SpectraTest FL1 Fluorescence 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 FL1 Fluorescence Validation Plate Overview

Molecular Devices® microplate readers are designed to provide consistent performance for many years. The SpectraTest® FL1 Fluorescence Validation Plate from Molecular Devices enables you to validate the optical performance of the following instruments:

- FlexStation® 3 Multi-Mode Microplate Reader
- Gemini™ EM Dual Scanning Microplate Spectrofluorometer
- Gemini™ XPS Dual Scanning Microplate Spectrofluorometer
- 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® M2 Multi-Mode Microplate Reader
- SpectraMax® M2e Multi-Mode 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 FL1 Fluorescence 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 FL1 Fluorescence 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 FL1 Fluorescence Validation Plate package contains the following items:

- Validation plate with the following features:
  - Fluorescent strips certified at four different signal intensity levels [Certificate Fluorescent Unit (CFU Magnitudes 1–4)]
  - Non-fluorescent strips (CFU Magnitude 0)
  - NIST or NMI-traceable didymium glass (National Institute of Standards and Technology or National Metrology Institute)
  - Fluorescent reference strips with and without didymium glass
- Certificate of Calibration
- Validation Plate User Guide
- Protective Sleeve and Case

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 FL1 Fluorescence 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 33.

Available Tests

The SpectraTest FL1 Fluorescence Validation Plate enables you to qualify the performance of the system by testing optical specifications that are critical to achieve quality results. Each FL1 validation protocol is specific to the instrument you test and can do the following tests:

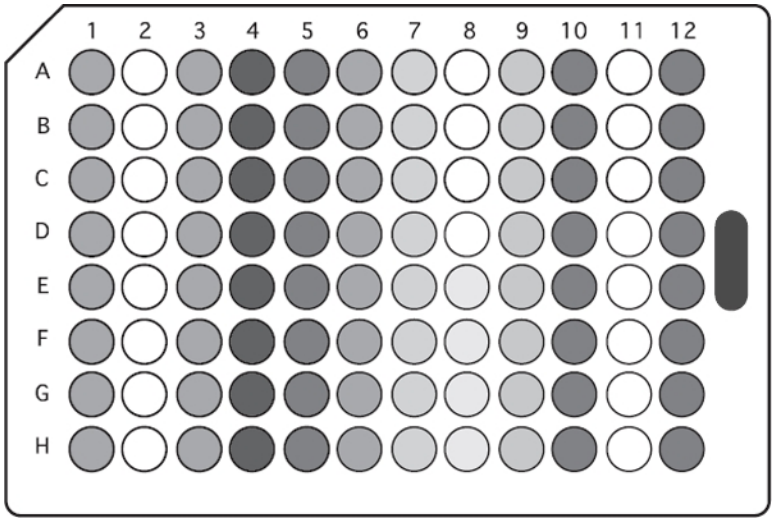
TestsTop or **Tests** provide the following top-read mode tests:

- Fluorescein Lower Limit of Detection (LLD)
- Excitation Wavelength Accuracy
- Emission Wavelength Accuracy
- Excitation Wavelength Precision
- Emission Wavelength Precision
- PMT Matching (high vs. medium PMT settings)
- PMT Matching (low vs. medium PMT settings) (SpectraMax i3, SpectraMax i3x, SpectraMax iD3, and SpectraMax iD5)
- Top-to-Bottom Bias (FlexStation 3, Gemini EM, Gemini XPS, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, and SpectraMax M5e)
- Kinetic Noise (low signal)
- Kinetic Noise (high signal)
- Well-to-Well Precision
- RFU Linearity
- Relative Fluorescence Unit (RFU) Scale Ratio (FlexStation 3, Gemini EM, Gemini XPS, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, and SpectraMax M5e)

TestsBottom or **TestsBot** provide bottom-read mode tests for components that are not shared with the top-read mode for instruments with bottom-read capability.

- Fluorescein Lower Limit of Detection (LLD)
- Excitation Wavelength Accuracy
- Emission Wavelength Accuracy
- Top-to-Bottom Bias (FlexStation 3, Gemini EM, Gemini XPS, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, and SpectraMax M5e)
- Well-to-Well Precision
- RFU Linearity
- Relative Fluorescence Unit (RFU) Scale Ratio (FlexStation 3, Gemini EM, Gemini XPS, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, and SpectraMax M5e)

The following indicates the columns related to the available tests.



SpectraTest FL1 Fluorescence Validation Plate Configuration

Test	Columns	Configuration
Fluorescein LLD	2,11 1,3,10,12	CFU Magnitude 0 CFU Magnitude 3
Wavelength accuracy, fluorescence technique	8 (E–H) 9	Fluorescent ref Fluorescent ref + didymium
Wavelength precision	9	Fluorescence ref + didymium
*Wavelength accuracy, reflectance technique	7 8 (A–D)	Didymium Glass
PMT matching	5 - FlexStation 3, Gemini EM, Gemini XPS, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, and SpectraMax M5e 4 and 5 - SpectraMax i3, SpectraMax i3x, SpectraMax iD3, and SpectraMax iD5	CFU Magnitude 2
*Top-to-bottom bias	1,3,10,12	CFU Magnitude 3
Kinetic noise (low signal)	2	CFU Magnitude 0
Kinetic noise (high signal)	3	CFU Magnitude 3
Well-to-well precision	1,3,10,12	CFU Magnitude 3
RFU Linearity	4 5 1, 3, 10, 12 6	CFU Magnitude 1 CFU Magnitude 2 CFU Magnitude 3 CFU Magnitude 4
*RFU Scale Ratio	1, 3, 10, 12	CFU Magnitude 3

* Not applicable for SpectraMax i3, SpectraMax i3x, SpectraMax iD3, and SpectraMax iD5.

