



# SpectraTest® ABS1 Absorbance Validation Plate

## User Guide

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## Chapter 1: SpectraTest ABS1 Validation Plate Overview

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® ABS1 Absorbance Validation Plate from Molecular Devices enables you to validate the optical performance of the following instruments:

- SpectraMax® iD3 Multi-Mode Microplate Reader
- SpectraMax® iD5 Multi-Mode Microplate Reader
- SpectraMax® i3x Multi-Mode Microplate Reader
- SpectraMax® i3 Multi-Mode Microplate Reader
- FlexStation® 3 Multi-Mode Microplate Reader
- SpectraMax® M5e Multi-Mode Microplate Reader
- SpectraMax® M5 Multi-Mode Microplate Reader
- SpectraMax® M4 Multi-Mode Microplate Reader
- SpectraMax® M3 Multi-Mode Microplate Reader
- SpectraMax® M2 and M2e Multi-Mode Microplate Readers
- SpectraMax® Plus384 Absorbance Microplate Reader
- SpectraMax® 340PC 384 Absorbance Microplate Reader
- SpectraMax® 190 Absorbance Microplate Reader
- VersaMax™ Microplate Reader

The SpectraTest ABS1 Absorbance 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.

The SpectraTest ABS1 Absorbance Validation Plate package contains the following items:

- Validation plate with NIST-traceable filters (National Institute of Standards and Technology)
- Certificate of Calibration
- Validation Plate User Guide
- Protective Sleeve and Case



**CAUTION!** Treat the optical standards with care to retain their validity. The SpectraTest ABS1 Absorbance 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 not blow on the plate with your mouth to clean it. See [Maintenance and Troubleshooting on page 20](#).

#### Validation Packages Part Numbers

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

## 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
- Values for the neutral density filters used to determine photometric accuracy
- 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 exclusively for SpectraTest ABS1 Absorbance Validation Plate calibration and is checked for accuracy at fixed intervals of time. Molecular Devices recommends that you have the SpectraTest ABS1 Absorbance Validation Plate recertified yearly. See [Recertification on page 21](#).

## ISO-17025

SpectraTest ABS1 Absorbance 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.

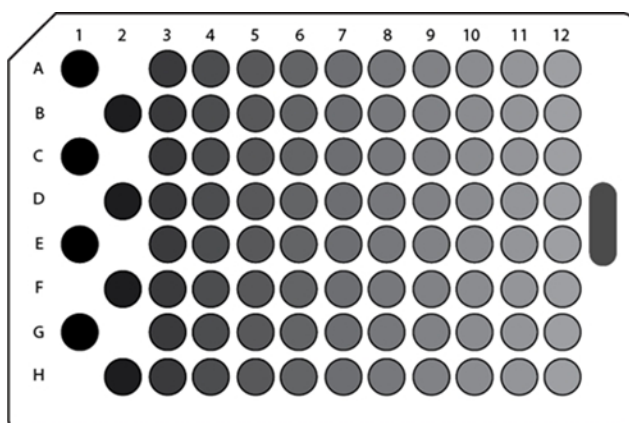
## Available Tests

The SpectraTest ABS1 Absorbance Validation Plate enables you to qualify the performance of the system by testing optical specifications that are critical to achieve quality results. See [Validation Tests on page 14](#).

Each ABS1 validation protocol is specific to the instrument you test and includes the following tests:

- **Baseline Noise Endpoint and Kinetic** - Measures the stability of the optical detection system (100% Transmission).
- **Optical Alignment** - Determines whether the carriage is aligned and the light beam passes through the center of the well.
- **Ultimate Dark** - Measures the signal when the light beam is completely obstructed (0% Transmission).
- **Photometric Accuracy (Linearity)** - Measures the accuracy or linearity of the optical density measurement.
- **Photometric Precision (Reproducibility)** - Measures the precision or reproducibility of the optical density measurement.
- **Stray Light** - Measures the purity of the monochromatic light that passes through the sample.
- **Wavelength Precision** - Measures the precision or reproducibility of the wavelength selection.
- **Wavelength Accuracy** - Measures the accuracy of the assigned wavelength.

The following indicates the columns related to the available tests.



