SpectraTest® Multi-Mode Validation Plate

User Guide

MOLECULAR DEVICES

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Molecular Devices, LLC is ISO 9001 registered.

Contents

Chapter 1: SpectraTest Multi-Mode Validation Plate Overview

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Molecular Devices® microplate readers are designed to provide consistent performance for many years. You must periodically validate and document the instrument performance to fulfill regulatory requirements.

The SpectraTest® Multi-Mode Validation Plate (validation plate) from Molecular Devices is a comprehensive optical validation package. The SoftMax® Pro Data Acquisition and Analysis Software Protocol Library includes instrument specific protocols that read the validation plate, perform the required test measurements, and make the required calculations (software version 7.0 and later). You can customize the test report format.

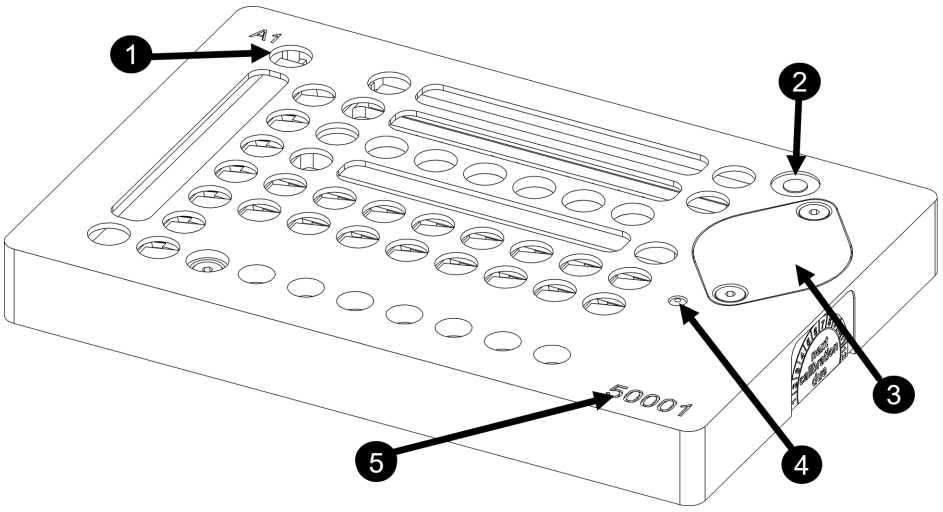
The validation plate enables you to validate the performance of the following instruments:

- FilterMax™ F3 Multi-Mode Microplate Reader
- FilterMax™ F5 Multi-Mode Microplate Reader
- SpectraMax® iD5 Multi-Mode Microplate Reader*
- SpectraMax® i3 Multi-Mode Microplate Reader*
- SpectraMax® i3x Multi-Mode Microplate Reader*
- SpectraMax® Paradigm® Multi-Mode Microplate Reader*

* Only for specific read modes or cartridges. To validate the basic read modes for the SpectraMax iD5, i3, and i3x instruments, use the SpectraTest ABS1 Absorbance Validation Plate, SpectraTest FL1 Fluorescence Validation Plate, and SpectraTest LM1 Luminescence Validation Plate.

Validation Packages Part Numbers

Part Number	Item Name	Compatible Instruments
0200-6117	SpectraTest ABS1 Absorbance Validation Plate	SpectraMax iD3, iD5, i3x, i3, M2, M2e, M3, M4, M5, M5e, Plus 384, 340PC 384, 190, ABS, ABS Plus, VersaMax, FlexStation 3
0200-5060	SpectraTest FL1 Fluorescence Validation Plate	Gemini EM, Gemini XPS, SpectraMax iD3, iD5, i3x, i3, M2, M2e, M3, M4, M5, M5e, FlexStation 3
0200-6186	SpectraTest LM1 Luminescence Validation Plate	SpectraMax iD3, iD5, i3x, i3, M3, M4, M5, M5e, SpectraMax L, FlexStation 3
0200-2420	Cuvette Absorbance Validation Set	SpectraMax Plus 384, ABS Plus, M2, M2e, M3, M4, M5, M5e
0200-7200	Multi-Mode Validation Plate	FilterMax F3, FilterMax F5, SpectraMax Paradigm, iD5*, i3*, i3x* * Specific read modes or cartridges.



SpectraTest Multi-Mode Validation Plate Configuration

Item	Description
1	Well A1
2	Validation plate luminescence On button
3	Battery compartment cover
4	Power On/Battery OK indicator. LED flashes when powered on.
5	Hardware ID number

The validation plate package contains the following items:

- Validation plate with the following features:
  - Fluorescent standard curve certified at eight different signal intensity levels wells F3 through F10
  - Non-fluorescent sample well F2 (blank)
  - NIST-traceable neutral density filters wells E3 through E10 (National Institute of Standards and Technology)
  - Glow luminescent standard curve wells H4 through H10
- FilterMax Certificate of Calibration
- Paradigm Certificate of Calibration
- CD that contains a Certificate.xlsx file
- Protective Toolbox

CAUTION! Treat the optical standards with care to retain their validity. The validation plate is vulnerable to ambient contamination. 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 blow on the plate with your mouth to clean it. See Maintenance and Troubleshooting on page 56.

Multi-Mode Protocols

The validation plate enables you to qualify the performance of the system by testing optical specifications that are critical to achieve quality results. The SoftMax Pro Software Protocol Library includes a Reader Validation Plate Multi-Mode folder that contains the protocols for the validation plate that are specific for the instruments to validate.

SoftMax Pro Software Protocols for the SpectraTest Multi-Mode Validation Plate

Instrument	Protocol Name	Included Tests
FilterMax F3 FilterMax F3 Protocol on page 13	F3 or DTX 800 Instrument	Absorbance, Fluorescence, Luminescence
FilterMax F5 FilterMax F5 Protocol on page 23	F5 or DTX 880 Instrument	Absorbance, Fluorescence, Luminescence, Time-Resolved Fluorescence, Fluorescence Polarization
SpectraMax iD5 SpectraMax iD5 Protocol on page 31	SpectraMax iD5 Multi-Mode TRF FP Std	Time-Resolved Fluorescence, Fluorescence Polarization
SpectraMax i3 and SpectraMax i3x SpectraMax i3x Protocols on page 34	LUM ALPHA Cartridges TRF FPOL HTRF cartridges	LUM ALPHA and TRF FPOL HTRF
SpectraMax Paradigm SpectraMax Paradigm Protocols on page 40	12 cartridge-specific protocols	Cartridge-specific tests

The Note sections in each protocol provide directions and describe what to expect. For further information, see the *SoftMax Pro Data Acquisition and Analysis Software User Guide* or the application help.

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) or from the protocol sharing web site (www.softmaxpro.com).

1. Create a new folder (sub-directory) on the hard drive to contain the protocol file, and give it a name of your choice.
2. Locate the protocol file to download. The protocol file name includes the instruments for which it is intended, such as FilterMax F3. Select the protocol that is for the instrument you plan to validate.
3. Save the protocol file in the folder you create.

Chapter 2: Getting Started

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You should read all Note sections in each experiment for information and instructions.

Select the **Home** tab, click **Protocol Manager** and navigate to: **Protocol Library** > **Reader Validation-Plate Multi-Mode** > (instrument name) > (protocol name).

The first experiment in every SpectraTest Multi-Mode Validation Plate protocol contains a Note section named START that contains instructions for what to do. Several other Note sections provide additional details.

- **This Protocol**: Contains a description of the protocol.
- **Revision**: Displays the revisions made to the protocol.
- **START**: Contains instructions for how to start using the protocol.
- Additional Note sections in the first experiment describe additional protocol-specific information related to plates, slides, cartridges etc.

Certificate of Calibration

The validation plate comes with two Certificates of Calibration that contain information specific to the individual validation plate for which they are created. The following information is included:

- Serial Number
- * Calibration Record ID
- Certification Date
- Protocol-Specific Values

* The Calibration Record ID is located at the bottom of the printout next to the signatures. The ID is a concatenation of the Plate ID, Calibration Date, and Printout Date. The ID changes when a copy is issued on another day.

The validation plate also comes with a CD that contains a Certificate.xlsx file. The Certificate.xlsx file contains two worksheets that enable you to enter the Certificate of Calibration information into the SoftMax Pro Software.

- **In Array Format** - Contains a yellow highlighted section that you copy and then paste into the EZinCert Note section in each protocol to enter certificate information into the protocol.
- **MM Certificate** - Contains certificate information that you use if the EZinCert method of certificate entry does not work. The manual method to enter information from the Certificate of Calibration into the Multi-Mode Validation Plate protocols is cumbersome and prone to user error. You should contact Molecular Devices support before you attempt to manually enter certificate information.

All validation protocols require you to use the information contained in Certificate.xlsx file to enter information that is specific to the Certificate of Calibration into the SoftMax Pro Software. Copy and paste the information into each protocol one time before the initial use and then again each time Molecular Devices recertifies the validation plate and sends you a new Certificate of Calibration.

Molecular Devices recommends that you have the validation plate recertified yearly. See Recertification on page 56.

Certificate Entry Sections

You must enter the Certificate of Calibration information into a Note section named  EZinCert that is in either in the first experiment or in the last experiment. Locate and expand the experiment named  **CertInfo**,  **MM Validation Plate**,  **Plate Certificate Values**, or  **Appendix**.

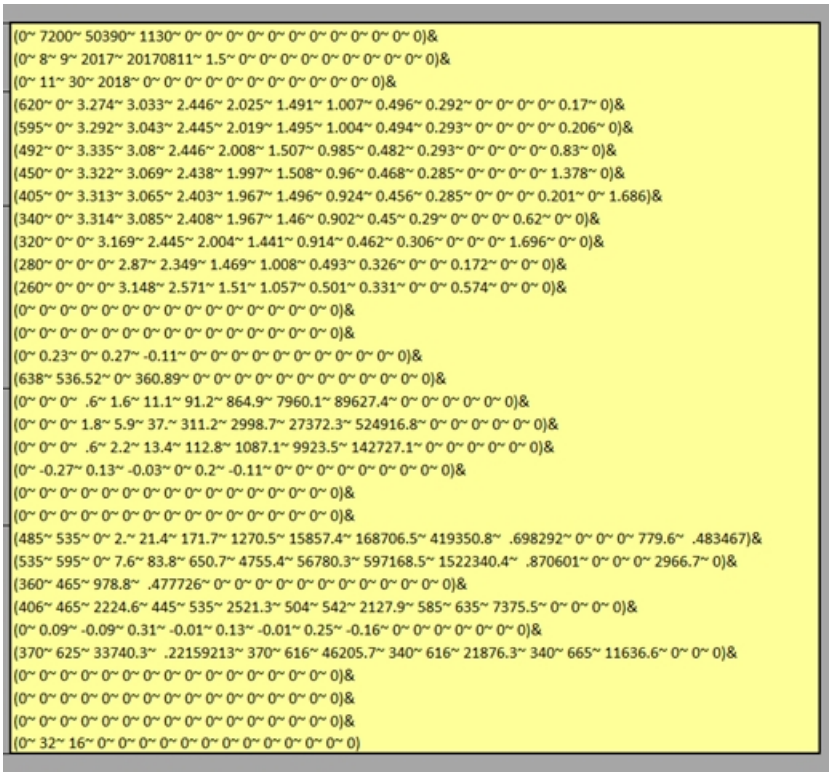
-  **First Use**: Contains instructions to get you started with the protocol.
-  **EZinCert**: Contains the field that enables you to use the EZinCert method to enter the Certificate of Calibration information. This is the recommended certificate information entry method.
-  Remaining Note sections contain protocol information or result information, or perform calculations. You should read and become familiar with the information in the Note sections.

