SoftMax® Pro

Data Acquisition and Analysis Software

Cuvette Port Validation Protocol

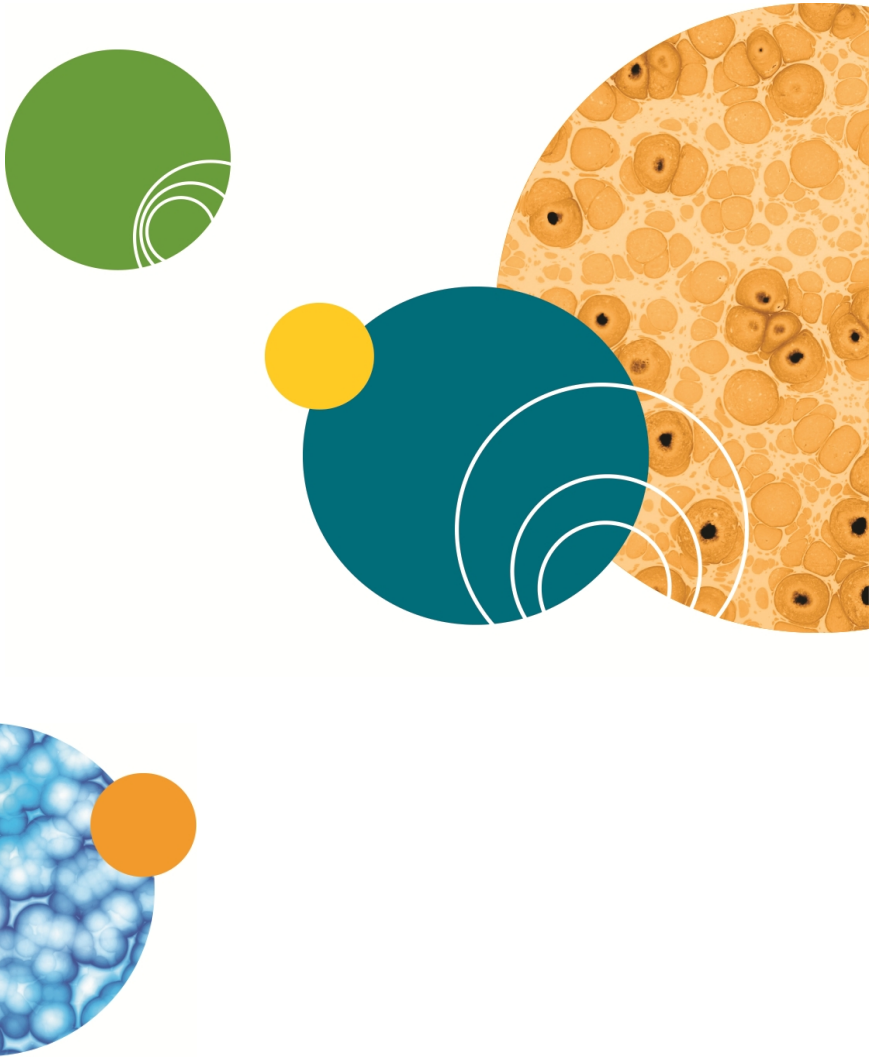
User Guide

MOLECULAR DEVICES

0112-0044 B
February 2016

Product manufactured by Molecular Devices, LLC.
1311 Orleans Drive, Sunnyvale, California, United States of America 94089.
Molecular Devices, LLC is ISO 9001 registered.

Contents



Chapter 1: Introduction

1

SpectraMax® Microplate Readers are designed to provide consistent performance for many years. However, to fulfill regulatory requirements, the performance of an instrument must periodically be validated and documented. The Hellma Secondary Spectrometric Calibration Standards (also called “cuvette standards”) are NIST traceable. When the cuvette standards are used with a SoftMax® Pro Software Cuvette Port Validation protocol, they provide a means of validating the optical performance of the SpectraMax reader cuvette port.

The SoftMax Pro Software Cuvette Port Validation protocols are compatible with cuvette ports on the SpectraMax Plus 384 Absorbance Microplate Reader and with SpectraMax M2, M2e, M3, M4, M5, and M5e Multi-Mode Microplate Readers.

Note: To validate the optical performance of the microplate drawer of your SpectraMax instrument, use the SpectraTest® ABS1 Absorbance Validation Plate.

The SoftMax Pro Software Cuvette Port Validation protocols automate data collection and test-result reporting.

This guide describes how to use a protocol with the cuvette standards, and the acceptability criteria for each of the tests in the protocol.

