



# SpectraMax® Paradigm®

Multi-Mode Microplate Reader

## User Guide

# Contents

<b>Safety Information</b> .....	<b>5</b>
Warnings, Cautions, Notes, and Tips .....	5
<b>Chapter 1: Introduction</b> .....	<b>11</b>
Computer Integration .....	11
Applications .....	12
Dual Photomultiplier Tubes .....	12
Plate Controls .....	12
Environmental Controls .....	13
<b>Chapter 2: Setting Up the Instrument</b> .....	<b>15</b>
Package Contents .....	15
Unpacking the Instrument .....	16
Removing Transport Locks .....	19
Connecting Instrument Cables .....	24
<b>Chapter 3: Getting Started</b> .....	<b>27</b>
Status Indicators .....	28
Removing Software Locks .....	29
Front Panel Controls and Indicators .....	30
Loading and Unloading Plates .....	31
Detection Cartridges .....	33
<b>Chapter 4: Detection Cartridges</b> .....	<b>39</b>
Absorbance Detection Cartridge .....	40
Tunable Wavelength (TUNE) Detection Cartridge .....	42
Multi-Mode (MULTI) Detection Cartridge .....	46
AlphaScreen Detection Cartridges .....	50
Cisbio HTRF Detection Cartridge .....	53
Time Resolved Fluorescence (TRF-EUSA) Detection Cartridge .....	55
Fluorescence Intensity (FI) Detection Cartridges .....	57
Fluorescence Polarization (FP) Detection Cartridges .....	59
Glow Luminescence (LUM) Detection Cartridges .....	61
Dual Color Luminescence (LUM) (BRET2) Detection Cartridge .....	64
ScanLater Western Blot (WB) Detection Cartridge .....	68

<b>Chapter 5: Read Modes and Read Types</b> .....	<b>71</b>
Supported Read Types .....	71
Absorbance Read Mode .....	74
Fluorescence Intensity Read Mode .....	79
Luminescence Read Mode .....	83
Time-Resolved Fluorescence Read Mode .....	86
FRET Read Mode .....	90
HTRF Read Mode .....	91
Fluorescence Polarization Read Mode .....	94
AlphaScreen Read Mode .....	97
ScanLater Western Blot TRF Read Mode .....	100
<b>Chapter 6: Maintenance and Troubleshooting</b> .....	<b>103</b>
Preventive Maintenance .....	103
Before You Move the Instrument .....	107
Troubleshooting .....	117
Obtaining Support .....	118
<b>Appendix A: Instrument Specifications</b> .....	<b>119</b>
Environmental Controls .....	120
<b>Appendix B: System Diagrams and Dimensions</b> .....	<b>121</b>
<b>Appendix C: Electromagnetic Compatibility</b> .....	<b>123</b>
<b>Glossary</b> .....	<b>124</b>

## Chapter 1: Introduction

# 1

The SpectraMax Paradigm Multi-Mode Microplate Reader is a user-upgradeable, multi-mode microplate reader that can perform absorbance, fluorescence, time-resolved fluorescence (including HTRF), fluorescence polarization, AlphaScreen®, AlphaLISA®, and luminescence measurements.

Detection cartridge modularity enables you to configure the system to meet your needs and provides flexibility to address future applications. You can install up to six detection cartridges in each of the two detection cartridge drawers. For information about detection cartridges, see [Detection Cartridges on page 39](#).

Depending on the application and the cartridge installed, the instrument can read 6, 12, 24, 48, 96, 384, and 1536-well plates. For micro-volume measurements, the instrument supports SpectraDrop 24-well Micro-Volume Microplates and SpectraDrop 64-well Micro-Volume Microplates. See [Plate Recommendations on page 32](#).



**CAUTION!** To prevent damage to the instrument, the height of the plate must not exceed 17 mm, including the lid if the plate is lidded.

You can integrate the SpectraMax Paradigm with the StakMax® Microplate Handling System.

### Computer Integration

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

You should install the SoftMax Pro Software on the computer before you set up the instrument. Please be aware that some updates to the SoftMax Pro Software require a purchase. Contact Molecular Devices before you update the software. To download the latest version of the software, visit:

<https://www.moleculardevices.com/products/microplate-readers/acquisition-and-analysis-software/softmax-pro-software#Order>.



**Note:** For information about the computer specifications that are required to run the software, the software installation and licensing instructions, and the directions to create the software connection between the computer and the instrument, see the *SoftMax Pro Data Acquisition and Analysis Software Installation Guide*.

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 you do not use the computer to control an instrument. You can set these options in Windows Control Panel.

## Applications

The high sensitivity and flexibility of the SpectraMax Paradigm 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 are in the Information Center and Knowledge Base on the Molecular Devices web site at [www.moleculardevices.com](http://www.moleculardevices.com).

## Dual Photomultiplier Tubes

The SpectraMax Paradigm is equipped with two photomultiplier tubes (PMTs). The dual PMTs let the instrument measure two separate emissions successively or simultaneously, resulting in faster read times and increased throughput.

## Plate Controls

Plate controls include Shake and On-the-Fly Detection. The instrument can also detect the height and position of a plate in the plate drawer.

### Shake

The Shake feature of the instrument permits the contents of the wells in a plate to be mixed inside the plate chamber before each read cycle, making it possible to do kinetic analysis of solid-phase, enzyme-mediated reactions.

The following shake settings are available:

- **Intensity:** Low, Medium, or High. The actual shake speed is based on the plate format.
- **Direction:** Linear or Orbital patterns.
- **Duration:** Length of time in seconds (1 to 60).

You should use the shake feature for ELISAs and other solid-phase, enzyme-mediated reactions to enhance accuracy.

### On-the-Fly Detection

Some detection cartridges support On-the-Fly Detection that enable the instrument to read plates as the plate drawer moves within the chamber instead of pausing the plate drawer to read each well. This results in shorter read times.

There are two On-the-Fly Detection modes:

- **Performance** - A faster read time than not using On-the Fly Detection. This setting is not as fast as Speed in order to provide considerably better results than Speed for demanding assays.
- **Speed** - The fastest possible read time per plate. There is a trade-off between the data quality and read speed because Speed samples each well for shorter integration times.

The following table compares the read time for different plate types in each of the On-the-Fly detection modes. These read times do not include the time needed for the plate 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.

#### Plate Read Times For On-The-Fly Detection ( $\pm 5$ seconds)

Mode	96-Well	384-Well	1536-Well
Optimized for speed	12 seconds	25 seconds	50 seconds
Optimized for performance	20 seconds	40 seconds	80 seconds

#### Plate Height Sensing

You can use plates up to a height of 25 mm. A sensor detects the height of a plate and confirms that the height is consistent with the plate type you select in the software and that you positioned the plate properly on the plate drawer.



**CAUTION!** To prevent damage to the instrument, the height of the plate must not exceed 17 mm, including the lid if the plate is lidded.

## Environmental Controls

The instrument environmental controls include temperature regulation and a gas inlet.

### Temperature Regulation

The instrument can maintain the temperature inside the plate chamber at ambient plus 5°C (7.2°F) above ambient to 45°C (113°F)  $\pm 1^\circ\text{C}$  up to 45°C. When you use a detection cartridge that has a flash lamp, the instrument can maintain the temperature at ambient plus 5°C  $\pm 1^\circ\text{C}$  up to 45°C. You can use the instrument touchscreen or the software to set and control the temperature. See the *SoftMax Pro Data Acquisition and Analysis Software User Guide* or the application help.



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

## Gas Inlet

The gas inlet enables you to apply partial pressure of CO<sub>2</sub>, nitrogen, or other gas inside the plate chamber. This is useful for a cell-based assay in which you need to control the CO<sub>2</sub> environment to keep cell cultures alive. Neither the instrument nor the software controls or monitors the gas supply.



**Note:** The combination of temperature and CO<sub>2</sub> environment does not create a true CO<sub>2</sub> incubator environment in the instrument.

---

Use a compressed gas supply in a well-ventilated area. The instrument is not air-tight. Gas can escape into the atmosphere surrounding the instrument. When you use potentially toxic gas, observe the cautionary procedures your safety officer defines to maintain a safe work environment.

## Chapter 3: Getting Started

# 3

Now that you installed the SoftMax Pro Software software on the computer, unpacked the instrument, removed the physical transport locks, and connected the cables, it is time to get started. The power switch and power connection are on the rear of the instrument.

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

To turn the instrument on or off, press the power switch to place the rocker in the on or off position.



### Power Switch

Item	Description
1	Power switch
2	On
3	Off



## Status Indicators

The color and activity of the LED status indicators on the front of the instrument provide information about the instrument status.

### LED Status Indicators

Color Bar Activity	Instrument Status
Green LED - solid glow	The instrument is in the ready state.
Orange LED - blinking	The instrument is not ready due to an error occurring during initialization. View the error message in the software.
Green LED - solid glow Orange LED - solid glow or blinking	The instrument is communicating with the software. Optics, drawers, or other mechanical items are moving within the instrument.
Red LED - solid glow	The front panel is removed from a detection cartridge drawer, or the plate chamber door is not closed properly.
All LEDs - circulating horizontally	The instrument is performing a read operation.
Amber LED	The instrument drawers software lock is engaged. You must remove the software lock before you install detection cartridges.

### Standby Mode

When you power on the instrument, the Standby button on the lower right front of the instrument is illuminated to indicate the instrument is in Standby mode.

- In Standby mode, the Standby button is illuminated and all LEDs on the status indicator panel turn off. Power is removed from the internal components to prevent movement or operation of the instrument.
- In Operation mode, the Standby button is not illuminated and the LEDs on the status indicator panel turn on. Power is applied to the internal components to permit normal operation of the instrument.

Press the Standby button to place the instrument in Operation mode.

When the instrument is not in use, you can power off the instrument or you can press the Standby button a second time to place the instrument back into Standby mode.

## Removing Software Locks

At this point you have removed the physical transport locks, installed the SoftMax Pro Software, connected the cables, and powered on the instrument. As a safety precaution, the SoftMax Pro Software controls internal locks to prevent the drawers from opening until the software detects and initializes the instrument.



**CAUTION!** The instrument can be damaged if you do not remove all physical transport locks before you use the software to unlock the drawers and initialize the instrument.

---



**Note:** Do not install detection cartridges into the instrument until after you complete the following instructions to unlock the drawers and initialize the instrument.

---

To remove the software locks and initialize the instrument:

1. If the Standby button on the front lower-right corner of the instrument is illuminated, press the **Standby** button to take the instrument out of standby mode.  
The LEDs on the status panel flash and turn off, and then the amber LED turns on to indicate that the instrument drawers are locked.
2. Start the SoftMax Pro Software. See the *SoftMax Pro Data Acquisition and Analysis Software Installation Guide*.  
The amber LED on the status panel turns off and the green LED turns on to indicate a successful connection between the instrument and the computer.
3. After the computer and the instrument successfully connect, the Instrument Unlocking Procedure wizard displays.
4. If the Instrument Unlocking Procedure wizard does not display, use the Instrument Connection dialog to select and connect to the instrument.



**Note:** If the instrument does not appear in the Available Instruments list on the Instrument Connections dialog, click **Refresh** above the list. See the *SoftMax Pro Data Acquisition and Analysis Software Installation Guide*.

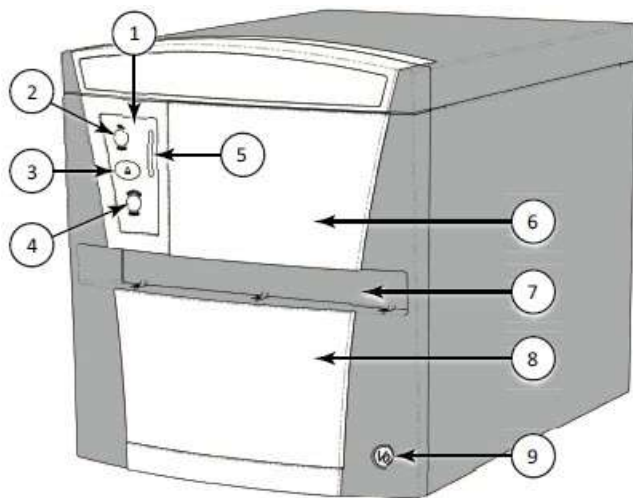
---

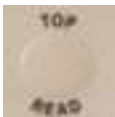


5. Follow the prompts in the Instrument Unlocking Procedure wizard to unlock the drawers and initialize the instrument.

After you complete the Instrument Unlocking Procedure wizard, the instrument does an initialization procedure that moves the optics and plate drawers to home positions. The green LED on the status panel turns on, the amber LED flashes and turns off, and then the green LED remains on. You can now install detection cartridges in the instrument.

## Front Panel Controls and Indicators


The instrument front panel has three buttons and an LED status indicator that provides information about instrument status. The buttons enable you to open and close the detection cartridge drawers and the plate drawer.



Item	Description
1	Key pad
2	 - Open and close top read detection cartridge drawer
3	 Open and close plate drawer
4	 Open and close bottom read detection cartridge drawer
5	Status LEDs
6	Top read detection cartridge drawer
7	Plate drawer
8	Bottom read detection cartridge drawer
9	Standby button

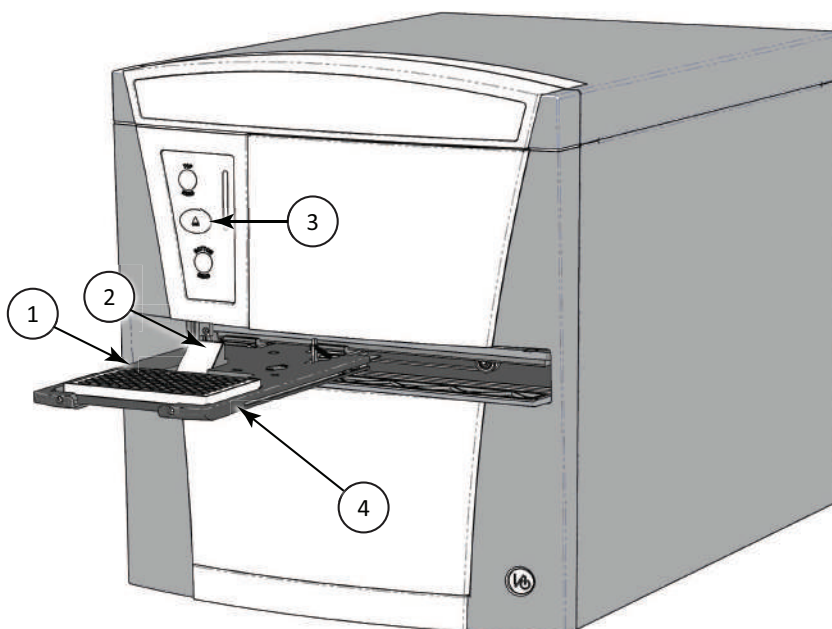
## Loading and Unloading Plates

To load or unload a plate:


1. On the front panel of the instrument, press  to open the plate drawer.
2. Place the plate on or remove it from the plate drawer.




**Note:** You can place plates on the plate drawer in either landscape or portrait orientation.



**Plate Drawer With Plate Loaded in Landscape Orientation**

Item	Description
1	Well A1 on the plate in landscape orientation
2	Plate height sensor
3	 - Press to open or close the plate drawer
4	Plate drawer

3. Press  to close the plate drawer.



**CAUTION!** To prevent damage to the instrument, set the plate height and read height accurately before you start a read. See the *SoftMax Pro Data Acquisition and Analysis Software User Guide* or the application help.

## Plate Orientation

Insert the plate in the orientation that matches the orientation you select in the software. See the *SoftMax Pro Data Acquisition and Analysis Software User Guide* or the application help.

- Select **Landscape** to put the A1 location in the upper-left corner closest to the instrument.
- Select **Portrait** to put the A1 location in the upper-right corner closest to the instrument.
- Select **Opposite Landscape** to put the A1 location in the lower-right corner farthest from the instrument.
- Select **Opposite Portrait** to put the A1 location in the lower-left corner farthest from the instrument.

## Plate Recommendations

Depending on the application, the instrument can read 6, 12, 24, 48, 96, and 384-well plates and strip wells. For micro-volume measurements, the instrument supports SpectraDrop 24-well Micro-Volume Microplates and SpectraDrop 64-well Micro-Volume Microplates. The instrument is capable of reading 1536-well microplates when used with specific detection cartridges.

The SoftMax Pro Software includes a plate list and you can use the software to define additional plate types with the manufacturer's specifications for well size, spacing, and distance from the plate edge.

The type of plate and the way you handle it can have an effect on the measurement performance of the instrument. Select a plate type with properties suited for the application and for use with multi-mode microplate readers.



**CAUTION!** To prevent damage to the instrument, the height of the plate must not exceed 17 mm, including the lid if the plate is lidded.

---

General plate handling guidelines:

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

## Detection Cartridges

The SpectraMax Paradigm supports user-installable detection cartridges to expand its read capabilities. Each detection cartridge contains the light source, optics, and electrical components needed to do specific read modes.

Application notes with specific application protocol suggestions are in the Information Center and Knowledge Base on the Molecular Devices web site at [www.moleculardevices.com](http://www.moleculardevices.com).

You install detection cartridges in either the top read detection cartridge drawer or the bottom read cartridge detection drawer. There are six slots in each of the two detection cartridge drawers. A detection cartridge occupies one or more slots. Installation and removal of a detection cartridge is the same regardless of the number of slots it occupies or whether you install it in the top or bottom detection cartridge drawer.



**CAUTION!** To prevent dust from collecting inside the instrument, the detection cartridge drawer should be open only for as long as necessary to install or remove detection cartridges. Keep the detection cartridge drawer closed whenever possible.

---

### Installing Detection Cartridges

When you install detection cartridges in the instrument, the SoftMax Pro Software detects the cartridge and displays the related data acquisition settings on the Settings dialog and enables the applicable read modes. There are six slots available to install detection cartridges in each of the two detection cartridge drawers.



**Note:** When you use the software in offline mode, all detection cartridges display on in the Settings dialog.

---



**CAUTION!** To prevent damage to the installed detection cartridges and the instrument, do not manually slide the detection cartridge drawer in or out when the instrument is powered on or when one or more detection cartridges are installed in the drawer.

---





**Note:** Some detection cartridges occupy more than one slot, and some cannot be installed in the rear-most slot. For the installation requirements for a specific detection cartridge, see [Detection Cartridges on page 39](#).

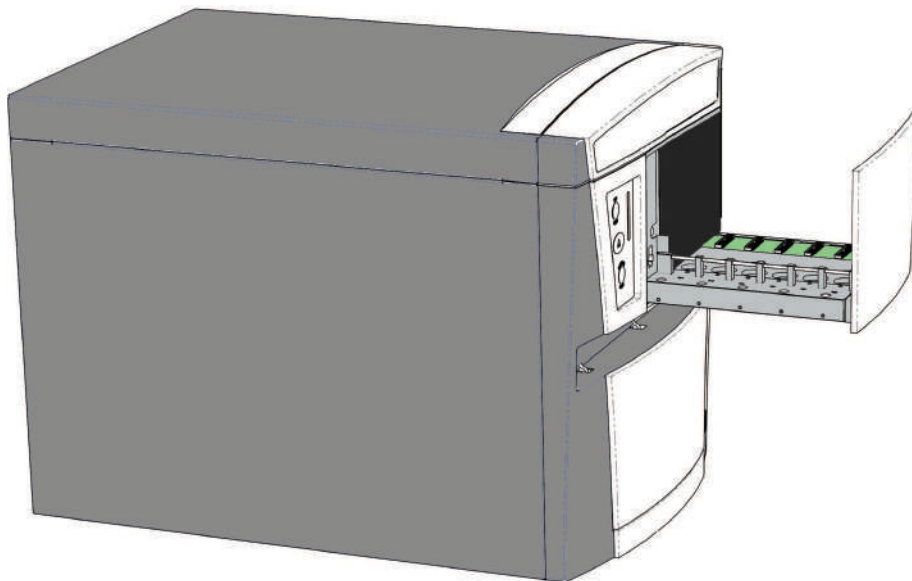
---

To install a detection cartridge:

1. On the instrument front panel, press:

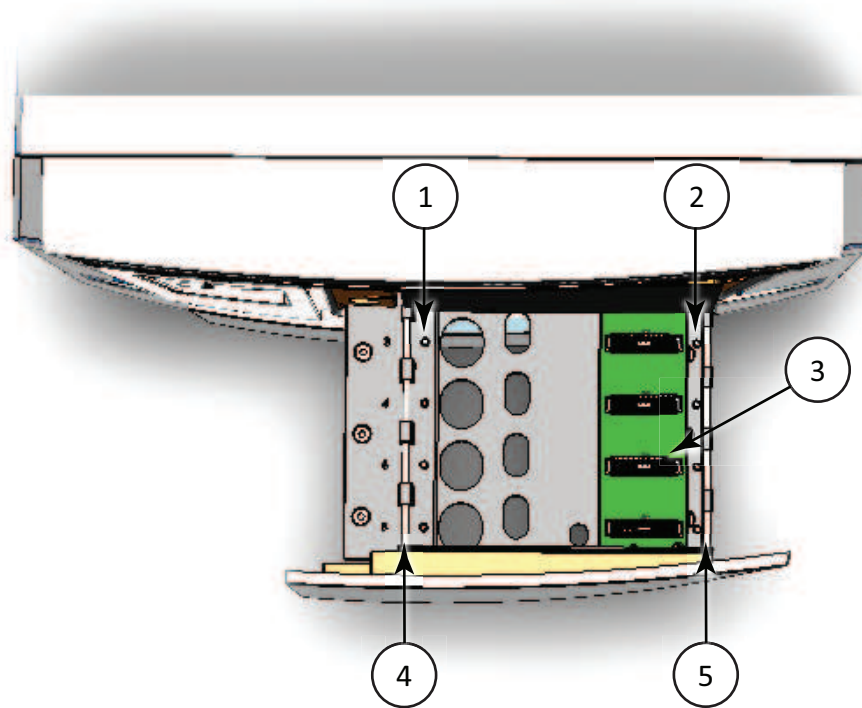
-  to open the top detection cartridge drawer.