EZinCert Certificate Entry

You must enter information from the Certificate of Calibration into the SoftMax Pro Software before you run a validation protocol. All relevant Certificate of Calibration information is in the Certificate.xlsx file that is on the CD that is included in the validation plate package. You should use the EZinCert method to enter the certificate information.

1. Insert the CD from the validation package into the computer CD Drive.
2. Locate the **Certificate.xlsx** file and save the file to a location from where you can copy and paste the contents into the protocol file in the SoftMax Pro Software.

3. Open the **Certificate.xls** file, select the values with the yellow background, and copy this information to the computer clipboard (Ctrl+C).



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(0~ 11~ 30~ 2018~ 0~ 0~ 0~ 0~ 0~ 0~ 0~ 0~ 0~ 0~ 0~ 0)&
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(492~ 0~ 3.335~ 3.08~ 2.446~ 2.008~ 1.507~ 0.985~ 0.482~ 0.293~ 0~ 0~ 0~ 0~ 0.83~ 0)&
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(485~ 535~ 0~ 2.~ 21.4~ 171.7~ 1270.5~ 15857.4~ 168706.5~ 419350.8~  .698292~ 0~ 0~ 0~ 779.6~  .483467)&
(535~ 595~ 0~ 7.6~ 83.8~ 650.7~ 4755.4~ 56780.3~ 597168.5~ 1522340.4~  .870601~ 0~ 0~ 0~ 2966.7~ 0)&
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(370~ 625~ 33740.3~  .22159213~ 370~ 616~ 46205.7~ 340~ 616~ 21876.3~ 340~ 665~ 11636.6~ 0~ 0~ 0)&
(0~ 0~ 0~ 0~ 0~ 0~ 0~ 0~ 0~ 0~ 0~ 0~ 0~ 0~ 0~ 0)&
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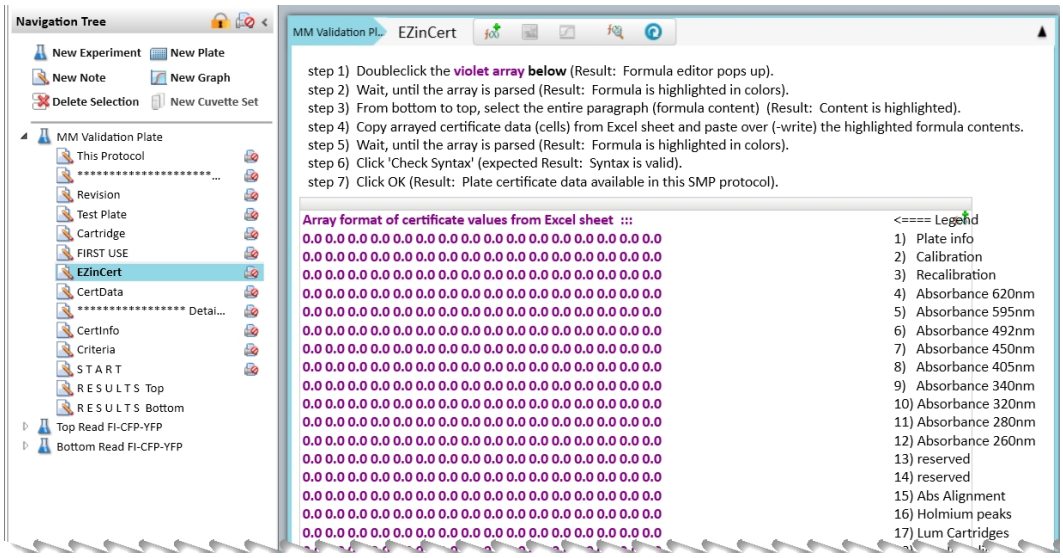
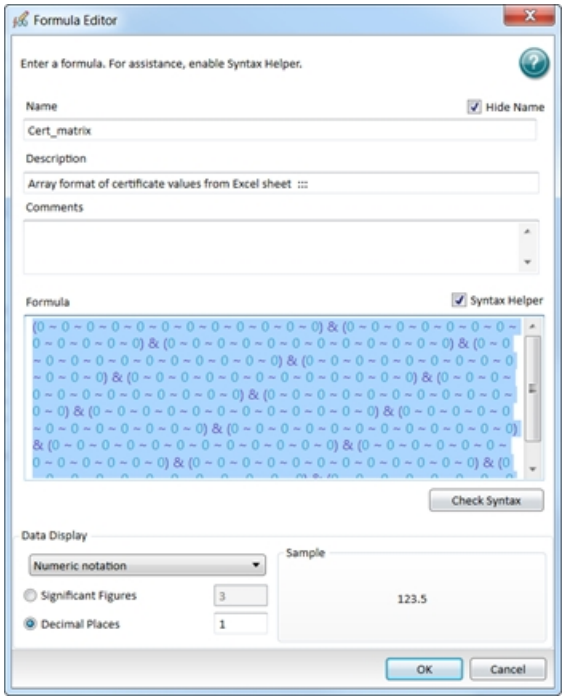
(0~ 32~ 16~ 0~ 0~ 0~ 0~ 0~ 0~ 0~ 0~ 0~ 0~ 0~ 0~ 0)
```

4. In the SoftMax Pro Software, select the **Home** tab, click **Protocol Manager** and navigate to: **Protocol Library** > **Reader Validation-Plate Multi-Mode** > (instrument name) > (protocol name).
5. In the Navigation Tree, expand the experiment that contains the **EZinCert** Note section.

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



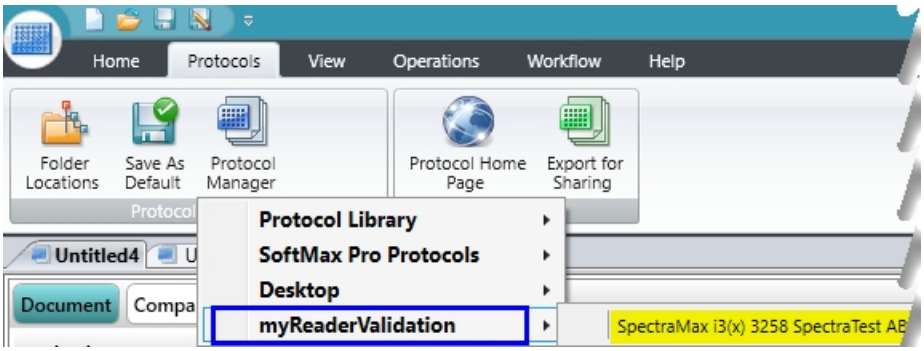
7. Wait until the content of the Formula field loads and displays colors. Then, starting at the bottom of the **Formula** field, drag the cursor upward to highlight the contents of the Formula field.



8. Paste data from the Certificate.xlsx 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** and enter a new file name to save the certificate information without over writing the original protocol.

Tip: Name the file with the validation expiration date and instrument type, for example, SpectraMax i3x 2019-Jan-22. You can save the file to the folder of your choice.



12. In the Save As dialog, click the **Save As Type** drop-down and select **Protocol Files**.

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

Chapter 3: FilterMax F3 Protocol

3

The SoftMax Pro Protocol Library contains one validation protocol for the FilterMax F3. You must use the Certificate.xlsx file to enter the Certificate of Calibration information into the SoftMax Pro Software and save the protocol file with a new name before you run the following tests. See EZinCert Certificate Entry on page 9.

For a description of the available tests, how to interpret the test results, and acceptability criteria, see FilterMax F3 (and F5) Tests on page 15.

Run FilterMax F3 Protocol Tests

Now that you have entered the data from the Certificate of Calibration and renamed the protocol, do the following to run the validation plate protocol for the FilterMax F3.

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.
4. Open the protocol file that contains the certification data you entered.
5. Expand the **Test ABS (EX-3 Filter Slide)** experiment:
   - **Check Filter Slide EX-3**: Instructions to check the EX-3 filter slide.
   - **Check Filter Slide EM**: Instructions to check the emission filter slide.
   - **START**: Instructions for the Test ABS (EX-3 Filter Slide) test.
6. Click **Save As** to save the file as a data file with a name of your choice.
7. Select the **620 nm Alignment** Plate section.
8. Select the **Operations** tab, click **AutoRead**, and confirm that all Plate sections are selected.
9. Place the validation plate in the instrument drawer with well A1 in the A1 drawer position.
10. Click **Read**. The instrument reads all Plate sections in the experiment.
11. Expand the **Results** experiment and select the **Status** Note section to view the read progress.
12. When all plate sections are read, click **Save** to save the data file.
13. Expand the **Test FL Top (Ex-3, Em-3)** experiment:
    - **Check Filter Slide EX-3**: Instructions to check the EX-3 filter slide.
    - **Check Filter Slide EM-3**: Instructions to check the EM-3 filter slide.
    - **START**: Instructions for the Test FL Top (Ex-3, Em-3) test.

14. Select the **FL-Top Fluor. Alignment X** Plate section.
15. Select the **Operations** tab, click **AutoRead**, and confirm that all Plate sections are selected.
16. Place the validation plate in the instrument drawer with well A1 in the A1 drawer position.
17. Click **Read**. The instrument reads all Plate sections in the experiment.
18. Expand the **Results** experiment and select the **Status** Note section to view the read progress.
19. When all plate sections are read, click **Save** to save the data file.
20. Expand the **Test LUM (EM-3 Filter Slide)** experiment:
    - **Check Filter Slide EX**: Instructions to check the EX filter slide.
    - **Check Filter Slide EM-3**: Instructions to check the EM-3 filter slide.
    - **Switch ON the Plate**: Instructions to power on the validation plate.
    - **START**: Instructions for the Test LUM (EM-3 Filter Slide) test.
21. Select the **LUM Alignment 2d (X)** Plate section.
22. Select the **Operations** tab, click **AutoRead**, and confirm that all Plate sections are selected.
23. Press the button at well position A11 to power on the validation plate.
24. Place the validation plate in the instrument drawer with well A1 in the A1 drawer position.
25. Click **Read**. The instrument reads all Plate sections in the experiment.
26. Expand the **Results** experiment and select the **Status** Note section to view the read progress.
27. When all plate sections are read, click **Save** to save the data file.
28. When all plate sections are read, remove the validation plate from the drawer and return it to the storage case.
29. After you run all tests, expand the **Results** experiment and select the **Report** Note section. Two fields in the Report Note section enable you to enter the name of the person who ran the test and the name of the person who verified the test.

FilterMax F3 (and F5) Tests

The Acceptable/Out of Specification limits for the tests are based on instrument specifications plus other applicable tolerances. When you use NIST-traceable didymium glass, the tolerance is determined from the tolerances quoted by the NIST on the primary standard they supply, plus a different tolerance for the secondary standard.

FilterMax F3 (and F5) Absorbance Tests

Align:

- Optical Alignment tests whether the carriage is aligned and the light beam passes through the center of the well.
- Align X
- Align Y
- Hysteresis tests the difference in left to right movement for odd numbered rows, and right to left movement for even numbered rows after carriage returns.

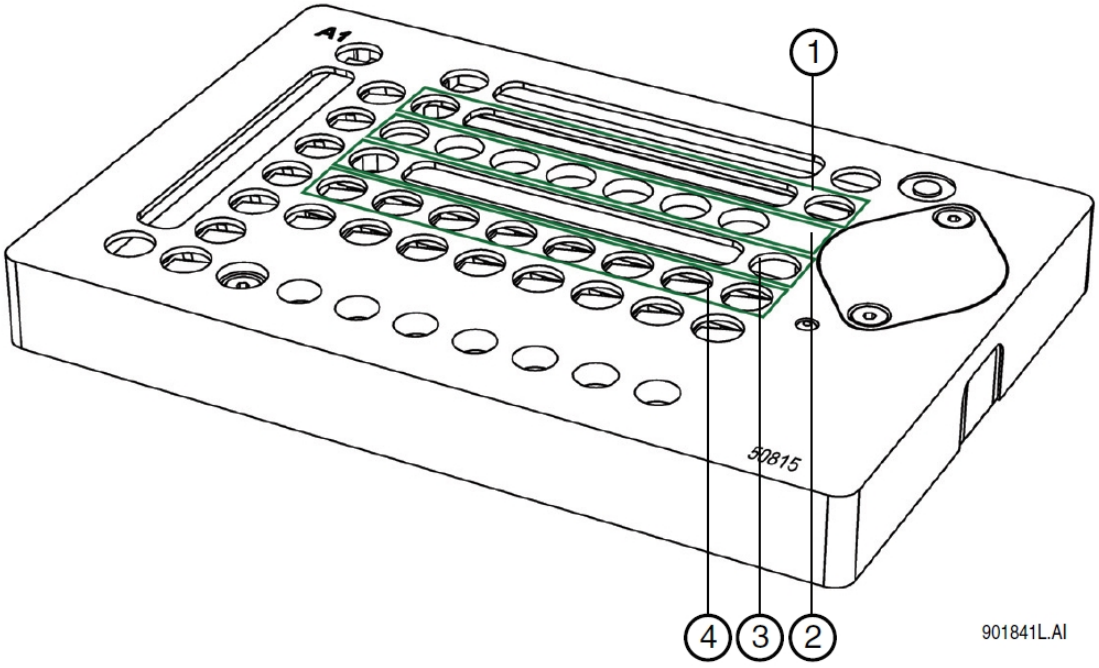
Range:

- Photometric Accuracy (Linearity) tests the accuracy or linearity of the optical density measurement.
- Photometric Precision (Reproducibility) tests the precision or reproducibility of the optical density measurement.

Signal:

- Wavelength Plausibility tests if the filter matches the selected wavelength.
- Absorbance Low (open hole, well C9)
- Absorbance High (beam stop, well C10)
- Filter Integrity

The following indicates the plate rows related to the available Absorbance tests.



SpectraTest Multi-Mode Validation Plate Absorbance Tests Well Configuration

Item	Description
1	Absorbance X alignment check
2	Absorbance functional checks
3	Absorbance Y alignment check
4	Absorbance standard curve

Absorbance Parameters

The Certificate of Calibration provides the standard curve of NIST-traceable absorbance values (in absorbance units) as determined on equipment regularly calibrated using NIST-traceable absorbance standards.

Absorbance Parameters

Parameter	Details
Signal	Filter Integrity - Validates the absorbance filter's proper out-of-band rejection. Wavelength Plausibility - Validates the absorbance filter slide configuration to ensure filter definitions are assigned to the correct slots. High Low - Validates the extreme high and low limits of the measurement range.
Align	Quantifies the alignment between the validation plate and the absorbance read head in the instrument. Measured values are compared to values calibrated at the factory, which represent the proper alignment of a Society of Biomolecular Screening (SBS) standard plate.