Note: The SoftMax Pro Data Acquisition and Analysis Software is required to run the SoftMax Pro Software Cuvette Port Validation protocol.

The Hellma Cuvette Set Validation Package

The Hellma Cuvette Set Validation Package includes:

- Hellma Secondary Spectrometric Calibration Standards (5)
- Storage case for the cuvette standards
- Hellma certificate of calibration
- CD with protocol files
- User guide

The SoftMax Pro Software Cuvette Port Validation protocols are installed with the SoftMax Pro Software and can be found in the **Reader Validation-Cuvette Abs** folder. Make sure that you use the correct protocol for your reader. See Using Cuvette Port Validation Protocols on page 7.

The Cuvette Validation Standards

There are five cuvette standards:

- **F0:** Solid black, open wall insert used for the Ultimate Dark Test
- **F1:** Holmium oxide standard used for Wavelength Accuracy
- **F2:** NG11 neutral density glass used for Photometric Accuracy and Precision
- **F3:** NG5 neutral density glass used for Photometric Accuracy and Precision
- **F4:** NG4 neutral density glass used for Photometric Accuracy and Precision

During the validation tests, the beam travels from left to right.

Cuvette **F0** must be inserted so that when you stand in front of the instrument the **F0** label is facing you, such that the opaque walls block the beam. See Figure 2-1: The F0 cuvette in the cuvette port on page 10.

Cuvettes **F1** through **F4** must be inserted so that the writing on top of the standard faces the microplate drawer, such that the glass filter is closer to the right side. See Figure 2-2: The F1 cuvette in the cuvette port on page 11 and Figure 2-3: The F2 cuvette in the cuvette port on page 12.

CAUTION! The cuvette standards must be treated with care if they are to retain their validity. When you are not using them, store the standards in their case to protect the optical surfaces from dust, scratches, and corrosion. Never touch the glass filters of the standards with your fingertips.

Chapter 2: Using Cuvette Port Validation Protocols

2

Two Cuvette Port Validation protocols are installed with the SoftMax Pro Software. They can be found in the **Reader Validation-Cuvette Abs** folder. Make sure that you use the correct protocol for your reader.

Table 2-1: Cuvette Port Validation Protocols

Microplate Readers	Validation Protocol
SpectraMax Plus 384 Absorbance Microplate Reader	Plus Hellma
SpectraMax M2, M2e, M3, M4, M5, and M5e Multi-Mode Microplate Readers	M Series Hellma

Note: If you are using SoftMax Pro Software version 5.4.1 or older, the Cuvette Port Validation protocols installed with the software are no longer valid. Use the updated protocol files on the CD that came with the validation kit.

Each Cuvette Port Validation protocol contains three experiment sections that perform the following functions:

- **Overview and Results** contains an introduction, the **CertInfo** section into which information from the certificate of calibration must be entered, and the **Results** section to contain the results of all tests after they have been run.
- **Baseline Tests** contains the Baseline Noise Tests and an introductory section with instructions on how to run the tests. All tests in **Baseline Tests** must be run with nothing in the cuvette port.
  - **Baseline Noise Test** measures the sensitivity and stability of the optical system (100% Transmission).
    (EndBaseNoise, KinBaseNoise)
- **Cuvette Tests** contains the four tests used with the cuvette standards and an introductory section with instructions for running the tests:
  - **Ultimate Dark** measures the optical and electronic signal-to-noise ratio (0% Transmission).
    (UltDrk)
  - **Photometric Accuracy** tests the accuracy or linearity of the optical density measurement.
    (PhotoAcc F2, PhotoAcc F3, PhotoAcc F4)
  - **Photometric Precision** tests the precision or reproducibility of the optical density measurement.
    (PhotometricPrec)
  - **Wavelength Accuracy** tests the accuracy of the optical density measurement at the designated wavelength.
    (WavelengthAcc)

To use the following instructions, you must be familiar with the SoftMax Pro Software. If you have questions about using SoftMax Pro Software, see the SoftMax Pro Software application help or user guide.

Preparing the Cuvette Port Validation Protocol

The standards provided in the Hellma Cuvette Set are NIST traceable. For the Cuvette Port Validation protocol to calculate test results properly, you must open the protocol and type the values found on the inside of the lid of the box containing the standards, or on the Certificate of Calibration.

These values need to be entered only one time before the first use of the protocol, until after the cuvette standards are factory recalibrated. After factory recalibration, the new values need to be typed into the protocol.

