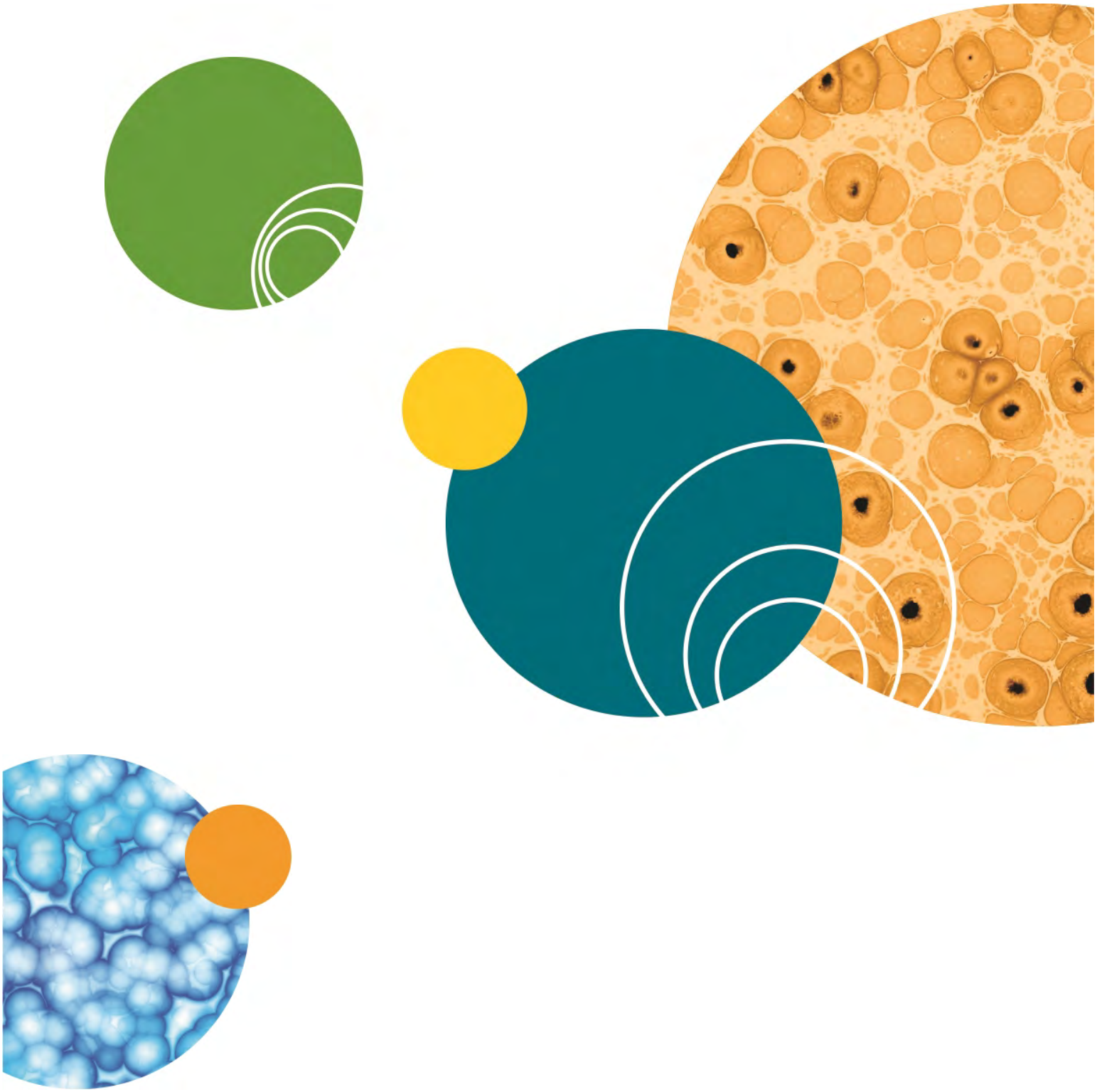
The value of Z´ can be determined using the following formula:

$$Z' = 1 - \frac{3(SD_{c+}) + 3(SD_{c-})}{| Mean_{c+} - Mean_{c-} |}$$

where **SD** is the standard deviation, **c+** is the positive control, and **c–** is the negative control.

A Z´ value greater than or equal to 0.4 is the generally acceptable minimum for an assay. Higher values might be desired when results are more critical.

Z´ is not linear and can be made unrealistically small by outliers that skew the standard deviations in either population. To improve the Z´ value, you can increase the quantity of label in the sample, if acceptable for the assay, or increase the read time per well.



Chapter 6: Maintenance and Troubleshooting

6

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 Obtain Support on page 124.

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

The following topics describe maintenance and troubleshooting procedures that can be done by users to ensure optimal operation of the instrument.

WARNING! Service or maintenance procedures other than those specified in this guide can be done only by Molecular Devices qualified personnel. When service is required, contact Molecular Devices technical support.

Clean Injector and Accessories

CAUTION! Do not clean the inside of the injector other than the inside of the bottle holder. Cleaning the inside can cause damage.

Periodically clean the outside surfaces, the inside and outside of the bottle holder, the snorkel clamps, and the snorkel end of the injector tubing with a lint-free cloth that has been lightly dampened with water. You can remove the bottle holder for cleaning. See Injector Assembly and Maintenance on page 37.

If decontamination is required, use a lint-free cloth that has been lightly dampened with a decontaminating solution, such as 70% ethanol or 3% sodium hypochlorite. See List of Compatible Solutions on page 132.

Note: After you use a decontamination solution, always wipe the areas with a lint-free cloth that has been lightly dampened with water to remove the residue. If you use sodium hypochlorite, wipe the areas with a lint-free cloth that has been lightly dampened with 70% alcohol before you wipe again with water.

CAUTION! Do not use abrasive cleaners. Do not spray cleaner directly onto the instrument. Do not immerse the injector.

To clean the waste plate, strip wells, bottles, and adapters, use a lightly dampened, lint-free cloth. After you clean these accessories, let them air dry on absorbent paper or cloth. Invert the waste plate, strip wells, and bottles so that they drain as they dry. These accessories can be replaced if cleaning is no longer practical. See Injector Specifications on page 130.

To clean the injector tips, remove the bottles from the bottle holder. Dab the surface of the injectors with a lightly dampened, lint-free cloth. Do not insert anything into the injector tips as this can damage their internal non-stick coating.

To clean the inside of the injector tubing, use the Wash operation. See and Wash Injector Tubing on page 51.

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 displayed by the software.

Molecular Devices recommends turning the power off when the instrument is not in use.

Replace Fuses

If the instrument does not seem to get power after you switch it on, check to see whether the power cord is securely plugged into a functioning power outlet and to the power port on the rear of the instrument.

If the power failed while the instrument was on, verify 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, replace the fuses. You can obtain replacement fuses from Molecular Devices. For fuse specifications and part numbers, see Physical Specifications on page 129

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 fuses are located in the fuse carrier which is part of the power outlet on the rear of the instrument.

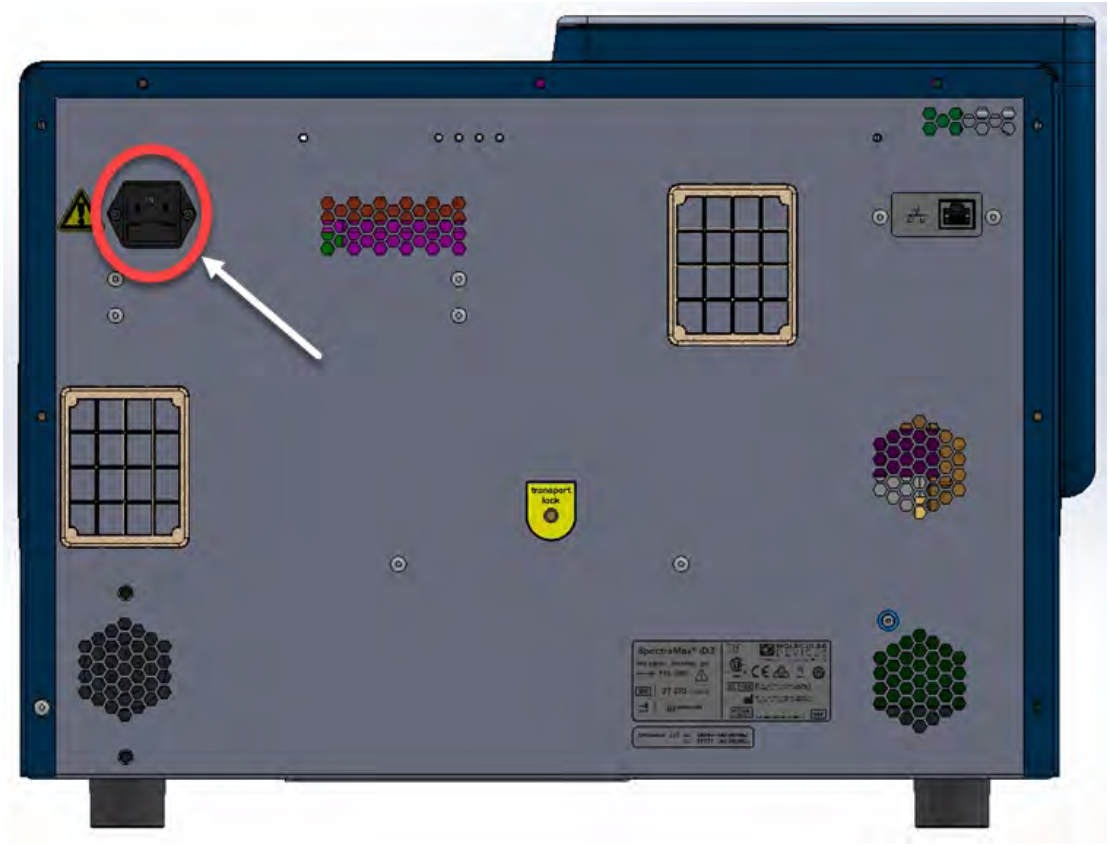


Figure 6-1: Fuse holder below power port

To replace the fuses:

WARNING! HIGH VOLTAGE Always turn off the power and disconnect the power cord from the main power source before you do a maintenance procedure that requires removal of a panel or cover or disassembly of an interior instrument component.