Precision	Validates repeatability between kinetic cycles. Precision is reported as the standard deviation of the repeated reads. The validation plate features a standard curve that covers the absorbance measurement range for the standard wavelengths supported by the instrument. This feature is required to determine precision for the entire measurement range.
Linearity	Validates the linearity of optical density (OD) measurements by comparing the measured values to NIST-traceable standard values. The validation plate features a standard curve that covers the absorbance measurement range for the standard wavelengths supported by the instrument. This feature is required to determine linearity.
Accuracy	Validates the read accuracy of OD measurements by comparing the measured values to NIST-traceable standard values.

FilterMax F3 Fluorescence Tests

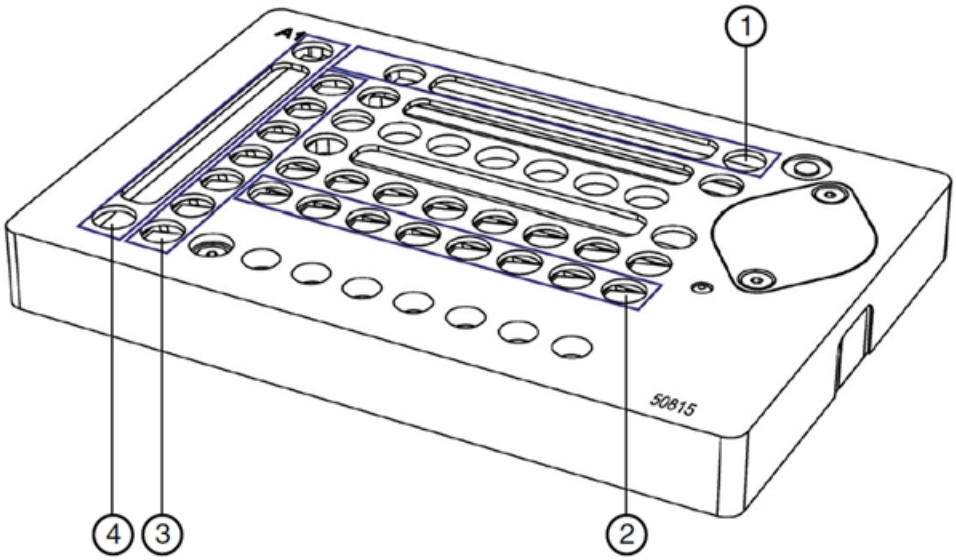
Align:

- Optical Alignment tests whether the carriage is aligned and the light beam passes through the center of the well.
- Align X
- Align Y

Fluorescein and Coumarin:

- Noise
- Background
- Standard Curve
- Range

The following indicates the columns related to the available Fluorescence tests.



SpectraTest Multi-Mode Validation Plate Fluorescence Tests Well Configuration

Item	Description
1	Fluorescence X alignment check
2	Fluorescence standard curve
3	Fluorescence functional checks and extended testing
4	Fluorescence Y alignment check

Fluorescence Parameters

The Certificate of Calibration provides the standard curve of fluorescein in units of an equivalent fluorescein concentration measured under standard conditions using NIST SRM 1932 (available through ThermoFisher.com, article number F36915).

Fluorescence Parameters

Parameter	Details
Alignment	Quantifies the alignment between the validation plate and the fluorescence read head of the detector. Measured values are compared to values calibrated at the factory, which represent the proper alignment of a Society of Biomolecular Screening (SBS) standard plate.
Measurement Range	The validation plate features a standard curve the includes high signal intensity. This feature is required to determine linearity.
Precision	Validates the precision of fluorescence top read measurements by determining the kinetic cycle-to-cycle repeatability for each standard of the curve. Precision is reported as a coefficient of variation (CV) value. The validation plate features a standard curve that covers the fluorescence measurement range for the standard wavelengths supported by the instrument. This feature is required to determine precision for the entire measurement range.
Linearity	Compares values measured in the test with calibration values determined at the factory. The validation plate features a standard curve for the fluorescence top read wavelengths. This feature is required to determine linearity.
Signal	Determines the signal to background (S/B) ratio for a type of standard measured with a particular filter set. Using the equivalent label concentration of the standard (see the printed certificate), the reciprocal S/B is transformed into background equivalent label concentration. A small reciprocal S/B value indicates the proper combination of label, filter set, and light source is present. Determines the signal to background noise ratio (S/N) for a type of standard measured with a particular filter set. Using the equivalent label concentration of the standard (see printed certificate), the reciprocal S/N is scaled to the detectable equivalent label concentration. A small reciprocal S/N value indicates the baseline of the instrument is stable. Note: Noise (N) is measured as the precision value of the blank standard on the validation plate. In the assay used to determine instrument specifications, the detection limit is based on replicate uniformity of assay blanks. Passing the Validation Plate Signal test provides supporting evidence, but does not guarantee, that the instrument can achieve the published detection limits.
Dynamic Range	Quantifies the useful measurement range between the noise level N (three times the standard deviation of repeated blank reads) and signal S at the higher end of the standard curve of the validation plate. Dynamic range equals LOG10(S/N). Note: When parameter Linearity passes (indicated as PASS in the validation plate results), the Dynamic Range may be regarded as the Linear Dynamic Range.

FilterMax F3 (and F5) Luminescence Tests

Align:

- Optical Alignment tests whether the carriage is aligned and the light beam passes through the center of the well.
- Align X
- Align Y

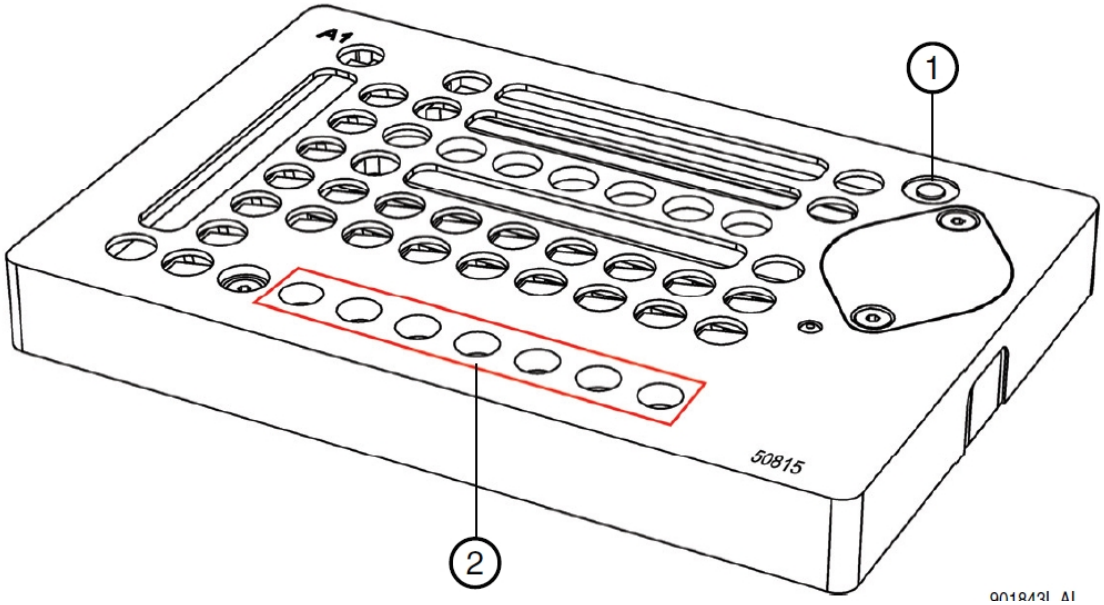
Signal and Background:

- Indicator for Lower Limit of Detection (LLD)
- Measures stability of the optical system at bright wells with the plate ON.
- Precision as % Coefficient of Variation (%CV) = standard deviation/average RLUs x 100

Linearity:

- RLU Linearity measures linearity of signal spanning light output of five orders of magnitude or more.
- Measured light outputs from wells H04 to H10 are assigned equivalent fmol/well of ATP (glow luminescence assay units).

The following indicates the columns related to the available Luminescence tests.



SpectraTest Multi-Mode Validation Plate Luminescence Tests Well Configuration

Item	Description
1	Validation Plate Luminescence On button
2	Luminescence standard curve (row H)

Luminescence Parameters

Parameters	Description
Alignment	Quantifies the alignment between the plate and the luminescence read head of the instrument. Measured values are compared to values calibrated at the factory, which represent the proper alignment of an SBS standard plate.
Precision	Validates the precision of luminescence measurements by determining the kinetic cycle-to-cycle repeatability for each standard. Precision is reported as a CV value. The validation plate features a standard curve that covers the luminescence measurement range for the standard wavelengths supported by the instrument. This feature is required to determine precision for the entire measurement range.
Linearity	Compares values measured in the test with linear calibration values determined at the factory. The validation plate features a standard curve that covers the luminescence measurement range for the standard wavelengths supported by the instrument. This feature is required to determine linearity.
Signal	Determines the signal to background ratio (S/B) for the luminescence standard. Using the equivalent label concentration of the standard (see the printed certificate), the reciprocal S/B is transformed into background equivalent label concentration. The reciprocal S/B value (smaller values are better) indicates proper light collection and detection efficiency, which are prerequisites to achieving the detection limit of the instrument. Determines the signal to background noise ratio (S/N) for the luminescence standard. Using the equivalent label concentration of the standard (see printed certificate), the reciprocal S/N is transformed into the detectable equivalent label concentration. The reciprocal S/N value (the smaller the better) indicates the baseline of the instrument is properly stable, which is a prerequisite to achieve the detection limit of the instrument. Note: Noise (N) is measured as the precision value of the blank standard on the plate. In the assay used to determine instrument specifications, the detection limit is based on replicate uniformity of assay blanks. Passing the plate signal test provides supporting evidence, but does not guarantee, that the instrument can achieve the published detection limits. The PerkinElmer ATPLite™ glow assay was used to determine the detection limit specification of the instrument.
Dynamic Range	Quantifies the useful measurement range between the noise level N (three times the standard deviation of repeated blank reads) and signal S at the higher end of the standard curve of the plate. Dynamic range equals LOG10(S/N). Note: When parameter Linearity passes (indicated as PASS in the validation plate results), the Dynamic Range may be regarded as the Linear Dynamic Range.

FilterMax F3 Acceptability Criteria

The acceptability criteria for the FilterMax F3 tests 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.

Acceptability Criteria Fluorescence Tests

Test	Validation Plate Wells	Acceptable/Out of Specification Criteria
Fluorescein LLD Top read	Well F07, (blank: F02)	LLD ≤ 5 fmol (<=> 25 pM)
Kinetic noise (high signal)	Wells F06 through F10	CV of measurements < 6.0% with a correction added when approaching the blank
R2	Wells F06 through F09	R2 of standard curve fit ≥ 0.95

Acceptability Criteria Absorbance Tests

Test	Validation Plate Wells	Acceptable/Out of Specification Criteria
Accuracy	Well C08	≤ 1%
Linearity	Wells E03 through 10	≤ 0.75%
Precision	Wells E03 through 10	≤ 0.5%
Alignment	Wells B10, D10	≤ 0.75 mm

Acceptability Criteria Luminescence Tests

Test	Validation Plate Wells	Acceptable/Out of Specification Criteria
Alignment		≤ 2.5 mm
Signal	Well H10	> 10^6 RLU
Background	Wells A01 through D12	≤ 10^3 RLU
Linearity	Wells H04 through 10	R^2 > 0.95
Precision	Wells H04 through 10	≤ 2%

Chapter 4: FilterMax F5 Protocol