### SpectraTest ABS1 Absorbance Validation Plate Configuration

Test	Columns	Configuration
Baseline Noise Tests	No plate in drawer	100% Transmittance
Optical Alignment and Ultimate Dark	1 and 2	Checkerboard strip alternating 100% T and 0% T. Ultimate Dark uses the wells with 0% T. Optical Alignment uses the wells with 100% T.
Photometric Accuracy and Photometric Precision	3 through 6	3: NG11, light gray glass, 2 mm thick (0.25 A) 4: NG5, gray glass, 2 mm thick (0.50 A) 5: NG4, dark gray glass, 2 mm thick (1.00 A) 6: NG4, dark gray glass, 3 mm thick (1.50 A)
Stray Light	7 through 10	7: WG320, clear glass, 2 mm thick (250 nm) (not used when testing the SpectraMax 340PC 384 or VersaMax instruments) 8: GG400, light green glass, 2 mm thick (340 nm) 9: GG475, yellow glass, 3 mm thick (405 nm) 10: RG715, dark red glass, 3 mm thick (650 nm)
Wavelength Precision	9	GG475, yellow glass, 3 mm thick (475 nm) FlexStation 3, SpectraMax M Series, SpectraMax Plus384, SpectraMax 340PC 384, SpectraMax 190, VersaMax
Wavelength Precision	11 and 12	11: HY1, light pink glass, 2 mm thick (Holmium oxide) 12: V30, purple glass, 2 mm thick (Didymium) SpectraMax iD3, SpectraMax iD5, SpectraMax i3x, SpectraMax i3
Wavelength Accuracy	11 and 12	11: HY1, light pink glass, 2 mm thick (Holmium oxide) 12: V30, purple glass, 2 mm thick (Didymium) FlexStation 3, SpectraMax M Series, SpectraMax Plus384, SpectraMax 340PC 384, SpectraMax 190, VersaMax

## Chapter 2: Validation Protocols

The SoftMax Pro Software Protocol Library contains protocols for use with the SpectraTest ABS1 Absorbance 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.



**Note:** SoftMax Pro Software version 6.x and 7.x protocols have a .spr file extension.

### Instrument Software Compatibility

Instrument	SoftMax Pro Minimum Version
SpectraMax iD3 and SpectraMax iD5	7.0.3
SpectraMax i3, SpectraMax i3x - protocol name includes "i3(x)"	6.5.1
SpectraMax i3 - protocol name does not include "i3(x)"	6.4.2
Most other instruments	4.8

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](http://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 FlexStation 3 ABS1. 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 ABS1 Absorbance Validation Plate protocol file contain settings that are for a specific instrument. Validation protocols require manual entry of certificate information. See [Certification Entry on page 9](#).

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



## Validation Plate Certification


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.


All protocol files enable you to manually enter the certification information.





## Certification Entry


Protocols require manual entry of the certification information and contain three instrument specific experiments. You should read all Note sections in each experiment for additional information and instructions.




 The **SpectraTest ABS1** 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 10](#).
-  **Result:** Displays the result of the validation protocol, including Data Point diagnostics, after you run the protocol.
-  **Data Diagnostics or Criteria:** Displays the definition of test parameters and their limiting values.
-  **Revision:** Displays the changes made to the protocol.

 The **No Plate Inserted** or **Expt#2** experiment contains the Baseline Noise tests that you run with the instrument plate drawer empty (no validation plate in the drawer):

-  Note sections contain information to get you started with this experiment.
-  **Endpoint** (three plates): Contains plate settings for Endpoint read type data acquisition.
-  **Kinetic** (three plates): Contains plate settings for Kinetic read type data acquisition.
-  Group sections perform calculations.

 The **ABS1 Plate Inserted** or **Expt#3** experiment contains the tests you run with the validation plate inserted in the instrument plate drawer:

-  Note sections contain information to get you started with this experiment.
-  **Optical Alignment:** Plate settings for data acquisition.
-  **Ultimate Dark:** Plate settings for data acquisition.