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 Fluorescence Unit (CFU) values for the fluorescent strips (Magnitudes 1 through 4)
- Wavelength peak values used to determine wavelength accuracy

Factory certification of the validation plate's secondary standards is done using a reference instrument that is reserved for SpectraTest FL1 Fluorescence Validation Plate calibration and is checked for accuracy at fixed intervals of time. Molecular Devices recommends that you have the SpectraTest FL1 Fluorescence Validation Plate recertified yearly. See Recertification on page 33

Chapter 2: Validation Protocols

The SoftMax Pro Software Protocol Library contains protocols for use with the SpectraTest FL1 Fluorescence 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 FL1.

2. Locate the protocol file to download. The protocol file name includes the instruments for which it is intended, such as FlexStation 3 FL1. 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 FL1 Fluorescence 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 13.

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 FL1** 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 14.
- **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.
- **Data Point Diagnostic**: Displays result diagnostic information and contains software “hooks” to enable you to update the instrument firmware (NVRAM) parameters for the FlexStation 3, Gemini EM, Gemini XPS, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, and SpectraMax M5e.
- **Revision**: Displays the revisions made to the protocol.

 The **TestsTop** or **Tests** experiment contains the following top-read mode tests:

- **Read Me**: Contains information to get you started with the experiment. The SpectraMax i3, SpectraMax i3x protocol contains other Note sections with instructions.
- **Start Tests**: Contains plate settings for data acquisition.
- **AccPlate**: Contains plate settings for data acquisition.
- **Exc <n>**: Multiple Plate sections to test excitation wavelengths.
- **Em <n>**: Multiple Plate sections to test emission wavelengths.
- **WavePrec**: Contains plate settings for data acquisition.
- **PMT**: Multiple Plate sections to verify smooth transitions in signal between the PMT voltage settings.

- **BiasPlate**: Contains plate settings to measure top-bottom bias.
- **Kin1**: Contains plate settings for data acquisition.
- **Kin2**: Contains plate settings for data acquisition.
- **Water Plate**: Run this test when applicable. See Run Water Read Tests on page 24.
- Group sections perform calculations.

The **TestsBottom** or **TestsBot** experiment is included for instruments with bottom-read capability. It contains bottom-read mode tests for components that are not shared with the top-read mode:

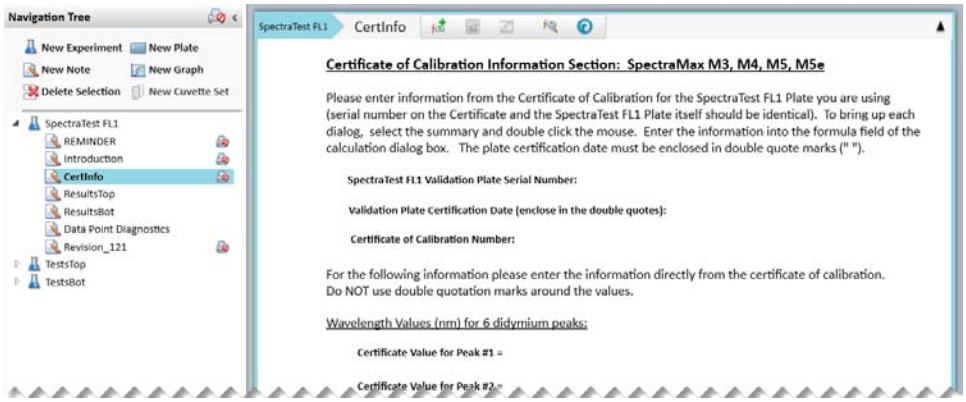
- **Read Me - Remove Adapter**: Contains information to get you started with the experiment
- **Start Tests**: Contains plate settings for data acquisition.
- **AccPlate**: Contains plate settings for data acquisition.
- **Exc <n>**: Multiple Plate sections to test excitation wavelengths.
- **Em <n>**: Multiple Plate sections to test emission wavelengths.
- **BiasPlate**: Contains plate settings for data acquisition.
- **Water Plate**: Run this test when applicable. See Run Water Read Tests on page 24.
- Group sections perform calculations.

Enter Certificate Information

All protocol files enable you to enter the certification information.

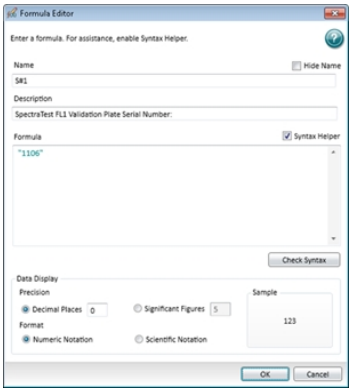
To enter certificate information:

1. In the SoftMax Pro Software, select the **Home** tab and click **Protocol Manager** > **Protocol Library** > **Reader Validation-Plate FI** > **<instrument>** to open the instrument-specific validation protocol.
2. In the Navigation Tree, expand the **SpectraTest FL1** experiment and select the **CertInfo** Note section. For the SpectraMax i3, SpectraMax i3x, SpectraMax iD3, and SpectraMax iD5, beginning with the SoftMax Pro Software version 7.1, expand the **Appendix** experiment and then select the **CertInfo** Note section.