The SoftMax Pro Protocol Library contains one validation protocol for the FilterMax F5. You must use the Certificate.xlsx file to enter the Certificate of Calibration information into the SoftMax Pro Software and save the protocol file with a new name before you run the following tests. See EZinCert Certificate Entry on page 9.

For a description of the available tests, how to interpret test results, and acceptability criteria, see FilterMax F5 Tests on page 25.

Run FilterMax F5 Protocol Tests

For the Absorbance read mode test, the FilterMax F5 requires any emission slide to protect the PMT against stray light. The filter slide moves to a position in between the emission filter rings.

Now that you have entered the data from the Certificate of Calibration and renamed the protocol, do the following to run the validation protocol for the FilterMax F5.

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.
4. Open the protocol file that contains the certification data you entered.
5. Expand the **Test ABS (EX-2 Filter Slide)** experiment:
   - **Check Filter Slide EX-2**: Instructions to check the EX-2 filter slide.
   - **Check Filter Slide EM**: Instructions to check the emission filter slide.
   - **START**: Instructions for the Test ABS (EX-2 Filter Slide) test.
6. Click **Save As** to save the file as a data file with a name of your choice.
7. Select the **620 nm Alignment** Plate section.
8. Select the **Operations** tab, click **AutoRead**, and confirm that all Plate sections are selected.
9. Place the validation plate in the instrument drawer with well A1 in the A1 drawer position.
10. Click **Read**. The instrument reads all Plate sections in the experiment.
11. Expand the **Results** experiment and select the **Status** Note section to view the read progress.
12. When all plate sections are read, click **Save** to save the data file.

13. Expand the **Test FL, TRF, FP (Exp-1, EMP-1)** experiment.
    - **Check Filter Slide EXP-1**: Instructions to check the EX-1 filter slide.
    - **Check Filter Slide EMP-1**: Instructions to check the EMP-1 filter slide.
    - **START**: Instructions for the Test FL, TRF, FP (Exp-1, EMP-1) test.
14. Select the **FL-Top Fluor. Alignment X** Plate section.
15. Select the **Operations** tab, click **AutoRead**, and confirm that all Plate sections are selected.
16. Place the validation plate in the instrument drawer with well A1 in the A1 drawer position.
17. Click **Read**. The instrument reads all Plate sections in the experiment.
18. Expand the **Results** experiment and select the **Status** Note section to view the read progress.
19. When all plate sections are read, click **Save** to save the data file.
20. Expand the **Test LUM (EMP-1 Filter Slide)** experiment:
    - **Check Filter Slide EX**: Instructions to check the EX filter slide.
    - **Check Filter Slide EM-1**: Instructions to check the EM-1 filter slide.
    - **Switch ON the Plate**: Instructions to power on the validation plate .
    - **START**: Instructions for the Test LUM (EM-3 Filter Slide) test.
21. Select the **LUM Alignment 2d (X)** Plate section.
22. Select the **Operations** tab, click **AutoRead**, and confirm that all Plate sections are selected.
23. Press the button at well position A11 to power on the validation plate.
24. Place the validation plate in the instrument drawer with well A1 in the A1 drawer position.
25. Click **Read**. The instrument reads all Plate sections in the experiment.
26. Expand the **Results** experiment and select the **Status** Note section to view the read progress.
27. When all plate sections are read, click **Save** to save the data file.
28. Optionally, repeat the steps to run the **Test Gen (optional EX-5,6 Slide)** test.
29. When all plate sections are read, remove the validation plate from the drawer and return it to the storage case.
30. After you run all tests, expand the **Results** experiment and select the **Report** Note section. Two fields in the Report Note section enable you to enter the name of the person who ran the test and the name of the person who verified the test.

FilterMax F5 Tests

The Acceptable/Out of Specification limits for the tests are based on instrument specifications plus other applicable tolerances. When you use NIST-traceable didymium glass, the tolerance is determined from the tolerances quoted by the NIST on the primary standard they supply, plus a different tolerance for the production of the secondary standard.

The Absorbance tests and Luminescence tests for the FilterMax F5 are the same as for the FilterMax F3. See FilterMax F3 (and F5) Tests on page 15.

FilterMax F5 Fluorescence Tests

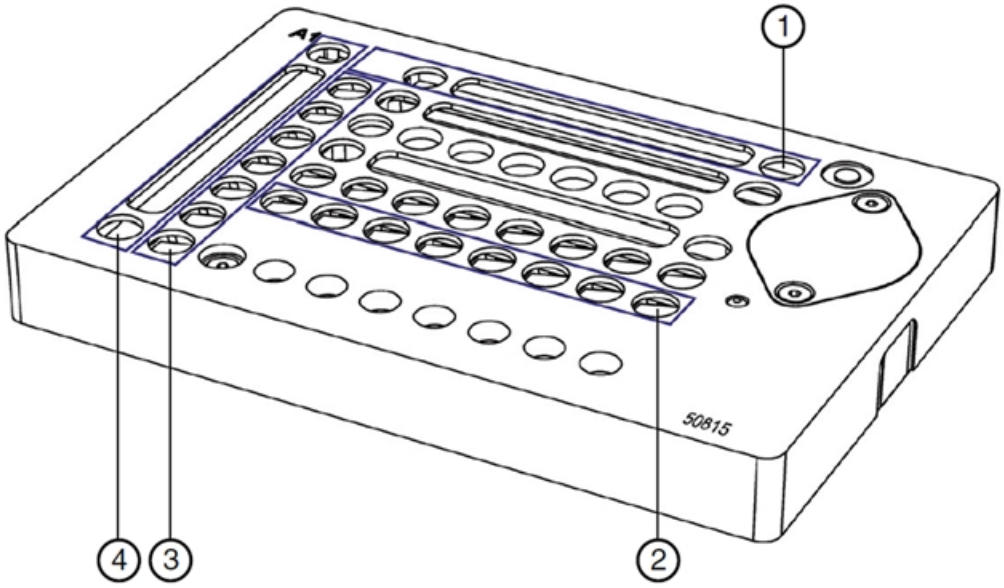
Align:

- Optical Alignment tests whether the carriage is aligned and the light beam passes through the center of the well.
- Align X
- Align Y

Fluorescein and Rhodamine:

- Noise
- Background
- Standard Curve
- Range

The following indicates the columns related to the available Fluorescence tests.



SpectraTest Multi-Mode Validation Plate Fluorescence Tests Well Configuration

Item	Description
1	Fluorescence X alignment check
2	Fluorescence standard curve
3	Fluorescence functional checks and extended testing
4	Fluorescence Y alignment check

Fluorescence Parameters

The Certificate of Calibration provides the standard curve of fluorescein in units of an equivalent fluorescein concentration measured under standard conditions using NIST SRM 1932 (available through ThermoFisher.com, article number F36915).

Fluorescence Parameters

Parameter	Details
Functionality	The Blocking parameter validates the integrity of polarizing filters installed on FilterMax F5 filter slides.
Alignment	Quantifies the alignment between the validation plate and the fluorescence read head of the detector. Measured values are compared to values calibrated at the factory, which represent the proper alignment of a Society of Biomolecular Screening (SBS) standard plate.
Measurement Range	The validation plate hardware features a standard curve the includes high signal intensity. This feature is also required to determine linearity.

Fluorescence Parameters (continued)

Parameter	Details
Precision	Validates the precision of fluorescence top read measurements by determining the kinetic cycle-to-cycle repeatability for each standard of the curve. Precision is reported as a coefficient of variation (CV) value. Also validates the precision of fluorescence polarization measurements. The validation plate features a standard curve that covers the fluorescence measurement range for the standard wavelengths supported by the instrument. This feature is required to determine precision for the entire measurement range.
Linearity	Compares values measured in the test with calibration values determined at the factory. The validation plate features a standard curve for the fluorescence top read wavelengths. This feature is required to determine linearity.
Signal	Determines the signal to background (S/B) ratio for a type of standard measured with a particular filter set. Using the equivalent label concentration of the standard (see the printed certificate), the reciprocal S/B is transformed into background equivalent label concentration. A small reciprocal S/B value indicates the proper combination of label, filter set, and light source is present. Determines the signal to background noise ratio (S/N) for a type of standard measured with a particular filter set. Using the equivalent label concentration of the standard (see printed certificate), the reciprocal S/N is scaled to the detectable equivalent label concentration. A small reciprocal S/N value indicates the baseline of the instrument is stable. Note: Noise (N) is measured as the precision value of the blank standard on the validation plate. In the assay used to determine instrument specifications, the detection limit is based on replicate uniformity of assay blanks. Passing the Validation Plate Signal test provides supporting evidence, but does not guarantee, that the instrument can achieve the published detection limits.
Dynamic Range	Quantifies the useful measurement range between the noise level N (three times the standard deviation of repeated blank reads) and signal S at the higher end of the standard curve of the validation plate. Dynamic range equals LOG10(S/N). Note: When parameter Linearity passes (indicated as PASS in the validation plate results), the Dynamic Range may be regarded as the Linear Dynamic Range.

FilterMax F5 Fluorescence Polarization Tests

Fluorescein:

- Background Signal (Par and Perp)
- Fluorescein Signals (Par and Perp)
- Dynamic Range (Min mP and Max mP)

Fluorescence Polarization Parameters

Parameter	Details
Functionality	The Blocking parameter validates the integrity of polarizing filters installed on FilterMax F5 filter slides.
Precision	Validates the precision (in mP) of fluorescence top read measurements by determining the kinetic cycle-to-cycle repeatability (in mP). Precision is reported as the standard deviation in mP.
Dynamic Range	Measures the mP in wells having the highest and lowest polarization and reports the Max-Min difference.