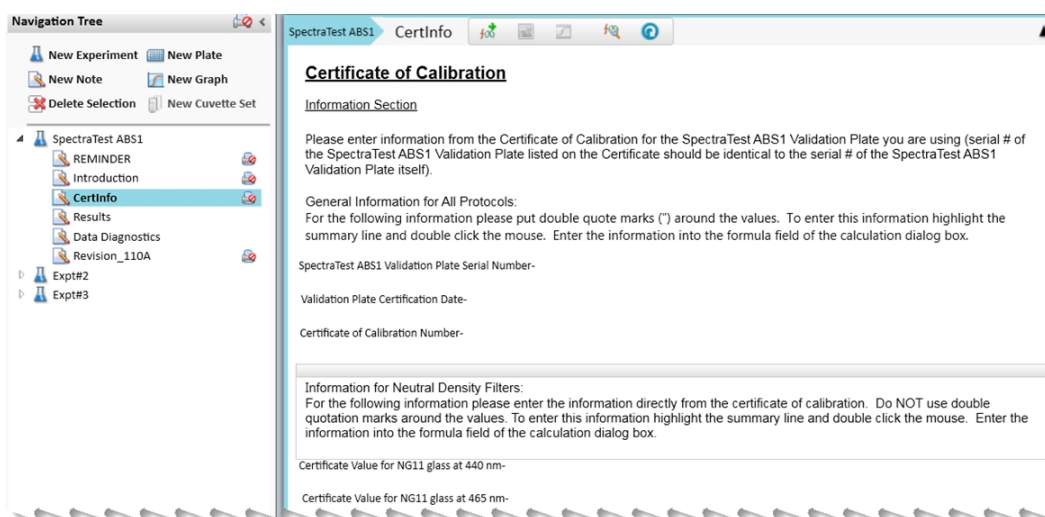
-  **Photometric Accuracy:** Plate settings for data acquisition.
-  **Photometric Precision:** Plate settings for data acquisition.
-  **Stray Light:** Plate settings for data acquisition.
-  **Wavelength Precision:** Plate settings for data acquisition.
-  **Wavelength Accuracy:** Plate settings for data acquisition.
-  Group sections perform calculations.
-  Graph sections to display results graphically.

## 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 Abs > <instrument>** to open the instrument-specific validation protocol.
2. In the Navigation Tree, expand the **SpectraTest ABS1** experiment and select the **CertInfo** Note section. For the new protocols, expand the **Appendix** experiment and then select the **CertInfo** Note section.



3. Double-click the **SpectraTest ABS1 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**.

The screenshot shows a 'Formula Editor' window. At the top, it says 'Enter a formula. For assistance, enable Syntax Helper.' Below this are fields for 'Name' (containing 'SR1'), 'Description' (containing 'SpectraTest ABS1 Validation Plate Serial Number'), and 'Formula' (containing '"1106"'). There is a 'Hide Name' checkbox (checked) and a 'Syntax Helper' checkbox (checked). A 'Check Syntax' button is below the formula field. At the bottom, there is a 'Data Display' section with 'Precision' set to 'Decimal Places' (0) and 'Format' set to 'Numeric Notation'. A 'Sample' field shows the value '123'. 'OK' and 'Cancel' buttons are at the very bottom.


5. Double-click the **SpectraTest ABS1 Certificate Date** field. In the **Formula** field, enter the certificate date in the double quotes, for example: "2018-05-15" and click **OK**.
6. Double-click the **ABS1 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 the wavelength values and the optical density values shown on the certificate into the corresponding fields.

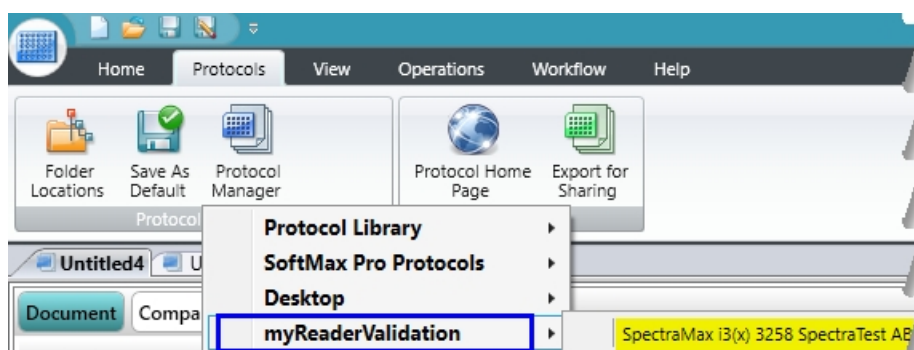


**Note:** Do not use quotation marks around the wavelength values and optical density values.

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 overwriting the original protocol.