3. Double-click the **SpectraTest FL1 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**.

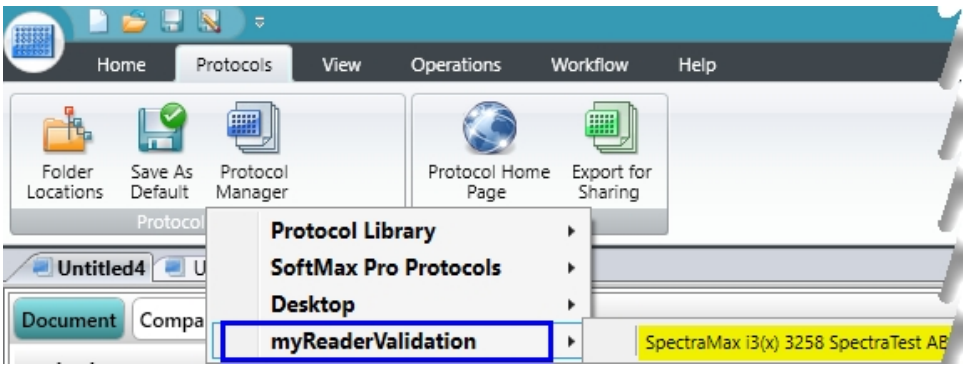


5. Double-click the **SpectraTest FL1 Certificate Date** field. In the **Formula** field, enter the certificate date in the double quotes, for example: “2025-01-22” and click **OK**.
6. Double-click the **FL1 Certificate of Calibration Number** field. In the **Formula** field, enter the Certificate of Calibration number in the double quotes, for example: “1” and click **OK**.
7. Enter each certificate value for each wavelength into the corresponding fields.

> **Note:** Do not use quotation marks around the wavelength values and Certificate Fluorescence 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 FL1 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 enable 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 FL1 <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 **TestsTop** experiment contains the following top-read mode tests:

- **Start**: Contains information to get you started with the experiment
- **Plate**: Contains plate settings for data acquisition.
- **KinLow**: Contains plate settings for data acquisition.
- **KinHigh**: Contains plate settings for data acquisition.
- **Exc <n>**: Multiple Plate sections to test excitation wavelengths.
- **Em <n>**: Multiple Plate sections to test emission wavelengths.
- **PMT**: Multiple Plate sections to determine the PMT setting.
- **Water Plate - Optional**: Run this test when applicable. See Run Water Read Tests on page 24.
- Group sections perform calculations.
- Graph sections to display results graphically.
- **Supplementary**: For internal use.
- **CalAndFactors**: For internal use.

 The **TestsBottom** experiment is included for instruments with bottom-read capability. It contains bottom-read mode tests for components that are not shared with the top-read mode:

- **Start**: Contains information to get you started with the experiment
- **Plate**: Contains plate settings for data acquisition.
- **Kin1**: Contains plate settings for data acquisition.
- **Water Plate - Optional**: Run this test when applicable. See Run Water Read Tests on page 24.
- Group sections perform calculations.
- 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 16.
- **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 14
- Remaining Note sections perform calculations or 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.

Note: Contact Molecular Devices Technical Support to have the EZinCert certification information file emailed to you. See Obtaining Support on page 36.

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.

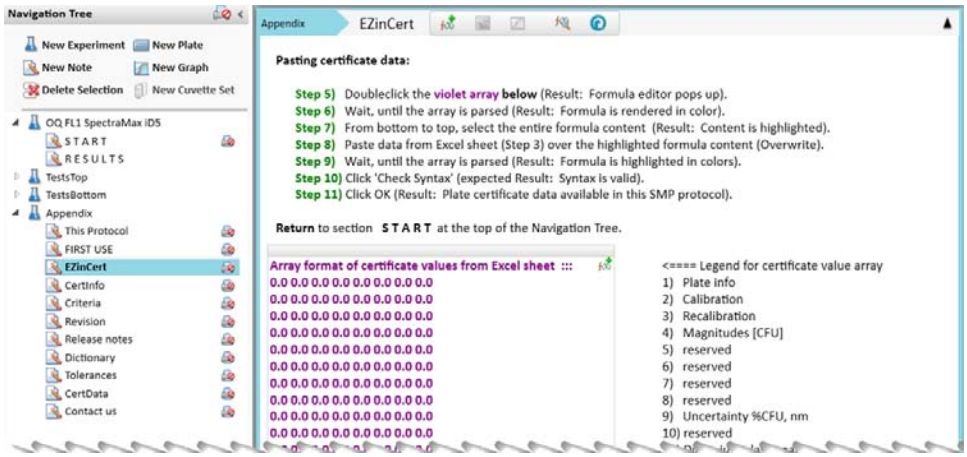
   Note: Do not open with an Internet browser as the data does not copy correctly.

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).