FilterMax F5 Time-Resolved Fluorescence Tests

Europium:

- Background
- Signal

Time-Resolved Fluorescence Parameters

Time-Resolved Fluorescence Parameters

Parameter	Details
Signal	Determines the signal to background (S/B) ratio for a type of standard measured with a particular filter set. Using the equivalent label concentration of the standard (see the printed certificate), the reciprocal S/B is transformed into background equivalent label concentration. A small reciprocal S/B value indicates the proper combination of label, filter set, and light source is present. Determines the signal to background noise ratio (S/N) for a type of standard measured with a particular filter set. Using the equivalent label concentration of the standard (see printed certificate), the reciprocal S/N is scaled to the detectable equivalent label concentration. A small reciprocal S/N value indicates the baseline of the instrument is stable. Note: Noise (N) is measured as the precision value of the blank standard on the validation plate. In the assay used to determine instrument specifications, the detection limit is based on replicate uniformity of assay blanks. Passing the Validation Plate Signal test provides supporting evidence, but does not guarantee, that the instrument can achieve the published detection limits.

FilterMax F5 Acceptability Criteria

The acceptability criteria for the FilterMax F5 tests 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.

Acceptability Criteria: Fluorescein and Kinetic Noise Tests

Test	Validation Plate Wells	Acceptable/Out of Specification Criteria
Fluorescein LLD Top read	Well F07, (blank: F02)	LLD ≤ 0.8 fmol (<=> 4 pM)
Fluorescein LLD Bottom read	Well B02, (plate bottom; blank: A02)	LLD ≤ 25 fmol (<=> 125 pM)
Kinetic noise (high signal)	Wells F06 through F10	CV of measurements < 6.0% with a correction added when approaching the blank
R2	Wells F06 through F09	R2 of standard curve fit ≥ 0.95
Signal /Background Top read		100 fmol/well
Signal /Background Bottom read		500 fmol/well
Signal/Noise Top read		0.8 fmol/well
Signal/Noise Bottom read		50 fmol/well

Acceptability Criteria: FP Fluorescein Test

Test	Validation Plate Wells	Acceptable/Out of Specification Criteria
Precision at 10 nM (1x stdev)	Interpolated between wells F05, F06, F07	≤ 3 mP
Dynamic Range		≥ 1200mP (Max.Pos.Pol.Std. minus Max.Neg.Pol.Std)
Max. Pos. Pol. Std.	Wells G02, H02	≥ 800 mP
Max. Neg. Pol. Std.		≤ -700 mP

Acceptability Criteria: TRF Europium Test

Test	Validation Plate Wells	Acceptable/Out of Specification Criteria
Signal Group Table (Using Exc. 370 nm, Em. 616 nm (red; PMT1))		
1/ (Signal/Background)	Wells E02, (blank: F02)	≤ 1000 amol/well
1/ (Signal/Noise)	Wells E02, (blank: F02)	≤ 100 amol/well

Chapter 5: SpectraMax iD5 Protocol

5

The SoftMax Pro Protocol Library contains one validation protocol for the SpectraMax iD5. You must use the Certificate.xlsx file to enter the Certificate of Calibration information into the SoftMax Pro Software and save the protocol file with a new name before you run the following tests. See EZinCert Certificate Entry on page 9.

For a description of the available tests, how to interpret test results, and acceptability criteria, see SpectraMax iD5 TRF and FP Tests on page 32.

Run SpectraMax iD5 Multi-Mode TRF and FP Protocol Tests

Now that you have entered the data from the Certificate of Calibration and renamed the protocol, do the following to run the validation protocol for the SpectraMax iD5.

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.
4. Open the protocol file that contains the certification data you entered.
5. Expand the **Standard Slides (EX, EM)** experiment and select the **START** Note section that contains instructions for the Standard Slides (EX, EM) test:
6. Click **Save As** to save the file as a data file with a name of your choice.
7. Select the **Opens Report TRF** Plate section.
8. Select the Operations tab, click **AutoRead**, and confirm that all Plate sections are selected.
9. Place the validation plate in the instrument drawer with well A1 in the A1 drawer position.
10. Click **Read**. The instrument reads all Plate sections in the experiment.
11. Expand the **Results** experiment and select the **Status** Note section to view the read progress.
12. When all plate sections are read, click **Save** to save the data file.
13. When all plate sections are read, remove the validation plate from the drawer and return it to the storage case.
14. After you run all tests, expand the **Results** experiment and select the **Report** Note section. Two fields in the Report Note section enable you to enter the name of the person who ran the test and the name of the person who verified the test.

SpectraMax iD5 TRF and FP Tests

SpectraMax iD5 Time-Resolved Fluorescence Tests

Europium:

- Background
- Signal

Time-Resolved Fluorescence Parameters

Parameter	Details
Functionality	The Blocking parameter validates the integrity of polarizing filters.
Precision	Validates the precision of TRF top read measurements by determining the kinetic cycle-to-cycle repeatability for signal and background
Signal	Determines the signal to background (S/B) ratio for a type of standard measured with a particular filter set. Using the equivalent label concentration of the standard (see the printed certificate), the reciprocal S/B is transformed into background equivalent label concentration. A small reciprocal S/B value indicates the proper combination of label, filter set, and light source is present. Determines the signal to background noise ratio (S/N) for a type of standard measured with a particular filter set. Using the equivalent label concentration of the standard (see printed certificate), the reciprocal S/N is scaled to the detectable equivalent label concentration. A small reciprocal S/N value indicates the baseline of the instrument is stable. Note: Noise (N) is measured as the precision value of the blank standard on the validation plate. In the assay used to determine instrument specifications, the detection limit is based on replicate uniformity of assay blanks. Passing the Validation Plate Signal test provides supporting evidence, but does not guarantee, that the instrument can achieve the published detection limits.

SpectraMax iD5 Fluorescence Polarization Tests

Fluorescein:

- Background Signal (Par and Perp)
- Fluorescein Signals (Par and Perp)
- Dynamic Range (Min mP and Max mP)

Fluorescence Polarization Parameters

Parameter	Details
Functionality	The Blocking parameter validates the integrity of polarizing filters installed on FilterMax F5 filter slides.
Precision	Validates the precision (in mP) of fluorescence top read measurements by determining the kinetic cycle-to-cycle repeatability (in mP). Precision is reported as the standard deviation in mP.
Dynamic Range	Measures the mP in wells having the highest and lowest polarization and reports the Max-Min difference.

SpectraMax iD5 Acceptability Criteria

The acceptability criteria for the TRF FP Std protocol tests 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.

Acceptability Criteria TRF Europium Test

Test	Validation Plate Wells	Acceptable/Out of Specification Criteria
Signal Group Table (Using Exc. 370 nm, Em. 616 nm (red; PMT1))		
1/ (Signal/Background)	Wells E02, (blank: F02)	≤ 100 amol/well
1/ (Signal/Noise)	Wells E2, (blank: F02)	≤ 10 amol/well

Acceptability Criteria FP Fluorescein Test

Test	Validation Plate Wells	Acceptable/Out of Specification Criteria
Precision at 10 nM	Interpolated between wells F05, F06, F07	≤ 5 mP
Dynamic Range	Wells F02, G02, H02	≥ 1200mP (Max.Pos.Pol.Std. minus Max.Neg.Pol.Std)
Max. Pos. Pol. Std.	Wells G02 (Max Pos), H02 (Max Neg)	≥ 800 mP
Max. Neg. Pol. Std.		≤ -700 mP

Chapter 6: SpectraMax i3x Protocols

6

The SoftMax Pro Protocol Library contains two validation protocols for the SpectraMax i3 and SpectraMax i3x. You must use the Certificate.xlsx file to enter the Certificate of Calibration information into the SoftMax Pro Software and save the protocol file with a new name before you run the following tests. See EZinCert Certificate Entry on page 9.

Run SpectraMax i3(x) LUM ALPHA Cartridges Protocol Tests

Now that you have entered the data from the Certificate of Calibration and renamed the protocol, do the following to run the LUM ALPHA Cartridges protocol tests. Each experiment in this protocol is for a specific cartridge. Only select the experiments for each cartridge that you want to qualify and ignore the experiments for cartridges that you do not want to qualify.

For a description of the available tests, how to interpret test results, and acceptability criteria, see SpectraMax i3(x) LUM ALPHA Tests on page 35.

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.
4. Open the protocol file that contains the certification data you entered.
5. To qualify the 0200-7012 LUM cartridge, expand the **0200-7012 LUM** experiment and select the **START** Note section that contains instructions for the 0200-7012 LUM experiment.
6. Click **Save As** to save the file as a data file with a name of your choice.
