SpectraTest LM1 Luminescence Validation Plate

User Guide

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MOLECULAR DEVICES

Product manufactured by Molecular Devices, LLC.
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Molecular Devices, LLC is ISO 9001 registered.

Contents

Chapter 1: SpectraTest LM1 Luminescence Validation Plate Overview

Molecular Devices® microplate readers are designed to provide consistent performance for many years. The SpectraTest® LM1 Luminescence Validation Plate from Molecular Devices enables you to validate the optical performance of the following instruments:

- FlexStation® 3 Multi-Mode Microplate Reader
- SpectraMax® i3 Multi-Mode Microplate Reader
- SpectraMax® i3x Multi-Mode Microplate Reader
- SpectraMax® iD3 Multi-Mode Microplate Reader
- SpectraMax® iD5 Multi-Mode Microplate Reader
- SpectraMax® L Luminescence Microplate Reader
- SpectraMax® M3 Multi-Mode Microplate Reader
- SpectraMax® M4 Multi-Mode Microplate Reader
- SpectraMax® M5 Multi-Mode Microplate Reader
- SpectraMax® M5e Multi-Mode Microplate Reader

The SpectraTest LM1 Luminescence Validation Plate is a comprehensive optical validation package. The SoftMax® Pro Data Acquisition and Analysis Software Protocol Library includes instrument specific protocols that automatically read the validation plate, perform the required test measurements, and make the required calculations. The software also enables you to customize the test report format.

ISO-17025

SpectraTest LM1 Luminescence Validation Plate is a tool of metrology. The American Association for Laboratory Accreditation (A2LA) has granted accreditation to the Laboratory Quality System under ISO/IEC 17025 for validation plate calibration and re-calibration processes.

Package Contents

The SpectraTest LM1 Luminescence Validation Plate package contains the following items:

- Validation Plate
  - Lamp
  - 20 wells of OD2 filters
  - Wells with filters that range from OD0.5 to OD5
  - One bright well
- Certificates of Calibration
  Each certificate is created for a specific instrument. You must enter the values from the certificate that is for the instrument you plan to validate.
- Validation Plate User Guide
- Protective Sleeve and Case
- Screwdriver (to change the battery)

Validation Packages Part Numbers

Part Number	Item Name	Compatible Instruments
n/a*	SpectraTest ABS1 Absorbance Validation Plate	FlexStation 3, SpectraMax 190, SpectraMax 340PC384, SpectraMax ABS, SpectraMax ABS Plus, SpectraMax i3, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, SpectraMax M5e, SpectraMax Plus 384, VersaMax
0200-6191	SpectraTest ABS2 Absorbance Validation Plate	FlexStation 3, SpectraMax ABS, SpectraMax ABS Plus, SpectraMax i3, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, SpectraMax M5e, SpectraMax Plus 384
0200-5060	SpectraTest FL1 Fluorescence Validation Plate	FlexStation 3, Gemini EM, Gemini XPS, SpectraMax i3, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, SpectraMax M5e
0200-6186	SpectraTest LM1 Luminescence Validation Plate	FlexStation 3, SpectraMax i3, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, SpectraMax L, SpectraMax M3, SpectraMax M4, SpectraMax M5, SpectraMax M5e
0200-2420	Cuvette Absorbance Validation Set	SpectraMax ABS Plus, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, SpectraMax M5e, SpectraMax Plus 384
0200-7200	Multi-Mode Validation Plate	FilterMax F3, FilterMax F5, SpectraMax i3*, SpectraMax i3x*, SpectraMax iD5*, SpectraMax Paradigm * Specific read modes or cartridges.

* Discontinued. Use the SpectraTest ABS2 Absorbance Validation Plate.

Care and Handling

Treat the optical standards with care to retain their validity. The SpectraTest LM1 Luminescence Validation Plate is vulnerable to ambient contamination. Do not expose to extreme temperatures and do not expose to direct sunlight for an extended period of time. When the plate is subjected to significant temperature changes, leave the plate in the storage sleeve until it reaches the ambient temperature to avoid condensation issues.

When not in use, keep the plate in the plastic storage sleeve in the storage case to protect the optical surfaces from dust, scratches, and corrosion. Do not touch the wells with your fingertips. Do not store the plate in the case without first putting the plate in the storage sleeve.

Inspect the plate before all plate runs to look for dust and dirt. If you observe dust on the plate, blow moisture-free, clean canned air across both sides of the plate. Do not use air from “house” air lines and do not blow on the plate with your mouth to clean it. See Maintenance and Troubleshooting on page 29.

Certificate of Calibration

Each validation plate comes with a Certificate of Calibration that contains information specific to the individual validation plate for which it is created. In addition to details that are relevant for ISO 17025 compliance, the following information is included:

- Serial Number
- Certificate Number
- Certification Date
- Certificate Luminescence Unit (CLU) values for the luminescent wells

Note: The Certificate of Calibration is unique for each validation plate and applies only to the instruments listed on the certificate. Each certificate is created for a specific instrument. You must enter the values from the certificate that is for the instrument you plan to validate.

Factory certification of the validation plate's secondary standards is done using a reference instrument that is reserved exclusively for SpectraTest LM1 Luminescence Validation Plate calibration and is checked for accuracy at fixed intervals of time. Molecular Devices recommends that you have the SpectraTest LM1 Luminescence Validation Plate recertified yearly. See Recertification on page 29.

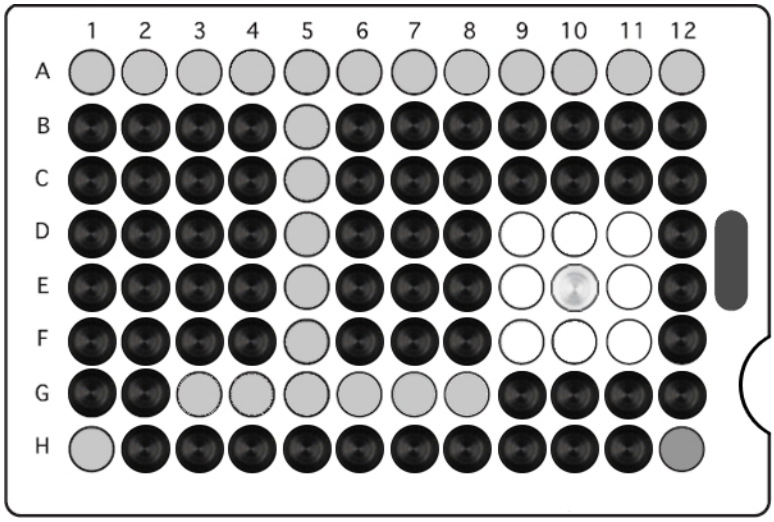
Available Tests

The SpectraTest LM1 Luminescence Validation Plate enables you to qualify the performance of the system by testing optical specifications that are critical to achieve quality results. Instruments with bottom-read capability use these same tests for both top-read mode and bottom-read mode. See Validation Tests on page 23.