1. Press and hold the power button to power off the instrument.
2. Unplug the power cord from the power port.
3. Use a small slot-head screwdriver to gently press on the carrier-release tab and then pull the fuse carrier to remove it from the instrument.
4. Gently pull the old fuses from the carrier by hand.
5. Gently place new fuses into the carrier by hand.
6. Press the fuse carrier into the instrument until the carrier snaps into place.
7. Plug the power cord into the power port.
8. Turn on power to the instrument.

Note: If the instrument still does not power on after you change the fuses, contact Molecular Devices technical support. See Obtain Support on page 124.

Clean the Instrument

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.

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

- Wipe up all spills immediately.
- Periodically clean the outside surfaces of the instrument using a cloth or sponge that has been lightly dampened with water.
- 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 all residue.
- If needed, clean the microplate drawer using a cloth or sponge that has been lightly dampened with water.
- If a bleach solution has been used, wipe the instrument using a lint-free cloth that has been lightly dampened with water to remove the bleach residue.

CAUTION! Do not use abrasive cleaners. Do not spray cleaner directly onto the instrument or into any openings. Do not let water or other fluids drip inside the instrument.

Before You Move the Instrument

When you move the instrument from one location to a new location, there are several things you must do before you power off the instrument.

This procedure requires the following tool included in the accessories tool box:

Table 6-1: Required Tool

Illustration	Part Number	Description
	YW 000 012	Holex HEXAGON ballhead bolt driver 3 mm

CAUTION! When transporting the instrument, warranty claims are void if damage during transport is caused by improper packing.

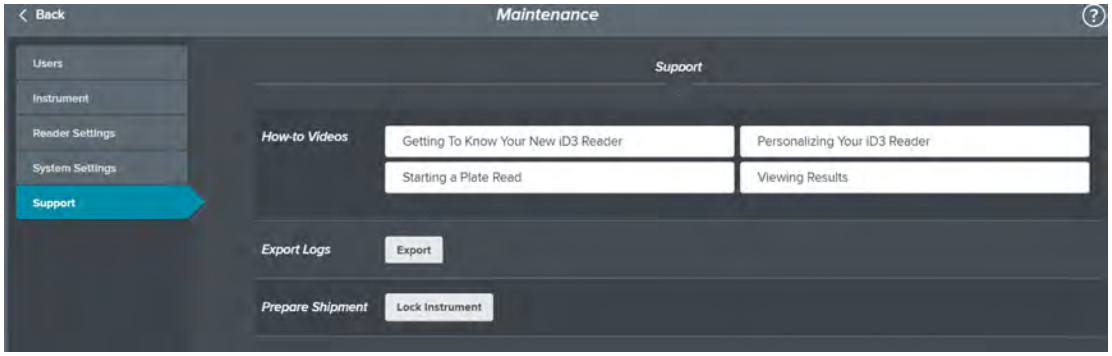


Figure 6-2: Maintenance page - Support tab - Lock Instrument button

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

1. Touch  and remove the microplate from the microplate drawer, if present.
2. Touch  to display the Maintenance page.
3. Touch **Support** to display the Support tab.
4. Touch **Lock Instrument** to move the transport slide into a position that can accept the transport lock.
5. You are prompted to confirm that there is no plate in the drawer. When there is no plate in the drawer, touch **OK**.

> **Note:** Most iD3 instruments have a Transport Lock sticker in the center of the instrument rear. If the Transport Lock sticker is on the top right of the instrument rear, skip this step.

6. Move to the rear of the instrument, insert the 3.0 mm Holex HEXAGON ballhead bolt driver into the Transport Lock opening and tighten the interior screw into the transport slide.

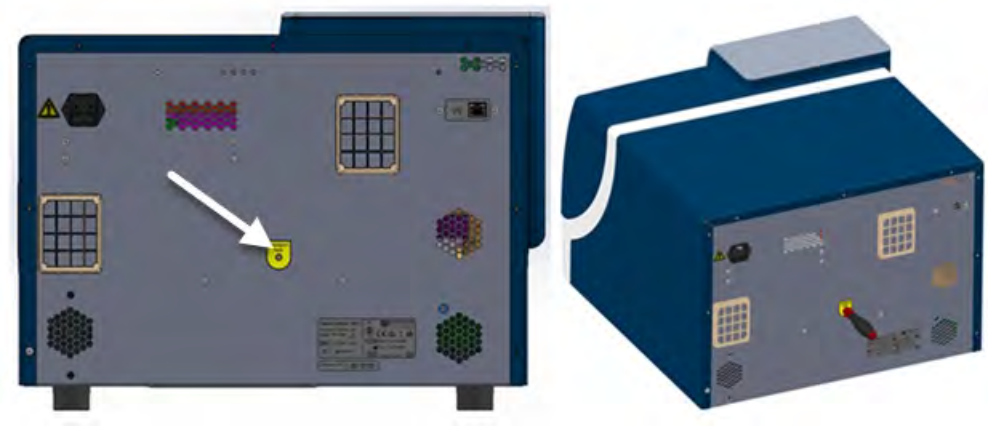


Figure 6-3: Transport lock opening

Note: The screw remains inside the instrument. The screw is spring mounted and cannot get lost within the instrument. Tighten the screw until it is snug. This locks the transport slide.

7. Move to the front of the instrument and touch **OK** to confirm that the transport slide has been locked. The microplate drawer opens to enable you to install the transport lock on the microplate drawer and the instrument proceeds to shut down. See Install Transport Locks on page 108.
8. If you use a laptop or computer to operate the instrument, make sure that the SoftMax Pro Software is not running and turn off the connected computer. Then disconnect the Ethernet cable from the rear of the instrument and from the computer.
9. Unplug the power cord from the rear of the instrument and from the wall outlet.
10. Disconnect the Ethernet cable from the rear of the instrument and from the wall outlet.
11. Store the power cord and Ethernet cable in the accessories tool box.
12. Install the transport lock on the microplate drawer. See Install Transport Locks on page 108.
13. If you plan to store the instrument, ship the instrument, or transport the instrument to a different building, pack the instrument in the original packaging. See Pack the Instrument on page 121.
14. Make sure that the new location is a dry, flat work area that has sufficient space for the instrument and required cables. See Instrument Specifications on page 125.

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

Install Transport Locks

Before you move or pack the instrument, do all the steps in Before You Move the Instrument on page 106 to move the transport slide and the microplate drawer into the position to accept the transport locks and to remove the cables from the instrument. The transport locks protects the instrument from damage during a move or shipment.

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

Note: If the rear of your instrument does not have the transport lock opening in the center, see Install Transport Lock Alternate on page 110.

This procedure requires the following tool included in the accessories tool box:

Table 6-2: Required Tool

Illustration	Part Number	Description
	YW 000 006	Hex key, 2.0 mm
	YW 000 012	Holex HEXAGON ballhead bolt driver 3 mm

When you do all the steps in Before You Move the Instrument on page 106 section, the transport slide moves to the correct position, you lock the transport slide, the microplate drawer door opens, and the microplate drawer moves into the position to accept the transport lock.

If you did not insert the 3.0 mm Holex HEXAGON ballhead bolt driver into the Transport Lock opening and tighten the interior screw into the transport slide you must power on the instrument and perform the steps in the Before You Move the Instrument on page 106 topic.

To install the transport lock on the microplate drawer:

1. Place the microplate drawer transport lock on the end of the microplate drawer.
2. Use the 2.0 mm hex key to tighten screws #2 and #3 until the lock is attached to the microplate drawer.