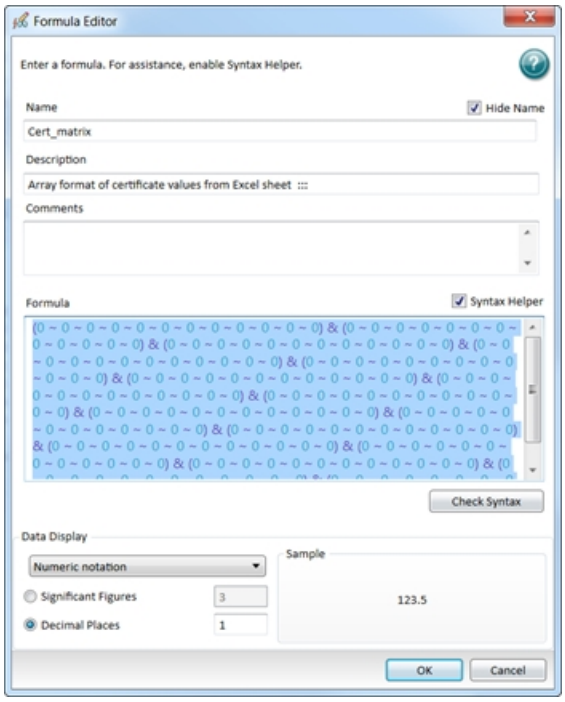
```
(0~ 0~ 00001~ 0~ 0~ 561839442)&
(0~ 8~ 15~ 2019~ 2)&
(0~ 8~ 15~ 2020~ 3)&
(405~0.312~ 1.193~ 2.239~ 2.929)&
(440~0.314~ 1.138~ 2.141~ 2.943)&
(465~0.28~ 1.057~ 1.981~ 2.954)&
(490~0.289~ 1.097~ 2.067~ 2.958)&
(546~0.285~ 1.061~ 2.004~ 2.948)&
(590~0.302~ 1.129~ 2.145~ 2.941)&
(635~0.302~ 1.089~ 2.074~ 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** Fl > **<instrument>** to open the instrument-specific validation protocol.
5. In the Navigation Tree, expand the **Appendix** experiment and select the **EZinCert** Note section.
6. Double-click the violet **Array Format of Certificate Values ...** field to display the Format Editor dialog.



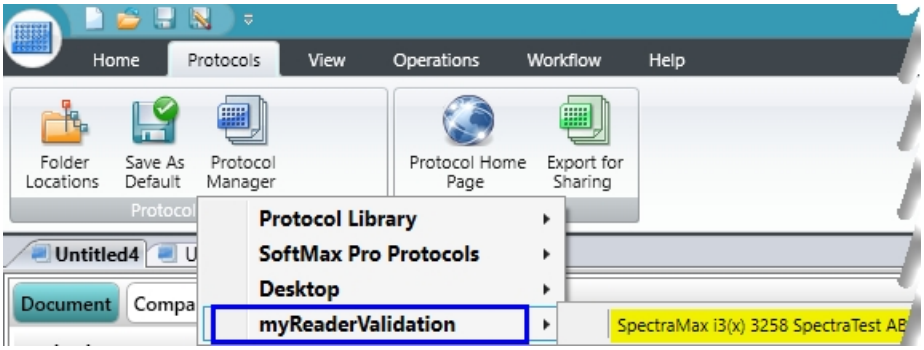
7. Wait until the content of the Formula field loads and displays colors. Then, starting at the bottom of the **Formula** field, drag the cursor upward to highlight the contents of the Formula field.



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. 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.
12. 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 FL1 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.



13. In the Navigation Tree, expand the **OQ** FL1 **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

Run Top-Read Validation Protocol Tests

Now that you have entered the data from the Certificate of Calibration and renamed the protocol, you are ready to run the validation protocol.

1. Power on the instrument and wait for the instrument to complete its 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 FL1** or **OQ FL1 <instrument>** experiment and select the **ResultsTop** 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. Place the validation plate in the instrument drawer with well A1 in the A1 drawer position.

    **Note:** For the FlexStation 3, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, and SpectraMax M5e you must first insert an adapter plate.

9. Expand the **TestsTop** or **Tests** experiment and select the **StartTests** or **Plate** Plate section.
10. Click  **Read**. The instrument reads all Plate sections in the experiment except the Water Plate section that you use only if the LLD outcome is ambiguous.
11. When all plate sections are read (except the Water Plate section), remove the validation plate from the drawer, return it to its protective plastic sleeve, and 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.

12. Click  **Save** to save the data file.

13. Expand the **SpectraTest FL1** experiment and select the **ResultsTop** or the **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 23.
    - 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 outcome of the LLD of fluorescein indicates Please Read Water-Filled Plate, then the LLD result is ambiguous and you need to verify the result.
      In general, the reflective glass surfaces of the validation plate are imperfect surrogates for a clean water-filled plate. An ambiguous read can often be due to ambient dust contamination of the Magnitude 0 (non-fluorescent) wells of the validation plate. The variability of the background wells is a major component in the calculation of detection limits. You can blow clean canned air on the top and bottom of the plate and repeat the test to re-read the AccPlate or LLDPlate Plate section. A message prompts you to verify the deletion of the existing data. If the test fails after the second read, use a water filled plate to verify the LLD of fluorescein. See Run Water Read Tests on page 24.
    - If the data is Out of Specification, see Troubleshooting on page 35.
    - If a failure in excitation wavelength accuracy, emission wavelength accuracy, or PMT matching occurs, some instruments enable you to update the instrument firmware parameters to correct these failures. See Update Instrument Firmware Parameters on page 34. Although the RFU Scale Factor does not have a Pass/Fail criterion, you can update it.

Run Bottom-Read Validation Protocol Tests

After you run the top-read tests, you can run the bottom-read 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 from the top-read tests.
5. Expand the **SpectraTest FL1** experiment, select the **Results (Bot)** Note section, and then enter the following information. This step is not applicable for the SpectraMax iD3 and SpectraMax iD5.
   - **Instrument Serial Number**: Enter the instrument serial number. For the SpectraMax i3 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 instrument because this instrument requires the adapter plate when you run both top-read and bottom-read tests.
8. 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.
9. Expand the **TestsBot** or **TestsBottom** experiment section and select the **StartTests** or **Plate** Plate section.
10. Click **Read**. The instrument reads all Plate sections in the experiment.
11. When all Plate sections are read, remove the validation plate from the drawer, return it to its protective plastic sleeve, and place the 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.

12. Click **Save** to save the data file.

13. Expand the **SpectraTest FL1** experiment and select the **ResultsBot** 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 outcome of the LLD of fluorescein indicates Please Read Water-Filled Plate, then the LLD result is ambiguous and you need to verify the result.
      In general, the reflective glass surfaces of the validation plate are imperfect surrogates for a clean water-filled plate. An ambiguous read can often be due to ambient dust contamination of the Magnitude 0 (non-fluorescent) wells of the validation plate. The variability of the background wells is a major component in the calculation of detection limits. You can blow clean canned air on the top and bottom of the plate and repeat the test to re-read the AccPlate or LLDPlate Plate section. A message prompts you to verify the deletion of the existing data. If the test fails after the second read, use a water filled plate to verify the LLD of fluorescein. See Run Water Read Tests on page 24.