Each LM1 validation protocol is specific to the instrument you test and can enable the following tests:

- Background Noise
- Background Spike
- Lower Limit of Detection (LLD)
- Cross-talk
- Linearity
- Kinetic Noise, Low RLU
- Kinetic Spike, Low RLU
- Kinetic Drift, Low RLU
- Kinetic Noise, High RLU
- Kinetic Drift, High RLU
- Well-to-Well Precision
- Left-to-Right Bias
- Top-to-Bottom Bias

The following indicates the columns related to the available tests.



SpectraTest LM1 Luminescence Validation Plate Configuration

Test	Wells
Background Noise (plate turned off)	All wells except E10 and G03
Background Spike (plate turned off)	All wells except E10 and G03
Lower Limit of Detection (LLD)	G03 through G06 for FlexStation 3, SpectraMax i3, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, SpectraMax M3, SpectraMax M4, SpectraMax M5, and SpectraMax M5e G03 through G08 for SpectraMax L
Cross-talk	D09 through D11, E09 through E11, and F09 through F11
Linearity	G03 through G06 for FlexStation 3, SpectraMax i3, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, SpectraMax M3, SpectraMax M4, SpectraMax M5, and SpectraMax M5e G03 through G08 for SpectraMax L
Kinetic Noise, Low RLU	D02
Kinetic Spike, Low RLU	D02
Kinetic Drift, Low RLU	D02
Kinetic Noise, High RLU	G03
Kinetic Drift, High RLU	G03
Well-to-Well Precision	A01 through A12, B05 through G05, H01, and H12
Left-to-Right Bias	A01, H01, A12, and H12
Top-to-Bottom Bias	A01 through A12, H01, and H12

Chapter 2: Validation Protocols

The SoftMax Pro Software Protocol Library contains protocols for use with the SpectraTest LM1 Luminescence Validation Plate. In the Protocol Library, there is a Reader Validation Plate folder that contains a protocol that is specific for the instrument to validate.

Before you run the validation protocol, confirm that the time and date settings on the computer are correct. The SoftMax Pro Software uses the computer system settings for the time and date stamps.

Download Validation Protocols

If needed, you can obtain the latest version of the validation protocols by contacting Molecular Devices support via the web site https://www.moleculardevices.com/support.html

1. Create a new folder (sub-directory) on the hard drive to contain the protocol file and give it a name of your choice.

   Note: To be consistent with the current Protocol Library naming convention, the location and name would be the following C:/Program Data/Molecular Devices/SMP<n.n>/Protocol Library/Reader Validation-Plate LUM.

2. Locate the protocol file to download. The protocol file name includes the instruments for which it is intended, such as FlexStation 3 LM1. Select the protocol that is for the instrument you plan to validate.
3. Save the protocol file in the folder you create.

Protocol Files

The experiments and sections in each SpectraTest LM1 Luminescence Validation Plate protocol file contain settings that are for a specific instrument. You should read all Note sections in each experiment. For additional information and instructions, see the *SoftMax Pro Data Acquisition and Analysis Software User Guide* or the application help.

Older validation protocols require manual entry of certificate information. See Manual Certification Entry on page 11.

You can use the EZinCert method to enter certificate information into newer validation protocols. See EZinCert Certificate Entry on page 16.

Chapter 3: Entering Certificate Information

Before you run a validation protocol you must enter information from the Certificate of Calibration that accompanies the validation plate into the SoftMax Pro Software. Enter the information one time before the initial use of the protocol and then again each time Molecular Devices recertifies the validation plate and sends you a new Certificate of Calibration.

Manual Certification Entry

Some validation protocols require manual entry of the certification information. You should read all Note sections in each experiment for additional information and instructions.

Manual Entry Protocol Sections

The following is an example of the sections in a validation protocol that requires manual certification entry:

 The **SpectraTest LM1** experiment contains the following Note sections:

-  **Reminder**: Contains recertification information.
-  **Introduction**: Contains a copy of the instructions found in this document.
-  **CertInfo**: You must enter the information from the Certificate of Calibration into this section. See Enter Certificate Information on page 12.
-  **Result (Top)**: Displays the result of the top read validation protocol after you run the protocol.
-  **Result (Bot)**: Displays the result of the bottom read validation protocol after you run the protocol.
-  **Revision**: Displays the results of the validation protocol when applicable.

 The **Background, Plate Off (Top)** experiment contains the following sections:

-  **Dark Adapt**: Contains the plate settings for the time delay acquisition.
-  **Plate Off**: Contains the plate settings for data acquisition.
-  Group sections perform calculations.

 The **Plate On (Top)** experiment contains the following sections:

-  **Dark Adapt**: Contains plate settings for the time delay acquisition.
-  **Kin1**: Contains plate settings for data acquisition.
-  **Plate**: Contains plate settings for data acquisition.
-  **Kin2**: Contains plate settings for data acquisition.
-  Group sections perform calculations.

 The **Background, Plate Off (Bot)** experiment for instruments with bottom-read capability contains the following sections:

- **Dark Adapt**: Contains plate settings for time delay acquisition.
- **Plate Off**: Contains plate settings for data acquisition.
- Group sections perform calculations.

The **Plate On (Bot)** experiment for instruments with bottom-read capability contains the following sections:

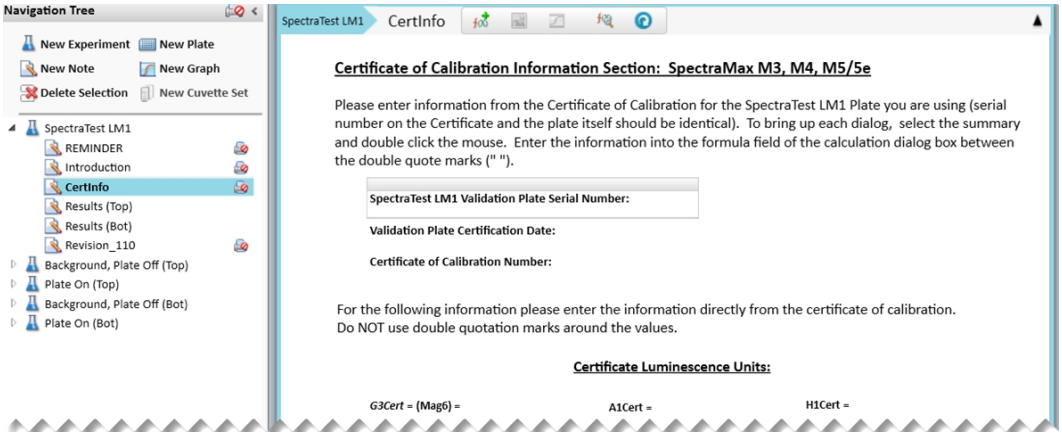
- **Dark Adapt**: Contains plate settings for time delay acquisition.
- **Kin1**: Contains plate settings for data acquisition.
- **Plate**: Contains plate settings for data acquisition.
- **Kin2**: Contains plate settings for data acquisition.
- Group sections perform calculations.