-  to open the bottom detection cartridge drawer.



2. Remove the red cap from the detection cartridge.

- Position the two small holes and the connector pins on the detection cartridge over the holder pins and connector for the detection cartridge slot.



**Top View of Detection Cartridge Drawer**

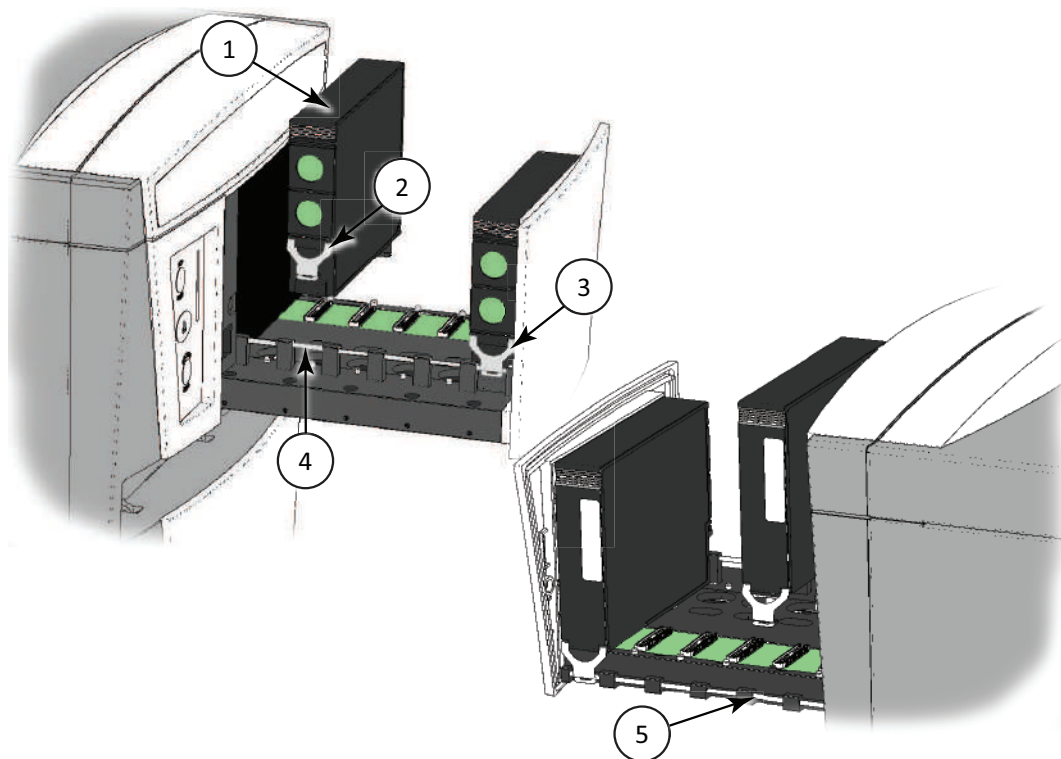
Item	Description
1	Holder pin
2	Holder pin
3	Detection cartridge connector
4	Retaining rod
5	Retaining rod

- Gently but firmly push the detection cartridge onto the holder pins and connector so that the detection cartridge is fully seated in the detection cartridge slot.
- Push the two retaining clips on either side of the detection cartridge so that they fasten to the retaining rods on each side of the detection cartridge drawer.

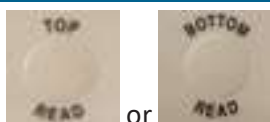


**Note:** Detection cartridges that occupy more than one slot have two retaining clips on each side. Fasten all retaining clips to the retaining rods.





Detection Cartridge Retaining Clips and Retaining Rods	
1	Detection cartridge
2	Retaining clip unattached
3	Retaining clip attached
4	Retaining rod
5	Retaining rod



6. Press  or  to close the detection cartridge drawer.

7. Start the SoftMax Pro Software and connect to the instrument.

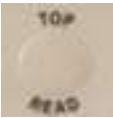
If the software is running and connected to the instrument, select the Operations tab and click **Refresh** to let the software detect the detection cartridges.


## Removing Detection Cartridges

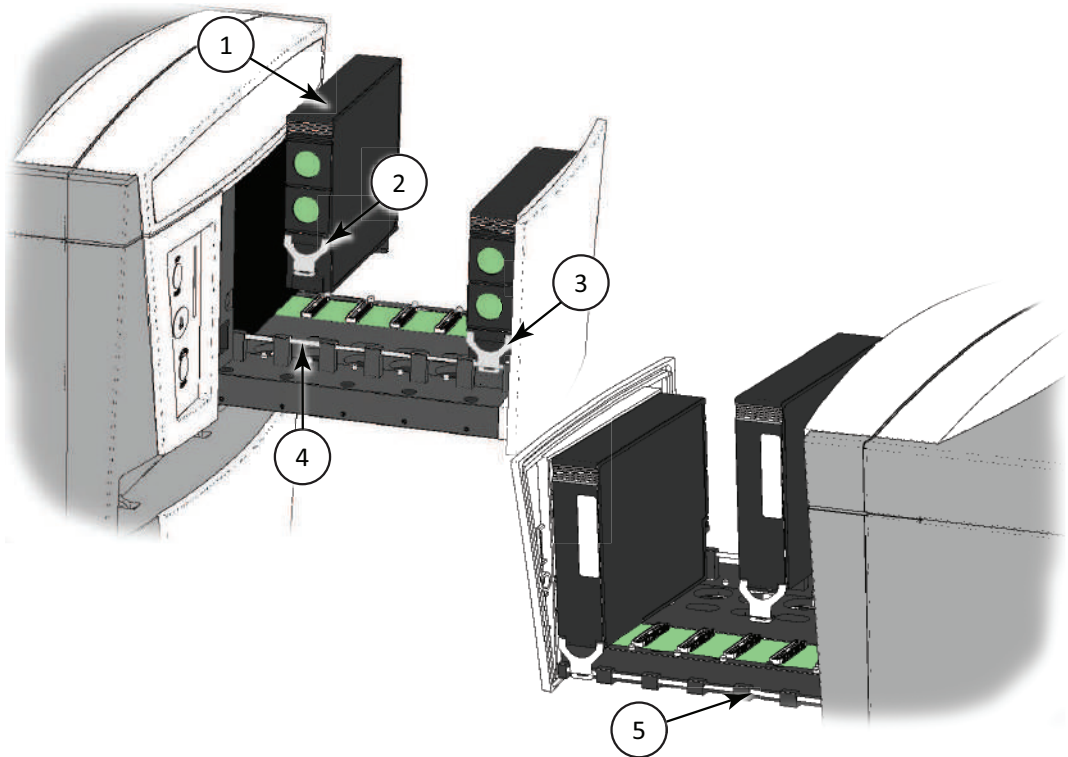
You do not need to remove a detection cartridge when it is not in use. You must remove detection cartridges when you pack the instrument for shipment. Store each detection cartridge in the box in which it was shipped in a dry, dust-free, controlled environment.

To remove a detection cartridge from the instrument:

1. On the instrument front panel, press:

-  to open the top detection cartridge drawer.

-  to open the bottom detection cartridge drawer.



Item	Detection Cartridge Retaining Clips and Retaining Rods
1	Detection cartridge
2	Retaining clip unattached
3	Retaining clip attached
4	Retaining rod
5	Retaining rod

2. Place the end of a flat head screwdriver in the slot on the retaining clip and use it as a lever to unfasten the retaining clips on either side of the detection cartridge.



**Note:** Detection cartridges that occupy more than one slot have two retaining clips on each side. Unfasten all retaining clips from the retaining rods.

---



**Note:** When you remove a detection cartridge from the bottom detection cartridge drawer, keep a firm hold of the detection cartridge. When all retaining clips are released, the detection cartridge drops out.

---

3. Lift the detection cartridge straight up or pull it down off of the connector and holder pins on the detection cartridge slot.
4. Place the red cap on the detection cartridge.
5. Press **Top Read** or **Bottom Read** to close the detection cartridge drawer.

## Chapter 4: Detection Cartridges

The SpectraMax Paradigm supports user-installable detection cartridges expand its read capabilities. Each detection cartridge contains the light source, optics, and electrical components needed to do specific read modes.

Application notes with specific application protocol suggestions are in the Information Center and Knowledge Base on the Molecular Devices web site at [www.moleculardevices.com](http://www.moleculardevices.com).

For information about detection cartridges, see the following topics:

- Absorbance Detection Cartridge, see page 40
- Tunable Wavelength (TUNE) Detection Cartridge, see page 42
- Multi-Mode (MULTI) Detection Cartridge, see page 46
- AlphaScreen Detection Cartridges , see page 50
- Cisbio HTRF Detection Cartridge on page 53
- Time Resolved Fluorescence (TRF-EUSA) Detection Cartridge, see page 55
- Fluorescence Intensity (FI) Detection Cartridges, see page 57
- Fluorescence Polarization (FP) Detection Cartridges, see page 59
- Glow Luminescence (LUM) Detection Cartridges, see page 61
- Dual Color Luminescence (LUM) (BRET2) Detection Cartridge, see page 64
- ScanLater Western Blot (WB) Detection Cartridge on page 68

Contact Molecular Devices to have custom detection cartridges designed to meet the specific needs of your application.

## Absorbance Detection Cartridge

The Absorbance Detection Cartridge combines wavelength scanning and a broad spectrum wavelength range necessary to address a variety of nucleic acids, proteins, ELISAs, and immunoassays found in the laboratory. The Absorbance Detection Cartridge uses a monochromator to do absorbance endpoint, kinetic, well scan, and spectrum read types (wavelength scanning) measurements.

The Absorbance Detection Cartridge consists of two components: a detection component (ABS-DET) which measures the absorbance, and an excitation component (ABS-MONO) which sets the absorbance wavelengths. The detection component is installed in the top read detection cartridge drawer and occupies one (1) detection cartridge slot. The excitation component is installed in the bottom read detection cartridge drawer and occupies two (2) detection cartridge slots. The two components must be installed to do absorbance measurements with the SpectraMax Paradigm Multi-Mode Microplate Reader.

### Typical Applications

- Nucleic Acid Quantitation
- Protein Quantitation
- ELISA
- Immunoassay
- Proliferation/Viability

### Absorbance

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

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

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

The Absorbance Detection Cartridge uses a monochromator with wavelength range of 230 nm to 1000 nm and a 4 nm bandwidth to measure the absorbance of samples.

### Plate Recommendations

#### Plate Recommendations for the Absorbance Detection Cartridge

Read Mode	Plate Type	Other Considerations
Absorbance (ABS)	all clear, clear bottom, UV clear, flat bottom	When an application specifies a surface treatment, use only plates with the correct treatment. Molecular Devices recommends using an unidided plate for absorbance measurements.

## Measurement Specifications

### Measurement Specifications for the Absorbance Detection Cartridge

Item	Description
Detection cartridge name	Absorbance Detection Cartridge
Short name	ABS-MONO, ABS-DET
Part number	0200-7000
Weight	ABS-MONO: 2.4 lbs. (1.1 kg) ABS-DET: 0.9 lbs. (0.4 kg)
Read Modes	Absorbance (ABS)
Type	NA
Number of slots	ABS-MONO: 1 top ABS-DET: 2 bottom
Wavelength range	230 nm to 1000 nm Bandwidth: 4 nm
Wavelength Scan Speed	35 nm per second
Wavelength Accuracy	$\pm 1.5$ nm
Wavelength Repeatability	$\pm 0.5$ nm
Photometric Range	0 OD to 3.5 OD
Photometric Resolution	0.0001 OD
Photometric Accuracy at 2 OD, 405 nm	$\pm(2\% + 0.010)$ OD
Photometric Precision at 2 OD, 405 nm	$\pm(1\% + 0.005)$ OD

## Tunable Wavelength (TUNE) Detection Cartridge

The Tunable Wavelength (TUNE) Detection Cartridge enables several detection modes, including:

- [Fluorescence Intensity on page 42](#)
- [Time-Resolved Fluorescence on page 43](#)
- [Luminescence on page 43](#)

The spectral optimization feature of the Tunable Wavelength (TUNE) Detection Cartridge can help to get the maximum signal-to-background ratio for most fluorophores or luminescence labels that are compatible with the wavelength ranges.

Using a Spectrum read, you can define a fixed excitation wavelength and scan the emission wavelengths, or define a fixed emission wavelength and scan the excitation wavelengths. The spectral optimization helps to get the maximum signal-to-background ratio from a fluorescent or fluorescently labeled analyte. For more information on using the spectral optimization feature, see the *SoftMax Pro Data Acquisition and Analysis Software User Guide* or the application help.

The Tunable Wavelength (TUNE) Detection Cartridge occupies three slots in the detection cartridge drawer. You can install this cartridge in either the top detection cartridge drawer for top reads, or in the bottom detection cartridge drawer for bottom reads.



**Note:** You should install the Tunable Wavelength (TUNE) Detection Cartridge in the top detection cartridge drawer when you run time-resolved fluorescence or glow luminescence read modes.

---

The cartridge supports the following read types:

- [Endpoint Read Type on page 71](#)
- [Kinetic Read Type on page 71](#)
- [Well Scan Read Type on page 72](#)
- [Spectrum Read Type on page 72](#)

### Fluorescence Intensity

In fluorescence intensity read mode, the source light is directed through a tunable excitation filter and then focused by an objective lens from above or below the plate. The filter passes only the specified wavelength band necessary to excite samples. The objective lens collects the resulting fluorescence and directs it through a tunable emission filter to separate background light from the specific wavelengths generated by samples. This signal is detected by a photomultiplier tube.

The Tunable Wavelength (TUNE) Detection Cartridge contains filter sets for measuring fluorescence intensity of most fluorophores that are compatible with the available wavelength ranges. It uses high-power LEDs and tunable filters together with the photon counting detection of the instrument, resulting in excellent detection limits and linear dynamic range at short read times.

Using a Spectrum read, you can define a fixed excitation wavelength and scan the emission wavelengths, or define a fixed emission wavelength and scan the excitation wavelengths. The spectral optimization helps to get the maximum signal-to-background ratio from a fluorescent or fluorescently labeled analyte. For more information on using the spectral optimization feature, see the *SoftMax Pro Data Acquisition and Analysis Software User Guide* or the application help.

## Time-Resolved Fluorescence

Time-Resolved Fluorescence is a measurement technique that depends on three characteristics that lead to better discrimination between the specific signal, proportional to the quantity of label, and the unspecific fluorescence resulting from background and compound interference:

- Pulsed excitation light sources
- Time-gated electronics faster than the fluorescence lifetime
- Labels with prolonged fluorescence lifetime

The time-gating electronics introduce a delay between the cutoff of each light pulse and the start of signal collection. During the delay, the unspecific fluorescence (caused by test compounds, assay reagents, and the plate) vanishes, while only a small portion of the specific fluorescence from the label is sacrificed. Enough of the specific signal remains during the decay period, with the added benefit of reduced background.

The Tunable Wavelength (TUNE) Detection Cartridge uses an ultraviolet LED that emits in the range between 350 nm and 380 nm for excitation of europium chelates, and comes equipped with emission filters for europium. This enables single-label europium assays to be run.



**Note:** Europium cryptate and terbium require excitation wavelengths below 330 nm and cannot be measured with this detection cartridge.

---

## Luminescence

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.

The Tunable Wavelength (TUNE) Detection Cartridge contains the components for measuring the light intensity from luminescence. Since the light is emitted as a result of a chemical reaction, no excitation light and no excitation filters are required to measure luminescence. The luminescence can be measured with no emission filters, or an emission wavelength selected from within the specified range.



**Note:** This detection cartridge is not suitable for measurement of flash luminescence reactions, which require injectors.

---



## Plate Recommendations

### Plate Recommendations for the Tunable Wavelength (TUNE) Detection Cartridge

Read mode	Plate type	Other considerations
Fluorescence Intensity (FL) top read	Solid black	When an application specifies a surface treatment, use only plates with the correct treatment.
Fluorescence Intensity (FL) bottom read	Solid black, clear bottom	When an application specifies a surface treatment, use only plates with the correct treatment.
Time-Resolved Fluorescence (TRF)	Solid white	When an application specifies a surface treatment, use only plates with the correct treatment.
Luminescence (LUM)	Solid white	When an application specifies a surface treatment, use only plates with the correct treatment. You should use an unlidded plate for luminescence measurements.



**Note:** White plates provide significantly higher signal for luminescence than black plates, and are recommended if high sensitivity is required. However, white plates can exhibit some detectable phosphorescence that increases background after being exposed to light (in particular under neon lights). For maximum sensitivity, you should prepare plates under reduced ambient light conditions, and place the plate inside the instrument for 1 to 10 minutes to adapt the plates to darkness before you start the read.

## Measurement Specifications

### Measurement Specifications for the Tunable Wavelength (TUNE) Detection Cartridge

Item	Description
Detection cartridge name	Tunable Wavelength (TUNE) Detection Cartridge
Short name	TUNE
Part number	0200-7050
Weight	3.1 lbs (1.4 kg)
Read Modes	Fluorescence Intensity (FL) Time-Resolved Fluorescence (TRF) Luminescence (LUM)
Type	Single emission
Number of slots	3 slots, top or bottom
Light source	High-power LEDs

**Measurement Specifications for the Tunable Wavelength (TUNE) Detection Cartridge  
(continued)**

Item	Description
Wavelength range	EX: 360 nm to 790 nm EM: 400 nm to 850 nm Wavelength Increment: 1 nm Wavelength Accuracy: $\pm 2$ nm Wavelength Repeatability: 1 nm Luminescence can be used with no EM filter
Labels	Labels that are compatible with the wavelength ranges specified in the filter set above.
Detection Limit, Top optimized	<b>Fluorescence Intensity (Fluorescein):</b> 96-well (200 $\mu$ L): 0.100 fmol/well (0.5 pM) 384-well (75 $\mu$ L): 0.038 fmol/well (0.5 pM) 1536-well (8 $\mu$ L): 0.012 fmol/well (1.5 pM) <b>Time-Resolved Fluorescence (Europium):</b> 96-well (200 $\mu$ L): 30 amol/well (0.15 pM) 384-well (100 $\mu$ L): 10 amol/well (0.1 pM) 1536-well (8 $\mu$ L): 2.4 amol/well (0.3 pM) <b>Luminescence (ATP):</b> 96-well (200 $\mu$ L): 4.0 fmol/well (20 pM) 384-well (50 $\mu$ L): 2.5 fmol/well (50 pM)
Detection Limit, Top guaranteed	<b>Fluorescence Intensity (Fluorescein):</b> 96-well (200 $\mu$ L): 0.300 fmol/well (1.5 pM) 384-well (75 $\mu$ L): 0.225 fmol/well (3.0 pM) 1536-well (8 $\mu$ L): 0.048 fmol/well (6.0 pM) <b>Time-Resolved Fluorescence (Europium):</b> 96-well (200 $\mu$ L): 100 amol/well (0.5 pM) 384-well (100 $\mu$ L): 50 amol/well (0.5 pM) 1536-well (8 $\mu$ L): 20 amol/well (2.5 pM) <b>Luminescence (ATP):</b> 96-well (200 $\mu$ L): 16 fmol/well (80 pM) 384-well (50 $\mu$ L): 7.5 fmol/well (150 pM)
Linear dynamic range, Top	Fluorescein: >6 logs in a single plate read

## Multi-Mode (MULTI) Detection Cartridge

Optimized for 96-well and 384-well plates, the Multi-Mode (MULTI) Detection Cartridge enables several detection modes, including:

- [Fluorescence Intensity on page 46](#)
- [Time-Resolved Fluorescence on page 46](#)
- [Luminescence on page 47](#)

The Multi-Mode (MULTI) Detection Cartridge occupies three slots in the detection cartridge drawer. You can install this cartridge in either the top detection cartridge drawer for top reads, or in the bottom detection cartridge drawer for bottom reads.



**Note:** You should install the Multi-Mode (MULTI) Detection Cartridge in the top detection cartridge drawer when you run time-resolved fluorescence or glow luminescence read modes.

The cartridge supports the following read types:

- [Endpoint Read Type on page 71](#)
- [Kinetic Read Type on page 71](#)
- [Well Scan Read Type on page 72](#)

### Fluorescence Intensity

In fluorescence intensity read mode, the source light is directed through a tunable excitation filter and then focused by an objective lens from above or below the plate. The filter passes only the specified wavelength band necessary to excite samples. The objective lens collects the resulting fluorescence and directs it through an emission filter to separate background light from the specific wavelengths generated by samples. This signal is detected by a photomultiplier tube.

The Multi-Mode (MULTI) Detection Cartridge contains filter sets for measuring fluorescence intensity of coumarin, fluorescein, rhodamine, and Texas Red. The Multi-Mode (MULTI) Detection Cartridge uses high-power LEDs and optimized filter sets together with the photon counting detection of the instrument, resulting in excellent detection limits and linear dynamic range at short read times.