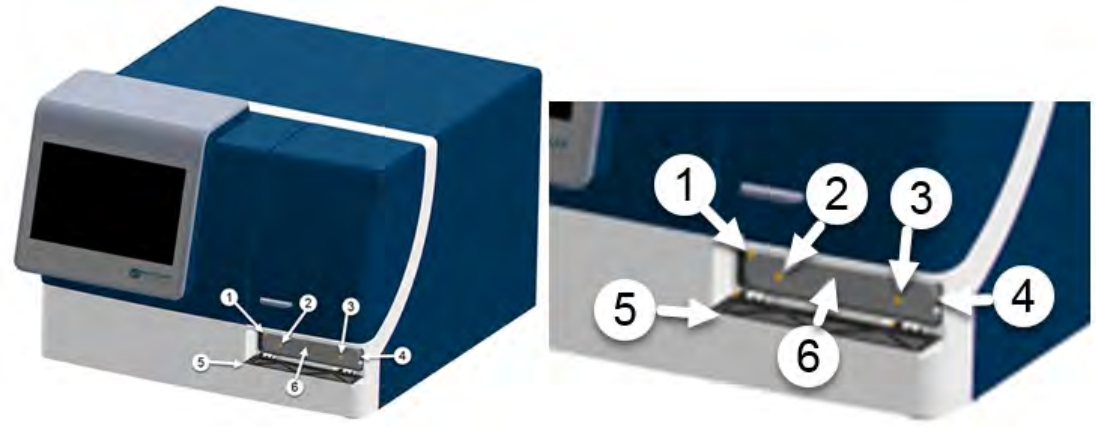


Figure 6-4: Microplate drawer transport lock

Item	Description
1	Screw #1 fastens the lock to the internal frame of the instrument
2	Screw #2 fastens the lock to the microplate drawer
3	Screw #3 fastens the lock to the microplate drawer
4	Microplate drawer
5	Microplate chamber door in open position
6	Microplate drawer transport lock

3. Gently push the microplate drawer into the instrument and as far to the left as possible until screw #1, which fastens the lock to the internal frame of the instrument, lines up with the hole on the internal frame. The microplate chamber door must be held open manually until you fasten the transport lock.
4. Tighten screw #1 until the microplate drawer is securely locked in place.
5. Route the yellow tab connected to the transport lock so that it will pass over the top of the microplate chamber door when the door is closed.
6. Gently close the microplate chamber door.

For information on how to pack the instrument in its original container, see Pack the Instrument on page 121.

Install Transport Lock Alternate

Before you move or pack the instrument, do all the steps in Before You Move the Instrument on page 106 to move the transport slide into position to accept the transport lock and to remove the cables from the instrument. The transport lock protects the instrument from damage during a move or shipment.

If the touchscreen is not accessible and you could not touch Lock Instrument For Shipment, see Install Transport Lock Manually on page 114.

CAUTION! Do not touch or loosen screws or parts other than those designated in the instructions. Changes to other screws or parts can cause misalignment and possibly void the warranty.

If the rear of your instrument has the transport lock opening in the center, see Install Transport Locks on page 108.

This procedure requires the following tool included in the accessories tool box:

Table 6-3: Required Tool

Illustration	Part Number	Description
	YW 000 044	Hex key, 4.0 mm

To install the transport lock on the transport slide:

CAUTION! The transport lock cannot be installed properly if you did not touch the **Lock Instrument For Shipment** button on the Maintenance page Support tab before you powered the instrument off. See Before You Move the Instrument on page 106. If the touchscreen is not accessible and you could not touch Lock Instrument For Shipment, see Install Transport Lock Manually on page 114.

1. Use the provided 4.0 mm hex key to loosen screw #1 in the upper-right corner on the back of the instrument. After a few turns, the touchscreen housing rises. You might hear a click when the housing is free for the next step. Do not loosen the screw after the

touchscreen is unlocked.

Figure 6-5: Instrument rear - release touchscreen housing

2. Walk to the front of the instrument and gently lift the touchscreen housing to access the transport lock.

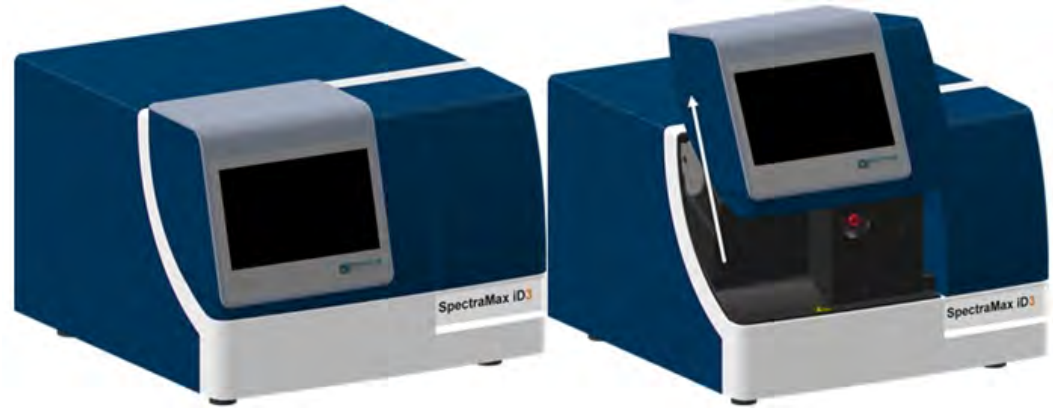


Figure 6-6: Instrument front - raise touchscreen housing

WARNING! You must hold the touchscreen housing in place for the following steps.

3. Loosen screw #2, by hand, to free the transport lock face plate. The screw is attached to the faceplate to prevent it from being misplaced.

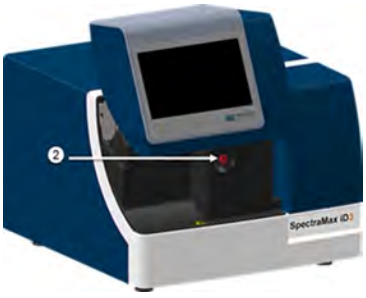


Figure 6-7: Transport lock face plate

4. Remove the transport lock face plate (with screw #2 attached) to access the transport locks.

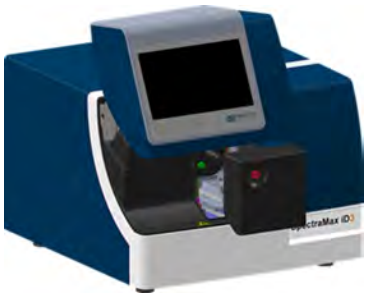


Figure 6-8: Remove transport lock face plate

5. By hand, remove the long transport lock #4 from the slot on the instrument case.

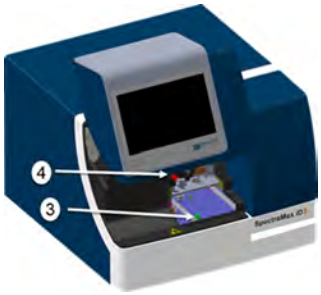


Figure 6-9: Remove transport locks

6. By hand, remove the short transport lock #3 from the slot below the transport slide.
7. Screw the short transport lock #3 into the slot on the instrument case from where you removed the long transport lock.

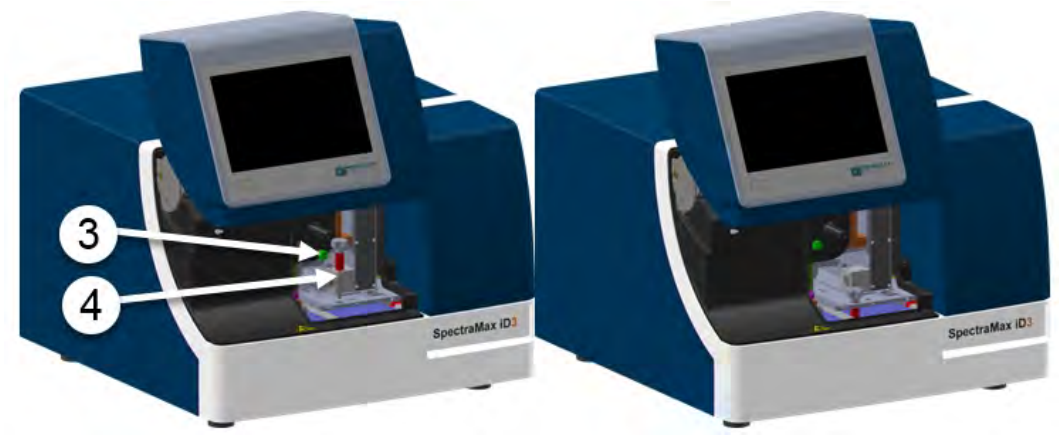


Figure 6-10: Switch transport locks

8. Thread the long transport lock #4 through the transport slide and into the slot from where you removed the short transport lock and screw transport lock #4 into place, by hand.

9. Align the transport lock faceplate with screw #2 to its original position.

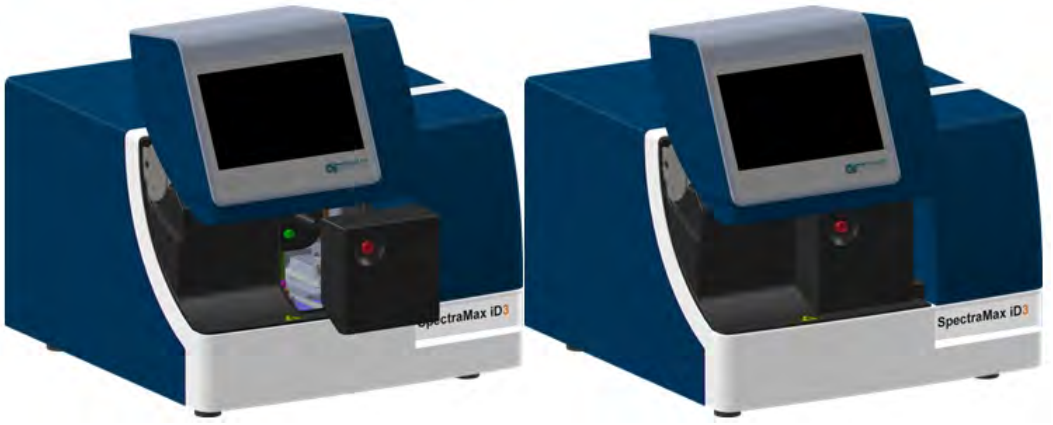


Figure 6-11: Replace transport lock face plate

10. Screw the transport lock faceplate back into place with screw #2 by hand.
11. Gently lower the touchscreen housing.

> **Note:** Do not push the touchscreen housing down manually. It lowers automatically as you turn the hex key in the next step.