7. Select the **Alignment 2d (x)** Plate section.
8. Select the **Operations** tab, click **AutoRead**, and confirm that all Plate sections are selected.
9. Click **Info** and confirm that the LUM cartridge is the only cartridge discovered in the top drawer.
10. Press the button on the validation plate at well position A11 to power on the validation plate.
11. Place the validation plate in the instrument drawer with well A1 in the A1 drawer position.
12. Click **Read**. The instrument reads all Plate sections in the experiment.
13. When all plate sections are read, click **Save** to save the data file.

14. Select the **Results** Note section. Two fields in the Result Note section enable you to enter the name of the person who ran the test and the name of the person who verified the test.
15. To qualify the 0200-7013 LUM-CHROM cartridge, expand the **0200-7013 LUM-CHROM** experiment and select the **START** Note section that contains instructions for the 0200-7013 LUM-CHROM experiment.
16. Select the **Alignment 2d (x)** Plate section.
17. Select the Operations tab, click **AutoRead**, and confirm that all Plate sections are selected.
18. Click **Info** and confirm that the LUM-CHROM cartridge is the only cartridge discovered in the top drawer.
19. Press the button at well position A11 to power on the validation plate
20. Place the validation plate in the instrument drawer with well A1 in the A1 drawer position.
21. Click **Read**. The instrument reads all Plate sections in the experiment.
22. When all plate sections are read, click **Save** to save the data file.
23. Select the **Results** Note section. Two fields in the Result Note section enable you to enter the name of the person who ran the test and the name of the person who verified the test.
24. Repeat the steps to run the tests for each of the experiments for the cartridges you want to qualify.
25. When all plate sections are read, remove the validation plate from the drawer and return it to the storage case.

SpectraMax i3(x) LUM ALPHA Tests

LUM ALPHA tests:

- Alignment
- Background
- Signal

LUM ALPHA Cartridges Protocol Tests Acceptability Criteria

The acceptability criteria for the LUM ALPA Cartridges protocol tests 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.

Acceptability Criteria

Test	Acceptable/Out of Specification Criteria
X Alignment	≤ 2.5 mm
Y Alignment	≤ 2.5 mm
Background (RLU)	SpectraMax i3x ≤ 150 SpectraMax i3 ≤ 1500
Linearity R2	R2 of standard curve fit ≥ 0.95
Precision	CV of measurements < 2.0% with a correction added when approaching the blank
0200-7012 LUM Test	
Signal (RLU)	≥ 1 Million
0200-7013 LUM-CHROM Test	
Signal (RLU)	≥ 0.4 Million
0200-7014 LUM 96 Test	
Signal (RLU)	≥ 3 Million
0200-7015 LUM 384 Test	
Signal (RLU)	≥ 2.5 Million
0200-7016 LUM-BRET2 Test	
Signal (RLU)	≥ 0.8 Million
0200-7017 ALPHA 384 std Test	
Signal (RLU)	≥ 2.5 Million
0200-7018 ALPHA 384 HTS Test	
Signal (RLU)	≥ 2.5 Million
0200-7019 ALPHA 1536 HTS Test	
Signal (RLU)	≥ 1 Million

Run SpectraMax i3(x) TRF FPOL HTRF Cartridges Protocol Tests

Now that you have entered the data from the Certificate of Calibration and renamed the protocol, do the following to run the TRF FPOL HTRF Cartridges protocol tests. Each experiment in this protocol is for a specific cartridge. Only select the experiments for each cartridge that you want to qualify and ignore the tests for cartridges that you do not want to qualify.

For a description of the available tests, how to interpret test results, and acceptability criteria, see SpectraMax i3(x) TRF FPOL HTRF Tests on page 38.

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.
4. Open the protocol file that contains the certification data you entered.
5. To qualify the 0200-7008 TRF Eu Sa cartridge, expand the **0200-7008 TRF Eu Sa** experiment and select the **START** Note section that contains instructions for the 0200-7008 TRF Eu Sa experiment.
6. Click **Save As** to save the file as a data file with a name of your choice.
7. Select the **Standard** Plate section.
8. Select the **Operations** tab, click **AutoRead**, and confirm that all Plate sections are selected.
9. Click **Info** and confirm that the TRF-EUSA cartridge is the only cartridge discovered in the top drawer.
10. Place the validation plate in the instrument drawer with well A1 in the A1 drawer position.
11. Click **Read**. The instrument reads all Plate sections in the experiment.
12. When all plate sections are read, click **Save** to save the data file.
13. Select the **Results** Note section. Two fields in the Result Note section enable you to enter the name of the person who ran the test and the name of the person who verified the test.
14. To qualify the 0200-7009 FP-FLUO cartridge, expand the **0200-7009, FP-FLUO** experiment and select the **START** Note section that contains instructions for the 0200-7009 FP-FLUO experiment.
15. Select the **Alignment x** Plate section.
16. Select the Operations tab, click **AutoRead**, and confirm that all Plate sections are selected.

17. Click **Info** and confirm that the FP-FLUO cartridge is the only cartridge discovered in the top drawer.
18. Place the validation plate in the instrument drawer with well A1 in the A1 drawer position.
19. Click **Read**. The instrument reads all Plate sections in the experiment.
20. When all plate sections are read, click **Save** to save the data file.
21. Select the **Results** Note section. Two fields in the Result Note section enable you to enter the name of the person who ran the test and the name of the person who verified the test.
22. Repeat the steps to run the tests for each of the experiments for the cartridges you want to qualify.
23. When all plate sections are read, remove the validation plate from the drawer and return it to the storage case.

SpectraMax i3(x) TRF FPOL HTRF Tests

Europium:

- Background
- Signal

TRF FPOL HTRF Cartridges Protocol Tests Acceptability Criteria

The acceptability criteria for the TRF FPOL HTRF Cartridges protocol tests 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.

Acceptability Criteria: 0200-7008 TRF Eu Sa Test

Test	Validation Plate Wells	Acceptable/Out of Specification Criteria
Signal Group Table (Using Exc. 370 nm, Em. 616 nm (red; PMT1))		
1/ (Signal/Background)	Wells E02, (blank: F02)	≤ 100 amol/well (or fM assuming 0.1 mL/well)
1/ (Signal/Noise)	Wells E02, (blank: F02)	≤ 10 amol/well (or fM assuming 0.1 mL/well)

Acceptability Criteria: 0200-7009 FP-FLUO Test

Test	Validation Plate Wells	Acceptable/Out of Spec. Criteria
X alignment	Wells A07 through A17	≤ 1.5 mm
Y alignment	Wells C01, D01, E01…M01	≤ 1.5 mm
X Fly minus Stop & Go mode	Wells A07 through A17	≤ 0.5 mm
X Fly reverse minus forward	Wells B07 through B17	≤ 0.5 mm
Precision at 1 nM (interpolated, q=)	Wells F05 through F07	≤ 4 mP
Max. Pos. Pol. Std.	Well G02	≥ 800 mP
Max. Neg. Pol. Std.	Well H02	≤ -700 mP

Acceptability Criteria: 0200-7010 FP-RHOD Test

Test	Validation Plate Wells	Acceptable/Out of Spec. Criteria
X alignment	Wells A07 through A17	≤ 1.5 mm
Y alignment	Wells C01, D01, E01…M01	≤ 1.5 mm
X Fly minus Stop & Go mode	Wells A07 through A17	≤ 0.5 mm
X Fly reverse minus forward	Wells B07 through B17	≤ 0.5 mm
Precision at 1 nM (interpolated, q=)	Wells F05 through F07	≤ 4 mP
Max. Pos. Pol. Std.	Well G02	≥ 800 mP
Max. Neg. Pol. Std.	Well H02	≤ -700 mP

Acceptability Criteria: 0200-7011 HTRF 340-620 Test

Test	Plate Wells	Acceptable/Out of Specification Criteria
620* Signal Group Table (Using Exc. 340 nm, Em. 616 nm (red; PMT1))		
1/ (Signal/Background) 620 nm	Well G02	≤ 100 amol/well (or fM assuming 0.1 mL/well)
1/ (Signal/Noise) 620 nm	Well H02	≤ 20 amol/well (or fM assuming 0.1 mL/well)
665 Signal Group Table (Using Exc. 340 nm, Em. 665 nm (far-red; PMT2))		
1/ (Signal/Background) 665 nm	Wells E02, F02	≤ 100 amol/well (or fM assuming 0.1 mL/well)
1/ (Signal/Noise) 665 nm	Wells E02, (blank: F02)	≤ 20 amol/well (or fM assuming 0.1 mL/well)

* Cartridge emission filter actually optimized to 616 nm.

Chapter 7: SpectraMax Paradigm Protocols

The SoftMax Pro Protocol Library contains twelve protocols for the SpectraMax Paradigm. You must use the Certificate.xlsx file to enter the Certificate of Calibration information into the SoftMax Pro Software and save the protocol file with a new name before you run the following tests. See EZinCert Certificate Entry on page 9.

Run SpectraMax Paradigm Protocol Tests

Now that you have entered the data from the Certificate of Calibration and renamed the protocol, do the following to run the validation plate protocols for the SpectraMax Paradigm. There are 12 cartridge-specific protocols for the SpectraMax Paradigm. These directions summarize the general workflow for all 12 protocols. Read the Note sections in each protocol and in each experiment for detailed instructions.

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.
4. Open the protocol file that contains the certification data you entered.
5. Expand the  first experiment that contains a  Plate section and select the  **START** Note section.
   - Read the instructions carefully.