### Time-Resolved Fluorescence

Time-Resolved Fluorescence is a measurement technique that depends on three characteristics that lead to better discrimination between the specific signal, proportional to the quantity of label, and the unspecific fluorescence resulting from background and compound interference:

- Pulsed excitation light sources
- Time-gated electronics faster than the fluorescence lifetime
- Labels with prolonged fluorescence lifetime

The time-gating electronics introduce a delay between the cutoff of each light pulse and the start of signal collection. During the delay, the unspecific fluorescence (caused by test compounds, assay reagents, and the plate) vanishes, while only a small portion of the specific fluorescence from the label is sacrificed. Enough of the specific signal remains during the decay period, with the added benefit of reduced background.

The Multi-Mode (MULTI) Detection Cartridge uses an ultraviolet LED that emits in the range between 350 nm and 380 nm for excitation of europium chelates, and comes equipped with emission filters for europium. This enables single-label europium assays to be run.



**Note:** Europium cryptate and terbium require excitation wavelengths below 330 nm and cannot be measured with this detection cartridge.

## Luminescence

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.

The Multi-Mode (MULTI) Detection Cartridge contains the components for measuring the light intensity from luminescence. Since the light is emitted as a result of a chemical reaction, no excitation light and no excitation filters are required to measure luminescence. The luminescence can be measured with no emission filters, or an emission wavelength selected from within the specified range.



**Note:** This detection cartridge is not suitable for measurement of flash luminescence reactions, which require injectors.

## Plate Recommendations

### Plate Recommendations for the Multi-Mode (MULTI) Detection Cartridge

Read mode	Plate type	Other considerations
Fluorescence Intensity (FL) top read	Solid black	When an application specifies a surface treatment, use only plates with the correct treatment.
Fluorescence Intensity (FL) bottom read	Solid black, clear bottom	When an application specifies a surface treatment, use only plates with the correct treatment.
Time-Resolved Fluorescence (TRF)	Solid white	When an application specifies a surface treatment, use only plates with the correct treatment.
Luminescence (LUM)	Solid white	When an application specifies a surface treatment, use only plates with the correct treatment. You should use an unidded plate for luminescence measurements.



**Note:** White plates provide significantly higher signal for luminescence than black plates, and are recommended if high sensitivity is required. However, white plates can exhibit some detectable phosphorescence that increases background after being exposed to light (in particular under neon lights). For maximum sensitivity, you should prepare plates under reduced ambient light conditions, and place the plate inside the instrument for 1 to 10 minutes to adapt the plates to darkness before you start the read.

## Measurement Specifications

### Measurement Specifications for the Multi-Mode (MULTI) Detection Cartridge

Item	Description
Detection cartridge name	Multi-Mode (MULTI) Detection Cartridge
Short name	MULTI
Part number	0200-7001
Weight	3.1 lbs (1.4 kg)
Read Modes	Fluorescence Intensity (FL) Time-Resolved Fluorescence (TRF) Luminescence (LUM)
Type	Single emission
Number of slots	3 slots, top or bottom
Light source	High-power LEDs TRF: pulse period = 10 X pulse length
Filter set	<b>Coumarin:</b> EX: 360-35; EM: 465-35 <b>Fluorescein:</b> EX: 485-20; EM: 535-25 <b>Rhodamine:</b> EX: 535-35; EM: 595-35 <b>Texas Red:</b> EX: 585-10; EM: 635-35 <b>Europium chelate (TRF):</b> EX: 370-80; EM: 616-10
Labels	<b>Fluorescence Intensity:</b> Coumarin, Fluorescein, Rhodamine, Texas Red <b>Time-Resolved Fluorescence:</b> Europium Chelate

**Measurement Specifications for the Multi-Mode (MULTI) Detection Cartridge (continued)**

Item	Description
Detection limit, Top optimized	<p><b>Fluorescence Intensity (Fluorescein):</b>            96-well (200 <math>\mu</math>L): 150 amol/well (0.75 pM)            384-well (75 <math>\mu</math>L): 100 amol/well (1.3 pM)</p> <p><b>Time-Resolved Fluorescence (Europium):</b>            96-well (200 <math>\mu</math>L): 20 amol/well (0.1 pM)            384-well (100 <math>\mu</math>L): 10 amol/well (0.1 pM)</p> <p><b>Luminescence (ATP):</b>            96-well (200 <math>\mu</math>L): 0.6 fmol/well (3 pM)            384-well (50 <math>\mu</math>L): 0.3 fmol/well (6 pM)</p>
Detection limit, Top guaranteed	<p><b>Fluorescence Intensity (Fluorescein):</b>            96-well (200 <math>\mu</math>L): 400 amol/well (2 pM)            384-well (75 <math>\mu</math>L): 300 amol/well (4 pM)</p> <p><b>Time-Resolved Fluorescence (Europium):</b>            96-well (200 <math>\mu</math>L): 25 amol/well (0.125 pM)            384-well (100 <math>\mu</math>L): 15 amol/well (0.15 pM)</p> <p><b>Luminescence (ATP):</b>            96-well (200 <math>\mu</math>L): 2 fmol/well (10 pM)            384-well (50 <math>\mu</math>L): 2 fmol/well (40 pM)</p>
Linear dynamic range, Top	Fluorescein: 5 logs in a single plate read

## AlphaScreen Detection Cartridges

The AlphaScreen® Detection Cartridges use a 680 nm laser diode to provide a sensitive read system for AlphaScreen assays. The design isolates each well and enables the optimal performance for AlphaScreen assays. The detection cartridges also capture the Europium emission line of AlphaLISA®.

The following AlphaScreen Detection Cartridges are available:

- AlphaScreen 384 Std Detection Cartridge
- AlphaScreen 384 HTS Detection Cartridge
- AlphaScreen 1536 HTS Detection Cartridge

Each AlphaScreen Detection Cartridge occupies one slot in the detection cartridge drawer. You can install this cartridge in either the top detection cartridge drawer for top reads, or in the bottom detection cartridge drawer for bottom reads.



**Note:** You cannot install this detection cartridge in the rear-most slot in either the top or bottom detection cartridge drawer.



**Note:** You should install the AlphaScreen Detection Cartridge in the top detection cartridge drawer when you run AlphaScreen read modes.

The cartridges support the following read types:

- [Endpoint Read Type on page 71](#)
- [Kinetic Read Type on page 71](#)
- [Well Scan Read Type on page 72](#)

ALPHASCREEN and ALPHALISA are registered trademarks of PerkinElmer, Inc.

## AlphaScreen

ALPHA stands for Amplified Luminescent Proximity Homogeneous Assay. AlphaScreen is a bead-based chemistry used to study molecular interactions between moieties A and B, for example. When a biological interaction between A and B moves beads—coated with A and B, respectively—together, a cascade of chemical reactions produce a greatly amplified signal.

An AlphaScreen measurement includes a light pulse, by turning on the laser diode for a specified time, turning off the laser diode, followed by the measurement of the AlphaScreen signal, as specified in the protocol parameters.

### AlphaScreen HTS Timing Parameters

Parameter	Value	Comment
Excitation time	40 ms	The period when the sample is exposed to the laser.
Integration time	80 ms	The period when the signal is read from the sample.

## Plate Recommendations

### Plate Recommendations for the AlphaScreen Detection Cartridge

Read Mode	Plate Type	Other Considerations
AlphaScreen (ALPHA)	Solid white	When an application specifies a surface treatment, use only plates with the correct treatment. White plates are recommended for reading AlphaScreen (such as, Optiplate-384 white opaque, Perkin Elmer #6007290). Black plates absorb light and lead to reduced signals.

## Measurement Specifications

### Measurement Specifications for the AlphaScreen Detection Cartridges

Item	Description	Description	Description
Detection cartridge name	AlphaScreen 384 Std Detection Cartridge	AlphaScreen 384 HTS Detection Cartridge	AlphaScreen 1536 HTS Detection Cartridge
Short name	ALPHA 384 Std	ALPHA 384 HTS	ALPHA 1536 HTS
Part number	0200-7017	0200-7018	0200-7019
Weight	1.5 lbs (0.7 kg)	1.5 lbs (0.7 kg)	1.5 lbs (0.7 kg)
Plate formats	Compatible with 6, 12, 24, 48, 96, and 384-well plates.	Compatible with 6, 12, 24, 48, 96, and 384-well plates.	Compatible with 6, 12, 24, 48, 96, 384, 1536-well plates.
Read Mode	AlphaScreen	AlphaScreen	AlphaScreen
Type	Single emission	Single emission	Single emission
Number of slots	1 slot, top or bottom, not in the rear-most slot	1, top or bottom, not in the rear-most slot	1, top or bottom, not in the rear-most slot
Light source	Laser diode (1 watt)	Laser diode (1 watt)	Laser diode (1 watt)
Filter Set	EX: 680 EM: 570-100	EX: 680 EM: 570-100	EX: 680 EM: 570-100
Labels	AlphaScreen and AlphaLISA reagents and assay kits	AlphaScreen and AlhpLISA reagents and assay kits	AlphaScreen and AlphaLISA reagents and assay kits
Detection limit, Top*	384-well (25 µl) <100 amol/well	384-well (25 µl) <100 amol/well	NA



\*AlphaScreen detection limit <100 amol of biotinylated-LCK-P peptide, 25  $\mu$ L/well in 384-well plate. AlphaScreen detection limit of biotinylated-LCK-P peptide was determined with 3 x SD over background method using AlphaScreen Phosphotyrosine (PT66) Assay Kit from Perkin Elmer (Cat. No. 6760602C). Serial dilutions were made into assay buffer by diluting 10 nM b-LCK-P reaction mix that contains Acceptor and Donor beads. Measurement was done after a 1 hour incubation.

## Cisbio HTRF Detection Cartridge

The Cisbio HTRF Detection Cartridge uses a high-energy Xenon flash lamp for sensitive reads of HTRF reagents. The standard dual-emission design gives the most accurate results in short overall read time. This detection cartridge enables Time-Resolved Fluorescence read modes for europium cryptate and europium acceptors.

The fluorescence ratio related to the HTRF readout is a correction method developed by Cisbio, for which Cisbio has granted a license to Molecular Devices. Its application is strictly limited to the use of HTRF reagents and technology, excluding other TR-FRET technologies such as IMAP TR-FRET calculations of acceptor to donor ratios.

The Cisbio HTRF Detection Cartridge is a dual-emission detection cartridge that occupies two slots in the detection cartridge drawer. You can install this cartridge only in the top detection cartridge drawer for top reading.

The cartridge supports the following read types:

- [Endpoint Read Type on page 71](#)
- [Kinetic Read Type on page 71](#)
- [Well Scan Read Type on page 72](#)

HTRF is a registered trademark of Cisbio Bioassays.

## Homogeneous Time-Resolved Fluorescence

Homogeneous Time-Resolved Fluorescence (HTRF) is a measurement technique based on fluorescence resonance energy transfer (FRET) that uses the advantages of Time-Resolved Fluorescence (TRF) read.

The Cisbio HTRF Detection Cartridge uses a Xenon flash lamp. The excitation filter selects wavelengths suitable for the excitation of Europium cryptates below 330 nm. Emission filters detect signal for Europium cryptate (616 nm) and typical Europium acceptor labels (665 nm). The Cisbio HTRF Detection Cartridge incorporates a dual-emission design to measure the two emission channels simultaneously, improving precision and reducing read times.

The Cisbio HTRF Detection Cartridge provides the optimal performance for key applications for drug discovery, bioprocess development, and others.

- GPCR Assays
- Cytokine Assays
- cAMP Quantitation
- Human mAb Screening
- Kinase Assays

## Plate Recommendations

### Plate Recommendations for the Cisbio HTRF Detection Cartridge

Read Mode	Plate Type	Other Considerations
Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET)	Solid white	When an application specifies a surface treatment, use only plates with the correct treatment.

## Measurement Specifications

### Measurement Specifications for the Cisbio HTRF Detection Cartridge

Item	Description
Detection cartridge name	Cisbio HTRF Detection Cartridge
Short name	HTRF
Part number	0200-7011
Weight	2.6 lbs (1.2 kg)
Read mode	Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET)
Type	Dual emission
Number of slots	2 slots, top only
Light source	Xenon Flash Lamp
Filter set	EX: HTRF EM1: 616-10 EM2: 665-10
Labels	Europium cryptate, Europium acceptors
Applications	HTRF certified by Cisbio Bioassays

## Time Resolved Fluorescence (TRF-EUSA) Detection Cartridge

The Time Resolved Fluorescence (TRF-EUSA) Detection Cartridge enables Time-Resolved Fluorescence read modes for Europium and Samarium. It is ideal for protein interaction, GPCR, and enzyme activity applications.

The Time Resolved Fluorescence (TRF-EUSA) Detection Cartridge occupies one slot in the detection cartridge drawer. You can install this cartridge in either the top detection cartridge drawer for top reads, or in the bottom detection cartridge drawer for bottom reads. However, for best results, you should install the cartridge in the top drawer and use solid black plates.



**Note:** You should install the Time Resolved Fluorescence (TRF-EUSA) Detection Cartridge in the top detection cartridge drawer for best results.

The cartridge supports the following read types:

- [Endpoint Read Type on page 71](#)
- [Kinetic Read Type on page 71](#)
- [Well Scan Read Type on page 72](#)

Well scan is not available for dual-label assays.

### Time-Resolved Fluorescence

Time-Resolved Fluorescence is a measurement technique that depends on three characteristics that lead to better discrimination between the specific signal, proportional to the quantity of label, and the unspecific fluorescence resulting from background and compound interference:

- Pulsed excitation light sources
- Time-gated electronics faster than the fluorescence lifetime
- Labels with prolonged fluorescence lifetime

The time-gating electronics introduce a delay between the cutoff of each light pulse and the start of signal collection. During the delay, the unspecific fluorescence (caused by test compounds, assay reagents, and the plate) vanishes, while only a small portion of the specific fluorescence from the label is sacrificed. Enough of the specific signal remains during the decay period, with the added benefit of reduced background.

The Time Resolved Fluorescence (TRF-EUSA) Detection Cartridge uses an ultraviolet LED that emits in the range between 350 nm and 380 nm for excitation of Europium chelates and comes equipped with emission filters for europium and samarium. This enables single-label europium and samarium assays as well as dual-label assays that involve europium and samarium to be run.



**Note:** Europium cryptate and terbium require excitation wavelengths below 330 nm and cannot be measured with this detection cartridge.

## Plate Recommendations

### Plate Recommendations: Time Resolved Fluorescence (TRF-EUSA) Detection Cartridge

Read Mode	Plate Type	Other Considerations
Time-Resolved Fluorescence (TRF)	Solid white	When an application specifies a surface treatment, use only plates with the correct treatment.

## Measurement Specifications

### Measurement Specifications: Time Resolved Fluorescence (TRF-EUSA) Detection Cartridge

Item	Description
Detection cartridge name	Time Resolved Fluorescence (TRF-EUSA) Detection Cartridge
Short name	TRF-EUSA
Part number	0200-7008
Weight	1.5 lbs (0.7 kg)
Read modes	Time-Resolved Fluorescence (TRF) Time-Resolved Fluorescence, Dual-Label (TRF-Dual)
Type	Dual emission
Number of slots	1 slot, top recommended
Light source	Ultra high power LED
Filter set	EX: 370-80 EM1: 616-10 EM2: 642-10
Labels	Europium chelate, Samarium chelate
Detection limit, Top optimized	384-well (100 $\mu$ L): 3 amol/0.03 pM 1536-well (8 $\mu$ L): 1 amol/0.125 pM
Detection limit, Top guaranteed	384-well (100 $\mu$ L): 10 amol/0.1 pM 1536-well (8 $\mu$ L): 3 amol/0.375 pM
Linear dynamic range, Top	6 logs in a single plate read

## Fluorescence Intensity (FI) Detection Cartridges

The Fluorescence Intensity (FI) Detection Cartridges use an ultra high power LED for the excitation of various fluorescent labels, enabling fluorescence intensity read modes. The standard dual-emission design enables simple, straightforward fluorescence resonance energy transfer (FRET) measurement.

The following Fluorescence Intensity (FI) Detection Cartridges are available:

- Fluorescence Intensity (FI) (coum-fluor) Detection Cartridge
- Fluorescence Intensity (FI) (fluor-rhod) Detection Cartridge
- Fluorescence Intensity (FI) (Cy3-Cy5) Detection Cartridge

Each Fluorescence Intensity (FI) Detection Cartridge occupies one slot in the detection cartridge drawer. You can install this cartridge in either the top detection cartridge drawer for top reads, or in the bottom detection cartridge drawer for bottom reads.

The Fluorescence Intensity (FI) Detection Cartridges support the following read types:

- [Endpoint Read Type on page 71](#)
- [Kinetic Read Type on page 71](#)
- [Well Scan Read Type on page 72](#)

### Fluorescence Intensity

In Fluorescence Intensity read mode, the source light is directed through an excitation filter and then focused by an objective lens from above or below the plate. The filter passes only the specified wavelength band necessary to excite samples. The objective lens collects the resulting fluorescence and directs it through an emission filter to separate background light from the specific wavelengths generated by samples. This signal is detected by a photomultiplier tube.

The Fluorescence Intensity (FI) Detection Cartridges use an ultra high power LED and an optimized filter set together with the photon counting detection capabilities of the instrument, resulting in superior detection limits and linear dynamic range at short read times.

### Plate Recommendations

#### Plate Recommendations for the Fluorescence Intensity (FI) Detection Cartridges

Read Mode	Plate Type	Other Considerations
Fluorescence Intensity (FL), top read	Solid black	When an application specifies a surface treatment, use only plates with the correct treatment.
Fluorescence Intensity (FL), bottom read	Solid black, clear bottom	When an application specifies a surface treatment, use only plates with the correct treatment.

## Measurement Specifications

### Measurement Specifications for the Fluorescence Intensity (FI) Detection Cartridges

Item	Description	Description	Description
Detection cartridge name	Fluorescence Intensity (FI) (coum-fluor) Detection Cartridge	Fluorescence Intensity (FI) (fluor-rhod) Detection Cartridge	Fluorescence Intensity (FI) (Cy3-Cy5) Detection Cartridge
Short name	FI-COFL	FI-FLRH	FI-CY3CY5
Part number	0200-7002	0200-7003	0200-7004
Weight	1.5 lbs (0.7 kg)	1.5 lbs (0.7 kg)	1.5 lbs (0.7 kg)
Read Mode	Fluorescence Intensity (FL) Fluorescence Resonance Energy Transfer (FRET)	Fluorescence Intensity (FL) Fluorescence Resonance Energy Transfer (FRET)	Fluorescence Intensity (FL) Fluorescence Resonance Energy Transfer (FRET)
Type	Dual emission	Dual emission	Dual emission
Number of slots	1 slot, top or bottom	1 slot, top or bottom	1 slot, top or bottom
Light source	Ultra high power LED	Ultra high power LED	Ultra high power LED
Filter Set	EX: 360-35 EM1: 465-35 EM2: 535-25	EX: 485-20 EM1: 535-25 EM2: 595-35	EX: 535-25 EM1: 595-35 EM2: LP655
Labels	FL: Coumarin FRET: Coumarin-Fluorescein	FL: Fluorescein FRET: Fluorescein-Rhodamine	FL: Rhodamine FRET: CY3-CY5
Detection limit, Top	384-well plate (75 $\mu$ L): 10 fmol/well 1536-well plate (8 $\mu$ L): 3 fmol/well	384-well plate (75 $\mu$ L): 0.10 fmol/well 1536-well plate (8 $\mu$ L): 0.03 fmol/well	384-well plate (75 $\mu$ L): 0.15 fmol/well 1536-well plate (8 $\mu$ L): 0.06 fmol/well
Linear dynamic range, Top	4 logs in a single plate read	6 logs in a single plate read	6 logs in a single plate read

\*Other labels compatible with the excitation and emission wavelengths of the cartridge can also be used. All trademarks are the property of their respective owner.

## Fluorescence Polarization (FP) Detection Cartridges

The Fluorescence Polarization (FP) Detection Cartridges enable fluorescence polarization read modes for specific labels, depending on the cartridge.

Fluorescence Polarization (FP) Detection Cartridges are available for the following labels:

- Fluorescence Polarization (FP) (Fluorescein) Detection Cartridge
- Fluorescence Polarization (FP) (Rhodamine) Detection Cartridge

Each Fluorescence Polarization (FP) Detection Cartridge occupies one slot in the detection cartridge drawer. You can install this cartridge in either the top detection cartridge drawer for top reads, or in the bottom detection cartridge drawer for bottom reads. However, for best results, you should install the cartridge in the top drawer and use solid black plates.



**Note:** You should install the Fluorescence Polarization (FP) Detection Cartridge in the top detection cartridge drawer for best results.

The cartridges support the following read types:

- [Endpoint Read Type on page 71](#)
- [Kinetic Read Type on page 71](#)

### Fluorescence Polarization

Fluorescence Polarization (FP) read mode measures the relative change of polarization of emitted fluorescent compared to excitation light.

Fluorescence Polarization detection is based on Fluorescence Intensity, with the important difference that it uses plane-polarized light, rather than non-polarized light. Microplate readers measure the Fluorescence Polarization of the sample by detecting light emitted both parallel and perpendicular to the plane of excitation.

By using a fluorescent dye to label a small molecule, the binding of a small molecule to an interaction partner of equal or greater size can be monitored through its speed of rotation.

When molecules are excited with polarized light, the polarization of the emitted light depends on the size of the molecule to which the fluorophore is bound. Larger molecules emit a higher percentage of polarized light, while smaller molecules emit a lower percentage of polarized light because of their rapid molecular movement. For this reason FP is generally used for molecular binding assays in high-throughput screening (HTS).