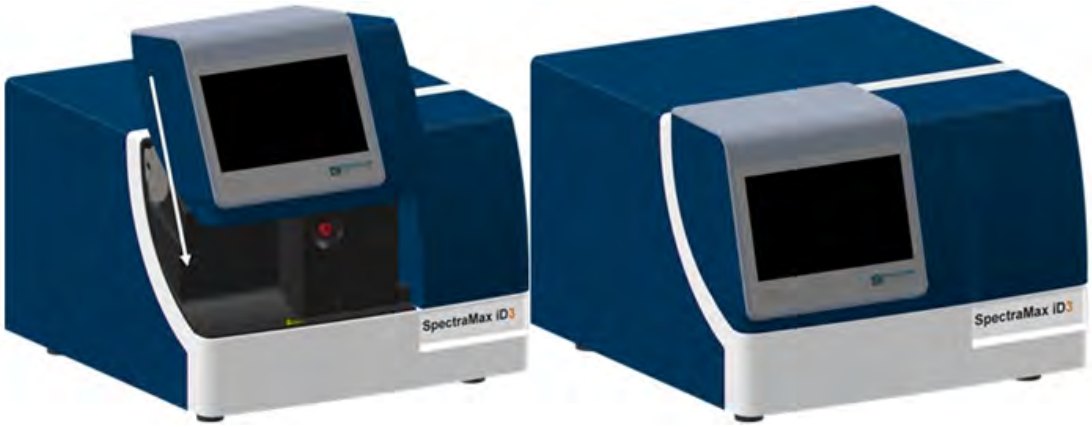


Figure 6-12: Lower touchscreen housing

12. Walk to the back of the instrument. Use the provided 4.0 mm hex key to tighten screw #1 in the upper-right corner on the back of the instrument until the touchscreen housing lowers and is locked in place. Do not over tighten the screw.

Figure 6-13: Lock touchscreen housing

For steps to pack the instrument in its original container, see Pack the Instrument on page 121.

Install Transport Lock Manually

If you cannot use the Maintenance page Lock Instrument for Shipment button and you need to move or ship the instrument, you must do the following to secure the microplate drawer and to move the transport slide into position to accept the transport lock. The transport lock protects the instrument from damage during a move or shipment.

CAUTION! Do not touch or loosen screws or parts other than those designated in the instructions. Changes to other screws or parts can cause misalignment and possibly void the warranty.

This procedure requires the following tool included in the accessories tool box:

Table 6-4: Required Tool

Illustration	Part Number	Description
	YW 000 044	Hex key, 4.0 mm

To install the transport lock on the transport slide:

CAUTION! These steps should only be done when you could not touch the **Lock Instrument For Shipment** button on the Maintenance page Support tab before you powered the instrument off. See Before You Move the Instrument on page 106.

1. Use the provided 4.0 mm hex key to loosen screw #1 in the upper-right corner on the back of the instrument. After a few turns, the touchscreen housing rises. You might hear a click when the housing is free for the next step. Do not over loosen screw after touchscreen housing is unlocked.

Figure 6-14: Instrument rear - release touchscreen housing

2. Walk to the front of the instrument and gently lift the touchscreen housing to access the transport lock.

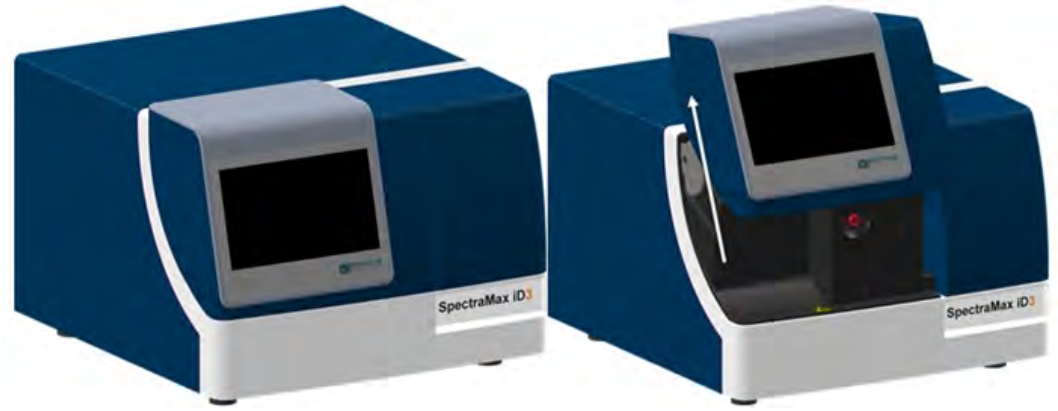


Figure 6-15: Instrument front - raise touchscreen housing

WARNING! You must hold the touchscreen housing in place for the following steps.

3. Loosen screw #2, by hand, to free the transport lock face plate. The screw is attached to the faceplate to prevent it from being misplaced.

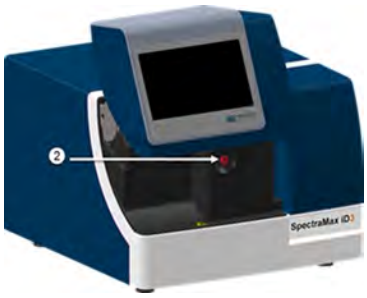


Figure 6-16: Transport lock face plate

4. Remove the transport lock face plate (with screw #2 attached) to access the transport locks.

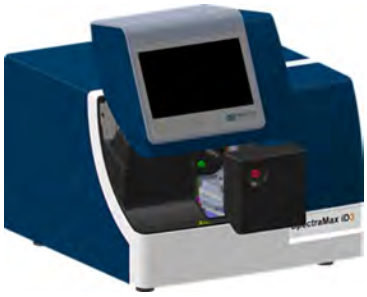


Figure 6-17: Remove transport lock face plate

5. By hand, remove the long transport lock #4 from the slot on the instrument case.

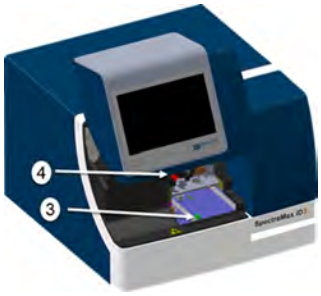


Figure 6-18: Remove transport locks

6. By hand, remove the short transport lock #3 from the slot below the transport slide.

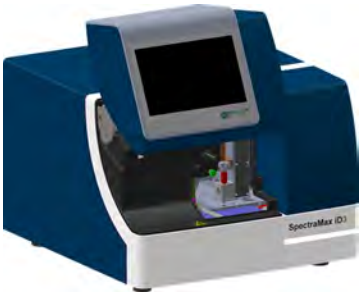


Figure 6-19: Switch transport locks

7. Screw the short transport lock #3 into the slot on the instrument case from where you removed the long transport lock. Before you screw the long transport lock into place, perform the following steps to align the transport slide and plate transport drawer.