Enter Certificate Information

All protocol files enable you to enter the certification information.

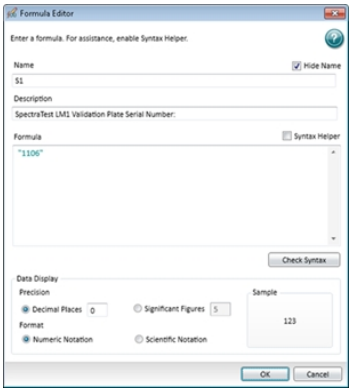
To enter certificate information:

1. In the SoftMax Pro Software, select the **Home** tab and click **Protocol Manager** > **Protocol Library** > **Reader Validation-Plate Lum** > **<instrument>** to open the instrument-specific validation protocol.
2. In the Navigation Tree, expand the **SpectraTest LM1** experiment and select the **CertInfo** Note section. For the new protocols, expand the **Appendix** experiment and then select the **CertInfo** Note section.



3. Double-click the **SpectraTest LM1 Validation Plate Serial Number** field to display the Formula Editor dialog.

4. In the **Formula** field, enter the Certificate of Calibration validation plate serial number within the double quotes, for example: “1106” and click **OK**.



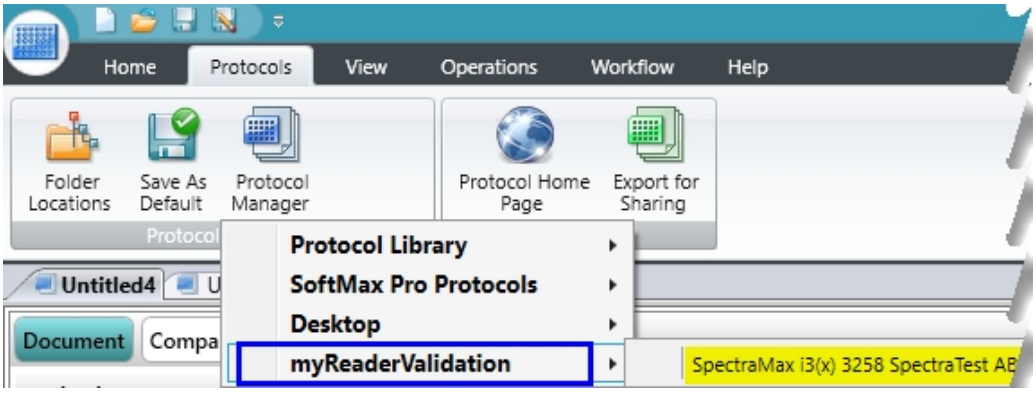
5. Double-click the **SpectraTest LM1 Certificate Date** field. In the **Formula** field, enter the certificate date in the double quotes, for example: “2025-01-22” and click **OK**.
6. Double-click the **LM1 Certificate of Calibration Number** field. In the **Formula** field, enter the Certificate of Calibration number in the double quotes, for example: “1” and click **OK**.
7. In the **Certificate Luminescence Unit** area, double-click each field to display the Formula Editor dialog and enter the corresponding value from the Certificate of Calibration into the Formula field.

Note: Do not use quotation marks around the Certificate Luminescence Unit values.

8. After you enter all certificate information, click **Save As** to save the file as a protocol file with a new name in order to save the certificate information and to prevent over writing the original protocol.
9. In the Save As dialog, click the **Save As Type** drop-down and select **Protocol Files** to save the file as a protocol.

Tip: Name the file with the validation expiration date and instrument type, for example LM1 Plate ID 2025-Jan-22 SpectraMax i3x and save the new protocol file.

You can use the Folder Locations feature to save the file to the folder of your choice.



The new protocol is now ready for use with the validation plate.

EZinCert Certificate Entry

Some new validation protocols allow you to use the EZinCert method to enter certificate information. You should read all Note sections in each experiment for additional information and instructions.

EZinCert Protocol Sections

The following is an example of the sections in a validation protocol for which you can use the EZinCert method to enter certification information:

The **OQ LM1 <instrument>** experiment contains the following Note sections:

- **Start**: Contains information to get you started with the protocol.
- **Results**: Displays the result of the validation protocol after you run the protocol.

The **Plate Off, Background** experiment contains the following sections:

- **Start**: Contains information to get you started with this experiment.
- **Dark Adapt**: Contains the plate settings for the time delay acquisition.
- **Plate Off**: Contains the plate settings for data acquisition.
- Group sections perform calculations.
- **Supplementary**: For internal use.
- **CalAndFactors**: For internal use.

The **Plate On, Signal** experiment contains the following sections:

- **Start**: Contains information to get you started with this experiment.
- **Dark Adapt**: Contains plate settings for the time delay acquisition.
- **Kin Low**: Contains plate settings for data acquisition.
- **Plate**: Contains plate settings for data acquisition.
- **Kin High**: Contains plate settings for data acquisition.
- **Result Details**: Section break title.
- Group sections perform calculations.
- **Appendix**: Section break title.
- Graph sections to display results graphically.
- **Supplementary**: For internal use.
- **CalAndFactors**: For internal use.

The **Option Bottom** experiment for instruments with bottom-read capability contains the following sections:

- **Plate On, Upside Down**: Contains information to get you started with this experiment.
- **Bottom Check**: Contains plate settings to check for bottom-read availability.

- **Dark Adapt**: Contains plate settings for time delay acquisition.
- **Plate**: Contains plate settings for data acquisition.
- **Result Details**: Section break title.
- Group sections perform calculations.
- **Appendix**: Section break title.
- Graph sections to display results graphically.
- **Supplementary**: For internal use.
- **CalAndFactors**: For internal use.

The **Appendix** experiment contains the following Note sections:

- **This Protocol**: Contains protocol information.
- **First Use**: Contains a copy of the instructions found in this document.
- **EZinCert**: Contains fields that enable you to use the EZinCert method to enter the Certificate of Calibration information into the protocol. See EZinCert Certificate Entry on page 14.
- **CertInfo**: Contains fields that enable you to manually enter the information from the Certificate of Calibration into this section. See Enter Certificate Information on page 12
- Remaining Note sections contain protocol information that you should read and become familiar with.

EZinCert Certificate Entry

In an effort to make the entry of certificate information more efficient, new validation protocols are designed to use the EZinCert method.