## Plate Recommendations

### Plate Recommendations for the Fluorescence Polarization (FP) Detection Cartridge

Read Mode	Plate Type	Other Considerations
Fluorescence Polarization (FP)	Solid black	When an application specifies a surface treatment, use only plates with the correct treatment. Molecular Devices recommends using unidilled plate for fluorescence polarization measurements.

## Measurement Specifications

### Measurement Specifications for the Fluorescence Polarization (FP) Detection Cartridges

Item	Description	Description
Detection cartridge name	Fluorescence Polarization (FP) (Fluorescein) Detection Cartridge	Fluorescence Polarization (FP) (Rhodamine) Detection Cartridge
Short name	FP-FLUO	FP-RHOD
Part number	0200-7009	0200-0710
Weight	1.5 lbs (0.7 kg)	1.5 lbs (0.7 kg)
Read Mode	Fluorescence polarization (FP)	Fluorescence polarization (FP)
Type	Dual emission	Dual emission
Number of slots	1 slot, top or bottom	1 slot, top or bottom
Light source	Ultra high power LED	Ultra high power LED
Filter Set	EXP: 485-20 EMP1: 535-25 EMP2: 535-25	EXP: 535-25 EMP1: 595-35 EMP2: 595-35
Labels	Fluorescein 1 nM Other labels compatible with the excitation and emission wavelengths of the cartridge can also be used.	Rhodamine 4 nM Other labels compatible with the excitation and emission wavelengths of the cartridge can also be used.
Detection limit, Top <sup>1</sup>	384-well (75 µl): 3 mP 1536-well (8 µl): 6 mP	384-well (75 µl): 4 mP 1536-well (8 µl): 6 mP

<sup>1</sup> Replicate standard deviation at the label concentration specified under Labels.

## Glow Luminescence (LUM) Detection Cartridges

The Glow Luminescence (LUM) Detection Cartridges enable Luminescence read modes.

The three Glow Luminescence (LUM) Detection Cartridges are individually optimized for 1536-well, 384-well, or 96-well plate formats:

- Glow Luminescence (LUM) Detection Cartridge
- Glow Luminescence (LUM) Detection Cartridge (384)
- Glow Luminescence (LUM) Detection Cartridge (96)

For dual-color luminescence, see the [Dual Color Luminescence \(LUM\) \(BRET2\) Detection Cartridge on page 64](#).

Each Glow Luminescence (LUM) Detection Cartridge occupies one slot in the detection cartridge drawer. You can install this cartridge in either the top detection cartridge drawer for top reads, or in the bottom detection cartridge drawer for bottom reads. However, for best results, you should install the cartridge in the top drawer and use solid white plates.



**Note:** This detection cartridge cannot be installed in the rear-most slot in either the top or bottom detection cartridge drawer for best results.



**Note:** You should install the Glow Luminescence (LUM) Detection Cartridge in the top detection cartridge drawer when you run luminescence (LUM) read modes.

The cartridges support the following read types:

- [Endpoint Read Type on page 71](#)
- [Kinetic Read Type on page 71](#)
- [Well Scan Read Type on page 72](#)

## Luminescence

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.

Each Glow Luminescence (LUM) Detection Cartridge contains the components to measure the light intensity from luminescence. Since the light is emitted as a result of a chemical reaction, no excitation light and no excitation filters are required to measure luminescence. The luminescence can be measured with no emission filters, or an emission wavelength selected from within the specified range.



**Note:** This detection cartridge is not suitable for measurement of flash luminescence reactions, which require injectors.

## Plate Recommendations

### Plate Recommendations for the Glow Luminescence (LUM) Detection Cartridges

Read Mode	Plate Type	Other Considerations
Luminescence (LUM)	Solid white	When an application specifies a surface treatment, use only plates with the correct treatment. You should use an unlidded plate for luminescence measurements.

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

## Measurement Specifications

### Measurement Specifications for the Glow Luminescence (LUM) Detection Cartridges

Item	Description	Description	Description
Detection cartridge name	Glow Luminescence (LUM) Detection Cartridge	Glow Luminescence (LUM) Detection Cartridge (384)	Glow Luminescence (LUM) Detection Cartridge (96)
Short name	LUM	LUM 384	LUM 96
Part number	0200-7012	0200-7015	0200-7014
Weight	1.5 lbs (0.7 kg)	1.5 lbs (0.7 kg)	1.5 lbs (0.7 kg)
Plate formats	6, 12, 24, 48, 96, 384, 1536-well plates	6, 12, 24, 48, 96, and 384-well plates	6, 12, 24, 48, 96-well plates
Read mode	Luminescence (LUM)	Luminescence (LUM)	Luminescence (LUM)
Type	Single emission	Single emission	Single emission
Number of slots	1 slot, top or bottom, not in the rear-most slot	1 slot, top or bottom, not in the rear-most slot	1 slot, top or bottom, not in the rear-most slot
Light source	None	None	None
Wavelength range	Visible to 650 nm	Visible to 650 nm	Visible to 650 nm
Labels/Substrates	Labels compatible with the wavelength range	Labels compatible with the wavelength range	Labels compatible with the wavelength range
Detection limit, Top (ATP) optimized	<b>96-well (200 <math>\mu</math>L)</b> 0.8 fmol/well (4 pM) <b>384-well (50 <math>\mu</math>L)</b> 0.35 fmol/well (7 pM) <b>1536-well (8 <math>\mu</math>L)</b> 0.32 fmol/well (40 pM)	<b>96-well (200 <math>\mu</math>L)</b> 0.5 fmol/well (2.5 pM) <b>384-well (50 <math>\mu</math>L)</b> 0.2 fmol/well (4 pM) <b>1536-well (8 <math>\mu</math>L)</b> NA	<b>96-well (200 <math>\mu</math>L)</b> 0.3 fmol/well (1.5 pM) <b>384-well (50 <math>\mu</math>L)</b> NA <b>1536-well (8 <math>\mu</math>L)</b> NA
Detection limit, Top (ATP) guaranteed	<b>96-well (200 <math>\mu</math>L)</b> 2.4 fmol/well (12 pM) <b>384-well (50 <math>\mu</math>L)</b> 1.5 fmol/well (30 pM) <b>1536-well (8 <math>\mu</math>L)</b> 0.8 fmol/well (100 pM)	<b>96-well (200 <math>\mu</math>L)</b> 1.4 fmol/well (7 pM) <b>384-well (50 <math>\mu</math>L)</b> 0.75 fmol/well (15 pM) <b>1536-well (8 <math>\mu</math>L)</b> NA	<b>96-well (200 <math>\mu</math>L)</b> 1.4 fmol/well (7 pM) <b>384-well (50 <math>\mu</math>L)</b> NA <b>1536-well (8 <math>\mu</math>L)</b> NA
Linear dynamic range, Top	5 logs in a single plate read	5 logs in a single plate read	5 logs in a single plate read

## Dual Color Luminescence (LUM) (BRET2) Detection Cartridge

The Dual Color Luminescence (LUM) (BRET2) Detection Cartridge enables read modes for luminescence and is designed to provide the optimal performance when used with Perkin Elmer BRET<sup>2</sup> reagents.

For luminescence measurements that do not require emission filters, see [Glow Luminescence \(LUM\) Detection Cartridges on page 61](#).

The Dual Color Luminescence (LUM) (BRET2) Detection Cartridge occupies one slot in the detection cartridge drawer. You can install this cartridge in either the top detection cartridge drawer for top reads, or in the bottom detection cartridge drawer for bottom reads. However, for best results, you should install the cartridge in the top drawer and use solid white plates.



**Note:** This detection cartridge cannot be installed in the rear-most slot in either the top or bottom detection cartridge drawer.



**Note:** You should install the Dual Color Luminescence (LUM) (BRET2) Detection Cartridge in the top detection cartridge drawer when you run luminescence (LUM) read modes for best results.

---

The cartridge supports the following read types:

- [Endpoint Read Type on page 71](#)
- [Kinetic Read Type on page 71](#)
- [Well Scan Read Type on page 72](#)

BRET<sup>2</sup> is a trademark of PerkinElmer, Inc.

### BRET Luminescence

Bioluminescence Resonance Energy Transfer (BRET) is a measurement technique that shares the key feature of Fluorescence Resonance Energy Transfer (FRET) while using the advantages of Luminescence reads.

BRET employs a luminescence substrate that can undergo cleavage due to an enzymatic group (donor label) resulting in luminescence emission (channel 1). Unlike other generic luminescence assays, the BRET luminescence can be quenched by a radiationless Energy Transfer to an acceptor label (a fluorophore) resulting in fluorescence emission at a longer wavelength (red shifted, channel 2). The acceptor must be chosen such that its excitation spectrum overlaps the emission spectrum of the luminescence (Resonance condition). In the presence of substrate the energy transfer can occur only when the enzymatic group and the acceptor are in close proximity. Because of this fact, when labels are applied to study protein-protein interactions for example, signal measured at the emission wavelength of the acceptor fluorophore indicates binding.

The Dual Color Luminescence (LUM) (BRET<sup>2</sup>) Detection Cartridge contains the components to measure the light intensity from BRET<sup>2</sup> reagents. Since the light is emitted as a result of a chemical reaction, no excitation light and no excitation filters are required to measure luminescence, and the detection of the acceptor fluorophore (channel 2) can be regarded as a luminescence also. Emission filters differentiate emissions from the primary luminescence and the acceptor fluorophore. So, a dual color luminescence read mode is applied. The incorporates a dual emission design to measure the two emission channels simultaneously, improving precision and reducing read times.

BRET<sup>2</sup> can be used to study various kinds of protein-protein interactions. It employs a luciferase label (where “label” generally means fused by the use of expression vectors) for the one protein and a Green Fluorescent Protein (GFP) variant label for the other. The type of luciferase (Renilla) metabolizes the luminescent substrate (a coelenterazine derivative) resulting in blue luminescence (emission filter 1). Given the substrate, when the other protein with the GFP label is in close proximity to the luciferase, it quenches the blue luminescence and emits a green luminescence (emission filter 2). A high green to blue ratio indicates binding. The energy transfer is possible by the selection of the labels, that is, the overlap of the blue luminescence spectrum with the excitation spectrum of the GFP (resonance condition). As a result, BRET<sup>2</sup> combines the advantage of being a homogeneous (no washing steps required) assay with the generic sensitivity of luminescence detection.

Energy Transfer data are generally normalized by taking the red shifted emission and dividing it by the blue shifted emission. In terms of the BRET<sup>2</sup> reagents, the normalization of the green signal with the blue signal mainly compensates for the decay of the signal due to the digestion of the substrate. The software supports data reduction in taking ratios of the two emission channels.

Because of the signal decay, you must measure the assay immediately after adding the luminescence substrate. The spectral separation of the green from the blue signal conveniently eliminates the need for preprocessing the raw data.

When you do a binding assay, controls are involved that represent a maximum (high) and a minimum (low) G:B (green to blue) ratio, and the Z' parameter can be applied. More than merely taking the ratio of high and low G:B values, the Z' includes replicate variability to validate the actual data quality.

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. You can use higher values 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.

The BRET<sup>2</sup> reagents can be applied in various ways to study other types of molecular interactions in cell based assays. For a list of applications, go to [www.perkinelmer.com](http://www.perkinelmer.com).

## Plate Recommendations

### Plate Recommendations: Dual Color Luminescence (LUM) (BRET2) Detection Cartridge

Read Mode	Plate Type	Other Considerations
Luminescence (LUM)	Solid white	When an application specifies a surface treatment, use only plates with the correct treatment. You should use an unlidded plate for luminescence measurements.
Luminescence, Dual Color (LUM-Dual)	Solid white	When an application specifies a surface treatment, use only plates with the correct treatment. You should use an unlidded plate for luminescence measurements.

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

## Measurement Specifications

### Measurement Specifications: Dual Color Luminescence (LUM) (BRET2) Detection Cartridge

Item	Description
Detection cartridge name	Dual Color Luminescence (LUM) (BRET2) Detection Cartridge
Short name	LUM-BRET2
Part number	0200-7016
Weight	1.5 lbs (0.7 kg)
Read modes	Luminescence (LUM) Luminescence, Dual Color (LUM-Dual)
Type	Dual emission
Number of slots	1 slot, top or bottom, not in the rear-most slot
Light source	None
Wavelength range	EM1: 410-80 EM2: 515-30
Labels	Labels compatible with the wavelength range
Application	Designed for use with PerkinElmer BRET <sup>2</sup> reagents



## ScanLater Western Blot (WB) Detection Cartridge

The ScanLater™ Western Blot (WB) Detection Cartridge enables Time-Resolved Fluorescence read mode for Western Blot membranes.

The Molecular Devices ScanLater™ Western Blot Assay Kit is a novel system for protein analysis that can be used with the SpectraMax Paradigm, SpectraMax i3x, and SpectraMax iD5. Membranes are incubated with Eu-chelate labeled secondary antibodies or streptavidin that bind specifically to the target protein-specific primary antibody. For more information, contact your representative or search the knowledge base for ScanLater or Western Blot at [www.moleculardevices.com/service-support](http://www.moleculardevices.com/service-support).

The ScanLater Western Blot (WB) Detection Cartridge occupies two slots in the detection cartridge drawer.



**Note:** The ScanLater Western Blot (WB) Detection Cartridge must be installed in the top detection cartridge drawer for Western Blot membrane reads.

Western Blot reads use time-resolved fluorescence read mode with a membrane read type. See [Membrane Read Type](#) on page 73.

### Loading the Membrane Holder

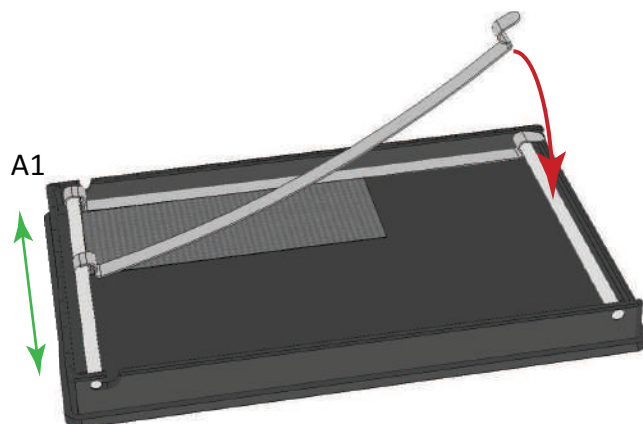


**Note:** Handle membranes only by the edges using clean forceps. Take care to not touch the membrane with gloved or bare hands.

To scan a membrane, you must first place it in a Molecular Devices ScanLater™ membrane holder. The maximum size of a membrane that will fit in the membrane holder is 109 mm x 77 mm.

To load the membrane holder:

1. Place the membrane on the membrane holder with one corner of the membrane aligned with the A1 corner of the membrane holder.



2. Slide the holder clips to place them over the membrane as close to the edges of the membrane as possible to expose the maximum scanning area of the blot.

3. Lower the holder clips into place and then gently press the end of each holder clip to snap it into place and secure the membrane to the holder.



**CAUTION!** To prevent damage to the instrument or the membrane holder, make sure that all the holder clips are securely snapped into place before you insert the membrane holder into the instrument.

4. Load the membrane holder into the plate drawer with the A1 corner in the A1 position of the drawer. See [Loading and Unloading Plates on page 31](#).

## Cleaning the Membrane Holder

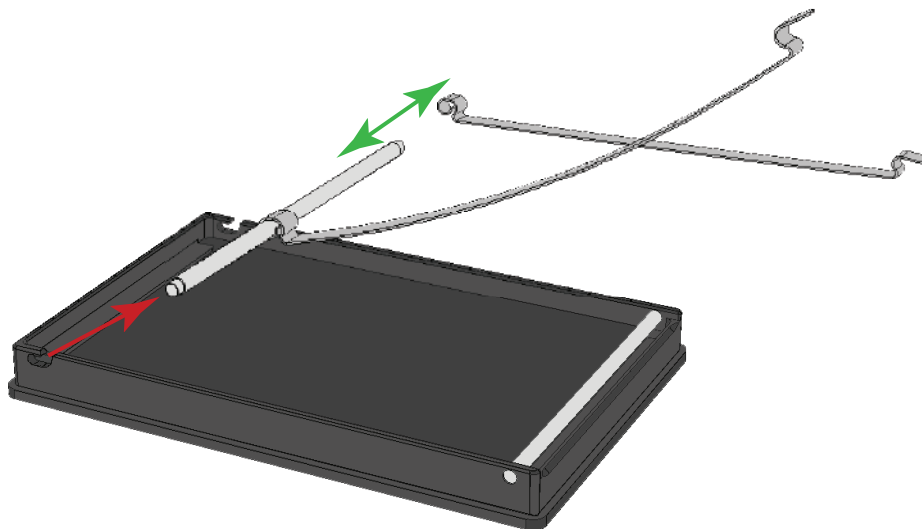
To remove dust, buffer residue, and smudges that can have a negative effect on image quality or contaminate the membrane, always clean the membrane holder before loading a new membrane for scanning.

The scanning surface can be wiped with methanol, rinsed with a small volume of water, followed by a final rinse with isopropanol to help prevent blot contamination. Pressurized “canned air” can be helpful in removing dust and lint.

## Membrane Holder Clips

You can add or remove the holder clips on the membrane holder as needed for your application.

1. If the clips are in the locked position, then unfasten the clips by pulling up on the tabs.
2. Slide the retainer bar on the "A" column side of the membrane holder out from its slots.



3. Slide the holder clip on or off the end of the retainer bar.



**Note:** When you add a holder clip, make sure that the tab is on top and that the center of the holder clip curves downward toward the plate.

4. Slide the retainer bar into the two slots and press the bar until it snaps into place.

## Measurement Specifications

### Measurement Specifications for the ScanLater Western Blot (WB) Detection Cartridge

Item	Description
Detection cartridge name	ScanLater Western Blot (WB) Detection Cartridge
Short name	SCANLATER WB
Part number	0200-7027
Weight	2.6 lbs (1.2 kg)
Read modes	Time-Resolved Fluorescence (TRF)
Type	Single emission
Number of slots	2 slots, top only
Light source	Ultra high power LED
Filter set	EX: 340-80 EM: 616-10
Labels	Europium chelate
Detection limit, Top	50 amol
Linear dynamic range, Top	5 logs in a single membrane scan

## Chapter 5: Read Modes and Read Types

The detection capabilities of the SpectraMax Paradigm Multi-Mode Microplate Reader are determined by the installed detection cartridges. Up to six detection cartridges can be installed in each of the two detection cartridge drawers. For information about detection cartridges, see [Detection Cartridges on page 39](#).

The software detects the installed cartridge configuration and does all measurement types supported by the detection cartridges. Use the SoftMax Pro Software to define the parameters for the read mode and read type of your assay. See the *SoftMax Pro Data Acquisition and Analysis Software User Guide* or the application help.

Application notes with specific application protocol suggestions are in the Information Center and Knowledge Base on the Molecular Devices web site at [www.moleculardevices.com](http://www.moleculardevices.com).

- [Absorbance Read Mode on page 74](#)
- [Fluorescence Intensity Read Mode on page 79](#)
- [Luminescence Read Mode on page 83](#)
- [Time-Resolved Fluorescence Read Mode on page 86](#)
- [FRET Read Mode on page 90](#)
- [HTRF Read Mode on page 91](#)
- [Fluorescence Polarization Read Mode on page 94](#)
- [AlphaScreen Read Mode on page 97](#)
- [ScanLater Western Blot TRF Read Mode on page 100](#)

### Supported Read Types

For most read modes, you can use the Endpoint, Kinetic, Well Scan, and Spectrum read types.

#### Endpoint Read Type

For the Endpoint read type, a read of each plate 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

For the Kinetic read type, the instrument collects data over time with reads taken in the center of each well at regular intervals.

The values calculated based on raw kinetic data include VMax, VMax per Sec, Time to VMax, Onset Time, and more. Kinetic reads can be single-wavelength or multiple-wavelength reads. Kinetic analysis can collect data points in time intervals of seconds, minutes, hours, or days.

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

Peak Pro™ Analysis functions provide advanced peak detection and characterization for applicable kinetic reads. See the *SoftMax Pro Data Acquisition and Analysis Software Formula Reference Guide*.

## Well Scan Read Type

The Well Scan read type can take reads at more than one location within a well. The Well Scan read type takes multiple reads of a single well of a plate on an evenly spaced, user defined grid or other pattern inside of each well at single or multiple wavelengths.

Some applications involve the detection of cells in large area tissue culture plates. Use the Well Scan read type with such plates to permit maximum surface area detection in cell-based protocols. Since many cell lines tend to grow as clumps or in the corners of plate wells, you can choose from several patterns and define the number of points to scan to work best with your application.

The following scanning patterns are available:

- A horizontal line
- A vertical line
- A cross pattern
- A fill pattern

The fill pattern can be either round or square to match the shape of the well. The image in the Well Scan settings shows the shape of the well as defined for the selected plate.

You can set the density of the well scan to determine the number of points to read in a line pattern or the maximum number of horizontal and vertical points included in a cross or fill pattern.

Depending on the read mode selected, the values are reported as optical density (OD), %Transmittance (%T), relative fluorescence units (RFU), or relative luminescence units (RLU).

## Spectrum Read Type

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

Spectrum reads are available only for specific detection cartridges. See [Detection Cartridges on page 39](#).

## Membrane Read Type

The Membrane read type is used for a Time-Resolved Fluorescence read of a Western Blot membrane. The selected area is read, and a TIFF image is generated with the results of the read.