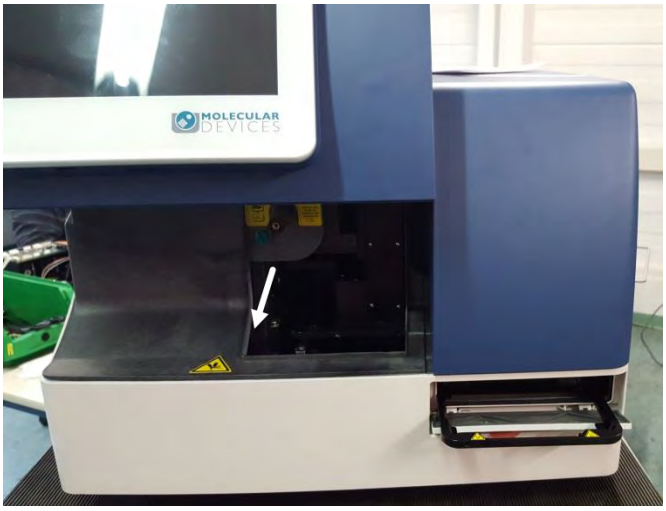


Figure 6-20: Pull transport slide forward

8. Pull the transport slide to the front position.

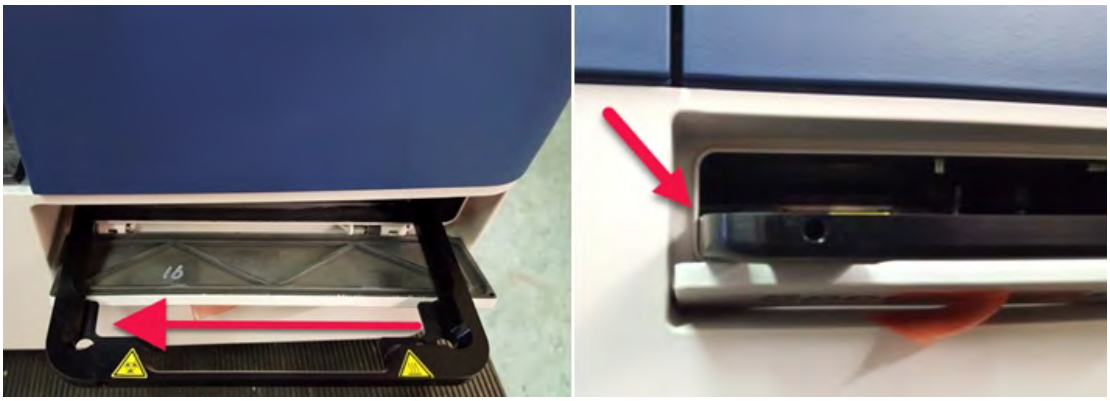


Figure 6-21: Push plate transport left

9. Push the plate transport to the left side of the opening.

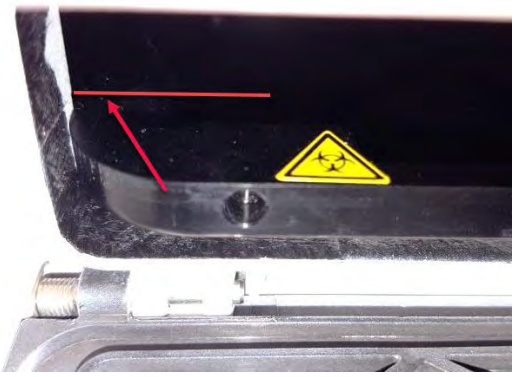
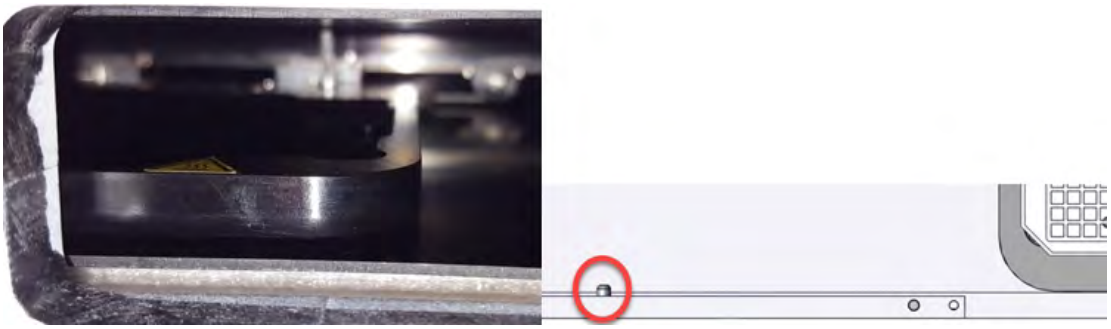


Figure 6-22: Align plate transport with chamber opening

10. Push the plate transport slightly into the transport chamber until the front of the plate transport aligns with the front of the chamber opening.

Figure 6-23: Push plate transfer left. Note the hole on the front. ^ Top view ^

11. Push the plate transport slightly into the chamber as well as to the left side of the device so that the transport slides along the front of the chamber in the left direction.



12. After about 10 cm, you will feel a resistance point which is a bolt.

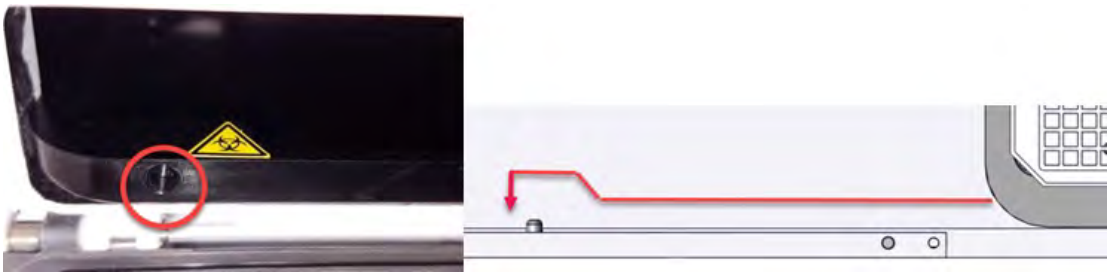


Figure 6-24: Set plate transport on bolt. ^ Top view ^

13. Move the plate transport a little bit inside and then to the left until you feel the hole on the front of the plate transport catch the bolt. Do not apply any downward pressure on the transport as you slide the plate over the bolt.

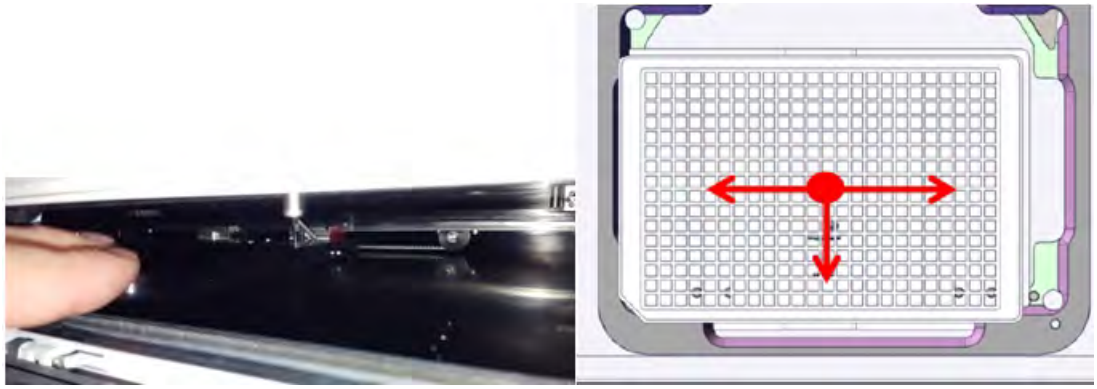


Figure 6-25: Plate transport should not move

14. Verify that the plate transport does not move freely.

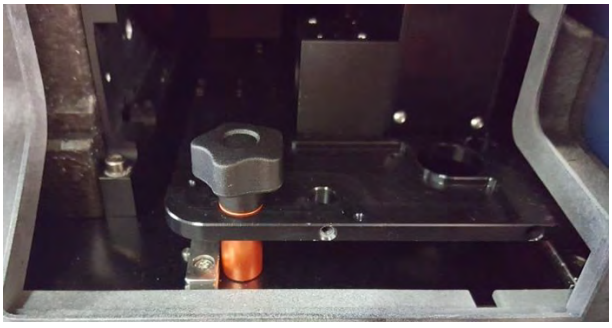


Figure 6-26: Insert long transport lock

15. Thread the long transport lock #4 through the transport slide and the plate transport into the slot from where you removed the short transport lock and screw transport lock #4 into place, by hand.

16. Align the transport lock faceplate with screw #2 to its original position.

Figure 6-27: Replace transport lock face plate

17. Screw the transport lock faceplate back into place with screw #2 by hand.
18. Gently lower the touchscreen housing.

> **Note:** Do not push the touchscreen housing down manually. It lowers automatically as you turn the hex key in the next step.

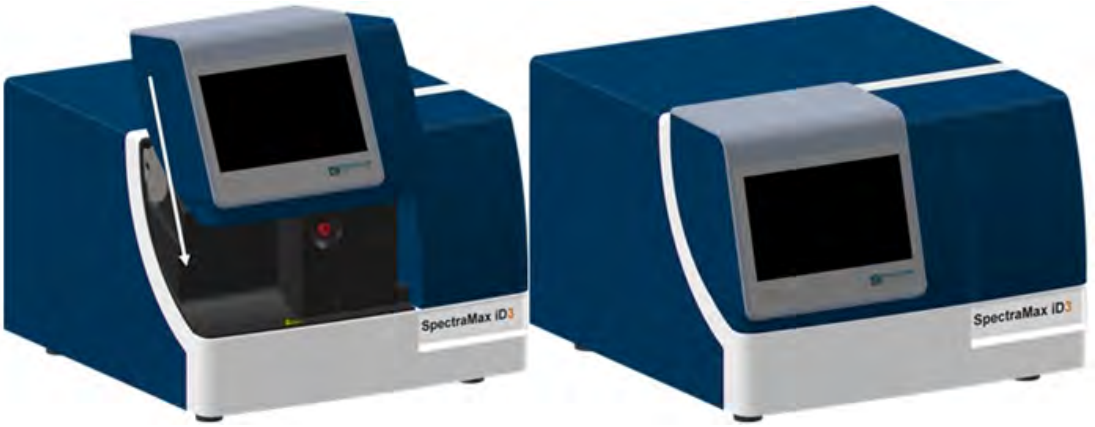


Figure 6-28: Lower touchscreen housing

19. Walk to the back of the instrument. Use the provided 4.0 mm hex key to tighten screw #1 in the upper-right corner on the back of the instrument until the touchscreen housing lowers and is locked in place. Do not over tighten screw.

Figure 6-29: Lock touchscreen housing

For steps to pack the instrument in its original container, see Pack the Instrument on page 121.

Pack the Instrument

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

CAUTION! When transporting the instrument, warranty claims are void if damage during transport is caused by improper packing.

Do all steps in Before You Move the Instrument on page 106 and Install Transport Locks on page 108 before you proceed with the following procedures. Correct packaging of the instrument also includes applicable decontamination procedures.

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

The instrument should be stored in a dry, dust-free, environmentally controlled area. For more information about acceptable storage environments, see Instrument Specifications on page 125.

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

To pack the instrument in the original packaging:

1. Make sure you have done all the steps in Before You Move the Instrument on page 106 and Install Transport Locks on page 108.
2. Store the power cord and Ethernet cable in the instrument accessories toolbox.
3. Wrap the instrument in static-free plastic.