To use EZinCert to enter certification information:

1. Insert the USB drive that shipped with the validation plate into a USB slot on the computer.
2. Locate and open the (SN_Date_Cert#)_EZinCert.pdf file using Adobe Acrobat.

> **Note:** Do not open with an Internet browser as the data does not copy correctly.

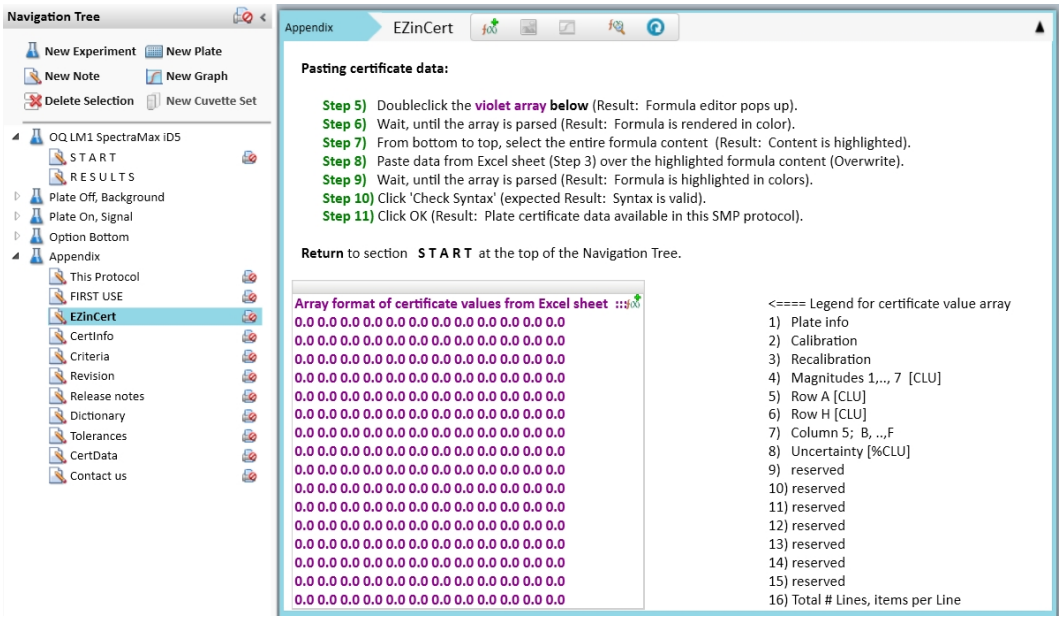
3. This file contains a group of values that have a shaded background. Select the values with the shaded background and copy this information to the computer clipboard (Ctrl+C).

```
(0~ 0~ 00001~ 0~ 0~ 561839442)&
(0~ 8~ 15~ 2019~ 2)&
(0~ 8~ 15~ 2020~ 3)&
(405~0.312~ 1.193~ 2.239~ 2.929)&
(440~0.314~ 1.138~ 2.141~ 2.943)&
(465~0.28~ 1.057~ 1.981~ 2.954)&
(490~0.289~ 1.097~ 2.067~ 2.958)&
(546~0.285~ 1.061~ 2.004~ 2.948)&
(590~0.302~ 1.129~ 2.145~ 2.941)&
(635~0.302~ 1.089~ 2.074~ 2.935)&
(650~0.306~ 1.087~ 2.065~ 2.934)&
(750~0.309~ 0.839~ 1.551~ 2.902)&
(0~0.0077~ 1.2~ 0~ 0)&
(0~360.4~ 445.6~ 536.1~ 0)&
(0~329.1~ 681~ 773.3~ 0)
```

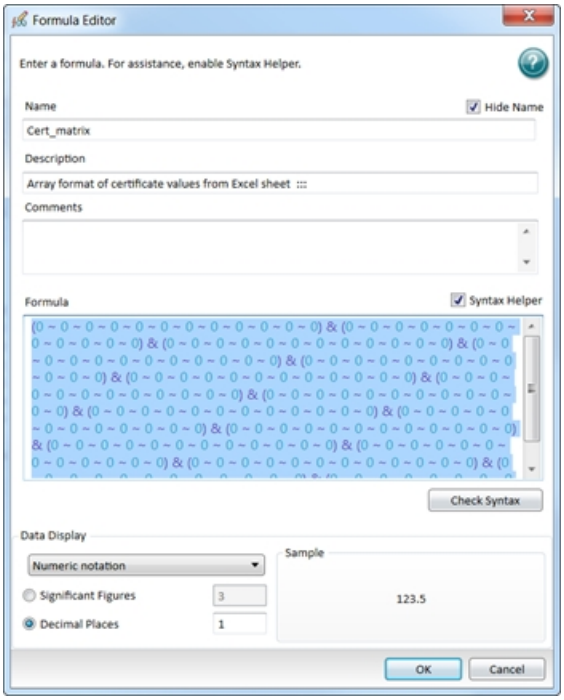
> **Note:** This image is a representation of the values in the file. Your section values will appear different.

4. In the SoftMax Pro Software, select the **Home** tab and click **Protocol Manager** > **Protocol Library** > **Reader Validation-Plate** Lum > **<instrument>** to open the instrument-specific validation protocol.
5. In the Navigation Tree, select the **EZinCert** Note section in either the first experiment or in the Appendix experiment.

6. Double-click the violet **Array Format of Certificate Values ...** field to display the Format Editor dialog.



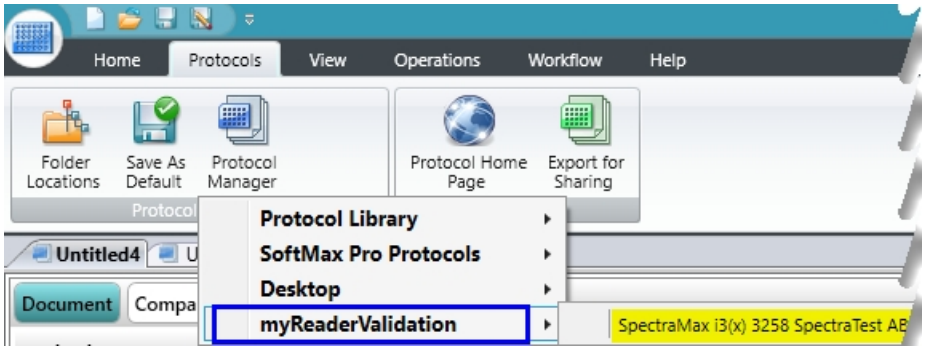
7. Wait until the content of the Formula field loads and displays colors. Then, starting at the bottom of the **Formula** field, drag the cursor upward to highlight the contents of the Formula field.