    - If the data is Out of Specification, see Troubleshooting on page 35.
    - If a failure in excitation wavelength accuracy, emission wavelength accuracy, or PMT matching occurs, some instruments enable you to update the instrument firmware parameters to correct these failures. See Update Instrument Firmware Parameters on page 34. Although the RFU Scale Factor does not have a Pass/Fail criterion, you can update it.

Run Water Read Tests

To run the water read test:

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 from the previous tests.
5. In a clean, solid-black, untreated 96-well plate, fill each well with 200 µL of water. For a bottom-read test, the plate must have a clear bottom. Do not use a clear-bottom plate for a top-read test.
   Molecular Devices recommends the use of the Greiner Bio-One Fluotrac 200 Microplate for the water test. For top reads, use a Greiner Bio-One 96-well, solid black plate (Item 655076). For bottom reads, use a Greiner Bio-One 96-well, clear-bottom, black-wall plate (Item 655090).
6. Place the water-filled plate in the instrument with well A1 in position A1 drawer position.

   Note: FlexStation 3, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, and SpectraMax M5e require the use of an adapter plate when you run a 96-well plate. For a bottom-read test, the adapter plate is required only for the FlexStation 3.

7. Expand the **TestsTop**, **TestsBot**, **TestBottom**, or **Tests** experiment and select the **WaterPlate** Plate section.
8. Click **Read**.
9. When the water-filled plate is read, remove the plate from the drawer.
10. Click **Save** to save the data file.

If the outcome is Out of Specification, see Troubleshooting on page 35.

Chapter 5: Interpreting 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. When you use NIST or NMI-traceable didymium glass, the tolerance is determined from the tolerances quoted by the NIST or accredited laboratory on the primary standard they supply.

Validation Tests

Note: Only the tests that are relevant to the instrument you test are included in the validation protocol for that instrument.

Fluorescein lower limit of detection (LLD) estimates the LLD of fluorescein in picomolar (pM) assuming 200 µL/well. If the LLD based on the validation plate is questionable, you should read a water-filled plate. Molecular Devices estimates the LLD from the variability (standard deviation) of the background and the gain (slope), which is the signal/fluorescein concentration (RFU/pM).

For the Gemini EM, Gemini XPS, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, and SpectraMax M5e, when you run the protocol, the software calculates a “surrogate” fluorescein concentration for the Magnitude 3 strips, using the CertInfo Certificate Fluorescence Units (CFU) value and assumes a scaling factor of 100 nM/2000 CFU.

The protocols for the SpectraMax i3, SpectraMax i3x, SpectraMax iD3, and SpectraMax iD5 use a fluorescein pM/CFU scaling factor to translate the standard curve x-axis from CFU into pM fluorescein units.

The LLD calculation for the Gemini EM, Gemini XPS, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, and SpectraMax M5e is:

$$\frac{3 \times SD_{background}}{Gain} = 3 \times SD_{background} \times \frac{Mag3\ CFU}{Mag3\ RFU} \times \frac{100\ nM}{2000\ CFU} \times 1000 \frac{pM}{nM}$$

where $SD_{background}$ is the standard deviation of the background (nonfluorescent) wells, **Mag3 CFU** is the Magnitude 3 value on the Certificate of Calibration and **Mag3RFU** is the mean RFU value of the Magnitude 3 strips the instrument measures.

The most common cause of an unacceptable LLD is a high standard deviation of the background. A less likely cause could be an erroneous RFU Scale Factor (a scale factor in the firmware of the instrument) or some other problem that causes low RFU values.

Historically, Molecular Devices has recommended that the LLD of fluorescein be estimated using buffer-filled and fluorescein-filled plates. Unfortunately, fluorescein is far from an ideal reference standard. It deteriorates when exposed to light. It is non-fluorescent below pH 7 and reaches maximal fluorescence above pH 9, yet it is chemically unstable at high pH. Finally, extinction coefficients of fluorescein can vary because purity estimates are based on analytical tests for fluorescent impurities, rather than mass impurities. These factors have made it inconvenient for customers who want to reproduce the LLD tests.

The validation plate is designed to eliminate the problems related to the use of fluorescein solutions. It uses a stable fluorescent reference material to simulate fluorescein solutions that encompasses a concentration range that spans three orders of magnitude. It uses glass strips in columns 2 and 11 to simulate a water-filled or buffer-filled background plate. There is a reasonable correlation between a buffer plate and the glass strips of a clean validation plate. However, the plate is extremely sensitive to ambient contamination. A clean plate read in top-read mode produces an average of approximately 0.5 RFU for the glass strips (values are in the Accuracy group table), with a standard deviation of 0.05 to 0.1. These values are typical for the Gemini EM, Gemini XPS, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, and SpectraMax M5e. Other readers will give different RFU values.

If the standard deviation greatly exceeds 0.1, it could be due to a single dust particle in a single well. You can blow clean, dry canned air across the plate (both top and bottom) and re-read it. A water-filled or buffer-filled plate is less vulnerable to occasional contamination because of the greater number of wells factored into the calculation. Some plates autoflouresce, and therefore are not suitable for this usage.