4. Replace the molded foam packaging around the instrument.

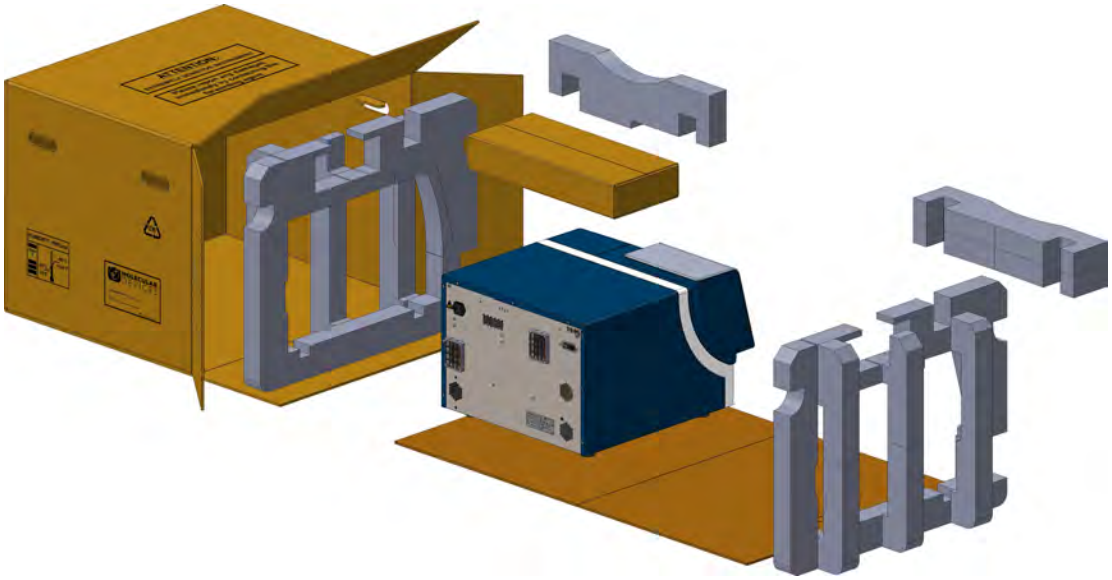


Figure 6-30: Accessory box and foam packaging

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

5. Place the accessories tool box in the foam packaging above the instrument.
6. Place the instrument and accessories tool box on the flat cardboard piece and slide it into the original box.

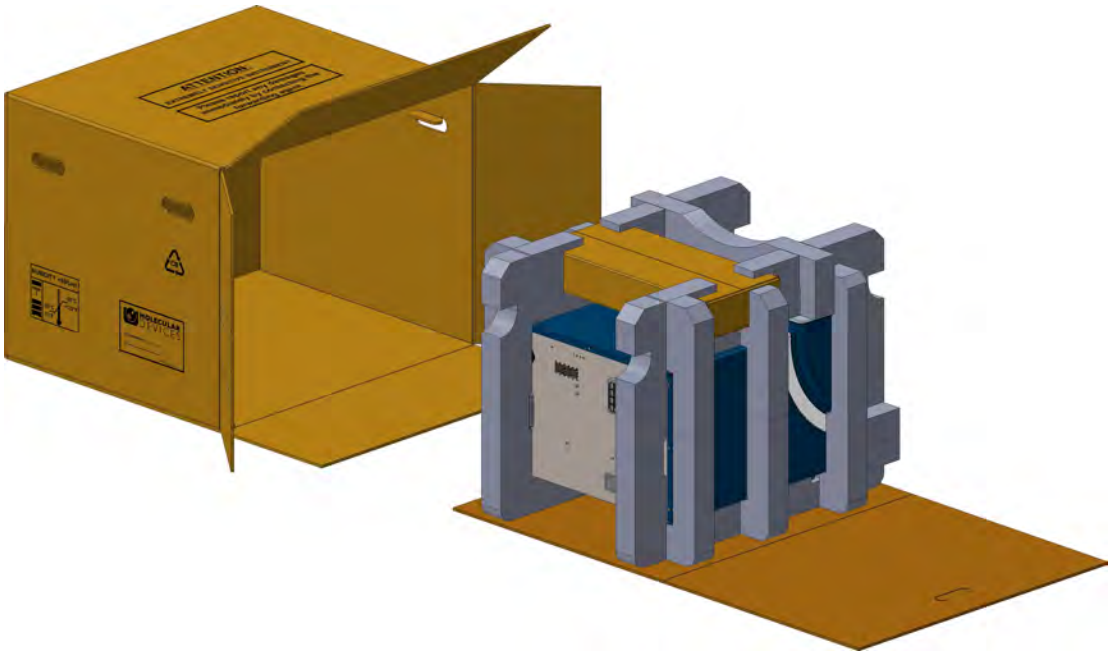


Figure 6-31: Slide the instrument into the box

7. Fold up the cardboard flap inside the box.

Figure 6-32: Fold the cardboard flap inside the box

8. Along the side labeled **Open Here**, close the box and seal it with packing tape.

Troubleshooting

WARNING! Service or maintenance procedures other than those specified in this guide can be done only by Molecular Devices qualified personnel. 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 9.

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 Obtain Support on page 124.

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:

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

Obtain 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 possible level of technical service.

Our support web site, www.moleculardevices.com/support, has a link to the Knowledge Base with technical notes, software upgrades, safety data sheets, and other resources. If you do not find the answers you seek, follow the links to the Technical Support Service Request Form to send an email to our technical support representatives.

You can contact your local representative or contact Molecular Devices Technical Support by telephone at 800-635-5577 (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.

Please have your instrument serial number or Work Order number, and your software version number available when you call. You can find this information on the Maintenance page - Instrument tab. See Instrument Maintenance on page 63.

Appendix A: Instrument Specifications

This appendix provides specifications for the iD3 basic instrument.

Measurement Specifications

The following tables list the instrument measurement specifications.

Table A-1: Read Times using Quick Read (Plate in/out may add 10-15 seconds)

	96 Wells	384 Wells
Absorbance	0.5 min	1.5 min
Fluorescence Intensity	0.5 min	1.5 min
Luminescence	0.5 min	1.5 min

Table A-2: Absorbance Measurement Specifications of the iD3 instrument

Item	Description
Wavelength Range	230 - 1000 nm
Wavelength Selection	Monochromator tunable in 1 nm increments
Wavelength Bandwidth	4.0 nm full width half maximum
Wavelength Accuracy	±2.0 nm across wavelength range
Wavelength Repeatability	±1.0 nm
Photometric Range	0 - 4.0 OD
Photometric Resolution	0.001 OD
Photometric Accuracy	< ±0.010 OD ±1.0%, 0 - 3 OD VIS 0 - 3 OD UV
Photometric Precision (repeatability)	< ±0.003 OD ±1.0%, 0 - 3 OD VIS 0 - 3 OD UV
Stray Light	≤ 0.05% at 260 nm, 280 nm
Photometric Stabilization	Instantaneous
Photometric Drift	None (continuous referencing of monochromatic input)
Calibration	Automatic before every endpoint read and before the first kinetic read
Optical Alignment	None required
Photodetectors	Silicon Photodiode

Table A-3: Fluorescence Intensity Measurement Specification of the iD3

Item	Description
Wavelength Range	EX 250 - 830 nm EM 270 - 850 nm
Wavelength Selection	Monochromators tunable in 1.0 nm increments
Wavelength Accuracy	±2 nm
Wavelength Precision	±1 nm
Bandwidth (EX/EM)	EX: 15 nm EM: 25 nm
Number of excitation/emission pairs per plate	4
Dynamic Range	>6 logs
Sensitivity Top Read Mono*	Fluorescein
96 Wells	4 pM - Guaranteed 1 pM - Optimized
384 Wells	6 pM - Guaranteed 1 pM - Optimized
Sensitivity Bottom Read Mono*	Fluorescein using glass bottom Greiner Sensoplate™ glass bottom multiwell plates
96 Wells	10 pM - Guaranteed 2 pM - Optimized
384 Wells	10 pM - Guaranteed 2.5 pM - Optimized
System Validation	Self-calibrating with built-in fluorescence calibrators
Light Source	High power xenon flash lamp
Average Lamp Lifetime	1 billion flashes or 2 years normal operation

Table A-4: Luminescence Measurement Specifications of the iD3

Item	Description
Wavelength Selection	Choice of simultaneous detection of All Wavelengths or selection in 1.0 nm increments
Wavelength Range	300 - 850 nm 300 - 650 nm for "All Wavelengths" setting
Wavelength Accuracy	±2 nm
Wavelength Precision	±1 nm
Dynamic Range	>7 decades
Sensitivity Top Read*	Perkin Elmer ATPlite 1step Luminescence Assay System
96 Well	10 pM - Guaranteed 2 pM - Optimized
384 Well	20 pM - Guaranteed 4 pM - Optimized
Crosstalk	<0.1% in white 96-well half area plate <0.2% in white 384-well Costar small volume

*For properly functioning, operating, and maintained equipment.

Computer System Specifications

All instrument software is accessible from the touchscreen. If you choose to operate the instrument from a laptop or computer, SoftMax Pro Software version 7.0.1 or higher can be installed on a computer with the following system specifications.