The Molecular Devices ScanLater™ Western Blot Assay Kit is a novel system for protein analysis that can be used with the SpectraMax Paradigm, SpectraMax i3x, and SpectraMax iD5. Membranes are incubated with Eu-chelate labeled secondary antibodies or streptavidin that bind specifically to the target protein-specific primary antibody. For more information, contact your Molecular Devices representative or search the knowledge base for ScanLater or Western Blot at [www.moleculardevices.com/service-support](http://www.moleculardevices.com/service-support).

For information about the detection cartridge for Western Blot membrane reads, see [ScanLater Western Blot \(WB\) Detection Cartridge on page 68](#).

## Absorbance Read Mode

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

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

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

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

The 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 instrument enables you to choose whether to display absorbance data as Optical Density (OD) or %Transmittance (%T) in the Reduction dialog.

### Optical Density

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

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

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

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

### % Transmittance

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

$$T = I / I_0$$

$$\%T = 100T$$

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

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

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

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

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

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

### Applications of Absorbance

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

To do absorbance reads, the SpectraMax Paradigm Multi-Mode Microplate Reader requires the [Absorbance Detection Cartridge](#), see [page 40](#).

### 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.

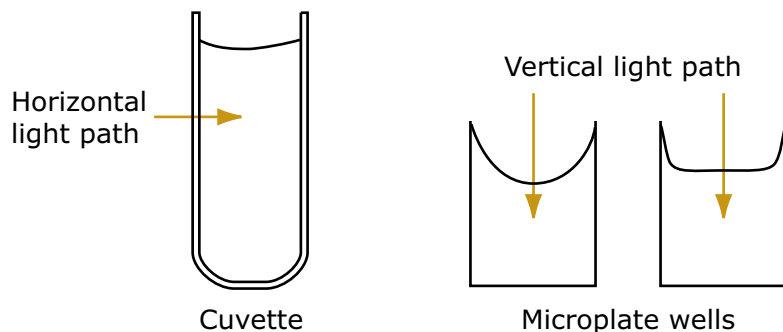
The Beer–Lambert law states that absorbance is proportional to the distance that light travels through the sample:

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

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

In a plate, pathlength is dependent on the liquid volume, so absorbance is proportional to both the concentration and the pathlength of the sample. Standard curves are often used to determine analyte concentrations in vertical-beam photometry of unknowns, yet errors can still occur from pipetting the samples and standards. The PathCheck technology determines the pathlength of aqueous samples in the plate and normalizes the absorbance in each well to a pathlength of 1 cm. This way of correcting the microwell absorbance values is accurate to within  $\pm 4\%$  of the values obtained directly in a 1 cm cuvette.





PathCheck technology normalizes the data acquired from an Absorbance read mode Endpoint read type to a 1 cm pathlength, correcting the OD for each well to the value expected if the sample were read in a 1 cm cuvette. The instrument uses the factory installed Water Constant to obtain the 1 cm values. For the SpectraMax you can read a cuvette that contains deionized water or buffer to use the Cuvette Reference correction method (typically not necessary when you use aqueous solutions with minimal alcohol, salt, or organic solvent content).

### Water Constant

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



**Note:** The Cuvette Reference correction method that the software uses with the PathCheck Pathlength Measurement Technology is different from the reference read of a cuvette that occurs when you click the Ref button in the Cuvette Set section tool bar.



**Note:** After you read a plate with PathCheck technology turned on, the software stores PathCheck information permanently in the document. You can apply or not apply PathCheck technology to the absorbance values. If you do select to use PathCheck technology for the plate read, you cannot apply the PathCheck Pathlength Measurement Technology feature after the read.

### Eliminating Pathlength Independent Component

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

#### Use a Plate Blank

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

1. Designate a minimum of one well (preferably several) as Plate Blank.
2. Pipette buffer (for example, your sample matrix) into those wells and read along with the samples. Do not use an empty well for a blank.

The instrument automatically subtracts the average of the blank wells from each of the samples. The OD of the plate material is subtracted as part of the blank.

3. Select the Use Plate Blank check box in the Data Reduction dialog.

### Use a Plate Background OD

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

To determine the Plate Background OD:

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

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



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

### Interfering Substances

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

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

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

To determine possible color interference:

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

Do the same for pure water.

If the delta OD for the sample differs significantly from the delta OD for water, then you should not use the PathCheck technology.

Organic solvents could interfere with the PathCheck technology if 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 the solvent absorbs between 900 nm and 1000 nm, the interference would be similar to the interference of highly colored samples. If you add an organic solvent other than ethanol or methanol, you should run a Spectrum scan between 900 nm and 1000 nm to determine if the solvent would interfere with the PathCheck technology.

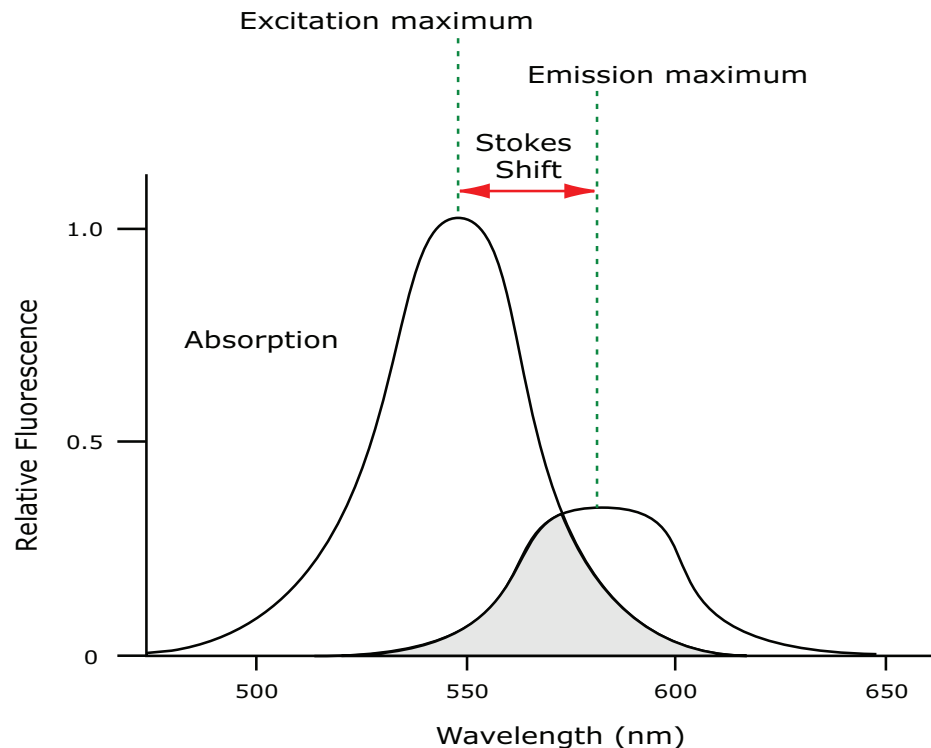
## Fluorescence Intensity Read Mode

Fluorescence occurs when absorbed light is re-radiated at a longer wavelength. In the Fluorescence Intensity (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:

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

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

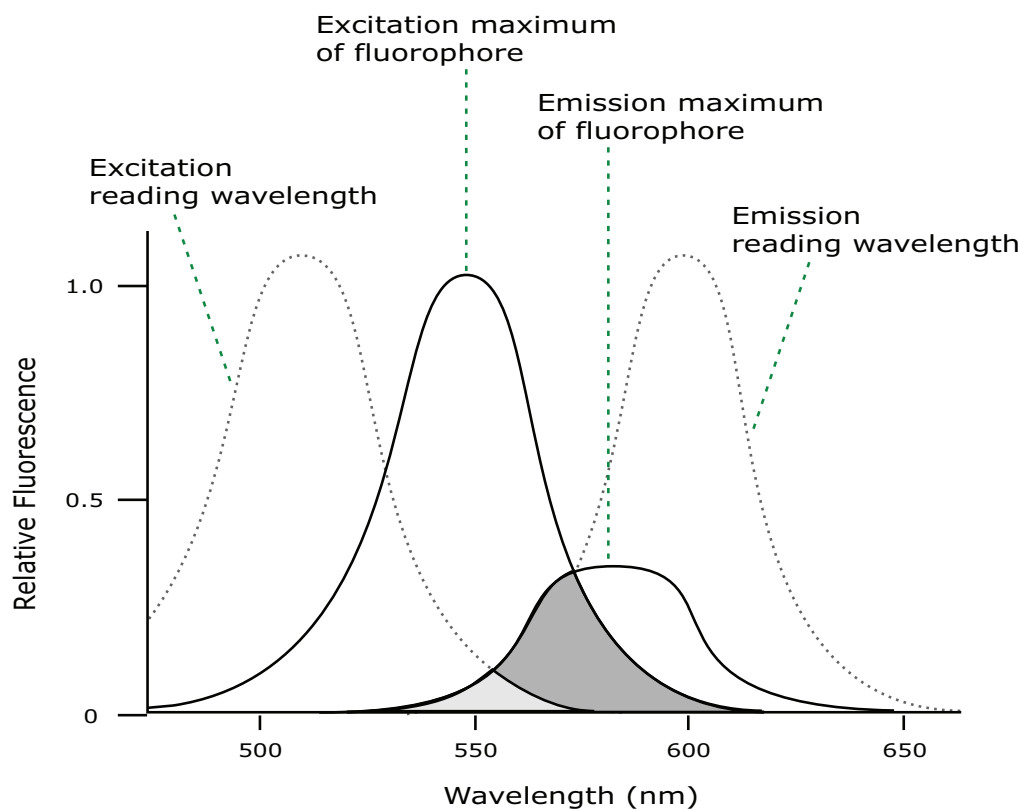


### Excitation and Emission Spectra

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

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

The Spectral Optimization Wizard provides the best settings for maximizing the signal to background window,  $(S-B)/B$ , while minimizing the optimization time. You can use this wizard with a Tunable Wavelength (TUNE) Detection Cartridge installed in the SpectraMax Paradigm Multi-Mode Microplate Reader. See the *SoftMax Pro Data Acquisition and Analysis Software User Guide* or the application help.



### Optimized Excitation and Emission Read Wavelengths

The previous figure shows that the best results are often obtained when the excitation and emission wavelengths you use for the read are not the same as the peak wavelengths of the excitation and emission spectra of the fluorophore. When the read 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, which results in a purer emission signal and more accurate data.

The instrument enables you to scan 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 several variables.

### Applications of Fluorescence Intensity

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

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

The following detection cartridges have fluorescence intensity read mode capability:

- [Tunable Wavelength \(TUNE\) Detection Cartridge](#), see page 42
- [Multi-Mode \(MULTI\) Detection Cartridge](#), see page 46
- [Fluorescence Intensity \(FI\) Detection Cartridges](#), see page 57

### 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.

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

where  $\text{conc}_{\text{std}}$  is the concentration of the *standard*, and *sample*, *blank*, and *std* 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,  $(\text{std} - \text{blank}) / \text{conc}_{\text{std}}$  is equivalent to the slope of the standard curve, and so the concentration of the label is determined by  $(\text{sample} - \text{blank}) / (\text{slope of standard curve})$ .

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

## Detection Limit

The detection limit is the smallest sample concentration that can be detected 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:

$$\text{Det Limit} = \frac{3 \text{ StDev}_{\text{blank}}}{\left( \frac{\text{std-blank}}{\text{conc}_{\text{std}}} \right)}$$

where  $\text{conc}_{\text{std}}$  is the concentration of the standard,  $\text{StDev}_{\text{blank}}$  is the standard deviation of the blank replicates, and  $\text{blank}$  and  $\text{std}$  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  $\text{LDR}$  is expressed as a log, and  $\text{max conc lin}$  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 measured species emit light naturally. For this reason, the lamp does not flash, so no background excitation interference occurs.

For the Luminescence (LUM) read mode, the instrument provides measurements in Relative Light 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 plate that has been exposed to light, you should dark adapt the plate by placing the sample-loaded plate inside the instrument for several minutes before you start the read.

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.

When maximum sensitivity is required, Molecular Devices recommends the use of dual-color luminescence detection cartridges. See [Dual Color Luminescence \(LUM\) \(BRET2\) Detection Cartridge on page 64](#).

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

### 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

The following detection cartridges have luminescence read mode capability:

- [Tunable Wavelength \(TUNE\) Detection Cartridge, see page 42](#)
- [Multi-Mode \(MULTI\) Detection Cartridge, see page 46](#)
- [Glow Luminescence \(LUM\) Detection Cartridges, see page 61](#)
- [Dual Color Luminescence \(LUM\) \(BRET2\) Detection Cartridge, see page 64](#)



## Analyzing 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 not a benchmark of sensitivity.

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

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

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

### Detection Limit

The detection limit is the smallest sample concentration that can be detected 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:

$$\text{Det Limit} = \frac{3 \text{ StDev}_{\text{blank}}}{\left( \frac{\text{std-blank}}{\text{conc}_{\text{std}}} \right)}$$

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. Even if the reaction begins with a high concentration of ATP, as it gets used up it can become rate-limiting. In this case, the non-linearity is an effect of the assay and not caused by the microplate reader.



**Note:** Very bright samples can exceed the linear dynamic range of the instrument. If so, the read can be done with an attenuation filter.

### Data Optimization

Measurement noise is dependent on the read time per sample (time per plate or time per well). The detection limit improves when the read time is increased. Therefore, it is important to specify the read time when you compare 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. You can use higher values 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.

## Time-Resolved Fluorescence Read Mode

The Time-Resolved Fluorescence read mode is a secondary read mode.

Time-Resolved Fluorescence is a measurement technique that depends on three characteristics that lead to better discrimination between the specific signal, proportional to the quantity of label, and the unspecific fluorescence resulting from background and compound interference:

- Pulsed excitation light sources
- Time-gated electronics faster than the fluorescence lifetime
- Labels with prolonged fluorescence lifetime

The time-gating electronics introduce a delay between the cutoff of each light pulse and the start of signal collection. During the delay, the unspecific fluorescence (caused by test compounds, assay reagents, and the plate) vanishes, while only a small portion of the specific fluorescence from the label is sacrificed. Enough of the specific signal remains during the decay period, with the added benefit of reduced background.

In Time-Resolved Fluorescence read mode, the instrument detects the extremely long emission half-lives of rare earth elements called lanthanides, such as europium (lifetime of about 700  $\mu$ s), samarium (lifetime of about 70  $\mu$ s), or terbium (lifetime of about 1000  $\mu$ s).

### Applications of Time-Resolved Fluorescence

Time-Resolved Fluorescence is widely used in high throughput screening applications such as kinase assays, and is useful in some fluorescence immunoassays, such as DELFIA (dissociation-enhanced enzyme linked fluorescence immunoassay). TRF is also useful in some assay variants of TR-FRET (Time-Resolved Fluorescence Resonance Energy Transfer) in which the FRET acceptor label acts as a quencher only and does not emit fluorescence. The proximity between donor label and acceptor (quencher) is then quantified by the intensity decrease of the donor label.

DELFIA requires washing steps as in an ELISA, but the TR-FRET assay involving quenching is a homogeneous plate assay technique and requires only mixing and measuring—no wash steps are required. It can also be miniaturized, which makes it useful for high-throughput screening applications.

The Cisbio Bioassays HTRF<sup>®</sup> (Homogeneous Time-Resolved Fluorescence) technology is a proprietary Time-Resolved Fluorescence technology that overcomes many of the drawbacks of standard Fluorescence Resonance Energy Transfer (FRET) techniques, such as the requirements to correct for autofluorescence and the fluorescent contributions of unbound fluorophores. See [HTRF Read Mode on page 91](#).

The following detection cartridges have time-resolved fluorescence read mode capability:

- [Tunable Wavelength \(TUNE\) Detection Cartridge, see page 42](#)
- [Multi-Mode \(MULTI\) Detection Cartridge, see page 46](#)

- [Cisbio HTRF Detection Cartridge on page 53](#)
- [Time Resolved Fluorescence \(TRF-EUSA\) Detection Cartridge, see page 55](#)

## Analyzing Time-Resolved Fluorescence Data

A time-resolved fluorescence (TRF) measurement includes a number of pulses. Each pulse consists of turning the light source on, then off (Excitation Time), pausing for a specified length of time (Measurement Delay), and measuring the fluorescence intensity of the sample for a specified length of time (Integration Time). The pulses are repeated several times, as specified in the protocol parameters.

### Blank Correction

Although background is significantly lower than with fluorescence intensity measurements, you should use blanks or assay controls.

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

### Data Normalization

TRF raw data changes in magnitude when the timing parameters are changed. However, TRF data are normalized for a number of 1000 pulses. This means that the sample raw data does not change when only the number of pulses is changed.

### Data Optimization

There are two timing parameters which can be optimized to adjust the performance of the measurement: time per well and integration time per cycle.

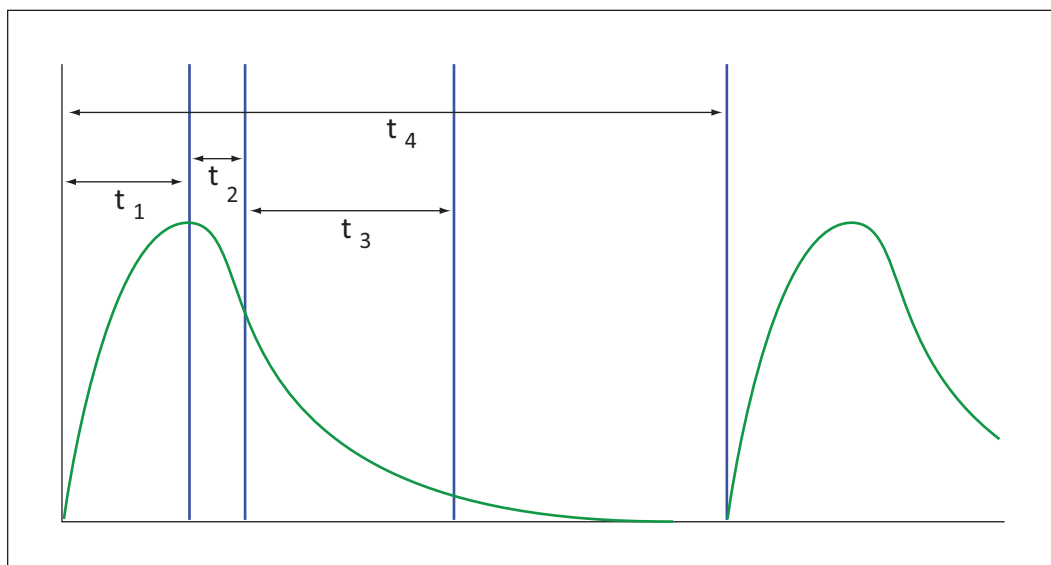
Measurement noise is dependent on the read time per sample (time per plate or time per well). The detection limit improves when the read time is increased. Therefore, it is important to specify the read time when you compare measurements. For TRF, the read time per well increases with the selected number of pulses. The time between pulses and the intensity of each pulse, however, can be different on various systems.

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.

To further optimize measurement results, optimize the timing parameters. Use the following table and figure as guidelines for the selection of timing parameters.

**Time-Resolved Fluorescence Timing Parameters Example**

Parameter	Value	Comment
Pulse length	0.100 ms	The period for excitation of the sample, shown as $t_1$ in the following figure. This is the suggested value for the TUNE, MULTI, and TRF detection cartridges.
Measurement delay	0.010 ms	The delay to ensure the excitation pulse is no longer detectable, shown as $t_2$ in the following figure. This is the suggested value for the TUNE, MULTI and TRF detection cartridges.
Integration time per cycle (pulse)	0.890 ms	The period for accumulating the signal, shown as $t_3$ in the following figure. This is the suggested value for the TUNE and MULTI detection cartridges.
Integration time per cycle (pulse)	1.890 ms	The period for accumulating the signal, shown as $t_3$ in the following figure. This is the suggested value for the TRF detection cartridge.
Total cycle time		The total cycle time is shown as $t_4$ in the following figure.

**Timing Parameters For Time-Resolved Fluorescence**

When neglecting the time delay  $t_2$  compared to the integration time window  $t_3$ , the accumulated signal  $A$  can be approximated with the following equation:

$$A / A_{\max} = (1 - \exp(-M)) \times 100\%$$

In the equation above,  $M$  is the size of the time window (or integration time) divided by the exponential decay time constant (or the fluorescence lifetime of the label).

$$M = (\text{integration time}) / (\text{fluorescence lifetime})$$

For example, using Europium, which has a fluorescence lifetime of 700  $\mu\text{s}$ , and the suggested integration time per cycle of 1.890 ms (or 1890  $\mu\text{s}$ ),  $M = 1890 / 700 = 2.7$ . Inserting this value of  $M$  into the first equation yields  $A / A_{\text{max}} = 93\%$ .

To optimize the integration time per cycle (pulse), the integration time should be set such that the value of  $M$  produces the desired signal. For example, to get more than 86% signal, select an integration time such that  $M$  is greater than 2.0. Using the previous Europium example and solving for the integration time, the integration time can be set to  $M$  (2.0) times the fluorescence lifetime (700  $\mu\text{s}$ ), or 1400  $\mu\text{s}$  (1.4 ms).

#### Achievable Accumulated Signal Percentage Compared to $M$

$M$	0.25	0.50	0.75	1.00	1.25	1.50	2.00	3.00
$A / A_{\text{max}}[\%]$	22	39	53	63	71	78	86	95

$M$  can be technically limited by the time between pulses. Further gain in signal above some value of  $M$  can be negligible to improve results.

When you do a dual-label Europium-Samarium measurement, there are more timing parameters. There is some residual cross-talk of the Samarium signal captured in the Europium emission channel. Samarium has a much shorter fluorescence lifetime. To reduce the cross-talk of Samarium in the Europium channel, Europium is measured in a time window shifted away from the time window for Samarium. This lets the Europium be quantified without cross contamination from the Samarium. The known Europium concentration can be used to remove the Europium cross-contamination in the Samarium channel.