   - For some protocols you need to verify the cartridge serial number.
   - Insert the applicable cartridge into the appropriate drawer in the assigned slot.
6. Click  **Save As** to save the file as a data file with a name of your choice.
7. Select the  first Plate section in the experiment.
8. Select the Operations tab, click  **AutoRead**, and confirm that all Plate sections are selected.
9. Click  **Info** and confirm that the correct cartridge is installed correctly in the correct drawer based on the instructions contained in the  **START** Note section.
10. Place the validation plate in the instrument drawer with well A1 in the A1 drawer position.
11. For the following protocol tests press the button on the validation plate at well position A11 to power on the validation plate. For all other protocols the plate is powered off.
    - LUM ALPHA Cartridges protocol - all tests
    - Multi Cartridge protocol - Test LUM test
    - TUNE Cartridge protocol - Test LUM test
12. Click  **Read**. The instrument reads all Plate sections in the experiment.

13. When all plate sections are read, click **Save** to save the data file.
14. For the LUM ALPHA Cartridge protocol, select the **Results** Note section. Two fields in the Result Note section enable you to enter the name of the person who ran the test and the name of the person who verified the test. All other Paradigm protocols have a Results experiment that contain the results.
15. Repeat the steps to run the tests in each experiment in the protocol.
16. When all plate sections are read, remove the validation plate from the drawer and return it to the storage case.
17. After you run all tests, expand the **Results** experiment and select the **Report** Note section. Two fields in the Report Note section enable you to enter the name of the person who ran the test and the name of the person who verified the test. For the LUM ALPHA Cartridge protocol, each experiment contains a **Results** Note section.

SpectraMax Paradigm Cartridges Protocol Tests Acceptability Criteria

The acceptability criteria for the SpectraMax Paradigm Cartridge protocol tests 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.

Acceptability Criteria: ABS MONO Cartridge Protocol (Cartridge part number 0200-7000)

Test	Validation Plate Wells	Acceptable/Out of Specification Criteria
Wavelength Accuracy	Well C07	< 1.5 nm
Wavelength Precision	Well C07	< 0.5 nm
Precision	Wells E03 through E10 when in specified range	< (0.005 plus 0.01 x MeanValue) / MeanValue x100% (up to 2.2 OD Vis, 1.2 OD UV)
Linearity	Wells E03 through E10 when in specified range	< (0.010 plus 0.0075 x MeanValue) / MeanValue x100% (up to 3.15 OD Vis, 2.65 OD UV)
Beam Clear (OD)	Well C09	< 0.01
Beam Stopped (OD)	Well C10	> 3
Filter Integrity (OD)	Well C06	> 2.5
Wavelength Plausibility	Wells C03 through C06 (depending on selected wavelength)	< 5 nm
Absorbance 405 nm		
Accuracy (%)	Well C08 validating magnitude at ~1.6 OD	< (0.010 plus 0.02 x CertValue) / CertValue x100% @ 405nm
Alignment		
Alignment X	Wells B04 through B09	< 1.25 nm
Hysteresis X	Wells B04 through B09	< 1.0 nm
Alignment Y	Wells D04 through D09	< 1.25 nm

Acceptability Criteria: FL-CFP-YFP Cartridge Protocol (Cartridge part number 0200-7005)

Test	Validation Plate Wells	Acceptable/Out of Specification Criteria
Results Top (Using Exc. 445 nm, Em. 535 nm (green; PMT2))		
X Alignment	Wells A04 through A09	≤ 1.5 mm
Y Alignment	Wells B01 through G01	≤ 1.5 mm
1 / (Signal/Background)	Wells F07, (blank: F02)	≤ 500 fmol/well assuming 0.075 ml/well
1 / (Signal/Noise)	Wells F07, (blank: F02)	≤ 10 fmol/well assuming 0.075 mL/well
Dynamic Range	Wells F04 through F10	≥ 5 logs
Linearity R2		≥ 0.85
Linearity Dynamic Range		≥ 5 logs
Precision		6% (high signal) plus a value depending the proximity to background
Results Bottom (Using Exc. 445 nm, Em. 535 nm (green; PMT2))		
X Alignment	Wells A04 through A09 from below the plate	≤ 1.5 mm
Y Alignment	Wells B01 through G01 from below the plate	≤ 1.5 mm
X Fly minus Stop & Go mode	Wells A04 through A09 from below the plate	≤ 0.5 nm
x Fly reverse minus forward		≤ 0.5 nm
1 / (Signal/Background)	Wells B02, (blank: A02) from below the plate	≤ 1000 fmol/well assuming 0.075 ml/well
1 / (Signal/Noise)		≤ 20 fmol/well assuming 0.075 mL/well

Acceptability Criteria: FL-COFL Cartridge Protocol (Cartridge part number 0200-7002)

Test	Validation Plate Wells	Acceptable/Out of Specification Criteria
Results Top		
X Alignment	Wells A04 through A09 using Exc. 360, Em. 535, nm (green; PMT2)	≤ 1.5 mm
Y Alignment	Wells B01 through G01	≤ 1.5 mm
1 / (Signal/Background)	Wells D02, (blank: F02) using Exc. 360 nm, Em. 465 nm (blue; PMT1)	≤ 1000 fmol/well assuming 0.075 ml/well
1 / (Signal/Noise)	Wells D02, F02 (blank)	≤ 10 fmol/well assuming 0.075 mL/well
Dynamic Range	Wells F04 through F10 using Exc. 360 nm, EM. 535 nm (green; PMT2)	≥ 4 logs
Linearity R2		≥ 0.95
Linearity Dynamic Range		≥ 4 logs
Precision		6% (high signal) plus a value depending the proximity to background
Results Bottom		
X Alignment	Using Exc. 360, Em. 535, nm (green; PMT2)	≤ 1.5 mm
Y Alignment		≤ 1.5 mm
X Fly minus Stop & Go mode		≤ 0.5 nm
x Fly reverse minus forward	Wells A02, (blank: B02)	≤ 0.5 nm
1 / (Signal/Background)	Using Exc. 360 nm, Em. 465 nm (blue; PMT1)	≤ 5000 fmol/well assuming 0.075 ml/well
1 / (Signal/Noise)		≤ 50 fmol/well assuming 0.075 mL/well

Acceptability Criteria: FL-Cy3Cy5 Cartridge Protocol (Cartridge part number 0200-7004)

Test	Validation Plate Wells	Acceptable/Out of Specification Criteria
Results Top (Using Exc. 535, Em. 595, nm (red-shifted; PMT1))		
X Alignment		≤ 1.5 mm
Y Alignment		≤ 1.5 mm
1 / (Signal/Background)	Wells F07, (blank: F02)	≤ 30 fmol/well assuming 0.075 ml/well
1 / (Signal/Noise)	Wells F07, (blank: F02)	≤ 0.15 fmol/well assuming 0.075 mL/well
Dynamic Range	Wells F04 through F10	≥ 6 logs
Linearity R2	Wells F02 through F10	≥ 0.95
Linearity Dynamic Range	Wells F02 through F10	≥ 6 logs
Precision		6% (high signal) plus a value depending the proximity to background
Results Bottom (Using Exc. 535, Em. 595, nm (red-shifted; PMT1))		
X Alignment		≤ 1.5 mm
Y Alignment		≤ 1.5 mm
X Fly minus Stop & Go mode		≤ 0.5 nm
x Fly reverse minus forward		≤ 0.5 nm
1 / (Signal/Background)	Wells A02, (blank: B02) from below the plate	≤ 300 fmol/well assuming 0.075 ml/well
1 / (Signal/Noise)		≤ 6 fmol/well assuming 0.075 mL/well

Acceptability Criteria: FL-FLRH Cartridge Protocol (Cartridge part number 0200-7003)

Test	Validation Plate Wells	Acceptable/Out of Specification Criteria
Results Top (Using Exc. 485, Em. 535, nm (green; PMT1))		
X Alignment		≤ 1.5 mm
Y Alignment		≤ 1.5 mm
1 / (Signal/Background)	Wells F07, (blank: F02)	≤ 20 fmol/well or nM assuming 0.075 ml/well
1 / (Signal/Noise)	Wells F07, (blank: F02)	≤ 0.1 fmol/well or pM assuming 0.075 mL/well
Dynamic Range	Wells F04 through F10	≥ 6 logs
Linearity R2	Wells F04 through F10	≥ 0.95
Linearity Dynamic Range	Wells F04 through F10	≥ 6 logs
Precision		6% (high signal) plus a value depending the proximity to background
Results Bottom (Using Exc. 485, Em. 535, nm (green; PMT1))		
X Alignment		≤ 1.5 mm
Y Alignment		≤ 1.5 mm
X Fly minus Stop & Go mode		≤ 0.5 nm
x Fly reverse minus forward		≤ 0.5 nm
1 / (Signal/Background)	Wells A02, (blank: B02) from below the plate	≤ 200 fmol/well or nM assuming 0.075 ml/well
1 / (Signal/Noise)		≤ 6 fmol/well or pM assuming 0.075 mL/well

Acceptability Criteria: G-BLAZER Cartridge Protocol (Cartridge part number 0200-7006)

Test	Validation Plate Wells	Acceptable/Out of Specification Criteria
Results Top Using Exc. 406, Em. 535, nm (green; PMT2)		
X Alignment	Using Exc. 406, Em. 535, nm (green; PMT2)	≤ 1.5 mm
Y Alignment		≤ 1.5 mm
1 / (Signal/Background)	Wells B02, (blank: F02) using Exc. 406, Em. 465, nm (blue; PMT1)	≤ 1000 fmol/well assuming 0.075 ml/well
1 / (Signal/Noise)		≤ 10 fmol/well assuming 0.075 mL/well
Dynamic Range	Wells F04 through F10 using Exc. 406, Em. 535, nm (green; PMT2)	≥ 4 logs
Linearity R2		≥ 0.95
Linearity Dynamic Range		≥ 4 logs
Precision		6% (high signal) plus a value depending the proximity to background
Results Bottom		
X Alignment	Using Exc. 406, Em. 535, nm (green; PMT2)	≤ 1.5 mm
Y Alignment		≤ 1.5 mm
X Fly minus Stop & Go mode		≤ 0.5 nm
x Fly reverse minus forward	Wells A02, (blank: B02)	≤ 0.5 nm
1 / (Signal/Background)	Using Exc. 406, Em. 465, nm (blue; PMT1)	≤ 5000 fmol/well or nM assuming 0.075 ml/well
1 / (Signal/Noise)	Using Exc. 406, Em. 465, nm (blue; PMT1)	≤ 50 fmol/well or pM assuming 0.075 mL/well

Acceptability Criteria: LUM-ALPHA Cartridge Protocol (Experiment name is the cartridge part number)

Test	Validation Plate Wells	Acceptable/Out of Specification Criteria
All Experiments		
X alignment	Well H10	≤ 2.5 mm
Y alignment	Well H10	≤ 2.5 mm
Background (RLU)	Rows A through D	≤ 1000
Linearity R2	Wells H04 through H10, if not too close to background	≥ 0.95
Precision	Wells H04 through H10, if not too close to background	2% high signal plus a value depending proximity to background
0200-7012 LUM Experiment		
Signal (RLU)	Well H10	≥ 1 Million
0200-7013 LUM-CHROM Experiment		