8. Paste data from the EZinCert certification information file (Step 3) over the highlighted formula content (Ctrl+V).

9. Wait until the array parses and the Formula displays highlighted in colors. Then click **Check Syntax** to verify that the certificate information formula syntax is valid. If the syntax is not valid, copy and paste the data from the worksheet into the Formula Editor until the syntax is valid.
10. Click **OK** to close the Formula Editor dialog.
11. In the Navigation Tree select the **CertInfo** Note section in either the first experiment or the Appendix experiment and confirm that the certificate values display correctly in each field.
12. Click **Save As** to save the file with a new name in order to save the certificate information and to prevent over writing the original protocol.
13. In the Save As dialog, click the **Save As Type** drop-down and select **Protocol Files** to save the file as a protocol.

> Tip: Name the file with the validation expiration date and instrument type, for example LM1 Plate ID 2025-Jan-22 SpectraMax i3x and save the new protocol file.
>
> You can use the Folder Locations feature to save the file to the folder of your choice.



14. In the Navigation Tree, expand the **OQ** LM1 **SpectraMax iDx** experiment and select the **Results** Note section. This section should indicate that certificate data has been successfully entered. If not, repeat the steps in this section. The Summary Result should display "Incomplete" because the protocol has yet to be run.

The new protocol is now ready for use with the validation plate.

Chapter 4: Running Validation Protocols

Power the Plate On or Off

The power button on the validation plate is located in the cut-out on the edge of the plate next to the green indicator light.

- To power on the plate, press the power button on the edge of the plate. The green indicator light illuminates for 30 seconds, to indicate that the plate is turning on. When the light turns off, the plate is ready to test.
- To power off the plate, press the power button on the edge of the plate. The green indicator light flashes to indicate that the plate is turning off.

To help preserve battery life, the plate automatically turns off after 24 minutes.

To check if the plate is on or off, press the power button on the edge of the plate. If the indicator light flashes, then it was on and you powered it off. If the indicator light remains on for 30 seconds, then it was off and you powered it on.

Run Top-Read Validation Protocol Tests

Now that you entered the data from the Certificate of Calibration and renamed the protocol, you are ready to run the validation protocol. The top-read protocol includes tests to run with the validation plate powered off and tests to run with the validation plate powered on.

Run Background Tests with the Validation Plate Powered Off

Run the first series of tests with the plate powered off to establish background noise reads for the validation plate.

1. Power on the instrument and wait for the instrument to complete the start-up routine.
2. Start the SoftMax Pro Software.
3. Confirm that the instrument and the software are connected and communicating properly. If not, a red X displays over the instrument icon in the upper-left corner of the software window.
4. Open the protocol file that contains the certification data you entered.
5. Expand the **SpectraTest LM1** or **OQ LM1 <instrument>** experiment and select the **Results (Top)** or the **Results** Note section.
6. Enter the following information:
   - **Instrument Name**: Enter the instrument name. For the SpectraMax i3, SpectraMax i3x, SpectraMax iD3, and SpectraMax iD5 this field will automatically populate when the software completes the read of the first plate.
   - **Instrument Serial Number**: Enter the instrument serial number. For the SpectraMax i3, SpectraMax i3x, SpectraMax iD3, and SpectraMax iD5 this field will automatically populate when the software completes the read of the first plate.
   - **Tests Run By**: Enter the name of the person to run the test. This is at the bottom of the Results section for some protocols.
   - **Test Verified By**: (Optional) Enter the name of a second person to verify the test. This is at the bottom of the Results section for some protocols.
7. Click  **Save As** to save the file as a data file with a name of your choice.

8. Power off the validation plate, if needed.
   To check if the plate is on or off, press the power button on the edge of the plate. If the indicator light flashes, then it was on and you powered it off. If the indicator light remains on for 30 seconds, then it was off and you powered it on.
9. Place the validation plate in the instrument drawer with well A1 in the A1 drawer position.

   Note: For the FlexStation 3, SpectraMax M3, SpectraMax M4, SpectraMax M5, and SpectraMax M5e you must first insert an adapter plate.

10. Expand the **Background Plate Off (Top)**, **Background Plate Off (Bottom)**, or **Plate Off Background** experiment and select the **DarkAdapt** Plate section.
11. Click **Read**. The instrument reads all Plate sections in the experiment. This should take about two minutes to complete.
12. When all Plate sections are read, remove the validation plate from the drawer.

Run Tests with the Validation Plate Powered On

Run the next series of tests with the plate powered on. The power button is located in the cut-out on the edge of the plate next to the green indicator light.

1. Press the power button on the edge of the plate to power on the validation plate. The green indicator light illuminates for 30 seconds, to indicate that the plate is turning on. When the light turns off, the plate is ready to test.
2. Place the validation plate in the instrument drawer with well A1 in the A1 drawer position.

   Note: For the FlexStation 3, SpectraMax M3, SpectraMax M4, SpectraMax M5, and SpectraMax M5e you must first insert an adapter plate.

3. Expand the **Plate On**, **Plate On (Top)**, or **Plate On, Signal** experiment and select the **DarkAdapt** Plate section.
4. Click **Read**. The instrument reads all plate sections in the experiment.
5. When all plate sections are read, remove the validation plate from the drawer and press the power button on the edge of the plate. The green indicator light flashes to indicate that the plate is has powered off.
6. Return the validation plate it to its protective plastic sleeve, and then place validation plate and sleeve in the storage case, unless you plan to continue with the bottom-read tests.

   CAUTION! To protect the optical surfaces from dust, scratches, and corrosion, do not store the plate in the case without its protective sleeve.

7. Click **Save** to save the data file.
8. Expand the **SpectraTestLM1** experiment and select the **Results (Top)** or **Results** Note section. After the instrument collects data, the SoftMax Pro Software calculates whether or not the instrument passes each test.
   - If the results are within acceptable limits and you are validating an instrument with bottom-read capability, then go to Run Bottom-Read Validation Protocol Tests on page 21.
   - If the results are within acceptable limits and your instrument does not have bottom-read capability, then save the data file and print the report.
   - If the data is Out of Specification, No Data, No Fit, No Result, or Completion =%, see Troubleshooting on page 30.

Run Bottom-Read Validation Protocol Tests

There are two bottom-read tests, the first with the validation plate powered off and the second with the validation plate powered on.

Run Background Tests with the Validation Plate Powered Off

Run the first series of tests with the plate powered off to establish background noise reads for the validation plate. You probably completed steps 1 - 4 when you ran the previous tests.