#### TRF Suggested Timing Parameters: Dual Label Europium-Samarium

Parameter	Value	Comment
Pulse length	0.100 ms	The time interval for flash monitoring This is the suggested value for the TRF detection cartridge.
Measurement delay (first window)	0.010 ms	The delay to ensure the excitation pulse is no longer detectable This is the suggested value for the TRF detection cartridge.
Integration time (first window)	0.100 ms	The period for accumulating the Samarium signal This is the suggested value for the TRF detection cartridge.
Measurement delay (second window)	0.140 ms	The read out of the Samarium signal This is the suggested value for the TRF detection cartridge.
Integration time (second window)	0.750 ms	The period for accumulating the Europium signal This is the suggested value for the TRF detection cartridge.

## FRET Read Mode

Fluorescence resonance energy transfer (FRET) is a distance-dependent interaction between the electronic excited states of two dye molecules in which excitation is transferred from a donor molecule to an acceptor molecule *without emission of a photon*.

FRET relies on the distance-dependent transfer of energy from a donor molecule to an acceptor molecule. Due to its sensitivity to distance, FRET has been used to investigate molecular interactions. FRET is the radiationless transmission of energy from a donor molecule to an acceptor molecule. The donor molecule is the dye or chromophore that initially absorbs the energy and the acceptor is the chromophore to which the energy is subsequently transferred. This resonance interaction occurs over greater than interatomic distances, without conversion to thermal energy, and without a molecular collision. The transfer of energy leads to a reduction in the fluorescence intensity and excited state lifetime of the donor, and an increase in the emission intensity of the acceptor. A pair of molecules that interact in such a manner that FRET occurs is often referred to as a donor/acceptor pair.

While there are many factors that influence FRET, the primary conditions that need to be met for FRET to occur are relatively few:

- The donor and acceptor molecules must be in close proximity to each other.
- The absorption or excitation spectrum of the acceptor must overlap the fluorescence emission spectrum of the donor.

The degree to which they overlap is referred to as the spectral overlap integral (J).

- The donor and acceptor transition must be approximately parallel.

The following detection cartridge has FRET read mode capability:

- [Cisbio HTRF Detection Cartridge, see page 53](#)

## HTRF Read Mode

Homogeneous time-resolved fluorescence (HTRF) is a measurement technique based on fluorescence resonance energy transfer (FRET) using the advantages of time-resolved fluorescence (TRF) read.

HTRF uses a donor fluorophore with a long fluorescence lifetime, such as Europium. The acceptor fluorophore acts as if it also has a long fluorescence lifetime. This lets the time-gating principle of time-resolved fluorescence be applied to the acceptor emission to separate specific signal from background and signal caused by compound interference.

Time-gating electronics introduce a delay between the flashes and the start of signal collection. During the delay, the unspecific fluorescence caused by test compounds, assay reagents, and the plate vanishes while only a small portion of the specific fluorescence from the acceptor fluorophore is sacrificed. Enough of the specific signal remains, with the benefit of reduced background.

### Applications of Homogeneous Time-Resolved Fluorescence

Homogeneous time-resolved fluorescence (HTRF) is used in competitive assays to quantify the binding between two labeled molecules, or the disintegration of a bound complex. Binding partners can have similar molecular weights as opposed to fluorescence polarization read modes. HTRF is a homogeneous assay that requires only mixing and measuring—no wash steps are required. It can also be miniaturized, which makes it useful for high-throughput screening applications.

The fluorescence ratio related to the HTRF readout is a correction method developed by Cisbio, for which Cisbio has granted a license to Molecular Devices. Its application is strictly limited to the use of HTRF reagents and technology, excluding other TR-FRET technologies such as IMAP TR-FRET calculations of acceptor to donor ratios.

To do HTRF reads, the instrument requires the [Cisbio HTRF Detection Cartridge](#), see page 53. HTRF is a registered trademark of Cisbio Bioassays.

### Analyzing HTRF Data

A Homogeneous Time-Resolved Fluorescence (HTRF) measurement includes a number of flash intervals. Each flash interval consists of flashing the lamp, pausing for a specified length of time, and measuring the fluorescence intensity of the sample. These flash intervals are repeated several times, as specified in the protocol parameters.

### Data Reduction

Data reduction for HTRF reads consists of two steps.

First, a ratio of the signal measured by the emission from the acceptor label at 665 nm to the signal measured by the emission of the donor label at 616 nm is calculated and multiplied by a factor of 10,000. This generates what is called the HTRF ratio.



In the second step, ratios are calculated that represent the relative change in the HTRF signal compared to that of the assay background, represented by assay controls potentially named negative or Standard 0. This relative response ratio is called the Delta F and is formatted as a percentage, although values greater than 100 can be generated.

## Data Optimization

Measurement noise is dependent on the read time per sample (time per plate or time per well). The detection limit improves when the read time is increased. Therefore, it is important to specify the read time when you compare measurements. For TRF, the read time per well increases with the number of pulses you enter. The time between pulses can be different on various systems.

### HTRF Timing Parameters Example

Parameter	Value	Comment
Number of pulses	30	The number of flashes per read.
Measurement delay	30 $\mu$ s	The delay to ensure the excitation pulse is no longer detectable.
Integration time per cycle (pulse)	400 $\mu$ s	The period for accumulating the signal.

Defining the number of flashes (pulses) cannot be used for comparative purposes because the flash and intensity rate varies from system to system.

There are two timing parameters which can be optimized to adjust the performance of the measurement: time per plate or time per well, and integration time per cycle.

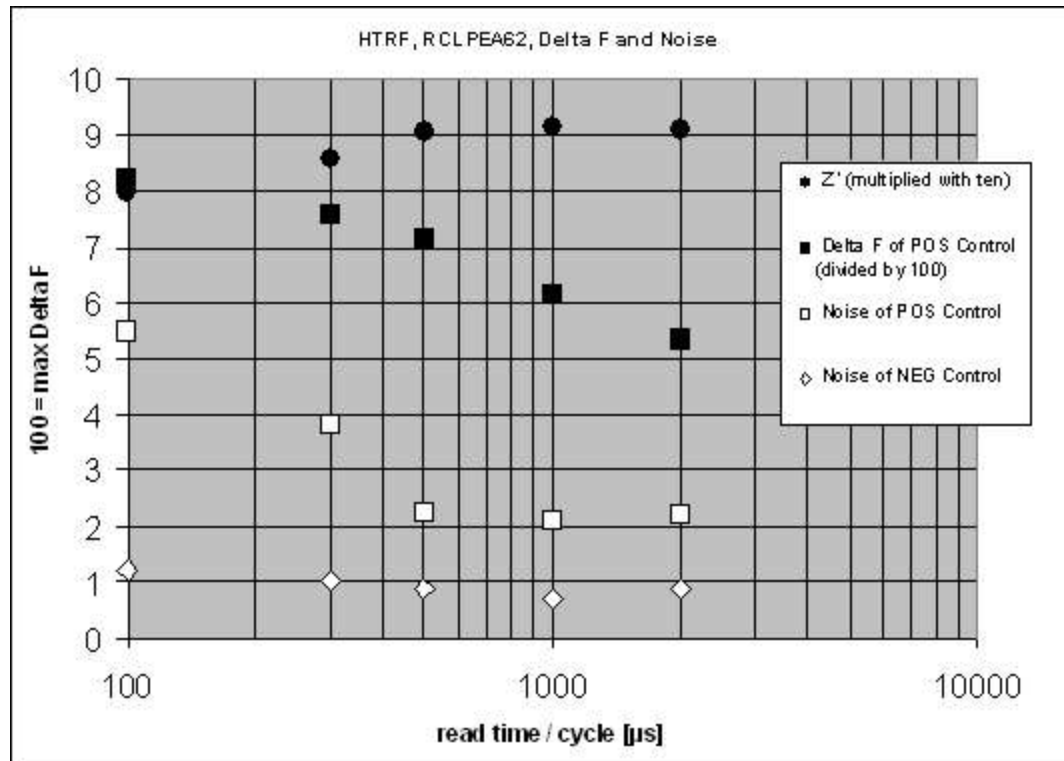
All low-light-level detection devices have some measurement noise in common. To average out the measurement noise, optimization of the time per well involves accumulating as many counts as possible. Within some range, you can reduce noise (CVs, detection limit) by increasing the read time per well, as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

As the number of flashes (read time per well) is increased, several aspects of the data improve:

- Delta F values show less variability (better CVs).
- Small Delta F values are better distinguished from noise.
- Noise of background is reduced.

The second timing parameter which can be optimized is the Integration time per cycle. Care must be taken in optimizing the integration time to consider noise. Delta F is higher at low integration times, but noise is also high at low integration times. The optimal integration time is where noise is minimized while maximizing Delta F.

In the following example, the optimal integration time (read time per cycle) is displayed to be in the 500  $\mu$ s to 1000  $\mu$ s range, as noise is minimized and Delta F is still relatively high. Going greater than 1000  $\mu$ s shows a sharp decline in Delta F without apparent improvement in noise.



#### Relationship Between Integration Time, Noise, and Delta F

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:

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

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. You can use higher values 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.

## Fluorescence Polarization Read Mode

The Fluorescence Polarization (FP) read mode measures the relative change of polarization of emitted fluorescence compared to excitation light.

Fluorescence Polarization detection is based on Fluorescence Intensity, with the important difference that it uses plane-polarized light, rather than non-polarized light. Microplate readers measure the Fluorescence Polarization of the sample by detecting light emitted both parallel and perpendicular to the plane of excitation.

By using a fluorescent dye to label a small molecule, the binding of a small molecule to an interaction partner of equal or greater size can be monitored through its speed of rotation.

When molecules are excited with polarized light, the change in the polarization of the emitted light depends on the size of the molecule to which the fluorophore is bound (the emitted light quickly depolarizes if the fluorescent molecule is unbound). Larger molecules yield a stronger polarization of the emitted light, while smaller molecules cause less polarization because of their rapid molecular movement. Fluorescence Polarization is used for molecular binding assays in high-throughput screening (HTS).

### Applications of Fluorescence Polarization

Fluorescence Polarization measurements provide information on molecular mobility and are generally used to quantify the success of a binding reaction between a smaller labeled ligand and a binding site at a much larger or immobilized molecule. Fluorescence Polarization can also be used to quantify the dissociation or cleavage of the labeled ligand from a binding site. Fluorescence Polarization is a homogeneous plate assay technique and requires only mixing and measuring—no wash steps are required as in an ELISA. It can also be miniaturized, which makes it useful for high-throughput screening applications.

The following detection cartridge has Fluorescence Polarization read mode capability:

- [Fluorescence Polarization \(FP\) Detection Cartridges](#), see page 59

### Analyzing Fluorescence Polarization Data

The Fluorescence Polarization read mode returns two sets of data: one for fluorescence intensity parallel (P) to the excitation plane, and the other for fluorescence intensity perpendicular (S) to the excitation plane. The software uses the S and P values to calculate the Polarization (mP) and Anisotropy (r) values.

Fluorescence Polarization assays in plates are generally designed with two control samples:

- LOW control sample: minimal polarization value resulting from unbound labeled ligand only
- HIGH control sample: maximum polarization value resulting from bound labeled ligand only

The Fluorescence Polarization data for a sample is evaluated based on its relative position between the low and high control values. Total intensity can also be determined from the raw data and is proportional to the quantity of label in a sample.

## Blank Correction

Many Fluorescence Polarization assays use small fluorescent label concentrations in the lower nm range. In this range, blank controls become significant when compared to samples.

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.

Background wells, which contain all assay components minus the fluorophore, should be tested. If the signal in the background wells is more than 1/10 the signal in the wells that contain fluorophore, then background wells should be run on each assay plate. The average raw signal from the background's parallel and perpendicular reads must be subtracted from the raw parallel and perpendicular reads of each sample well before the mP calculation is done.

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

## Data Reduction

Although the raw S and P values are the true actual values returned from the instrument, the calculated Polarization (mP) and Anisotropy (r) values are treated as the raw data and become the basis for further reduction calculations.

Polarization (mP) is calculated as follows:

$$\text{mP} = 1000 * \frac{(\text{parallel} - (G * \text{perpendicular}))}{(\text{parallel} + (G * \text{perpendicular}))}$$

Anisotropy (r) is calculated as follows:

$$r = \frac{(\text{parallel} - (G * \text{perpendicular}))}{(\text{parallel} + (2G * \text{perpendicular}))}$$

The G factor, or grating factor, is used in Fluorescence Polarization to correct polarization data for optical artifacts, converting relative mP data to theoretical mP data. Optical systems, particularly with reflective components, pass light of different polarization with different efficiency. G factor corrects for this instrument-based bias.

## Data Qualification and Validation

When you validate the data of a Fluorescence Polarization measurement and the assay, the two factors to look at are the precision value and the Z' factor.

The FP precision value is a measure of replicate uniformity determined by the standard deviation of replicates at a label concentration of 1 nM. Since the precision of a measured signal also depends on the read time, the read time must also be specified. A longer read time leads to a lower (better) precision value.

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. You can use higher values 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.

The assay window is dependent on the fluorophore lifetime and relative size of the receptor to the ligand. Precision values are better (lower) at higher signals, which normally come from higher label concentrations.

For a given assay window, Z' is a downward sloping linear function. That is, as precision values get higher (worse), the Z' value gets lower (worse).

Precision is dependent upon assay characteristics (sample volume, label concentration) and read time. In many assays, the characteristics are defined and cannot be changed. In this case, the only way to improve precision is to increase the read time per well.

## AlphaScreen Read Mode

ALPHA stands for Amplified Luminescent Proximity Homogeneous Assay. AlphaScreen® is a bead-based chemistry used to study molecular interactions between moieties A and B, for example. When a biological interaction between A and B moves beads (coated with A and B, respectively) together, a cascade of chemical reactions produce a greatly amplified signal.

The cascade finally resulting in signal is triggered by laser excitation (680 nm), making a photosensitizer on the A-beads convert oxygen to an excited (singlet) state. The energized oxygen diffuses away from the A-bead. When reaching the B-bead in close proximity, it reacts with a thioxene derivative on the B-bead generating chemiluminescence at 370 nm. Energy transfer to a fluorescent dye on the same bead shifts the emission wavelength into the 520 nm to 620 nm range. The limited lifetime of singlet oxygen in solvent (~4 microseconds) lets diffusion reach up to only around 200 nm distance. Thus, only B-beads in the proximity of A-beads yield signal, which indicates binding between moieties A and B.

An AlphaScreen measurement includes a light pulse, by turning on the laser diode for a specified time, turning off the laser diode, followed by the measurement of the AlphaScreen signal, as specified in the measurement protocol timing parameters.



---

**Note:** AlphaScreen beads are light sensitive. Beads are best handled under subdued (<100 lux) or green filtered (Roscolux filters #389 from Rosco, or equivalent) light conditions. Do incubation steps in the dark.

---

The raw data can be normalized to counts per second.

### Applications of AlphaScreen

AlphaScreen reagent and assays are used for drug discovery purposes. Examples of AlphaScreen assays include:

- G-protein coupled receptor (GPCR) assay kits, for cAMP quantification or IP3 quantification.
- Tyrosine Kinase assays.
- Cytokine detection kits, such as TNF-alpha detection (immunoassay).

AlphaScreen read mode can also capture the Europium emission line of AlphaLISA®.

To do AlphaScreen reads, the instrument requires [AlphaScreen Detection Cartridges](#), see [page 50](#).

For more information, go to [www.perkinelmer.com](http://www.perkinelmer.com).

ALPHASCREEN and ALPHALISA are registered trademarks of PerkinElmer, Inc.

## Analyzing AlphaScreen Data

The conversion rate of photons to counts and relative fluorescence units (RFU) is individual for each reader. Therefore, raw data from the same plate can seem to be different from one instrument to the next. Also, the data format used by instrument manufacturers might be counts normalized per second or not normalized counts, and therefore the raw data can be different by several orders of magnitude. It is important to know that the number of counts and the size of figures are not indicators of sensitivity.

The raw data can be normalized to counts per second by selecting the Normalization option on the Settings dialog.

### Background Correction

Although background is significantly lower than with fluorescence intensity measurements, you should use blanks or assay controls for background correction. The background can be effectively measured using blank replicates. When you read a sample with 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 plate. To prevent such interference, you can select the Interlaced Read option on the Settings dialog. This option reads only every other well in a checkerboard pattern, and then reads the plate again to read the previously omitted wells.

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.

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

### Detection Limit

The detection limit is the smallest sample concentration that can be detected 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:

$$\text{Det Limit} = \frac{3 \text{ StDev}_{\text{blank}}}{\left( \frac{\text{std-blank}}{\text{conc}_{\text{std}}} \right)}$$

where  $\text{conc}_{\text{std}}$  is the concentration of the standard,  $\text{StDev}_{\text{Blank}}$  is the standard deviation of the blank replicates, and  $\text{blank}$  and  $\text{std}$  are average values of the replicates for the blank and standard wells.

## Data Qualification and Validation

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:

$$Z' = 1 - \frac{3(SD_{c^+}) + 3(SD_{c^-})}{| \text{Mean}_{c^+} - \text{Mean}_{c^-} |}$$

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. You can use higher values 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.



**CAUTION!** The assay plate and the instrument should be kept at room temperature, since temperature variations cause fluctuations in signal.

---



## ScanLater Western Blot TRF Read Mode

Protein detection is an important task for pharmaceutical and clinical research, and Western Blots (WB), or protein immunoblots, are one of the most common methods employed for this purpose. Various techniques are used to detect proteins on Western Blot membranes including fluorescence, silver staining, and chemiluminescence. However, each technique has its limitations, and there is a continuous need to improve quantitation, accuracy, and dynamic range of Western Blots.

The Molecular Devices ScanLater™ Western Blot Assay Kit is a novel system for protein analysis that can be used with the SpectraMax Paradigm, SpectraMax i3x, and SpectraMax iD5. Membranes are incubated with Eu-chelate labeled secondary antibodies or streptavidin that bind specifically to the target protein-specific primary antibody. Europium has a long fluorescence lifetime, on the order of 1 msec, and detection is done in Time Resolved Fluorescence (TRF) mode which significantly reduces background from auto-fluorescence or other sources of short lifetime emissions. The membranes are placed into the instrument where they are scanned with the ScanLater Western Blot (WB) Detection Cartridge.

The method does not involve enzyme detection, and the Eu-chelates are resistant to photo-bleaching, so the signal remains stable for long periods of time (weeks to months). This enables you repeat the read of membranes and the potential for comparison of band intensities to known standards for more accurate quantitation. There is also no camera blooming, as can occur with chemiluminescence or fluorescence detection, so the system gives sharp bands and excellent image quality.

The TRF detection employs photon counting, so the theoretical dynamic range is  $>10^5$ . In practice, however, dynamic range is limited by saturation of binding sites on high-abundant bands and non-specific binding to background membrane.

### Applications

- Identify the nature of the protein or epitope effectively. Also, it can be used as a tool for quantitative analysis of protein.
- Use for chromatography components analysis, sucrose gradient analysis.
- Test the endogenous or exogenous expression of phosphoprotein to detect the phosphorylation signal.
- Protein resilience in the function experiment.
- Structure domain analysis.
- Analysis of the protein expression level.
- Analysis of protein content in the serum.
- Analysis of regulatory proteins expressed in the cell cycle.

The following detection cartridge has Western Blot TRF read mode capability:

- [ScanLater Western Blot \(WB\) Detection Cartridge on page 68](#)

The ScanLater Western Blot (WB) Detection Cartridge can be used for top reads only.

## Analyzing Western Blot TRF Data

After you scan a membrane for Western Blot data, the data displays in the software as an image. The image tools in the Plate section enable you to zoom, crop, colorize, and adjust the intensity of the image. You can select a region of interest (ROI) and rescan the membrane at a higher resolution.

Western Blot membrane data is saved as a TIFF image to enable you to use the image analysis tool of your choice for analysis. The SoftMax Pro Software includes a version of the ImageJ software from U.S. National Institute of Health (NIH).

For best results, use the Molecular Devices ScanLater™ Western Blot Assay Kit that matches your application.

## Blocking Nonspecific Binding

To reduce noise, use blocking buffer to reduce non-specific protein from binding with the membrane.

No single-blocking reagent is optimal for every antigen-antibody pair. Some primary antibodies can exhibit greatly reduced signal or different nonspecific binding in different blocking solutions. If you have difficulty detecting your target protein, changing the blocking solution can dramatically improve the performance. If the primary antibody has worked well in the past using chemiluminescent detection, try that same blocking solution for detection. Other commonly used blocking buffers other than BSA are 3% casein and 5% non-fat milk.

Milk-based blockers can contain IgG that can cross react with anti-goat antibodies. This can significantly increase background and reduce antibody titer. Milk-based blockers can also contain endogenous biotin or phosphoepitopes that can cause higher background.

To prevent background speckles on blots, use high-quality, ultra-pure water for buffers.

Do not over-block. Extended blocking times can cause loss of target protein from the membrane.

## Handling Membranes

To scan a membrane, you must first place it in a Molecular Devices ScanLater™ membrane holder. See [Loading the Membrane Holder on page 68](#).



**Note:** Handle membranes by their edges only, using clean forceps. Do not touch the membrane with gloved or bare hands.

---

The maximum size of a membrane that will fit in the membrane holder is 109 mm x 77 mm.

The Western Blot should be prepared using standard blotting procedures for the membrane being used. For optimal results, use Millipore Immobilon FL (IPFL00010). If using PVDF, pre-wet the membrane in 100% methanol.