Entering the Certificate Values

1. Start the SoftMax Pro Software and open the appropriate protocol for your reader. The original protocols can be found in the **Reader Validation-Cuvette Abs** folder:
   - For the SpectraMax Plus 384 Absorbance Microplate Reader, use the **Plus Hellma** protocol.
   - For the SpectraMax M2, M2e, M3, M4, M5, and M5e Multi-Mode Microplate Readers, use the **M Series Hellma** protocol.

   Note: If you are using SoftMax Pro Software version 5.4.1 or older, the Cuvette Port Validation protocols installed with the software are no longer valid. Use the updated protocol files on the CD that came with the validation kit.

2. Open the **Overview and Results** experiment in the protocol, and then click the **CertInfo** section.
3. For each of the cuvette standards, follow the instructions in the **CertInfo** section and type the appropriate values from the certificate of calibration.
   To enter this information double-click the summary line to open the **Formula Editor** dialog, and then type the value in the **Formula** field.
   - Type the **Certification Date** with double quotation (“ ”) marks around it.
   - Type the values for the filters (F1, F2, F3, and F4) as numbers with no quotation marks.

   Note: If double quotes are used around the filter values, or if no quotes are used around the **Certification Date**, the **Results** section will be filled with error messages, rather than labeling the results **Acceptable** or **Out of Specification**.

4. Save the edited protocol as a protocol file.
   You can either save the edited protocol using the original name to overwrite the original file, or using a new name. If you use a new name, do not use the original protocol file for subsequent validations. Since it does not have the calibration values in it, it will yield incorrect test results.

Running the Cuvette Port Validation Protocol

The **Acceptable** or **Out of Specification** limits for the tests are based on the instrument specifications for the SpectraMax instrument, plus any other applicable tolerances, such as the filter tolerances specified in the Hellma certificate of calibration.

Note: If your instrument fails one of the tests in the validation protocol, you do not need to continue with the remainder of the tests. The instrument must pass all of the tests to be validated. If you have questions about troubleshooting a failure to validate, or if you require immediate service, contact Devices technical support or your local Molecular Devices representative. See Obtaining Support on page 15.

Opening the Protocol

1. Turn on the microplate reader, and then start the SoftMax Pro Software.
2. Open the appropriate Cuvette Port Validation protocol file for your reader.
   The original protocols can be found in the **Reader Validation-Cuvette Abs** folder:
   - For the SpectraMax Plus 384 Absorbance Microplate Reader, use the **Plus Hellma** protocol.
   - For the SpectraMax M2, M2e, M3, M4, M5, and M5e Multi-Mode Microplate Readers, use the **M Series Hellma** protocol.

   Note: If you are using SoftMax Pro Software version 5.4.1 or older, the Cuvette Port Validation protocols installed with the software are no longer valid. Use the updated protocol files on the CD that came with the validation kit.
3. If this is the first time using the protocol, type the values from the Certificate of Calibration into the protocol before using it. See Entering the Certificate Values on page 8.

Note: The SoftMax Pro Software automatically time-and-date stamps each Cuvette Set as the data is collected. Confirm that the date and time settings on your computer are correct before running the Cuvette Port Validation protocol.

Running Baseline Noise Tests

1. Make sure that there is no cuvette in the cuvette port, and that the cuvette port door is closed.
2. Open the **Baseline Tests** experiment in the protocol.
3. Open the Cuvette Set named **EndBaseNoise**, and then click **Ref** in the toolbar.
4. One by one, select each of the cuvettes in the Cuvette Set, and then click **Read** in the toolbar to read each cuvette.
5. Open the Cuvette Set named **KinBaseNoise**, and then click **Ref** in the toolbar.
6. After the reference reading is completed, click **Read** in the toolbar.
7. Minimize the **Baseline Tests** experiment.

Validating the Cuvette Port Performance with the Hellma Spectrometric Secondary Calibration Standards

The four tests in the **Cuvette Tests** experiment can be run in any order, but all four tests must be completed to validate the cuvette port. Since the **Wavelength Accuracy** test uses a spectral scan that can last several minutes, you might want to run that test last.

CAUTION! The cuvette standards must be treated with care if they are to retain their validity. When you are not using them, store the standards in their case to protect the optical surfaces from dust, scratches, and corrosion. Never touch the glass filters of the standards with your fingertips.

To run the Cuvette Tests experiment:

1. Open the **Cuvette Tests** experiment in the protocol.
2. Run the four tests. See the following procedures:
   - Running the Ultra Dark Test on page 10
   - Running the Wavelength Accuracy Test on page 11
   - Running the Photometric Accuracy Test on page 12
   - Running the Photometric Precision Test on page 13
3. Minimize the **Cuvette Tests** experiment.
4. Review your results. See Interpreting the Test Results on page 14.