Excitation wavelength accuracy and **Emission wavelength accuracy** test the accuracy of the assigned wavelengths. The validation plate permits measurement of wavelength accuracy by two techniques: fluorescence and reflectance.

- The fluorescence technique uses a combination of the fluorescent reference material and NIST or NMI-traceable didymium glass to measure wavelength accuracy. The reference material has broad excitation and emission peaks. By inserting didymium glass into the optical path between the lamp or detector and the reference material, the didymium spectrum is superimposed onto the reference material excitation and emission spectra. In a different plate location, the reference material without the didymium is scanned separately and the ratio of the two scans plotted. The resultant ratioed spectrum is equivalent to the didymium transmission spectrum and so provides a NISTor NMI -traceable measurement of wavelength accuracy. The measured peaks are limited to the spectral region where the reference material is fluorescent.
- The reflectance technique is used for wavelengths outside the range of the fluorescence technique. It uses didymium glass with plain glass as the reference. Both the didymium and the plain glass are frosted on the bottom surface. The frosted surface reflects light transmitted through the didymium or glass back through the didymium or glass with a spatial distribution similar to that of fluorescent emission. The reader then collects the diffused reflected light just like fluorescence. As in the fluorescence technique, the didymium spectrum is impressed upon the reflected light spectrum. For the excitation spectrum, the emission monochromator is set to zero order (all wavelengths collected). The excitation scan (ratioed against the plain glass scan) corresponds to the transmission spectrum of the didymium. Similarly, for the emission spectrum, the excitation monochromator is set to zero order. The resultant emission scan (ratioed against the plain glass scan) also corresponds to the transmission spectrum of the didymium.

Note: The reflectance technique is not implemented for the SpectraMax i3, SpectraMax i3x, SpectraMax iD3, and SpectraMax iD5. The precision/repeatability is tested by comparing replicate (SpectraMax i3, SpectraMax i3x) or repeated reads (SpectraMax iD3, SpectraMax iD5) of spectral band peak positions. Therefore not as many wavelengths are tested compared to the SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, and SpectraMax M5e.

Excitation wavelength precision and **Emission wavelength precision** test the precision (reproducibility) of the assigned wavelengths. The test for the Gemini EM, Gemini XPS, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, and SpectraMax M5e take advantage of the fact measurements made on a spectral slope are very sensitive to wavelength precision, in contrast to measurements made on a peak. Wavelength precision is determined by making repeated measurements on a slope, while moving the monochromator to a new setting and back before each read. In practice, alternating excitation and emission pairs, one pair on an excitation peak slope and one pair on an emission peak slope, are repeatedly read and the min-to-max difference for each is recorded. The test for the SpectraMax i3, SpectraMax i3x, SpectraMax iD3, and SpectraMax iD5 involves repeated scans of a peak.

PMT matching tests the matching of signals at high and medium PMT (photomultiplier tube) settings. For some instruments, the protocol also includes matching of signals at low and medium PMT settings. The purpose of the PMT matching test is to ensure that the response of your instrument is linear over as wide a range as possible.

The Auto PMT mode of SpectraMax microplate readers enables you to read plates that contain samples with widely differing fluorophore concentrations. The software and the instrument adjust the gain on the PMT in accordance with the different signal intensities. At low fluorophore levels, a high gain is applied to maximize the signal above the background noise. At high fluorophore concentrations, a low gain is applied to prevent saturating the PMT. To make the response linear across wide differences in fluorescence intensity (PMT gain changes), slope adjustment factors are applied.

For some instruments, you can adjust the PMT sensitivity coefficient if the results of the PMT matching tests are out of the acceptable range. See Update Instrument Firmware Parameters on page 34. Contact Molecular Devices Technical Support for assistance. See Obtaining Support on page 36.

Top-to-bottom bias tests are for the Gemini EM, Gemini XPS, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, and SpectraMax M5e to test for significant RFU difference between the top rows (A, B) and bottom rows (G, H) of a plate. Occasionally, an instrument displays a systematic difference in response between the top and bottom rows. The Magnitude 3 strips are read and the software calculates whether there is a significant difference between the average of rows A and B and the average of rows G and H.

Kinetic noise (low signal) measures stability of the optical system at low signal. To test signal stability at low RFU levels, one 8-well glass strip in column 2 is read in kinetic mode and the software calculates the Coefficient of Variation (CV) of the repeated measurements for each well and linear drift (if present).

Kinetic noise (high signal) measures stability of the optical system at high signal. To test signal stability at high RFU levels, one 8-well strip in column 3 (Magnitude 3) is read in kinetic mode and the software calculates the coefficient of variation (CV) of the repeated measurements for each well and linear drift, if present.