Table A-5: Minimum and Recommended Requirements for SoftMax Pro Software

Item	Minimum	Recommended
Processor	Single-core, 2 GHz or faster	Quad core or faster
Operating system	Windows 7 (SP1 or newer), Professional 32-bit or 64-bit (x86 or x64) and .NET Framework 4.6 (.NET Framework 4.6 is installed automatically by the SoftMax Pro Software installer if required.)	- Windows 7 (SP1 or newer) Professional or Enterprise 32-bit or 64-bit (x86 or x64) - Windows 8 Professional or Enterprise, 32-bit or 64-bit (x86 or x64) - Windows 10 Professional or Enterprise, 32-bit or 64-bit (x86 or x64)
Data connection	Ethernet port	Ethernet port
Memory	2 GB RAM	4 GB RAM If running on a virtual machine, Molecular Devices recommends a minimum of 6 GB RAM. For automation, Molecular Devices recommends a minimum of 8 GB RAM.
Hard drive	500 MB of available space	1 GB of available space, or more
Graphics display	Graphics display adapter 1024 x 768 or higher-resolution display	32-bit graphics display with 256 MB video RAM 1280 x 1024 or higher-resolution display
Software activation	Internet connection or external USB drive	Internet connection or external USB drive

To prevent data loss, turn off all sleep and hibernation settings for the hard disk, the CPU, and the USB ports. Disable automatic Windows Updates. Update Windows manually when the instrument is not used by the software. You can set these options in Windows Control Panel.

The SpectraMax iD3 software version 1.0 is compatible with SoftMax Pro Software version 7.0.1.

The SpectraMax iD3 software version 1.1 is compatible with SoftMax Pro Software version 7.0.2.

To update the SpectraMax iD3 software, please contact Technical Support.

Physical Specifications

The following tables list the physical specifications of the SpectraMax iD3 instrument.

Table A-6: Physical Specifications of the iD3 Instrument

Item	Description
Environment	Indoor Use Only
Power Requirements	100-240 VAC ±10%, 2 A, 50/60 Hz
Dimensions	53.2cm W x 40.1cm H x 59.8cm D (20.94 in. W x 15.79 in. H x 23.54 in. D)
Front Clearance	11 cm (4.33 in.) for plate drawer
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	40 kg (88.1 lbs)
Plate Formats	6, 12, 24, 48, 96, 384 well microplates ANSI/SLAS conformant Maximum height: 22 mm
Reading Capability	Microplates, Cuvettes (with adapter)
Robotic Compatible	Yes
Shake	Orbital and double orbital
Temperature Control	5°C (7.2° F) above ambient up to 65°C (149° F) At temperature range from 55°C (131°F) up to 65°C (149°F) ambient temperature of 25°C (77°F) is required.
Temperature Uniformity	±0.75°C (1.35°F)
Temperature Accuracy	±1°C (1.8°F) at 37°C (98.6°F) Set Point
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)

Table A-6: Physical Specifications of the iD3 Instrument (continued)

Item	Description
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 Ethernet Port
NFC Antenna Reader/Writer	SANGOMA-MSMA 2V3 13.56 Mhz Multi Standard - Multi Antenna Reader/Writer Contains FCC ID: 2AKHW-SANGMSMA1 Contains IC: 22202-SANGMSMA1 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.

Injector Specifications

When your instrument has the SpectraMax Injector System, the specifications for measurements using the injector are shown in the following table.

Table A-7: Measurement Specifications for the Injector

Item	Description
Name	Injector
Weight	1.7 kg (3.7 lbs)
Microplate formats	6, 12, 24, 48, 96, and 384-well microplates
Read modes	The injector system is method independent. You can run injector protocols for the following read modes: Absorbance, Luminescence (all wavelength), Luminescence Monochromator, Fluorescence Intensity top, and Fluorescence Intensity bottom.
Type	Single emission
Light source	None
Labels/Substrates	Labels compatible with the wavelength range
Detection limit, optimized	20 amol ATP (“Flash” luminescence using Promega ENLITEN® ATP Assay System)
Detection limit, guaranteed	50 amol ATP (<=> 250 fM @ 0.2mL/well, “Flash” luminescence using Promega ENLITEN ATP Assay System)
Linear dynamic range	5 logs in a single microplate read

Table A-7: Measurement Specifications for the Injector (continued)

Item	Description
Injectors	2
Dispense volume	1 µL increments from 1 µL to the maximum allowable volume of the well, based on the selected microplate type
Dispense accuracy	±(4% of volume + 1 µL) / volume x 100%
Dispense precision	≤(2% of volume + 1 µL) / volume [µL] x 100% cv
Dispense speed	100 µL per second
Dead volume	**50 mL bottle:** 1 mL **Injector tubing:** 250 µL Fill the bottles with enough reagent for your experiment plus at least 2 mL to account for the prime operation and the quick-prime operation before the plate is read, and for the dead volume in the bottle and the tubing.
Minimum delay between injection and ABS	**Injector 1** 800 msec after injection ends **Injector 2** 800 msec after injection ends
Minimum delay between injection and LUM (top) read	**Injector 1:** 500 msec after injection ends **Injector 2:** 500 msec after injection ends
Minimum delay between injection and FL (bottom) read or FL (top)	**Injector 1:** 500 msec after injection ends **Injector 2:** 500 msec after injection ends

Table A-8: Microplate Selection Guidelines for the Injector

Read Mode	Microplate Type	Other Considerations
Luminescence (LUM), top read	Solid white If luminescence crosstalk is high, then using a black microplate can improve sensitivity.	When an application specifies a surface treatment, use only microplates with the correct treatment. For reads with injection, microplates must be unlidded.
Fluorescence Intensity (FL), bottom read	Black-sided, clear bottomed	When an application specifies a surface treatment, use only microplates with the correct treatment.

Note: White microplates provide significantly higher signal for luminescence than black microplates, and are recommended if high sensitivity is required. However, white microplates can exhibit some detectable phosphorescence that increases background after being exposed to light (in particular under neon lights). For maximum sensitivity, Molecular Devices recommends preparing microplates under reduced ambient light conditions, and adapting the microplates to darkness for 10 to 30 minutes before measurement.

The injector and the following accessories are available to order from Molecular Devices.

Table A-9: Injector and Accessories

Part Number	Description
ID3-INJ-UPG	Injector
5055251	Bottle Holder
5044163	Waste Plate
5044164	Tubing
5044165	Bottle Adapter
Cannot order from Molecular Devices	Wide-neck bottle, HDPE 50 mL capacity 36 mm square by 68 mm high 24 mm diameter inside neck Recommended supplier: VWR (215-0440)
Cannot order from Molecular Devices	Strip wells, polystyrene 1x8, clear, flat-bottomed Recommended supplier: Greiner Bio-One (762001)

List of Compatible Solutions

Use only compatible solutions with the injector.

The following table gives a partial list of commonly used compatible and incompatible solutions for dispensing through the injector tubing or for exterior cleaning of the injector and accessories. Most reagents are compatible with the injector, as long as the components used in the solution are in the compatible list. For a complete substance compatibility list, visit the knowledge base on the Molecular Devices technical support site.

Before you use a substance that is not listed, contact Molecular Devices technical support. See Obtain Support on page 124.

CAUTION! The information in this table is based on substance-compatibility information provided by suppliers of the materials used in the injector and other reputable sources. Before you run an assay, always test the behavior of substances under the specific conditions of your application.

Table A-10: List of Compatible and Incompatible Solutions

Compatible Solutions	Do Not Use
Alcohol, Ethyl (Ethanol), 70% solution or less	Acetone
Alcohol, Isobutyl (Isobutanol), 70% solution or less	Alcohol, Benzyl (Phenylcarbinol)
Alcohol, Methyl (Methanol), 70% solution or less	Hydrochloric Acid (HCl)
Ammonia, 10% solution or less	Ketones
Sodium Hypochlorite (NaClO), 3% solution or less	Sulfuric Acid (H_2SO_4)
Water (deionized, distilled, or fresh)	Water (salt or saline)

CAUTION! Always read the label or Safety Data Sheet (SDS) to determine the actual percentage of the substance in a solution. For example, household bleach generally contains approximately 5% sodium hypochlorite, so a 50% reduction yields less than a 3% solution of NaClO.

Appendix A: System Diagrams and Dimensions

In the following drawings, the dimensions are show in centimeters and inches.

Figure A-1: Front View of the iD3 with Dimensions

Item	Description
1	Width: 53.2 cm (20.94 in.)
2	Height: 40.1 cm (15.79 in.)

Figure A-1: Front View of the iD3 with Dimensions (continued)

Item	Description
3	Height of Microplate Drawer: 9.5 cm (3.7 in.)