- 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 ABS1 2019-Feb-28 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.

## Run Validation Protocols



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

- Power on the instrument and wait for the instrument to complete the start-up routine.
- Start the SoftMax Pro Software.
- 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.



**Note:** Do not insert the validation plate in the drawer. The first experiment section is done with an empty plate drawer.


- Open the protocol file that contains the certification data you entered.
- Expand the **SpectraTest ABS1** or **OQ ABS1 <instrument>** experiment and select 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. Expand the **No Plate Inserted** or **Expt#2** experiment and select the **Endpoint#1** Plate section.
9. Click  **Read**. The instrument reads all Plate sections in the experiment.
10. Place the validation plate in the instrument drawer with well A1 in the A1 drawer position.



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


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11. Expand the **ABS1 Plate Inserted** or **Expt#3** experiment and select the **OptAlign&UltDrk** Plate section.
12. Click  **Read**. The instrument reads all Plate sections in the experiment.
13. 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.

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14. Click  **Save** to save the data file.
15. Expand the **SpectraTestABS1** or **Expt#1** experiment and select the **Results** Note section and the **Data Point Diagnostics** or **Data Diagnostics** Note section (if included). 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 the Data Point Diagnostic or Data Diagnostics section indicates all data points have been reported, save the data file and print the report.
  - If the data is Out of Specification, see [Troubleshooting on page 21](#).

## Chapter 3: Interpret Test Results

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

### Validation Tests

The following validation tests are available:

- **Baseline Noise Endpoint and Kinetic** - Measures the stability of the optical detection system (100% Transmission).
- **Optical Alignment** - Determines whether the carriage is aligned and the light beam passes through the center of the well.
- **Ultimate Dark** - Measures the signal when the light beam is completely obstructed (0% Transmission).
- **Photometric Accuracy (Linearity)** - Measures the accuracy or linearity of the optical density measurement.
- **Photometric Precision (Reproducibility)** - Measures the precision or reproducibility of the optical density measurement.
- **Stray Light** - Measures the purity of the monochromatic light that passes through the sample.
- **Wavelength Precision** - Measures the precision or reproducibility of the wavelength selection.
- **Wavelength Accuracy** - Measures the accuracy of the assigned wavelength.

## Acceptability Criteria

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

**Table 3-1: Acceptability Criteria: SpectraMax iD3 and SpectraMax iD5 Tests**

Test	Validation Plate Columns	Acceptable/Out of Specification Criteria
Endpoint Baseline Noise	No plate	All values $\leq 0.007$ OD
Kinetic Baseline Noise	No plate	All values $\leq 0.007$ OD Drift $\leq 0.5$ mOD/min
Optical Alignment	1 and 2	Minimum values $\leq 0.015$
Ultimate Dark	1 and 2	Minimum OD $\geq 3.3$ when wavelength is between 400 nm and 650 nm Minimum OD $\geq 2.5$ when wavelength is $<400$ nm or $>650$ nm
Photometric Accuracy	3 through 6	$  \text{Average OD} - \text{Cert. Val.}   \leq (0.01 * \text{Cert. Val.} + 0.010)$
Photometric Precision	3 through 6	$\text{Stdev (Values of Replicates)} \leq (0.01 * \text{Cert. Val.} + 0.003)$
Stray Light	8 through 9	Minimum OD $\geq 2.3$
Stray Light	7 and 10	Minimum OD $\geq 2.8$
Wavelength Precision	11 and 12	$\text{Stdev (Peak Values of Replicates)} \leq 1.0$ nm
Wavelength Accuracy	11 and 12	$  \text{Average Peak Value} - \text{Cert. Val.}   \leq 3$ nm