Well-to-well precision tests well-to-well precision (reproducibility). The Magnitude 3 strips are read (32 wells total) and the software calculates the coefficient of variation (CV). Please note that these strips are vulnerable to ambient contamination, although not as susceptible as the glass strips.

RFU linearity measures linearity of signal spanning approximately three orders of magnitude. Magnitudes 1, 2, and 4 are normalized against Magnitude 3. The measured ratios (Mag 1/Mag 3, Mag 2/Mag 3, and Mag 4/Mag 3) are compared to the corresponding ratios calculated from the certificate.

Relative fluorescence unit (RFU) scale ratio is the ratio of the measured Mag3 RFU value to the Certificate Mag3 CFU value. The purpose of determining the ratio is to permit optional adjustment of the RFU Scale Factor in the instrument firmware. The RFU Scale Factor can be used to adjust instruments to give similar RFU values at fluorescein wavelengths.

The word “relative” in RFU means there is no absolute fluorescence metric, and so the fluorescence units from different fluorometers can (and do) vary by orders of magnitude. This is in contrast to spectrophotometers, where the metric is a ratio (I_T/I_0) and all properly functioning instruments give the same values. The ratio is adjusted in each individual instrument so that all models of a given SpectraMax microplate spectrofluorometer has similar responses at fluorescein wavelengths.

Note: The RFU Scale Ratio is not implemented for the SpectraMax i3, SpectraMax i3x, SpectraMax iD3, and SpectraMax iD5. There may be some variance in the RFU level even between instruments of the same type.

During manufacture, each validation plate is read on a certified instrument to determine its Certificate Fluorescence Units (CFU) values. When you run the protocol, the software calculates a “surrogate” fluorescein concentration for the Magnitude 3 strips. The surrogate value is used to estimate the LLD of fluorescein. The Gemini EM, Gemini XPS, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, and SpectraMax M5e are set to give a target value of 2000 RFU for a 100 nM fluorescein solution (pH 9, 200 µL/well in a solid black plate). The target values for the FlexStation 3 are 50,000 RFU (top-read mode) and 1,000,000 RFU (bottom-read mode). The SpectraMax i3, SpectraMax i3x, SpectraMax iD3, and SpectraMax iD5 are normalized for luminescence read out, and can yield slightly different RFUs on the same plate.

Some instruments enable you to adjust the RFU Scale Ratio. See Update Instrument Firmware Parameters on page 34.

Note: The RFU Scale Ratio has no effect on the quality of your results. It scales the RFU values. You can choose to adjust the RFU Scale Ratio. You are most likely to benefit from RFU Scale Ratio Normalization if you have multiple instruments and want those instruments all to give similar RFU results at fluorescein wavelengths.

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.

For bottom-read mode, several of the tests are not done because they use the same optical components as in top-read mode.

Note: Only the tests that are relevant to the instrument you test are included in the validation protocol for that instrument.

Acceptability Criteria: SpectraMax iD3 and SpectraMax iD5 Tests

Test	Validation Plate Columns	Acceptable/Out of Specification Criteria
Fluorescein LLD Top read	1 through 3 and 10 through 12	LLD ≤6 pM
Fluorescein LLD Bottom read	1 through 3 and 10 through 12	LLD ≤10 pM
Wavelength accuracy	7 through 9	| Average Peak Value – Cert.Val. | ≤3 nm
Wavelength precision	9	± 1.0 nm
PMT matching	5	Ratio (RFU at High PMT Gain/RFU at Medium PMT Gain) between 0.8 and 1.2 Ratio (RFU at Low PMT Gain/RFU at Medium PMT Gain) between 0.8 and 1.2
Kinetic noise (low signal)	2	CV of measurements < 10% and Maximum Drift < 0.2 LLD spec./min
Kinetic noise (high signal)	3	CV of measurements < 1.0% and Maximum Drift < 0.5%/min
Well-to-well precision	1, 3, 10, and 12	< 5.0%
RFU linearity	1, 3 through 6, 10 and 12	Measured ratios ±15% of ratios in certificate, except measured Mag1Ratio ±30% of Mag1Ratio in the certificate.

Acceptability Criteria: SpectraMax i3 and SpectraMax i3x Tests

Test	Validation Plate Columns	Acceptable/Out of Specification Criteria
Fluorescein LLD Top read	1 through 3 and 10 through 12	LLD ≤3 pM (SpectraMax i3) LLD ≤2 pM (SpectraMax i3x)
Fluorescein LLD Bottom read	1 through 3 and 10 through 12	LLD ≤10 pM
Wavelength accuracy	7 through 9	| Average Peak Value – Cert.Val. | < 3 nm
Wavelength precision	9	± 1.0 nm
PMT matching	5	Ratio (RFU at High PMT Gain/RFU at Medium PMT Gain) between 0.8 and 1.2 Ratio (RFU at Low PMT Gain/RFU at Medium PMT Gain) between 0.8 and 1.2
Kinetic noise (low signal)	2	CV of measurements < 10% and Maximum Drift < 0.1 LLD spec./min
Kinetic noise (high signal)	3	CV of measurements < 1.0% and Maximum Drift < 0.5%/min
Well-to-well precision	1, 3, 10, and 12	< 5.0%
RFU linearity	1, 3 through 6, 10 and 12	Measured ratios ±20% of ratios in certificate, except measured Mag1Ratio ±30% of Mag1Ratio in the certificate.

Acceptability Criteria: FlexStation 3, SpectraMax M3, SpectraMax M4, SpectraMax M5, and SpectraMax M5e Tests

Test	Validation Plate Columns	Acceptable/Out of Specification Criteria
Fluorescein LLD Top read	1 through 3 and 10 through 12	LLD ≤ 5 pM
Fluorescein LLD Bottom read	1 through 3 and 10 through 12	LLD ≤ 20 pM
Wavelength accuracy	7 through 9	| Average Peak Value – Cert.Val. | < 3 nm
Wavelength precision	9	± 0.2 nm
PMT matching	5	Ratio (RFU at High PMT Gain/RFU at Medium PMT Gain) between 0.9 and 1.1
Top-to-bottom bias	1, 3, 10, and 12	Difference between row AB average and row GH average < 3%
Kinetic noise (low signal)	2	CV of measurements < 100% and Maximum Drift < 0.1 RFU/min
Kinetic noise (high signal)	3	CV of measurements < 1.0% and Maximum Drift < 0.5%/min