Use enough antibody volume so that the entire membrane surface is sufficiently covered with liquid at all times. Use heat-seal bags if the volume is limiting. Do not let an area of the membrane dry out. Use agitation for all antibody incubations.

Small proteins can pass through the membrane during transfer (“blow-through”). To prevent this, use a membrane with a smaller pore size or reduce the transfer time.

Allow the blot to dry for a minimum of 1 hour before detection.

Do not wrap the membrane in plastic when scanning.

## Chapter 6: Maintenance and Troubleshooting

Perform only the maintenance tasks described in this guide. Contact a Molecular Devices service engineer to inspect and perform a preventive maintenance service on the instrument each year. See [Obtaining Support on page 118](#).

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



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

---

### Preventive Maintenance

To ensure optimal operation of the instrument, perform the following preventive maintenance procedures as needed:

- 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.
- Open the detection cartridge drawer only when installing or removing a detection cartridge.
- When a detection cartridge is not in use and removed from the instrument, always store the detection cartridge in the detection cartridge box.
- Follow applicable decontamination procedures as instructed by your laboratory safety officer.
- Contact Molecular Devices or an approved dealer for annual preventive maintenance. Molecular Devices recommends a yearly preventive maintenance be done on the instrument by a trained and qualified service engineer.
- Respond as required to all error messages displayed by the software.
- Power off the instrument when not in use.
- Use an applicable Validation Plate to regularly test the functionality of the instrument and detection cartridges. For more details, contact sales or service.

**Validation Packages Part Numbers**

Part Number	Item Name	Compatible Instruments
0200-6117	SpectraTest ABS1 Absorbance Validation Plate	SpectraMax iD3, iD5, i3x, i3, M2, M2e, M3, M4, M5, M5e, Plus 384, 340PC 384, 190, ABS, ABS Plus, VersaMax, FlexStation 3
0200-5060	SpectraTest FL1 Fluorescence Validation Plate	Gemini EM, Gemini XPS, SpectraMax iD3, iD5, i3x, i3, M2, M2e, M3, M4, M5, M5e, FlexStation 3
0200-6186	SpectraTest LM1 Luminescence Validation Plate	SpectraMax iD3, iD5, i3x, i3, M3, M4, M5, M5e, SpectraMax L, FlexStation 3
0200-2420	Cuvette Absorbance Validation Set	SpectraMax Plus 384, ABS Plus, M2, M2e, M3, M4, M5, M5e
0200-7200	Multi-Mode Validation Plate	FilterMaxF3, FilterMaxF5, SpectraMax Paradigm, iD5*, i3, i3x* * Specific read modes or cartridges.

**Cleaning the Instrument**

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



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



**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.

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

## Replacing Fuses

If the instrument does not seem to be getting power after switching it on, check to see whether the supplied 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, check that the power cord is not loose or disconnected and that power to the power outlet is functioning properly.

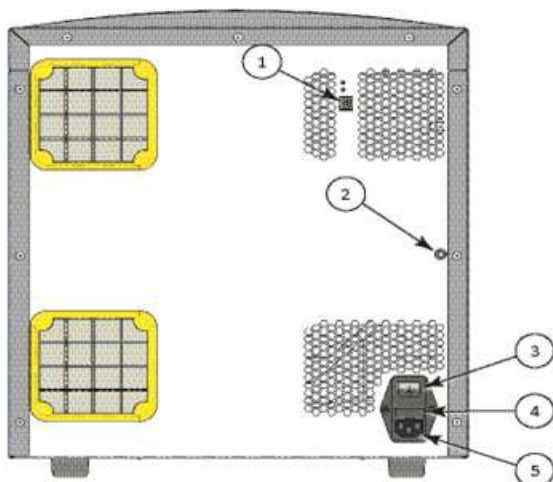
If these checks fail to remedy the loss of power, replace the fuses. You can obtain replacement fuses from Molecular Devices. For fuse specifications and part numbers, see [Environmental Controls on page 120](#).



**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 on the rear of the instrument.



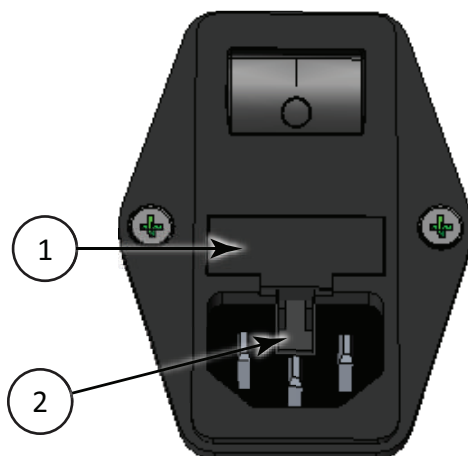
**Power Switch, Fuses, and Connection Ports**

Item	Description
1	USB port
2	Gas inlet quick connect fitting
3	Power switch
4	Fuse carrier
5	Power port

To replace the fuses:

**⚠️ WARNING! HIGH VOLTAGE** Power off the instrument and disconnect the power cord before you do maintenance procedures that require removal of a panel or cover or disassembly of an interior instrument component.

1. Power off the instrument.
2. Unplug the power cord from the power port.
3. Use a small flat head screwdriver to gently press on the carrier release tab and then pull the fuse carrier to remove it from the instrument.



#### Release Fuse Carrier

Item	Description
1	Fuse carrier
2	Carrier release tab

4. Gently pull the old fuses from the carrier by hand.
5. 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. Power on the instrument.



**Note:** If the instrument still does not power on after you change the fuses, contact Molecular Devices technical support. See [Obtaining Support on page 118](#).

## Before You Move the Instrument



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

Before you move the instrument, make sure that the new location is a dry, flat work area that has sufficient space for the instrument, host computer, and required cables. See [Instrument Specifications on page 119](#).



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

To minimize the possibility of damage during storage or shipment, you should pack the instrument in the original packaging materials. Correctly repacking the instrument includes following applicable decontamination procedures and installing the transport locks on the plate drawer, detection cartridge drawers, and the PMT shutter.

Store the instrument in a dry, dust-free, environmentally controlled area.



## Installing Software Locks

Before you move or pack the instrument, you must use the SoftMax Pro Software to install the software locks on the plate drawer and the detection cartridge drawers to protect the instrument from damage during shipping.

To install the software locks:

1. Power on the instrument and press the Standby button to put the instrument in Operation mode.
2. Make sure that all detection cartridges have been removed and that there is no plate in the plate drawer.
3. Power on the host computer and start the SoftMax Pro Software.
4. On the Instrument Connection dialog, connect the software to the instrument.
5. Select the Operations tab and click the **Info** button to display the Instrument Information dialog.
6. Click **Lock Instrument for Shipment**. The instrument proceeds to lock the detection cartridge drawers and the plate drawer. Leave the plate drawer open to install the hardware transport locks as described in the next section
7. Power off the instrument.
8. Shut down the SoftMax Pro Software and power off the host computer.

## Installing Transport Locks

Before you move or pack the instrument, install the hardware transport locks on the plate drawer and the detection cartridge drawers to protect the instrument from damage during shipping. You already removed all detection cartridges from the detection cartridge drawers and any plates from the plate drawer. You also installed the software locks and left the plate drawer open.

### Required tool

Part Number	Description
YW 000 006	2.0 mm hex key



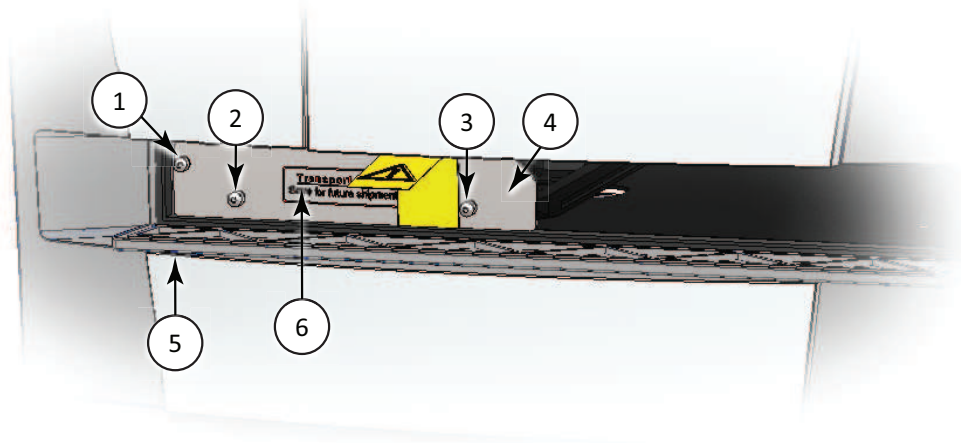
**CAUTION!** Do not touch or loosen screws or parts other than those specifically designated in the instructions. Doing so could cause misalignment and possibly void the warranty.



**CAUTION!** The front cover is held onto the front of the instrument by powerful magnets. Keep magnetic storage devices or strips, such as hard drives, key cards, and credit cards, away from the instrument covers.

To install the transport locks:

1. Make sure the power switch is in the off position.
2. Unplug the power cord from the power port on the rear of the instrument.
3. Place the plate drawer transport lock on the end of the plate drawer.
4. Use the 2.0 mm hex key to tighten screws #2 and #3 until the lock is attached to the plate drawer.

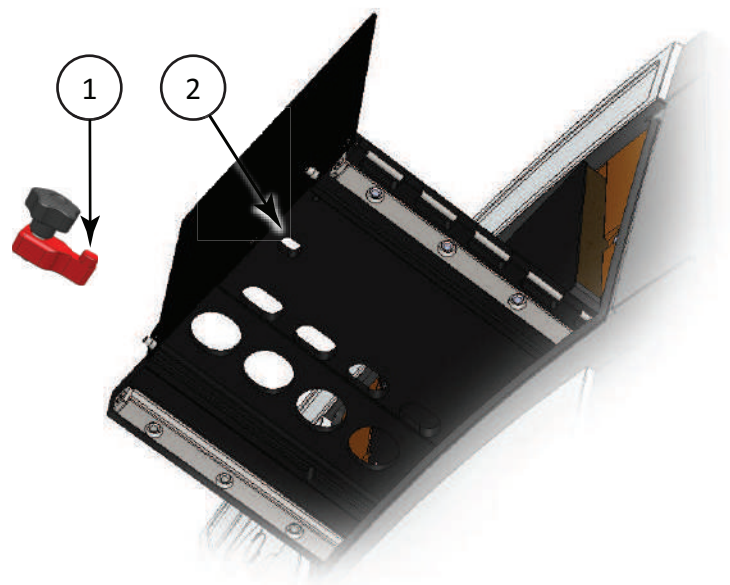


#### Plate Drawer Transport Lock

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

5. Gently push the plate 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, is lined up with the hole on the internal frame. You must hold the plate chamber door open until you fasten the transport lock.
6. Tighten screw #1 until the plate drawer is securely locked in place.
7. Route the yellow tab connected to the transport lock so that it will pass over the top of the plate chamber door when closed.
8. Gently close the plate chamber door.
9. Firmly pull on the bottom of the cover on the top detection cartridge drawer to remove it. The cover is held in place by powerful magnets.
10. Firmly pull on the top of the cover on the bottom detection cartridge drawer to remove it. The cover is held in place by powerful magnets.

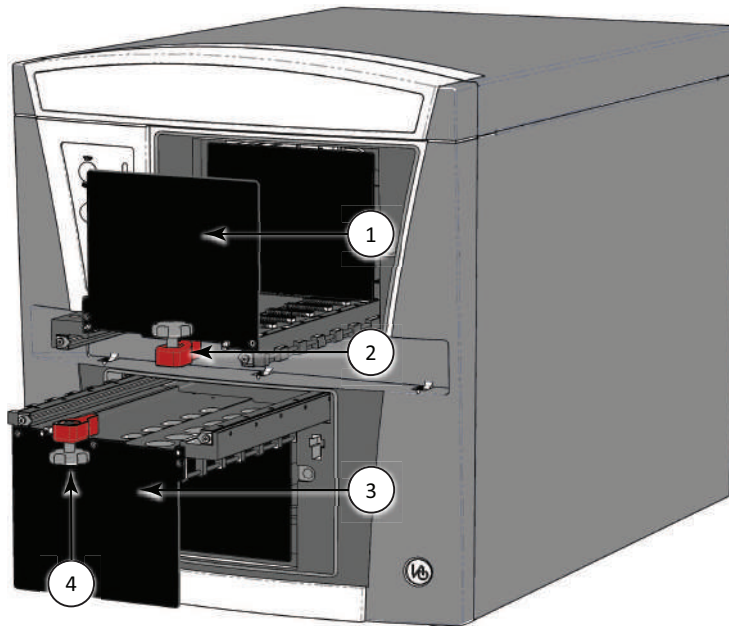
11. Slide the top detection cartridge drawer forward until it is outside the main instrument.
12. Place the tab on the detection cartridge drawer transport lock into the slot on the bottom of the top detection cartridge drawer.



**Top Detection Cartridge Drawer Bottom View**

Item	Description
1	Detection cartridge drawer transport lock tab
2	Detection cartridge drawer transport lock slot

13. Gently push the top detection cartridge drawer back inside the instrument.

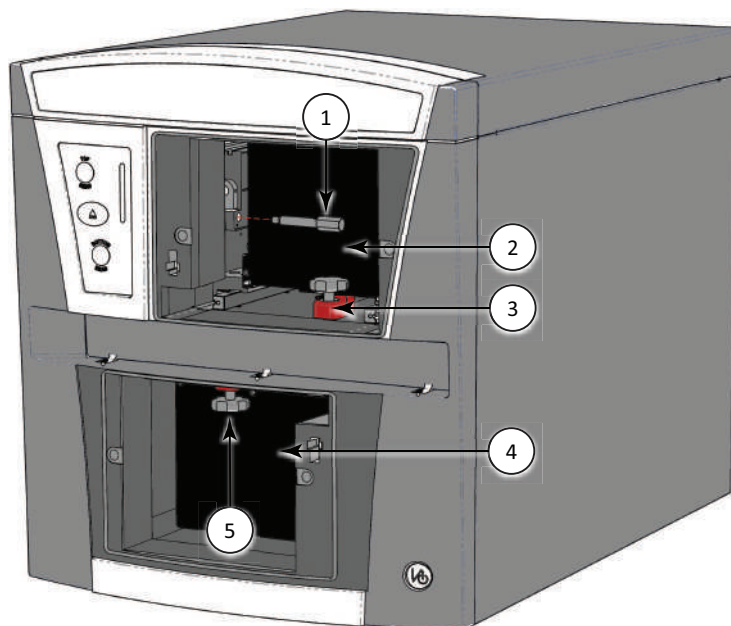


#### Detection Cartridge Drawer Transport Lock

Item	Description
1	Top detection cartridge drawer
2	Top detection cartridge drawer transport lock
3	Bottom detection cartridge drawer
4	Bottom detection cartridge drawer transport lock

14. Align the detection cartridge transport lock with the threaded hole on the floor of the top detection cartridge drawer chamber, and then turn the transport lock clockwise to lock the top detection cartridge drawer in place.

15. Insert the PMT transport lock into place and turn it clockwise to fasten it.



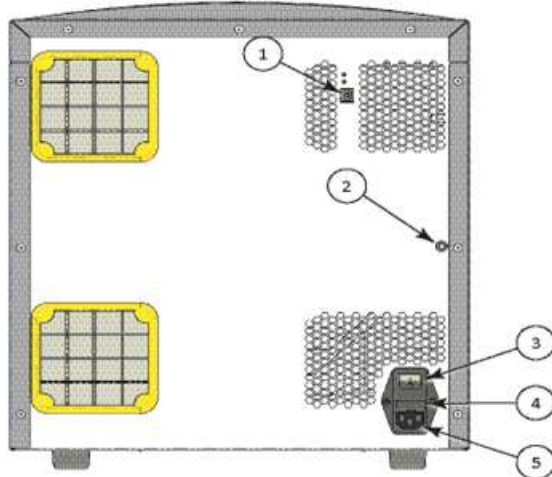
#### PMT Shutter and Detection Cartridge Drawer Transport Locks

Item	Description
1	PMT shutter transport lock
2	Top detection cartridge drawer
3	Top detection cartridge drawer transport lock
4	Bottom detection cartridge drawer
5	Bottom detection cartridge drawer transport lock

16. Place the tab on the detection cartridge drawer transport lock into the slot on the top of the bottom detection cartridge drawer.
17. Gently push the bottom detection cartridge drawer back inside the instrument.
18. Align the detection cartridge transport lock with the threaded hole on the roof of the bottom detection cartridge drawer chamber, and then turn the transport lock clockwise to lock the bottom detection cartridge drawer in place.
19. Align the magnets on the top detection cartridge cover with the magnets on the instrument to install the cover on the top detection cartridge drawer.
20. Align the magnets on the bottom detection cartridge cover with the magnets on the instrument to install the cover on the bottom detection cartridge drawer.

## Disconnecting Instrument Cables

Before you disconnect the cables, make sure that you have done all the steps to install the software locks and the hardware transport locks.



### Power Switch, Fuses, and Connection Ports

Item	Description
1	USB port
2	Gas inlet quick connect fitting
3	Power switch
4	Fuse carrier
5	Power port

To disconnect the instrument cables:

1. Disconnect the gas supply line, if applicable.
2. Unplug the power cord from the instrument and from the wall outlet.
3. Disconnect the USB cable from the instrument and from the computer.
4. Store the power cord and the USB cable in the accessories toolbox.


## Packing the Instrument

The original packaging is designed to protect the instrument during transportation. You must pack and ship detection cartridges separately from the instrument.



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

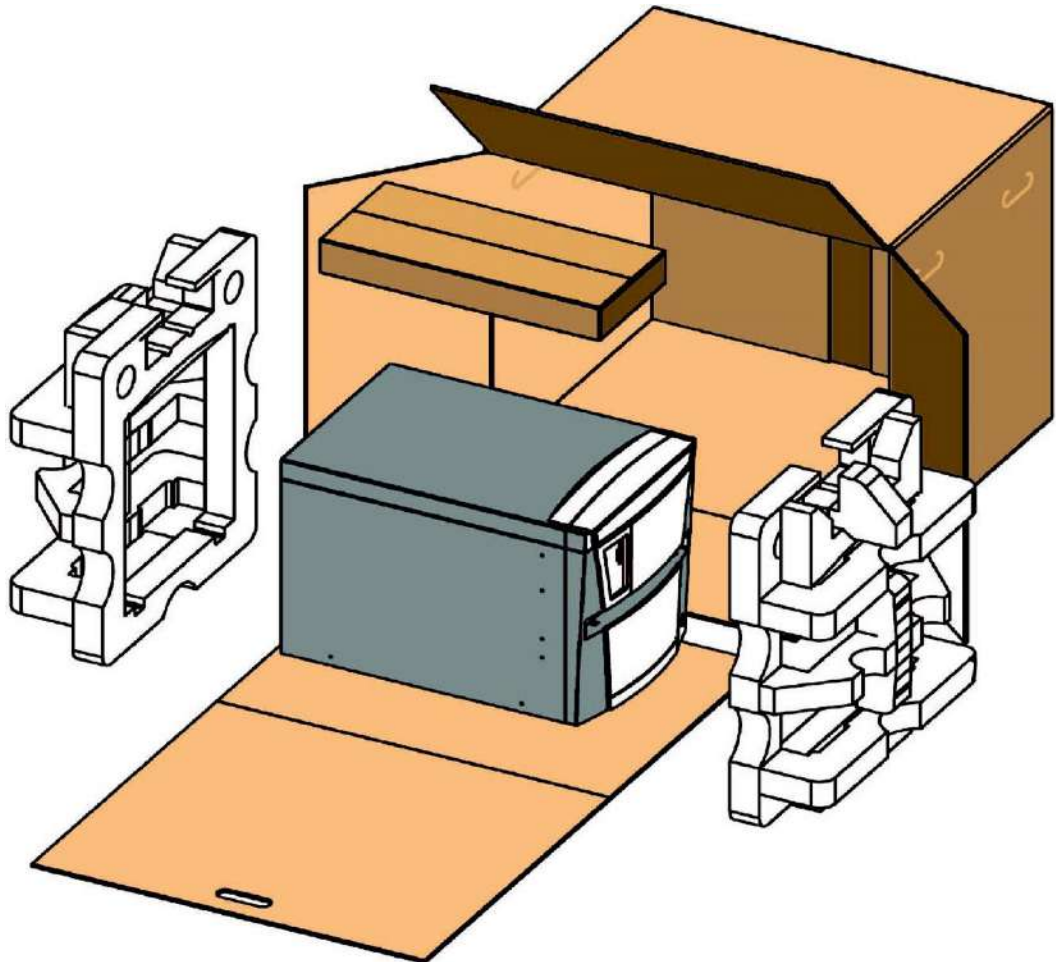
---

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


---

To pack the instrument:

1. Store the power cord and the USB cable in the instrument accessories toolbox.
2. Wrap the instrument in static-free plastic.
3. Replace the two pieces of molded foam packaging on each end of the instrument.