1. Power on the instrument and wait for the instrument to complete the start-up routine.
2. Start the SoftMax Pro Software.
3. Confirm that the instrument and the software are connected and communicating properly.
4. Open the data file that contains the certification data you entered and the results of the top-read tests.
5. Expand the **SpectraTest LM1** experiment, select the **Results (Bot)** Note section, and then enter the following information.
   - **Instrument Serial Number**: Enter the instrument serial number. For the SpectraMax iD3 and SpectraMax i3x this field will automatically populate when the software completes the read of the first plate.
   - **Tests Run By**: Enter the name of the person to run the test. This is at the bottom of the Results section for some protocols.
   - **Test Verified By**: (Optional) Enter the name of a second person to verify the test. This is at the bottom of the Results section for some protocols.
6. Click  **Save** to save the data file.
7. Remove the adapter plate from the instrument if you inserted the adapter plate for the top-read tests. Exception: Leave the adapter plate in for the FlexStation 3 because this instrument requires the adapter plate when you run both top-read and bottom-read tests.
8. Power off the validation plate, if needed.
9. Turn the validation plate upside down by rotating it from top-to-bottom so that column 1 remains on your left, and then place the validation plate face down in the instrument drawer so that well H1 is in the A1 drawer position.
10. For manual certification files, expand the **Background Plate Off (Bot)** experiment and select the **DarkAdapt** Plate section
    For EZinCert certification files, expand the **Option Bottom** experiment and select the **Bottom Check** Plate section.
11. Click  **Read**. The instrument reads all Plate sections in the experiment. This should take about two minutes to complete.
12. When all Plate sections are read, remove the validation plate from the drawer.

Run the Tests with the Validation Plate Powered On

Run the next series of tests with the plate powered on.

1. Press the power button on the edge of the plate to power on the validation plate. The green indicator light illuminates for 30 seconds, to indicate that the plate is turning on. When the light turns off, the plate is ready to test.
2. Turn the validation plate upside down by rotating it from top-to-bottom so that column 1 remains on your left, and then place the validation plate face down in the instrument drawer so that well H1 is in the A1 drawer position.
3. For manual certification files, expand the **Plate On (Bot)** experiment and select the **DarkAdapt** Plate section
   For EZinCert certification files, expand the **Option Bottom** experiment and select the **Bottom Check** Plate section.
4. Click **Read**. The instrument reads all Plate sections in the experiment. This should take about two minutes to complete.
5. When all Plate sections are read, remove the validation plate from the drawer and press the power button on the edge of the plate. The green indicator light flashes to indicate that the plate is turning off.
6. Return the validation plate it to its protective plastic sleeve, and then place validation plate and sleeve in the storage case.

   CAUTION! To protect the optical surfaces from dust, scratches, and corrosion, do not store the plate in the case without its protective sleeve.

7. Click **Save** to save the data file.
8. Expand the **SpectraTest LM1** experiment and select the **Results (Bot)** or **Results** Note section. After the instrument collects data, the SoftMax Pro Software calculates whether or not the instrument passes each test.
   - If the results are within acceptable limits, save the data file and print the report.
   - If the data is Out of Specification, see Troubleshooting on page 30.

Chapter 5: Interpret Test Results

This chapter gives detailed descriptions of the validation plate tests, their rationale, and interpretation. The Acceptable/Out of Specification limits for the tests are based on instrument specifications plus other applicable tolerances.

Validation Tests

Background Noise is an estimate of the instrument's measurement noise, determined by scanning the SpectraTest LM1 Luminescence Validation Plate with the plate turned OFF. The average noise and variation of that noise should be within expected limits to indicate proper functioning of the system. For some instruments, noise spikes over a fixed level are also counted since large numbers of spikes in noise might indicate a problem with the system.

Lower Limit of Detection (LLD) or **Detection Limit** estimates the minimum measurable optical signal, often converting that into an equivalent concentration of a luminescent material, such as luciferase. The units of the measurement are relative to the luminescence in well G08, and the LLD is estimated using the following formulas.

The nominal optical densities of the filters over the lamp range from 0.5 OD to 5 OD and the concentration relative to G08 are calculated by the differences in OD.

Let n = well ID, then:

- n=G08 and ODn=0.5
- n=G07 and ODn=OD 1
- n=G06 and ODn=OD 2
- n=G05 and ODn=OD 3
- n=G04 and ODn=OD 4
- n=G03 and ODn=OD 5

The relative concentration (in G08 units) for each well is

$$Conc_n = 10^{(OD_{G08} - OD_n)}$$

Published Certificate Values are relative to the luminescence counts from G05 and so the proper correction factor to apply to scale the detected counts to the certificate-corrected counts follows this formula.

$$RLUcorrected_n = \frac{RLU_n}{Cert_n \times 10^{(OD_n - OD_{G05})}}$$

The LLD is calculated as

$$LLD_n = \frac{3 \times \sigma_{bkg(RLU)}}{\left(\frac{RLUcorrected_n}{Conc_n}\right)}$$

Simple interpretation: LLD of 1.0 indicates that the luminescence of 1.0 x G08 is 3 times the standard deviation of the background noise, while LLD of 10.0 indicates that the luminescence of 10.0 x G08 is 3 times the standard deviation of the background noise.

The photon levels and test limits have been adjusted to correspond to the published sensitivity requirements for the different Molecular Devices luminometers.

Cross-talk is an estimate of light in adjacent wells from measurements of a different well and is determined using an isolated light source (in well E10). The maximum of one of 8 adjacent wells is taken as an estimate of cross-talk in the system, calculated as a percentage. The test limits correspond to the published cross-talk requirements for the different luminometers.

RLU Linearity measures linearity of signal spanning light output of 3 orders of magnitude or more, depending on the instrument. Measured light outputs from wells G03 to G08 are assigned Magnitudes 1 to 6. Magnitudes 1 to 6 are normalized against Magnitude 4 (G05) and the measured ratios (Mag1/Mag4, Mag2/Mag4, and so on) are compared to the corresponding ratios calculated from the certificate values to determine significant departure from expected measurement linearity. For the FlexStation 3, SpectraMax i3x, SpectraMax M3, SpectraMax M4, SpectraMax M5, and SpectraMax M5e the Mag1 and Mag2 values are not considered because the wells are too dim to measure a reliable value for linearity.

Kinetic Noise, Low RLU signal measures stability of the optical system at a background well with the plate ON. To test signal stability at low RLU levels, one well (D02) is read in kinetic mode and the SoftMax Pro Software calculates the following parameters for the repeated measurements over time.

- Average and standard deviation of RLUs
- Number of noise spikes above a pre-determined threshold (depends on instrument)
- kinetic drift (in RLUs per min)

Kinetic Noise, High RLU signal measures stability of the optical system at a bright well with the plate ON. To test signal stability at high RLU levels, one well (G03) is read in kinetic mode and the SoftMax Pro Software calculates the following parameters for the repeated measurements over time.

- Noise as % Coefficient of Variation (%CV) = standard deviation/average RLUs x 100
- Kinetic drift (in %/min)

Note: For the SpectraMax i3, SpectraMax i3x, SpectraMax iD3, and SpectraMax iD5 drift of background RLU is normalized to the detection limit.