Running the Ultra Dark Test

1. In the **Cuvette Tests** experiment, open the Cuvette Set named **UltDrk**.
2. Make sure there is no cuvette in the cuvette port and that the cuvette port door is closed.
3. Click **Ref** in the toolbar.
4. Place cuvette standard F0 in the cuvette port, aligning it so that when you stand in front of the instrument the F0 label is facing you. See Figure 2-1.

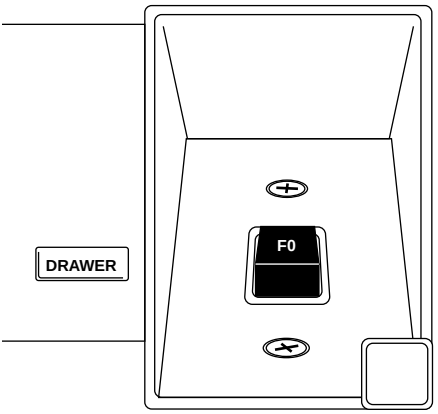


Figure 2-1: The F0 cuvette in the cuvette port

5. One by one, select each of the cuvettes in the **UltDrk** Cuvette Set, and then click **Read** in the toolbar to read each cuvette.
6. Remove cuvette standard F0 from the cuvette port and place it in the storage case.

Running the Wavelength Accuracy Test

Note: This read takes several minutes to complete because it uses a spectral scan.

1. In the **Cuvette Tests** experiment, open the Cuvette Set named **WavelengthAcc**.
2. Make sure there is no cuvette in the cuvette port and that the cuvette port door is closed.
3. Click **Ref** in the toolbar.
4. Place cuvette standard F1 in the cuvette port, aligning it so that the writing on top of the standard faces the microplate drawer. See Figure 2-2.

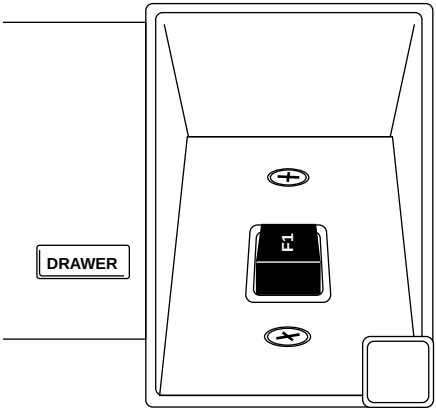


Figure 2-2: The F1 cuvette in the cuvette port

5. Click **Read** in the toolbar.
6. When the scan is complete, remove cuvette standard F1 from the cuvette port and place it in the storage case.

Running the Photometric Accuracy Test

1. In the **Cuvette Tests** experiment, open the Cuvette Set named **PhotoAcc F2**.
2. Make sure there is no cuvette in the cuvette port and that the cuvette port door is closed.
3. Click **Ref** in the toolbar.
4. Place cuvette standard F2 in the cuvette port, aligning it so that the writing on top of the standard faces the microplate drawer. See Figure 2-3.

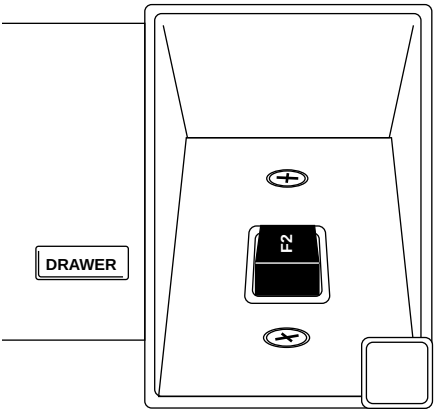


Figure 2-3: The F2 cuvette in the cuvette port

5. One by one, select each of the cuvettes in the **PhotoAcc F2** Cuvette Set, and then click **Read** in the toolbar to read each cuvette.
6. Remove cuvette standard F2 from the cuvette port and place it in the storage case.
7. Repeat the previous steps with the Cuvette Sets named **PhotoAcc F3** and **PhotoAcc F4** using the corresponding cuvette standards, and aligning them in the cuvette port in the same way as cuvette standard F2.

Running the Photometric Precision Test

1. In the **Cuvette Tests** experiment, open the Cuvette Set named **PhotometricPrec**.
2. Make sure there is no cuvette in the cuvette port and that the cuvette port door is closed.
3. Click **Ref** in the toolbar.
4. Place cuvette standard F2 in the cuvette port, aligning it so that the writing on top of the standard faces the microplate drawer. See Figure 2-4.