**Table 3-2: Acceptability Criteria: SpectraMax i3 and SpectraMax i3x Tests**

Test	Validation Plate Columns	Acceptable/Out of Specification Criteria
Endpoint Baseline Noise	No plate	All values $\leq 0.005$ OD
Kinetic Baseline Noise	No plate	All values $\leq 0.005$ OD Drift $\leq 0.5$ mOD/min
Optical Alignment	1 and 2	All values $\leq 0.015$
Ultimate Dark	1 and 2	Minimum OD $\geq 3.3$ when wavelength is between 400 nm and 650 nm Minimum OD $\geq 2.5$ when wavelength is $<400$ nm or $>650$ nm
Photometric Accuracy	3 through 6	$  \text{Average OD} - \text{Cert. Val.}   \leq (0.01 * \text{CertVal.} + 0.010)$
Photometric Precision	3 through 6	$\text{Stdev (Values of Replicates)} \leq (0.01 * \text{Cert. Val.} + 0.003)$
Stray Light	7 through 10	Minimum OD $\geq 2.2$
Wavelength Precision	11 and 12	$\text{Stdev (Peak Values of Replicates)} \leq 1.0$ nm
Wavelength Accuracy	11 and 12	$  \text{Average Peak Value} - \text{Cert. Val.}   \leq 3$ nm



**Table 3-3: Acceptability Criteria: SpectraMax M2, M2e, M3, M4, M5, M5e, and FlexStation 3 Tests**

Test	Validation Plate Columns	Acceptable/Out of Specification Criteria
Endpoint Baseline Noise	No plate	$-0.003 \leq \text{all values} \leq 0.003$
Kinetic Baseline Noise	No plate	$-0.003 \leq \text{all values} \leq 0.003$ Drift $\leq 0.2$ mOD/min
Optical Alignment	1 and 2	$-0.003 \leq \text{all values} \leq 0.015$
Ultimate Dark	1 and 2	Minimum OD $\geq 3.3$
Photometric Accuracy	3 through 6	$ \text{Average OD} - \text{Cert. Val.}  \leq (.01 * \text{Cert.Val.} + 0.006)$
Photometric Precision	3 through 6	Minimum OD $> (\text{Average OD} - (.01 * \text{Average OD}) + 0.003)$ <b>and</b> Minimum OD must be $\geq X$ Maximum OD $< (\text{Average OD} + (.01 * \text{Average OD}) + 0.003)$ <b>and</b> Minimum OD must be $\leq Y$ Where X and Y are constants that are specific to each of the neutral-density glasses: NG11: X = 0.1, Y = 0.5 NG5: X = 0.3, Y = 1.2 NG4 (2 mm): X = 0.5, Y = 2.0 NG4 (3 mm): X = 0.8, Y = 3.0
Stray Light	7 through 10	Minimum OD $\geq 2.2$
Wavelength Precision	9	Maximum OD – Minimum OD $\leq 0.042$ <b>and</b> Minimum OD $\geq 0.1$ <b>and</b> Maximum OD $\leq 1.5$
Wavelength Accuracy	11 and 12	$ \text{Average Peak Value} - \text{Cert. Val.}  \leq 3$ nm

**Table 3-4: Acceptability Criteria: SpectraMax ABS Plus and SpectraMax Plus 384 Tests**

Test	Validation Plate Columns	Acceptable/Out of Specification Criteria
Endpoint Baseline Noise	No plate	Minimum OD $\geq -0.003$ Maximum OD $\leq +0.003$
Kinetic Baseline Noise	No plate	Minimum OD $\geq -0.003$ Maximum OD $\leq +0.003$ Minimum Rate $\geq -0.2$ mOD/min Maximum Rate $\leq +0.2$ mOD/min
Optical Alignment	1 and 2	Minimum OD $\geq -0.003$ Maximum OD $\leq +0.015$
Ultimate Dark	1 and 2	Minimum OD $\geq 3.3$
Photometric Accuracy	3 through 6	Average OD – Cert.Val.   $\leq (.01 * \text{Cert.Val.} + 0.006)$
Photometric Precision	3 through 6	Minimum OD $> (\text{Average OD} - ((.01 * \text{Average OD}) + 0.003))$ <b>and</b> Minimum OD must be $\geq X$ Maximum OD $< (\text{Average OD} + ((.01 * \text{Average OD}) + 0.003))$ <b>and</b> Minimum OD must be $\leq Y$ Where X and Y are constants that are specific to each of the neutral-density glasses: NG11: X = 0.1, Y = 0.5 NG5: X = 0.3, Y = 1.2 NG4 (2 mm): X = 0.5, Y = 2.0 NG4 (3 mm): X = 0.8, Y = 3.0
Stray Light	7 through 10	Minimum OD $\geq 3.15$
Wavelength Precision	9	Maximum OD – Minimum OD $\leq 0.042$ <b>and</b> Minimum OD $\geq 0.1$ <b>and</b> Maximum OD $\leq 1.5$
Wavelength Accuracy	11 and 12	Average Peak Value – Cert. Val.   $\leq 2$ nm