Well-to-Well Precision tests measurement reproducibility across the plate. The 20 wells with nominally the same light output are measured and corrected for their known in-plate variations based on the certificate values. Resulting variations are estimates of the instrument measurement noise.

Left-to-Right Bias estimates the bias in measurement from the left edge of the plate to the right edge using two averaged values for each side.

Note: Detecting more complex biases can require use of a liquid-filled standard.

Top-to-Bottom Bias estimates the bias in measurement from the top edge of the plate to the bottom edge using two averaged values for the bottom edge and 12 averaged values for the top edge.

Note: Detecting more complex biases can require use of a liquid-filled standard.

Acceptability Criteria

The acceptability criteria for each test shown in the following tables are derived from a combination of the error of the instrument (or published specification for the instrument), the uncertainty of the measurement, and the uncertainty of the standard.

Table 5-1: Acceptability Criteria: SpectraMax iD3 and SpectraMax iD5 Tests

Test	Validation Plate Wells	Acceptable/Out of Specification Criteria
Background Noise (plate turned off)	All wells except E10 and G03	Mean < 20 RLU and SD < 10 RLU
Lower Limit of Detection (LLD) Detection Limit Top Read	G03 through G06	10 pM ATP* *Equivalent to using PerkinElmer ATPlite 1step assay system in white 96-well plate.
Cross-talk	D09 through D11, E09 through E11, and F09 through F11	< 0.15%
Linearity	G06 for Mag3Ratio G05 for Mag4Ratio G04 for Mag5Ratio G03 for Mag6Ratio	Mag3Ratio = 0.70 to 1.30 (±30%) Mag4Ratio = 0.85 to 1.15 (±15%) Mag5Ratio = 0.85 to 1.15 (±15%) Mag6Ratio = 0.85 to 1.15 (±15%)
Kinetic Noise, Low RLU	D02	Mean < 20 RLU and SD < 10 RLU
Kinetic Drift, Low RLU	D02	< 20% of LLD specification per minute
Kinetic Noise, High RLU	G03	< 1% CV
Kinetic Drift, High RLU	G03	< 1.0% per minute
Well-to-Well Precision	A01 through A12, B05 through G05, H01, and H12	< 5%
Left-to-Right Bias	A01, H01, A12, and H12	< 5%
Top-to-Bottom Bias	A01 through A12, H01, and H12	< 5%

Table 5-2: Acceptability Criteria: SpectraMax i3 and SpectraMax i3x Tests

Test	Validation Plate Wells	Acceptable/Out of Specification Criteria
Background Noise (plate turned off)	All wells except E10 and G03	Mean < 1500 RLU and SD < 150 RLU (SpectraMax i3) Mean < 150 RLU and SD < 30 RLU (SpectraMax i3x)
Lower Limit of Detection (LLD) Detection Limit Top Read	G03 through G06	75 pM ATP* (SpectraMax i3) 15 pM ATP* (SpectraMax i3x) ***Note:** Equivalent to use of PerkinElmer ATPlite 1step assay system in white 96-well plate.
Cross-talk	D09 through D11, E09 through E11, and F09 through F11	< 0.3% (SpectraMax i3) < 0.4% (SpectraMax i3x)
Linearity	G06 for Mag3Ratio G05 for Mag4Ratio G04 for Mag5Ratio G03 for Mag6Ratio	Mag3Ratio = 0.70 to 1.30 (±30%) Mag4Ratio = 0.85 to 1.15 (±15%) Mag5Ratio = 0.85 to 1.15 (±15%) Mag6Ratio = 0.85 to 1.15 (±15%)
Kinetic Noise, Low RLU	D02	Mean < 150 RLU and SD < 30 RLU
Kinetic Drift, Low RLU	D02	< 20% of LLD specification per minute (SpectraMax i3) < 50% of LLD specification per minute (SpectraMax i3x)
Kinetic Noise, High RLU	G03	< 1% CV
Kinetic Drift, High RLU	G03	< 0.5% per minute
Well-to-Well Precision	A01 through A12, B05 through G05, H01, and H12	< 5%
Left-to-Right Bias	A01, H01, A12, and H12	< 7%
Top-to-Bottom Bias	A01 through A12, H01, and H12	< 7%

Table 5-3: Acceptability Criteria: SpectraMax L Tests

Test	Validation Plate Wells	Acceptable/Out of Specification Criteria
Background Noise (plate turned off)	All wells except E10 and G03	Mean < 120 RLU and SD < 20 RLU
Background Spike (plate turned off)	All wells except E10 and G03	≤1, Limit at 180 RLU
Lower Limit of Detection (LLD) Detection Estimate	G08 for Mag1 (OD 0.5) G07 for Mag2 (OD 1) G06 for Mag3 (OD 2) G05 for Mag4 (OD 3) G04 for Mag5 (OD 4) G03 for Mag6 (OD 5)	Mag1 < 1.0 Mag2 < 1.0 Mag3 < 1.0 Mag4 < 1.0 Mag5 < 1.0 Mag6 < 1.0
Cross-talk	D09 through D11, E09 through E11, and F09 through F11	< 0.003%
Linearity	G08 for Mag1Ratio G07 for Mag2Ratio G06 for Mag3Ratio G05 for Mag4Ratio G04 for Mag5Ratio G03 for Mag6Ratio	Mag1Ratio = 0.7 to 1.3 Mag2Ratio = 0.8 to 1.2 Mag3Ratio = 0.9 to 1.1 Mag4Ratio = 1.0 Mag5Ratio = 0.9 to 1.1 Mag6Ratio = 0.9 to 1.1
Kinetic Noise, Low RLU	D02	Mean RLU < 120 and SD < 20
Kinetic Spike, Low RLU	D02	≤1
Kinetic Drift, Low RLU	D02	< 12 RLU per minute
Kinetic Noise, High RLU	G03	< 1% CV
Kinetic Drift, High RLU	G03	< 0.5% per minute
Well-to-Well Precision	A01 through A12, B05 through G05, H01, and H12	< 3%
Left-to-Right Bias	A01, H01, A12, and H12	< 5%
Top-to-Bottom Bias	A01 through A12, H01, and H12	< 5%

Table 5-4: Acceptability Criteria: FlexStation 3, SpectraMax M3, SpectraMax M4, SpectraMax M5, and SpectraMax M5e Tests