Well-to-well precision	1, 3, 10, and 12	< 3.0%
RFU linearity	1, 3 through 6, 10 and 12	Measured ratios 80% to 120% of ratios in certificate
RFU Scale Ratio	1, 3, 10, and 12	This value is retrieved from the instrument firmware

Acceptability Criteria: Gemini EM, Gemini XPS, SpectraMax M2, and SpectraMax M2e Tests

Test	Validation Plate Columns	Acceptable/Out of Specification Criteria
Fluorescein LLD Top read	1 through 3 and 10 through 12	LLD ≤15 pM
Fluorescein LLD Bottom read	1 through 3 and 10 through 12	LLD ≤40 pM Gemini EM LLD ≤25 pM SpectraMax M2e Gemini XPS and SpectraMax M2 do not have bottom read
Wavelength accuracy	7 through 9	| Average Peak Value – Cert.Val. | ≤3 nm
Wavelength precision	9	± 0.2 nm
PMT matching	5	Ratio (RFU at High PMT Gain/RFU at Medium PMT Gain) between 0.9 and 1.1
Top-to-bottom bias	1, 3, 10, and 12	Difference between row AB average and row GH average < 4% Difference between row AB average and row GH average < 3% for the SpectraMax M2
Kinetic noise (low signal)	2	CV of measurements < 100% and Maximum Drift < 0.1 RFU/min
Kinetic noise (high signal)	3	CV of measurements < 1.0% and Maximum Drift < 0.5%/min
Well-to-well precision	1, 3, 10, and 12	< 3.0%
RFU linearity	1, 3 through 6, 10 and 12	Measured ratios 80% to 120% of ratios in certificate
RFU Scale Ratio	1, 3, 10, and 12	This value is retrieved from the instrument firmware

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 for SpectraTest FL1 Fluorescence 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 36.

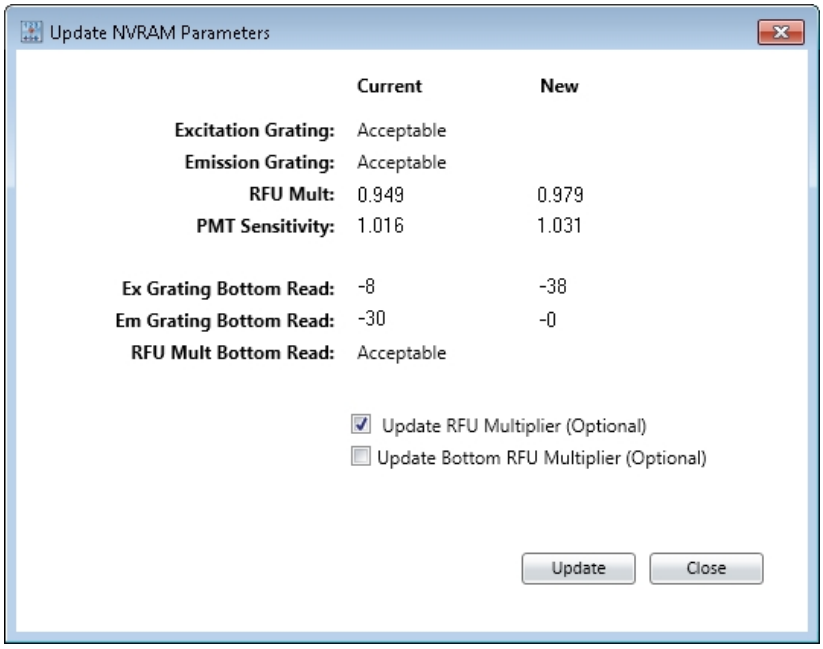
Note: Contact Molecular Devices well before the recertification date to reserve a place in the recertification program and for pricing. A minimum of one month is recommended.

Update Instrument Firmware Parameters

Note: Although some instruments enable you to use the SoftMax Pro Software to make adjustments to the instrument firmware you should contact technical support for assistance before you attempt to update the NVRAM.

The Gemini EM, Gemini XPS, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, and SpectraMax M5e can accept updates to several firmware parameters from the SoftMax Pro Software if the PMT matching test or a wavelength accuracy test reports Out of Specification you can update the NVRAM PMT calibration coefficient and hopefully correct the problem. You can enable the software to do this automatically. You can also have the software update the RFU Scale factor (RFU multiplier) to get a target RFU value of 2000 for 100 nM fluorescein. This is useful if you have multiple instruments and want similar signal levels between instruments at fluorescein settings.

1. In the SoftMax Pro Software, select the Operation tab and click **Update NVRAM Parameters** to display the Update NVRAM Parameters dialog.



2. View the following information:
   - Tests that are within specification display Acceptable.
   - Tests that are out of specification display the current value and the proposed new value.
3. Select the **Update RFU Multiplier** check box to update the RFU Scale Factor for top reads.
4. Select the **Bottom RFU Multiplier** check box to update the RFU Scale Factor for bottom reads.
5. Click **Update** to replace the current values with the proposed values.
6. Re-run the validation protocol to verify that the instrument now passes.
   For the Gemini XPS, you must restart the instrument to save the new parameters in the firmware.

Troubleshooting

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

- After you read the water-filled plate, if the outcome of the LLD of fluorescein displays Please Read Water-Filled Plate, prepare a new clean water-filled plate and re-read that section.
  Molecular Devices recommends that you use the Greiner Bio-One Fluotrac 200 Microplate for the water test. Tests have shown that these plates have lower background reads and more consistency in measurements than other plates.
  - For top reads, use a Greiner Bio-One 96-well, solid black plate (Item 655076).
  - For bottom reads, use a Greiner Bio-One 96-well, clear-bottom, black-wall plate (Item 655090).

  If the results are still unacceptable, see the discussion of fluorescein lower limit of detection (LLD) in the Validation Tests section and contact Molecular Devices Technical Support.
- Check that the information in the CertInfo Note section of the validation 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, ResultsTop, or ResultsBot section to see if the test results are within acceptable limits.
- 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 used the correct plate adapter, if applicable. The FlexStation 3, SpectraMax M2, SpectraMax M2e, 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 blow on it with your mouth. If the results are still unacceptable, you can try cleaning with alcohol. See Maintenance and Troubleshooting on page 33.
- Check the Data Point Diagnostics section, if included in the protocol. The summaries in this section check for errors that can be generated by missing data points. Data might be missing for one or more of the following reasons:
  - The data points are outside of the reduction limits set in this protocol for a specific test.
  - There is an instrument problem.
  - There is a problem with the transmission of information between the instrument and the software.
  - There is a problem with the computer.

  For all of these conditions, except the first one, the software displays the message: “data points are missing, please check your data.” If there is a section with missing data, you should re-run the Plate sections indicated in the Data Diagnostics report to check for an intermittent data transmission or computer problem.

If the results are still unacceptable, contact Molecular Devices Technical Support.

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.