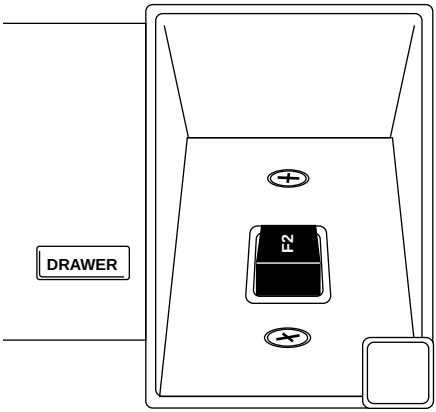


Figure 2-4: The F2 cuvette in the cuvette port

5. Select the first cuvette in the **PhotometricPrec** Cuvette Set, and then click **Read** in the toolbar.
6. Remove cuvette standard F2 from the cuvette port and place it in the storage case.
7. Place cuvette standard F3 in the cuvette port, aligning it so that the writing on top of the standard faces the microplate drawer. See Figure 2-4.
8. Select the second cuvette in the **PhotometricPrec** Cuvette Set, and then click **Read** in the toolbar.
9. Remove cuvette standard F3 from the cuvette port and place it in the storage case.
10. Place cuvette standard F4 in the cuvette port, aligning it so that the writing on top of the standard faces the microplate drawer. See Figure 2-4.
11. Select the third cuvette in the **PhotometricPrec** Cuvette Set, and then click **Read** in the toolbar.
12. Remove cuvette standard F4 from the cuvette port and place it in the storage case.

Interpreting the Test Results

The results of each test appear in the **Results** section of the **Overview and Results** experiment.

Note: If double quotes are used around the filter values, or if no quotes are used around the **Certification Date**, the **Results** section will be filled with error messages, rather than labeling the results **Acceptable** or **Out of Specification**. If you remove or add double quotes as necessary, the SoftMax Pro Software recalculates the test result and displays them correctly.

Acceptability Criteria

The acceptability criteria for each test are derived by summing the error of the instrument (or the published specification for the instrument) + the uncertainty of the measurement (where applicable) + quantization (where applicable). Table 2-2 lists each test, and the values needed for passing results.

Table 2-2: Acceptability Criteria for the Cuvette Port Validation Tests

Test	Cuvette Name	Acceptability Criteria
Endpoint Baseline Noise	None	Minimum OD ≥ –0.003 Maximum OD ≤ +0.003
Kinetic Baseline Noise	None	Minimum Rate ≥ –0.2 mOD/min Maximum Rate ≤ +0.2 mOD/min and Minimum OD ≥ –0.003 Maximum OD ≤ +0.003
Ultimate Dark	F0	Minimum OD ≥ 3.3
Wavelength Accuracy	F1	**SpectraMax Plus 384 reader:** Certificate Value – 2 ≤ Average Peak Value ≤ Certificate Value + 2 **SpectraMax M2, M2e, M3, M4, M5, and M5e readers:** Certificate Value – 3 ≤ Average Peak Value ≤ Certificate Value + 3
Photometric Accuracy	F2, F3, F4	Average OD = Certificate Value ± (Certificate Value(0.01) + 0.005)
Photometric Precision	F2, F3, F4	Minimum OD ≥ (Average OD – (Average OD(0.01) + 0.003)) and Maximum OD ≤ (Average OD + (Average OD(0.01) + 0.003))

Note: If you have questions about troubleshooting a failure to validate, or if you require immediate service, contact Molecular Devices technical support or your local Molecular Devices representative. See Obtaining Support on page 15.

Chapter 3: 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 possible level of technical service.

Our support web site, www.moleculardevices.com/support, has a link to the Knowledge Base with technical notes, software upgrades, safety data sheets, and other resources. If you do not find the answers you are seeking, follow the links to the Technical Support Service Request Form to send an email message to a pool of technical support representatives.

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

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

Contact Us

Phone: 800.635.5577
Web: www.moleculardevices.com
Email: info@moldev.com

Check our website for a current listing of worldwide distributors

Regional Offices

USA and Canada
+1.800.635.5577
United Kingdom
+44.118.944.8000
Europe*
00800.665.32860

China (Beijing)
+86.10.6410.8669
China (Shanghai)
+86.21.3372.1088
Hong Kong
+852.2248.6000

Japan (Osaka)
+81.6.7174.8831
Japan (Tokyo)
+81.3.6362.5260
South Korea
+82.2.3471.9534

Brazil
+55.11.3616.6607

Austria, Belgium, Denmark, Finland, France, Germany, Ireland, Netherlands, Spain, Sweden, and Switzerland

0112-0044 B