Test	Validation Plate Wells	Acceptable/Out of Specification Criteria
Background Noise (plate turned off)	All wells except E10 and G03	Mean < 50 RLU and SD < 20 RLU
Background Spike (plate turned off)	All wells except E10 and G03	≤1, Limit at 110 RLU
Lower Limit of Detection (LLD) Detection Limit Top Read	G06 for Mag3 (OD 2) G05 for Mag4 (OD 3) G04 for Mag5 (OD 4) G03 for Mag6 (OD 5)	Mag3 < 10.0 Mag4 < 10.0 Mag5 < 10.0 Mag6 < 10.0
Lower Limit of Detection (LLD) Detection Limit Bottom Read	G06 for Mag3 (OD 2) G05 for Mag4 (OD 3) G04 for Mag5 (OD 4) G03 for Mag6 (OD 5)	Mag3 < 20.0 Mag4 < 20.0 Mag5 < 20.0 Mag6 < 20.0
Cross-talk	D09 through D11, E09 through E11, and F09 through F11	< 0.3%
Linearity	G06 for Mag3Ratio G05 for Mag4Ratio G04 for Mag5Ratio G03 for Mag6Ratio	Mag3Ratio = 0.7 to 1.3 Mag4Ratio = 1.0 Mag5Ratio = 0.9 to 1.1 Mag6Ratio = 0.9 to 1.1
Kinetic Noise, Low RLU	D02	Average RLU < 50 and SD < 20
Kinetic Spike, Low RLU	D02	≤1
Kinetic Drift, Low RLU	D02	< 10 RLU per minute
Kinetic Noise, High RLU	G03	< 1% CV
Kinetic Drift, High RLU	G03	< 0.5% per minute
Well-to-Well Precision	A01 through A12, B05 through G05, H01, and H12	< 5%
Left-to-Right Bias	A01, H01, A12, and H12	< 7%
Top-to-Bottom Bias	A01 through A12, H01, and H12	< 7%

Chapter 6: Maintenance and Troubleshooting

At the time of delivery, all validation plates meet the specifications defined by Molecular Devices. You are responsible for maintaining the plates in a clean, dry, and covered environment. Validation plate maintenance requires the same care that you would give to all optical components.

- Store the plate in the plastic sleeve in the storage case when not in use.
- Inspect the plate before all plate reads. Look for dust and dirt.
- If you observe dust on the plate, you can blow moisture-free, clean canned air across both sides of the plate to clean it.

CAUTION! Do not use air from “house” air lines on the plate, and do not blow on it with your mouth.

- If a well needs more cleaning, you can use a high-purity ethanol or methanol, such as HPLC-grade reagent alcohol, and a tightly woven cotton swab. Loosely woven cotton swabs can leave behind fiber residues. The alcohol solution can contain methanol or isopropanol but must not contain more aggressive hydrocarbon solvents such as ethyl acetate or ketones.

CAUTION! Do not touch the inside of the plate wells with cleaning tools other than a clean, tightly woven swab. Do not use acetone or other nonpolar solvents to clean the plate.

Recertification

Molecular Devices recommends annual recertification of your validation plates in order to ensure that they meet specifications and to ensure data accuracy of your plate reader.

Factory certification of the validation plate’s secondary standards is done using a reference instrument that is reserved exclusively for SpectraTest LM1 Luminescence Validation Plate calibration and is checked for accuracy at fixed intervals of time.

You must return the validation plate to Molecular Devices to have it recertified. Only Molecular Devices has the necessary knowledge and equipment to recertify SpectraTest validation plates.

Each validation plate you return to Molecular Devices for recertification is measured as found.

- If the validation plate is found to be in tolerance, it is disassembled, cleaned, reassembled, and then returned with a new Certificate of Calibration.
- If the validation plate is found to be out-of-tolerance (OOT), you will be contacted to recommend and authorize the next steps.

The suggested recertification date (Next Calibration Date) is on the Certificate of Calibration. After you reserve a place in the recertification program, you will be notified when to return the validation plate to Molecular Devices. Contact us to schedule your recertification.

- North America: Customer.Relations@moldev.com
- Europe : Service.EU@moldev.com
- China: Support.China@moldev.com
- Rest of World: Contact your local sales representative. See Obtaining Support on page 31.

Note: Contact Molecular Devices well before the recertification date to reserve a place in the recertification program and for pricing. A minimum of one month is recommended.

Troubleshooting

Results for some instruments display Out of Specification by default as long as no data has been measured and until the plate read is done. After all plate reads are done the results eventually display Acceptable. Make sure all tests have completed.

If one or more test results are Out of Specification, perform the following troubleshooting procedures:

- Check that the information in the CertInfo section of the protocol matches the information on the Certificate of Calibration that accompanies the validation plate. If the information does not match, update the CertInfo section of the protocol with the information from the Certificate of Calibration, and then view the Results, Results (Top), or Results (Bot) section to see if the test results are Acceptable.
  Each certificate is created for a specific instrument. You must enter the values from the certificate that is for the instrument you plan to validate.
- Make sure you ran the tests with well A1 of the validation plate in the A1 drawer position and the plate was positioned such that the serial number and logo were visible. If the plate was positioned incorrectly, reposition it and repeat the test.
- Check that you use the correct plate adapter, if applicable. The FlexStation 3, SpectraMax M3, SpectraMax M4, SpectraMax M5, and SpectraMax M5e require the use of an adapter plate when you run the validation plate in top-read mode. In bottom-read mode, if you inserted an adapter plate for the top-read tests, remove it for the bottom-read tests. Exception: The FlexStation 3 requires an adapter plate for both top-read and bottom-read tests.
- Check the plate for dirt, dust, or other defects. Dust is not always visible. You can blow moisture-free, clean canned air across both sides of the plate to clean it and then repeat the test. Do not use air from “house” air lines on the plate, and do not blow on it with your mouth. If the results are still unacceptable, you can try cleaning with alcohol. See Maintenance and Troubleshooting on page 29.
- Check that the plate is either on or off for the applicable test protocol. To check if the plate is on or off, press the power button on the edge of the plate. If the indicator light flashes, then it was on and you powered it off. If the indicator light remains on for 30 seconds, then it was off and you powered it on.
- Check that the green light remains on for 30 seconds after you power on the SpectraTest LM1 Luminescence Validation Plate. A shorter duration or rapid blinking might indicate low battery life and require new batteries. See Change the Batteries on page 30.

Change the Batteries

To change the batteries installed in the rear of the plate, do the following steps :

1. Use the Phillips head screwdriver to remove the two screws that hold the battery cover on the bottom of the plate, and then remove the cover.
2. Remove the old batteries.
3. Insert three new AAAA batteries in the direction indicated.
4. Replace the battery cover and tighten the screws.
5. Press the power button on the edge of the plate and verify that the green indicator light turns on for 30 seconds. This light verifies that the batteries have been inserted correctly.

Obtaining Support

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

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

You can contact your local representative or Molecular Devices Technical Support at 800-635-5577 x 1815 (North America only) or +1 408-747-1700.
In Europe call +44 (0) 118 944 8000.

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

Please have the validation plate serial number, certificate number, and your software version number or Work Order number, available when you call.

WARNING! BIOHAZARD. It is your responsibility to decontaminate the plate before you return it to Molecular Devices for recertification. 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.

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Visit our website for a current listing of worldwide distributors.

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