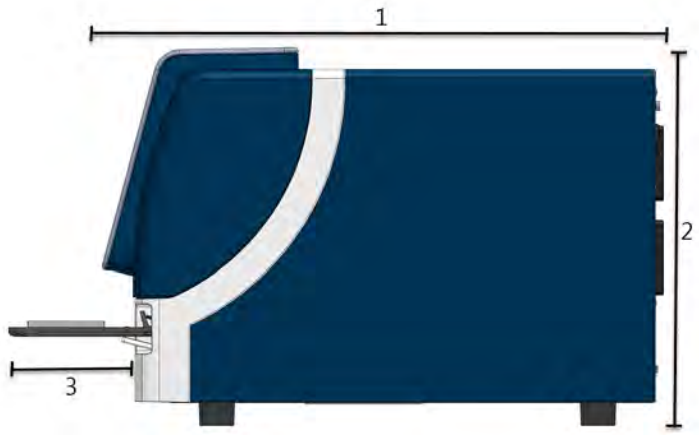
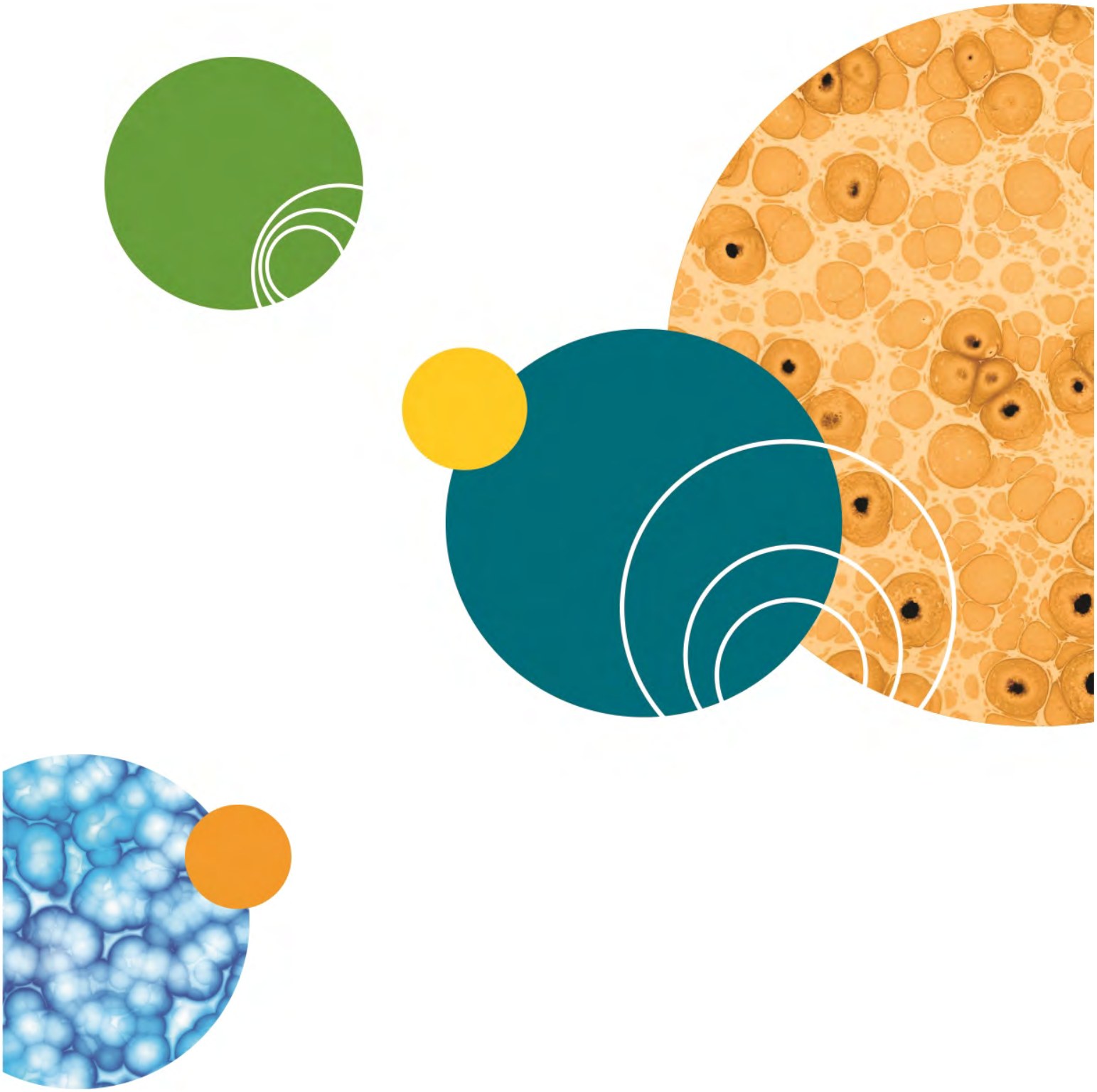


Figure A-2: Side View of the iD3 with Dimensions

Item	Description
1	Depth: 59.8 cm (23.54 in.)
2	Height: 40.1 cm (15.79 in.)
3	Maximum Length of Opened Microplate Drawer: 11 cm (4.33 in.)



Appendix B: Electromagnetic Compatibility

B

Regulatory for Canada (ICES/NMB-001:2006)

This ISM device complies with Canadian ICES-001.

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

ISM Equipment Classification (Group 1, Class A)

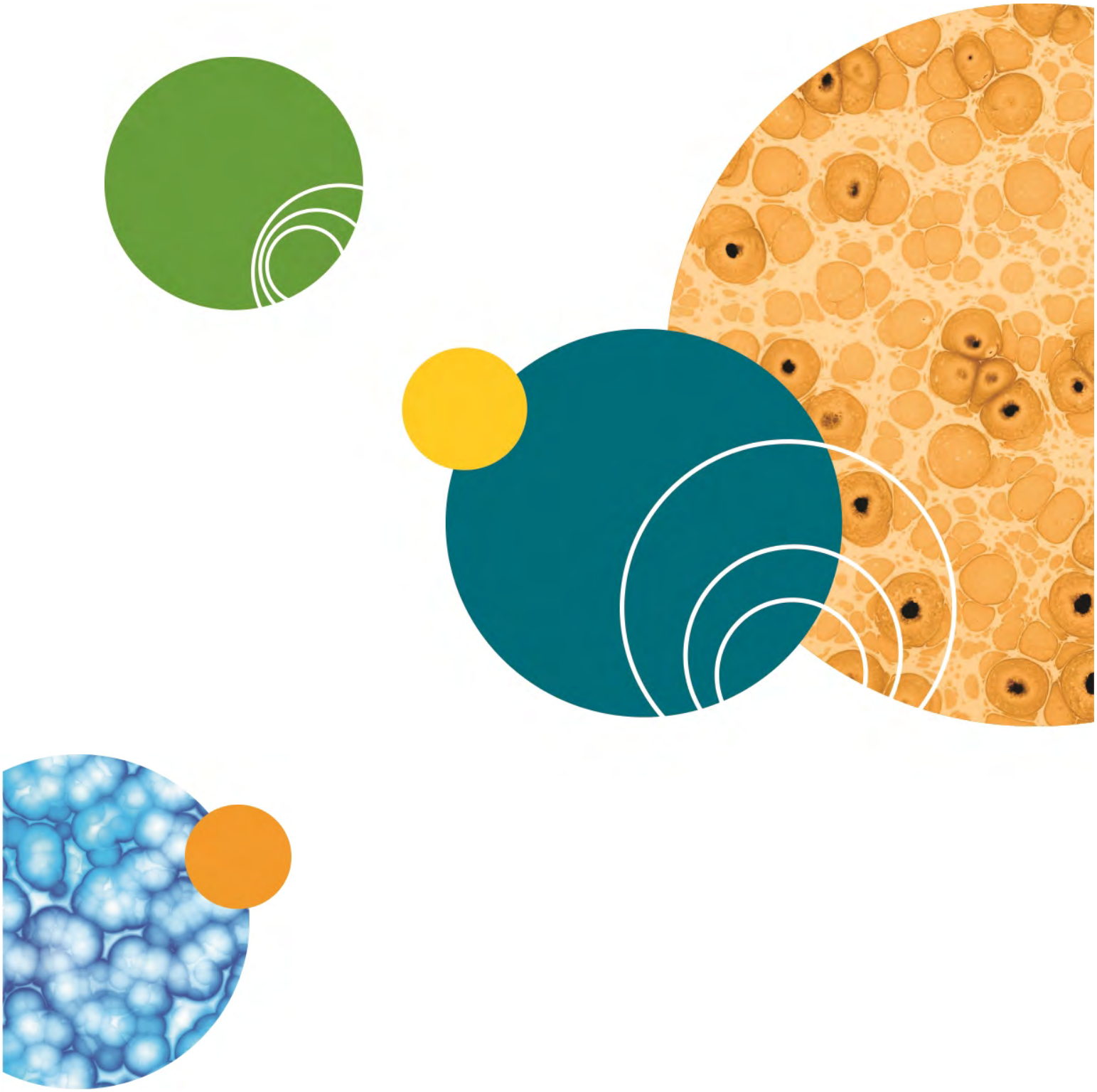
This equipment is designated as scientific equipment for laboratory use that intentionally generate and/or use conductively coupled radio-frequency energy for internal functioning, and are suitable for use in all establishments, other than domestic and those directly connected to a low voltage power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

Information to the User (FCC Notice)

This equipment has been tested and found to comply with the limits for non-consumer ISM equipment, pursuant to part 18 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a non-residential installation. This equipment generates, uses, and can radiate radio frequency energy and if not installed and used in accordance with the instructions, might cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- Reorient or relocate the receiving antenna.
- Increase the separation between the equipment and receiver.
- Connect the equipment into an outlet on a circuit different from that to which the receiver is connected.
- Consult the dealer or an experienced radio/TV technician for help.

In order to maintain compliance with FCC regulations, shielded cables must be used with this equipment. Operation with non-approved equipment or unshielded cables is likely to result in interference to radio and TV reception. The user is cautioned that changes and modifications made to the equipment without the approval of the manufacturer could void the user's authority to operate this equipment.



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Contact Us

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

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