**Table 3-5: Acceptability Criteria: SpectraMax ABS, 190, 340PC 384, and VersaMax Tests**

Test	Validation Plate Columns	Acceptable/Out of Specification Criteria
Endpoint Baseline Noise	No plate	$-0.003 \leq \text{all values} \leq 0.003$
Kinetic Baseline Noise	No plate	$-0.003 \leq \text{all values} \leq 0.003$ Drift $\leq 0.2$ mOD/min
Optical Alignment	1 and 2	$-0.003 \leq \text{all values} \leq 0.015$
Ultimate Dark	1 and 2	Minimum OD $\geq 3.3$
Photometric Accuracy	3 through 6	$ \text{Average OD} - \text{Cert. Val.}  \leq (.01 * \text{Cert.Val.} + 0.006)$
Photometric Precision	3 through 6	Minimum OD $> (\text{Average OD} - (.01 * \text{Average OD}) + 0.003)$ <b>and</b> Minimum OD must be $\geq X$ Maximum OD $< (\text{Average OD} + (.01 * \text{Average OD}) + 0.003)$ <b>and</b> Minimum OD must be $\leq Y$ Where X and Y are constants that are specific to each of the neutral-density glasses: NG11: X = 0.1, Y = 0.5 NG5: X = 0.3, Y = 1.2 NG4 (2 mm): X = 0.5, Y = 2.0 NG4 (3 mm): X = 0.8, Y = 3.0
Stray Light	7 through 10	Minimum OD $\geq 3.15$
Wavelength Precision	9	Maximum OD – Minimum OD $\leq 0.042$ <b>and</b> Minimum OD $\geq 0.1$ <b>and</b> Maximum OD $\leq 1.5$
Wavelength Accuracy	11 and 12	$ \text{Average Peak Value} - \text{Cert. Val.}  \leq 3$ nm

## Chapter 4: Maintenance and Troubleshooting

At the time of delivery, all validation plates meet the manufacturing specifications of 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.

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- 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.

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## Recertification

Factory certification of the validation plate's secondary standards is done using a reference instrument that is reserved exclusively for SpectraTest ABS1 Absorbance 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 22](#).



**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.

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## Troubleshooting

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

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

- Check that the information in the CertInfo section of the protocol matches the information on the Certificate of Calibration that accompanies the validation plate. If the information does not match, update the CertInfo section of the protocol with the information from the Certificate of Calibration, and then view the Results 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 SpectraMax M2, M2e, M3, M4, M5, M5e, and FlexStation 3 instruments require the use of an adapter plate when you run the SpectraTest ABS1 Absorbance Validation Plate.

- Check the plate for dirt, dust, or other defects. Dust is not always visible. You can blow moisture-free, clean canned air across both sides of the plate to clean it and then repeat the test. Do not use air from “house” air lines on the plate, and do not blow on it with your mouth. If the results are still unacceptable, you can try cleaning with alcohol. See [Maintenance and Troubleshooting on page 20](#).
- Check the Data Point Diagnostic or Data 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, [www.moleculardevices.com/service-support](http://www.moleculardevices.com/service-support), has a link to the Knowledge Base, which contains technical notes, software upgrades, safety data sheets, and other resources. If you still need assistance after consulting the Knowledge Base, you can submit a request to Molecular Devices Technical Support.

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

## Contact Us

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

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