Signal (RLU)	Well H10	≥ 0.4 Million
0200-7014 LUM96 Experiment		
Signal (RLU)	Well H10	≥ 3 Million
0200-7015 LUM384 Experiment		
Signal (RLU)	Well H10	≥ 2.5 Million
0200-7016 LUM-BRET2 Experiment		
Signal (RLU)	Well H10	≥ 0.8 Million
0200-7017 ALPHA-384 STD Experiment		
Signal (RLU)	Well H10	≥ 0.8 Million
0200-7018 ALPHA-384 HTS Experiment		
Signal (RLU)	Well H10	≥ 2.5 Million
0200-7019 ALPHA-1536 HTS Experiment		
Signal (RLU)	Well H10	≥ 1 Million

Acceptability Criteria: MULTI Cartridge Protocol (Cartridge part number 0200-7001)

Experiment Plate Section Name	Test	Acceptable/Out of Specification Criteria
Test FL Top Experiment		
Coumarin Plates Wells D02, (blank: F02)	1/(Signal/Background)	< 1000 fmol
Coumarin Plates	1/(Signal/Noise)	< 30 fmol
Fluorescein Plates	Alignment X	< 1.75 mm
Fluorescein Plates	Alignment Y	< 1.75 mm
Fluorescein Plates	Precision	6% (high signal) plus a value depending the proximity to background
Fluorescein Plates	Linearity R2	> 0.98
Fluorescein Plates Wells F07, (blank: F02)	1/(Signal/Background)	< 100 fmol
Fluorescein Plates	1/(Signal/Noise)	< 0.3 fmol
Fluorescein Plates	Dynamic Range	> 5 logs
Fluorescein Plates	Linear Dynamic Range	> 5 logs
Rhodamine Plates	Alignment X	< 1.75 mm
Rhodamine Plates	Alignment Y	< 1.75 mm
Rhodamine Plates	Precision	6% (high signal) plus a value depending the proximity to background
Rhodamine Plates	Linearity	> 0.98
Rhodamine Plates Wells F07, (blank: F02)	1/(Signal/Background)	< 200 fmol
Rhodamine Plates	1/(Signal/Noise)	< 10 fmol
Rhodamine Plates	Dynamic Range	> 5 logs
Rhodamine Plates	Linear Dynamic Range	> 5 logs
Texas Red Plates Wells C02, (blank: F02)	1/(Signal/Background)	< 1000 fmol
Texas Red Plates	1/(Signal/Noise)	< 30 fmol
TRF Europium Plates Wells E02, (blank: F02)	1/(Signal/Background)	< 500 amol

Acceptability Criteria: MULTI Cartridge Protocol (Cartridge part number 0200-7001) (continued)

Experiment Plate Section Name	Test	Acceptable/Out of Specification Criteria
TRF Europium Plates	1/(Signal/Noise)	< 25 amol
Test LUM Experiment		
All Tests Well H10	Alignment X	< 2.5 mm
All Tests Well H10	Alignment Y	< 2.5 mm
All Tests Wells H04 through H10, if not too close to background	Precision	3% (high signal) plus a value depending the proximity to background
All Tests Wells H04 through H10, if not too close to background	Linearity R2	> 0.98
All Tests Well H10	Signal (RLU)	> 1 Mio.
All Tests Rows A through D	Background (RLU)	< 1000
Test FL Bot Experiment		
Fluorescein Plates	Alignment X	< 1.75 mm
Fluorescein Plates	Alignment Y	<1.75 mm
Fluorescein Plates	1/(Signal/Background)	< 100 fmol
Fluorescein Plates	1/(Signal/Noise)	< 15 fmol
Rhodamine Plates	Alignment X	< 1.75 mm
Rhodamine Plates	Alignment Y	< 1.75 mm
Rhodamine Plates	1/(Signal/Background)	< 500 fmol
Rhodamine Plates	1/(Signal/Noise)	< 50 fmol

Acceptability Criteria: MULTI-TOX Label Blue Cartridge Protocol (Cartridge part number 0200-7007)

Test	Validation Plate Wells	Acceptable/Out of Specification Criteria
Test Top Read MULTI-TOX Experiment		
X Alignment	Using Exc. 406 nm, Em. 542 nm (green; PMT2)	< 1.5 mm
Y Alignment		< 1.5 mm
1/(Signal/Background)	Wells B02, (blank: F02) using Exc. 406 nm, Em. 465 nm (blue; PMT2)	< 1000 fmol
1/(Signal/Noise)		< 10 fmol
Dynamic Range	Wells F04 through F10 using Exc. 406 nm, Em. 542 nm (green; PMT2)	> 4 logs
Linearity		> 0.95
Linear Dynamic Range		> 4 logs
Precision		6% (high signal) plus a value depending the proximity to background
Bottom Read MULTI-TOX		
X Alignment	Using Exc. 406 nm, Em. 542 nm (green; PMT2)	≤ 1.5 mm
Y Alignment		≤ 1.5 mm
X Fly minus Stop & Go mode		≤ 0.5 mm
X Fly reverse minus forward		≤ 0.5 mm
1/(Signal/Background)	Using Exc. 406 nm, Em. 465 nm (blue; PMT1)	< 5000 fmol
1/(Signal/Noise)		< 50 fmol

Acceptability Criteria: MULTI-TOX Label Green Cartridge Protocol (Cartridge part number 0200-7007)

Test	Validation Plate Wells	Acceptable/Out of Specification Criteria
Test Top Read MULTI-TOX Experiment (Using Exc. 504 nm, Em. 542 nm (green; PMT2))		
X Alignment		< 1.5 mm
Y Alignment		< 1.5 mm
1/(Signal/Background)	Wells F07, (blank: F02)	< 500 fmol
1/(Signal/Noise)		< 5 fmol
Dynamic Range	Wells F04 through F10	> 4.5 logs
Linearity		> 0.95
Linear Dynamic Range		> 4.5 logs
Precision		6% (high signal) plus a value depending the proximity to background
Bottom Read MULTI-TOX (Using Exc. 504 nm, Em. 542 nm (green; PMT2))		
X Alignment		≤ 1.5 mm
Y Alignment		≤ 1.5 mm
X Fly minus Stop & Go mode		≤ 0.5 mm
X Fly reverse minus forward		≤ 0.5 mm
1/(Signal/Background)		< 200 fmol
1/(Signal/Noise)		< 6 fmol

Acceptability Criteria: TRF FPOL HTRF Cartridge Protocol (Experiment name is cartridge part number)

Test	Validation Plate Wells	Acceptable/Out of Specification Criteria
0200-7008 TRF_EUSA Experiment		
1/(Signal/Background)	Wells E02, (blank: F02)	< 100 fmol
1/(Signal/Noise)		< 10 fmol
0200-7009 FP-FLUO Experiment		
X alignment		≤ 1.5 mm
Y alignment		≤ 1.5 mm
X Fly minus Stop & Go mode		≤ 0.5 mm
X Fly reverse minus forward		≤ 0.5 mm
Precision at 1nM	Wells F05 through F07	3 mP
Max. Pos. Pol. Std.	Well G02	≥ 800 mP
Max. Neg. Pol. Std.	Well H02	≤ -700 mP
0200-7010 FP-RHOD Experiment		
X alignment		≤ 1.5 mm
Y alignment		≤ 1.5 mm
X Fly minus Stop & Go mode		≤ 0.5 mm
X Fly reverse minus forward		≤ 0.5 mm
Precision at 4 nM	Wells F05 through F07	4 mP
Max. Pos. Pol. Std.	Well G02	≥ 800 mP
Max. Neg. Pol. Std.	Well H02	≤ -700 mP
0200-7011 HTRF Experiment		
1/(Signal/Background) 620 nm	Wells E02, (blank: F02)	< 100 fmol
1/(Signal/Noise) 620 nm		< 20 fmol
1/(Signal/Background) 665 nm	Wells E02, (blank: F02)	< 100 fmol
1/(Signal/Noise) 665 nm		< 20 fmol

Acceptability Criteria: TUNE Cartridge Protocol (Cartridge part number 0200-7050)

Experiment Plate Section Name	Test	Acceptable/Out of Specification Criteria
Test FL Top Experiment		
Coumarin Background Wells D02, (blank: F02)	1/ (Signal/Background)	< 1000 fmol
Coumarin Background	1/(Signal/Noise)	< 1000 fmol
Fluorescein Plates Wells F07, (blank: F02)	Alignment X	< 1.75 mm
Fluorescein Plates	Alignment Y	< 1.75 mm
Fluorescein Plates	Precision	100%
Fluorescein Plates	Linearity R2	> 0.98
Rhodamine Plates Wells F07, (blank: F02)	Alignment X	< 1.75 mm
Rhodamine Plates	Alignment Y	< 1.75 mm
Rhodamine Plates	Precision	100%
Rhodamine Plates	Linearity	> 0.98
Rhodamine Plates	1/ (Signal/Background)	< 200 fmol
Rhodamine Plates	1/(Signal/Noise)	< 10 fmol
Rhodamine Plates	Dynamic Range	> 5 logs
Rhodamine Plates	Linear Dynamic Range	> 5 logs
Texas Red Plates Wells C02, (blank: F02)	1/ (Signal/Background)	< 1000 fmol
Texas Red Plates	1/(Signal/Noise)	< 30 fmol
TRF Europium Plates Wells E02, (blank: F02)	1/ (Signal/Background)	< 1000 amol
TRF Europium Plates	1/(Signal/Noise)	< 30 amol
Test Scan WLs Experiment		
Emission Wavelength Accuracy		< 3 nm
Emission Wavelength Precision		< 1 nm
Excitation Wavelength Accuracy		< 5 nm
Excitation Wavelength Precision		< 1.7 nm
Test LUM Experiment		
All Tests Well H10	Alignment X	< 2.5 mm

Acceptability Criteria: TUNE Cartridge Protocol (Cartridge part number 0200-7050) (continued)

Experiment Plate Section Name	Test	Acceptable/Out of Specification Criteria
All Tests Well H10	Alignment Y	< 2.5 mm
All Tests Wells H04 through H10, if not too close to background	Precision	100%
All Tests H04 through H10, if not too close to background	Linearity R2	> 0.98
All Tests H10	Signal (RLU)	> 1 Mio.
All Tests Rows A through D	Background (RLU)	< 1000
Test FL Bot Experiment		
Fluorescein Plates	Alignment X	< 1.75 mm
Fluorescein Plates	Alignment Y	<1.75 mm
Fluorescein Plates	1/ (Signal/Background)	< 100 fmol
Fluorescein Plates	1/(Signal/Noise)	< 15 fmol
Rhodamine Plates	Alignment X	< 1.75 mm
Rhodamine Plates	Alignment Y	< 1.75 mm
Rhodamine Plates	1/ (Signal/Background)	< 500 fmol
Rhodamine Plates	1/(Signal/Noise)	< 50 fmol
Rhodamine Plates	Dynamic Range	> 5 logs
Rhodamine Plates	Linear Dynamic Range	> 5 logs

Chapter 8: 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

Factory certification of the validation plate’s secondary standards is done using a reference instrument that is reserved exclusively for SpectraTest Multi-Mode 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. You should have the validation plate recertified annually.

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.

To have a validation plate recertified, contact Molecular Devices Technical Support. See Obtaining Support on page 57.

Note: Please contact Molecular Devices well before the recertification date to reserve a place in the recertification program. A minimum of one month is recommended.

Troubleshooting

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

- 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 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 56.

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

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

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

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

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Visit our website for a current listing of worldwide distributors.

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