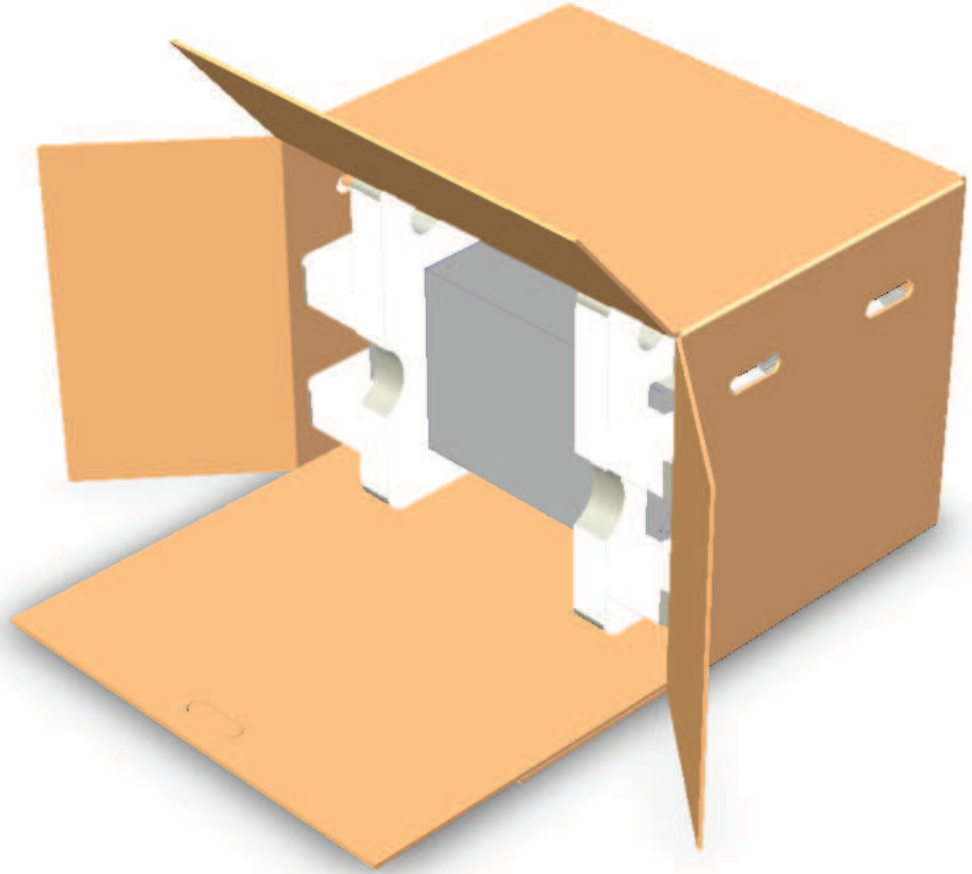
---

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

---

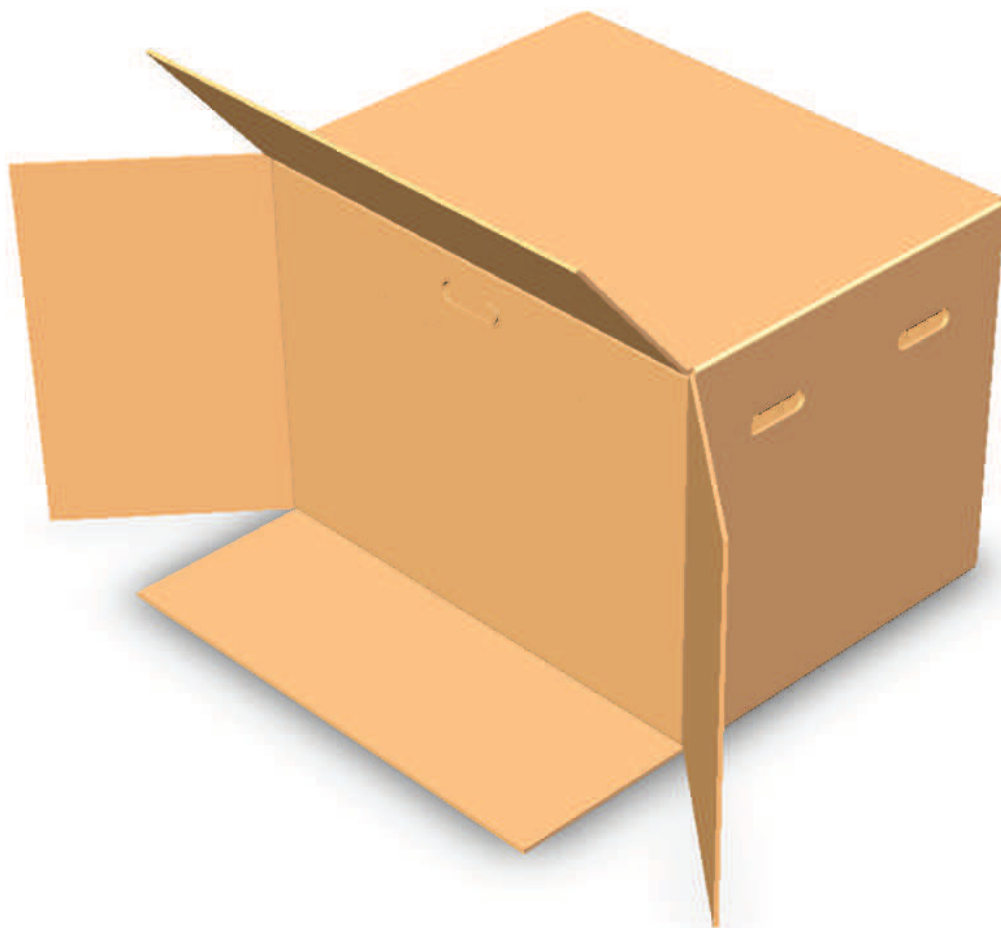
4. Place the accessories box in the foam packaging above the instrument.

5. Place the instrument and accessories box on the flat cardboard piece and slide it into the original box.





6. Fold the cardboard flap up inside the box.



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



## Troubleshooting



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

If	Then
All LEDs on the status indicator are out and the LED on the Standby button does not illuminate.	Make sure that the instrument is plugged in and powered on. Inspect the fuses. See <a href="#">Replacing Fuses on page 105</a> .
The fuses look burned or the filaments are broken.	Replace the fuses. See <a href="#">Replacing Fuses on page 105</a> .
All LEDs on the status indicator are out, the LED on the Standby button does not illuminate, the power is on, the fuses are okay, and the instrument does not operate.	Contact technical support. See <a href="#">Obtaining Support on page 118</a> .
The instrument does not respond to commands from the software.	Observe the status indicators and respond as required. See <a href="#">Status Indicators on page 28</a> . Inspect the USB connections to the instrument and the controlling computer. When the computer and instrument are both on and connected, a green LED illuminates above the USB port on the instrument. In the software, verify that the configured USB port matches the USB port connecting the instrument to the computer. See “Selecting an Instrument” in the <i>SoftMax Pro Data Acquisition and Analysis Software User Guide</i> or the application help. Contact technical support. See <a href="#">Obtaining Support on page 118</a> .
Functional failure of a detection cartridge	Install the detection cartridge in a different detection cartridge slot. Contact technical support. See <a href="#">Obtaining Support on page 118</a> .

## Obtaining Support

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

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

Please have your instrument serial number or Work Order number, and your software version number available when you call.



**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 it is applicable to do so. If parts are returned, they must be enclosed in a sealed plastic bag stating that the contents are safe to handle and are not contaminated.

---

## Appendix A: Instrument Specifications



### Physical Specifications: SpectraMax Paradigm Multi-Mode Microplate Reader

Item	Description
Environment	Indoor use only
Power requirements	100 VAC to 240 VAC $\pm$ 10%, 2 amps, frequency range: 50/60 Hz
Dimensions	39.20 cm W x 45.00 cm H x 63.00 cm D (15.43 in. W x 17.70 in. H x 23.84 in. D)
Weight	Without detection cartridges: 45.0 kg (99.2 lbs) For detection cartridge weights, see the detection cartridge chapters.
Power disconnect and ventilation clearance (rear)	20 cm to 30 cm (7.9 in. to 11.8 in.) between the rear of the instrument and the wall
Drawer clearance (front)	18 cm (7.1 in.) for the plate drawer and 15 cm (5.9 in.) for the detection cartridge drawer
Ambient operating temperature	15°C to 40°C (59°F to 104°F)
Ambient storage temperature	-5°C to 40°C (23°F to 104°F) continuous; -20°C to 50°C (-4°F to 122°F) transient (up to 10 hours)
Humidity restrictions	15% to 75% (non-condensing) at 30°C (86°F)
Altitude restrictions	Up to 2000 m (6562 ft)
Air pressure restrictions	54 kPa to 106 kPa (7.8 PSI to 15.4 PSI)
Sound pressure level	Maximum sound pressure: 73 dBA Maximum sound pressure at one meter: 68 dBA
Installation category	II
Pollution degree	2
Fuses	250 V, 4 amp, 5 x 20 mm, slow-blow, UL recognized/CSA/VDE (part number: S US 400 153)
Data connection	USB 2.0 port

## Environmental Controls

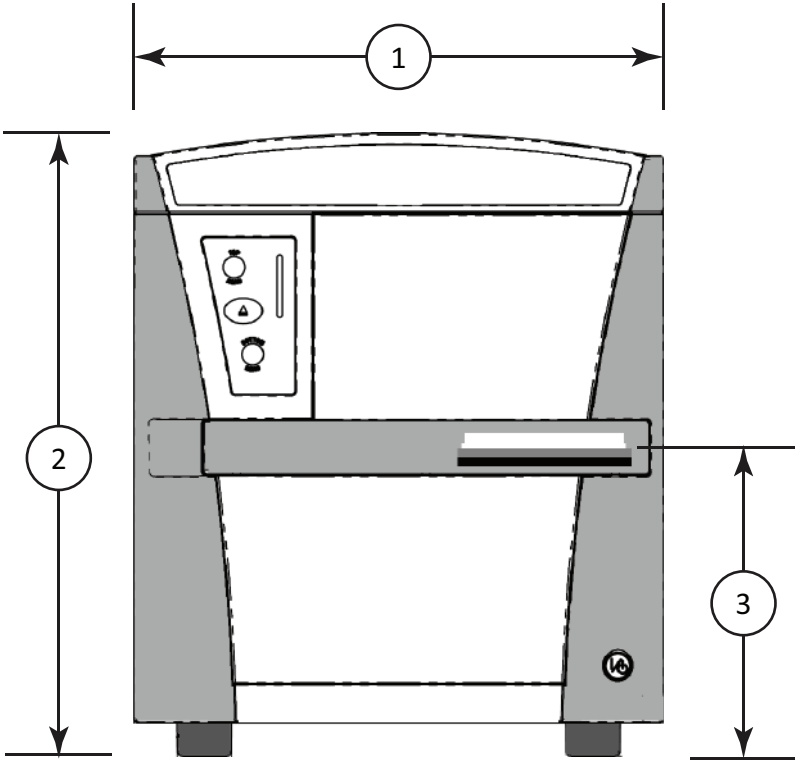
### Environmental Controls: SpectraMax Paradigm Multi-Mode Microplate Reader

Item	Description
Temperature range	LED-based detection cartridges: 4°C (7.2°F) above ambient to 45°C (113°F) Xenon flash lamp based detection cartridges: 5°C (7.2°F) above ambient to 45°C (113°F)
Displayed	1°C (1.8°F) increments
Accuracy	±1°C (1.8°F) at 37°C set point
Uniformity	±1.5°C (2.7°F)
Gas inlet specification	Maximum air supply pressure: 0.4 MPa
Gas tubing specification	Polyurethane tubing, outside diameter = 4.0 mm



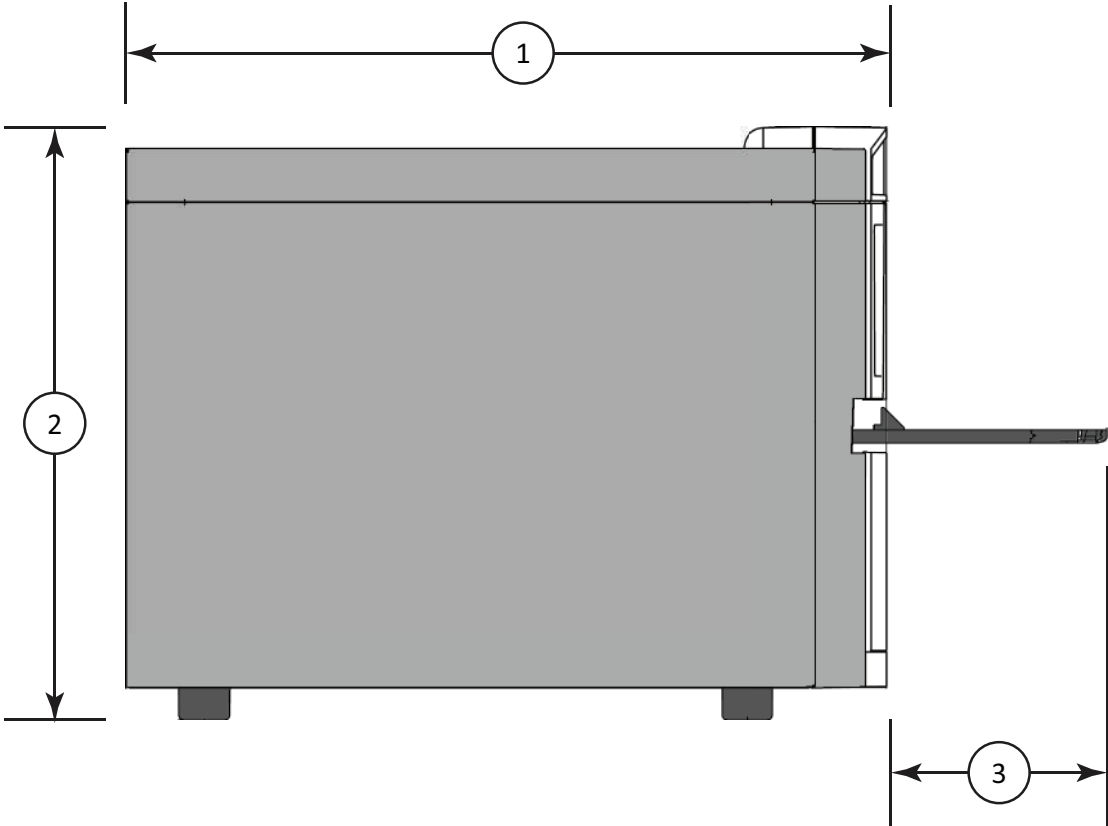
**WARNING!** Use a compressed gas supply in a well-ventilated area. The instrument is not air-tight. Gas can escape into the atmosphere surrounding the instrument. When you use potentially toxic gas, observe the cautionary procedures your safety officer defines to maintain a safe work environment.

# Appendix B: System Diagrams and Dimensions



**Front View: SpectraMax Paradigm Multi-Mode Microplate Reader**

Item	Description
1	Width: 39.20 cm (15.43 in.)
2	Height: 45.00 cm (17.70 in.)
3	Height of plate drawer: 22 cm (8.6 in.)



**Side View: SpectraMax Paradigm Multi-Mode Microplate Reader**

Item	Description
1	Depth: 63.00 cm ( 23.84 in.)
2	Height: 45.00 cm (17.70 in.)
3	Maximum length of open plate drawer: 17.5 cm (6.8 in.)

## Appendix C: Electromagnetic Compatibility



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

This ISM device complies with Canadian ICES-001.

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

### **ISM Equipment Classification (Group 1, Class A)**

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



# Glossary

## A

### Absorbance

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

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

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

See [Absorbance Read Mode on page 74](#).

### AlphaScreen

ALPHA stands for Amplified Luminescent Proximity Homogeneous Assay. AlphaScreen® is a bead-based chemistry used to study molecular interactions between moieties A and B, for example. When a biological interaction between A and B moves beads (coated with A and B, respectively) together, a cascade of chemical reactions produce a greatly amplified signal.

The cascade finally resulting in signal is triggered by laser excitation (680 nm), making a photosensitizer on the A-beads convert oxygen to an excited (singlet) state. The energized oxygen diffuses away from the A-bead. When reaching the B-bead in close proximity, it reacts with a thioxene derivative on the B-bead generating chemiluminescence at 370 nm. Energy transfer to a fluorescent dye on the same bead shifts the emission wavelength into the 520 nm to 620 nm range. The limited lifetime of singlet oxygen in solvent (~4 microseconds) lets diffusion reach up to only around 200 nm distance. Thus, only B-beads in the proximity of A-beads yield signal, which indicates binding between moieties A and B.

An AlphaScreen measurement includes a light pulse, by turning on the laser diode for a specified time, turning off the laser diode, followed by the measurement of the AlphaScreen signal, as specified in the measurement protocol timing parameters.



**Note:** AlphaScreen beads are light sensitive. Beads are best handled under subdued (<100 lux) or green filtered (Roscolux filters #389 from Rosco, or equivalent) light conditions. Do incubation steps in the dark.

---

See [AlphaScreen Read Mode on page 97](#).

## E

### Emission Spectrum Scan

An emission spectrum scan measures fluorescence or luminescence across a spectrum of wavelengths. Fluorescent reads use the emitted light from a fixed excitation wavelength. The default value reported for each well is the wavelength of maximum emission in either RFU for fluorescence mode or RLU for luminescence mode.

### Endpoint

For the Endpoint read type, a read of each plate 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).

### Excitation Spectrum Scan

An excitation spectrum scan measures fluorescence at a single emission wavelength for the emitted light across a spectrum of excitation wavelengths. The default value reported for each well is the wavelength of maximum fluorescence excitation in RFU.

## F

### Fluorescence

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:

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

See [Fluorescence Intensity Read Mode on page 79](#).

## Fluorescence Polarization

Fluorescence Polarization detection is based on Fluorescence Intensity, with the important difference that it uses plane-polarized light, rather than non-polarized light. Microplate readers measure the Fluorescence Polarization of the sample by detecting light emitted both parallel and perpendicular to the plane of excitation.

By using a fluorescent dye to label a small molecule, the binding of a small molecule to an interaction partner of equal or greater size can be monitored through its speed of rotation.

See [Fluorescence Polarization Read Mode on page 94](#).

## Fluorophore

A fluorophore is a material that absorbs light energy of a characteristic wavelength, undergoes an electronic state change, and emits light of a longer wavelength.

See [Fluorescence Intensity Read Mode on page 79](#).

## G

### G Factor

The G factor, or grating factor, is used in Fluorescence Polarization to correct polarization data for optical artifacts, converting relative mP data to theoretical mP data. Optical systems, particularly with reflective components, pass light of different polarization with different efficiency. G factor corrects for this instrument-based bias.

## K

### Kinetic

For the Kinetic read type, the instrument collects data over time with reads taken in the center of each well at regular intervals.

The values calculated based on raw kinetic data include VMax, VMax per Sec, Time to VMax, Onset Time, and more. Kinetic reads can be single-wavelength or multiple-wavelength reads.

## L

### Luminescence

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.

See [Luminescence Read Mode on page 83](#).

## M

### Membrane

The Membrane read type is used for a Time-Resolved Fluorescence read of a Western Blot membrane.

See [Membrane Read Type on page 73](#) and [ScanLater Western Blot TRF Read Mode on page 100](#).

## O

### Optical Density (OD)

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.

See [Absorbance Read Mode on page 74](#).

## P

### 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.

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

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

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

See [PathCheck Pathlength Measurement Technology on page 75](#).

### **Photomultiplier Tube (PMT)**

A Photomultiplier Tube (PMT) is a vacuum tube that can detect light from dim sources through the use of photo emission and successive instances of secondary emission to produce enough electrons to generate a useful current.

## **R**

### **Raw Data**

Raw data is the signal reported from the instrument with no changes made. This is reported as optical density (OD), relative fluorescence units (RFU), or relative luminescence units (RLU), depending on the read mode.

### **Reduced Data**

Data reduction causes the raw signal values reported by the instrument to be calculated and displayed based on user-defined formula settings.

## **S**

### **Spectrum**

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

See [Spectrum Read Type on page 72](#), [Emission Spectrum Scan on page 125](#), and [Excitation Spectrum Scan on page 125](#).

### **Stokes Shift**

The Stokes shift is the difference between the wavelengths of the excitation and emission maxima, or peaks.

See [Emission Spectrum Scan on page 125](#) and [Excitation Spectrum Scan on page 125](#).

## T

### Time-Resolved Fluorescence (TRF)

Time-Resolved Fluorescence is a measurement technique that depends on three characteristics that lead to better discrimination between the specific signal, proportional to the quantity of label, and the unspecific fluorescence resulting from background and compound interference:

- Pulsed excitation light sources
- Time-gated electronics faster than the fluorescence lifetime
- Labels with prolonged fluorescence lifetime

The time-gating electronics introduce a delay between the cutoff of each light pulse and the start of signal collection. During the delay, the unspecific fluorescence (caused by test compounds, assay reagents, and the plate) vanishes, while only a small portion of the specific fluorescence from the label is sacrificed. Enough of the specific signal remains during the decay period, with the added benefit of reduced background.

See [Time-Resolved Fluorescence Read Mode on page 86](#).

### %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.

## W

### Well Scan

The Well Scan read type can take reads at more than one location within a well. The Well Scan read type takes multiple reads of a single well of a plate on an evenly spaced, user defined grid or other pattern inside of each well at single or multiple wavelengths.

Some applications involve the detection of cells in large area tissue culture plates. Use the Well Scan read type with such plates to permit maximum surface area detection in cell-based protocols. Since many cell lines tend to grow as clumps or in the corners of plate wells, you can choose from several patterns and define the number of points to scan to work best with your application.

See [Well Scan Read Type on page 72](#).

## Z

### Z'

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. You can use higher values 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.

## Contact Us

Phone: [+1-800-635-5577](tel:+1-800-635-5577)  
Web: [moleculardevices.com](http://moleculardevices.com)  
Email: [info@moldev.com](mailto:info@moldev.com)

Visit our website for a current listing of worldwide distributors.