Cuvette Port Validation Protocol

SoftMax® Pro Data Acquisition and Analysis Software

User Guide

MOLECULAR DEVICES

0112-0044 C
April 2019

Product manufactured by Molecular Devices, LLC.
3860 N. First Street, San Jose, California, 95134, United States of America.
Molecular Devices, LLC is ISO 9001 registered.

Contents

Chapter 1: Cuvette Port Validation 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 Hellma® Secondary Spectrometric Calibration Standards (also called cuvette standards) are NIST traceable. When you use the cuvette standards with a SoftMax® Pro Software cuvette port validation protocol, they provide a means to validate the optical performance of the instrument cuvette port.

The SoftMax Pro Software cuvette port validation protocols are compatible with cuvette ports on the following instruments:

- SpectraMax® ABS Plus Absorbance Microplate Reader
- SpectraMax® Plus 384 Absorbance Microplate Reader
- SpectraMax® M2 Multi-Mode Microplate Reader
- SpectraMax® M2e Multi-Mode Microplate Reader
- SpectraMax® M3 Multi-Mode Microplate Reader
- SpectraMax® M4 Multi-Mode Microplate Reader
- SpectraMax® M5 Multi-Mode Microplate Reader
- SpectraMax® M5e Multi-Mode Microplate Reader.

The Hellma Cuvette Set Validation Package contains the following items:

- Hellma Secondary Spectrometric Calibration standards
  - F0 - Solid black, open wall insert for Ultimate Dark test
  - F1 - Holmium oxide for Wavelength Accuracy test
  - F2 - NG11 neutral density glass for Photometric Accuracy and Precision test
  - F3 - NG5 neutral density glass for Photometric Accuracy and Precision test
  - F4 - NG4 neutral density glass for Photometric Accuracy and Precision test
- Storage Case (for the cuvette standards)
- Hellma Certificate of Calibration
- Cuvette Port Validation Protocol User Guide

CAUTION! Treat the cuvette standards with care to retain their validity. When not in use, 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.

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.

Certificate of Calibration

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

- Serial Number
- Certification Date
- Wavelength peak values used to determine wavelength accuracy
- Optical densities used to determine photometric accuracy and precision

Factory certification of the Hella Cuvette Set Validation Package standards is done directly by Hellma. Please contact Hellma directly for recertification recommendations. See Recertification on page 19.

ISO-17025

The Hellma Cuvette Set Validation Package is a tool of metrology. Please contact Hellma directly for ISO-17025 accreditation information.

Available Tests

The SoftMax Pro Software Protocol Library contains cuvette port validation protocols in the Reader Validation-Cuvette Abs folder.

Each cuvette port validation protocol provides the following tests:

- Baseline tests are run with nothing in the cuvette port.
  - Baseline Noise Test measures the sensitivity and stability of the optical system (100% Transmission).
- Cuvette tests are run with the cuvette standards in the cuvette port for the following tests:
  - Ultimate Dark measures the optical and electronic signal-to-noise ratio (0% Transmission).
  - Wavelength Accuracy tests the accuracy of the optical density measurement at the designated wavelength.
  - Photometric Accuracy tests the accuracy or linearity of the optical density measurement.
  - Photometric Precision tests the precision or reproducibility of the optical density measurement.

Chapter 2: Use Validation Protocols

The SoftMax Pro Software Protocol Library contains the protocols for use with the validation package. The standards provided in the Hellma Cuvette Set Validation Package are NIST traceable. You must use the protocols included with SoftMax Pro Software version 5.4.1 or higher.

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

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: M Series Hellma or Plus Hellma.
3. Save the protocol file in the folder you create.

Protocol Files

The experiments and sections in each protocol file contain the instrument settings and formulas for the calculations. You must enter the Certification of Calibration information into the protocol. Enter Certificate Information on page 9.

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

Note: If the 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, contact Molecular Devices technical support. See Obtaining Support on page 19.

Protocol File Sections

The SoftMax Pro Software Protocol Library contains cuvette port validation protocols in the Reader Validation-Cuvette Abs folder. The following table lists which validation protocol is applicable for each instrument.

Cuvette Port Validation Protocols

Validation Protocol	Microplate Readers
M Series Hellma	SpectraMax M2, M2e, M3, M4, M5, and M5e
Plus Hellma	SpectraMax Plus 384
SpectraMax ABSPlus Hellma Cuvette	SpectraMax ABS Plus

Each cuvette port validation protocol contains three experiment sections that perform the following functions:

 The **Overview and Results** experiment contains the following Note sections.

-  **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 9.
-  **Results**: Displays the test results after you run the protocol.
-  **Revision**: Displays the revisions made to the protocol.

 The **Baseline Tests** experiment contains the Baseline Noise Tests you run with nothing in the cuvette port.

-  **Introduction**: Contains information to get you started with the experiment.
-  **End Baseline Noise**: Measures the sensitivity and stability of the optical system (100% Transmission).
-  **Kin Baseline Noise**: Measures the sensitivity and stability of the optical system (100% Transmission).
-  Group sections perform calculations.

 The **Cuvette Tests** experiment contains four tests you run with the cuvette standards in the cuvette port.

-  **Instructions**: Contains a copy of the instructions found in this document.
-  **UltDrk**: Measures the optical and electronic signal-to-noise ratio (0% Transmission).
-  **WavelengthAcc**: Measures the accuracy of the optical density at the designated wavelength.
-  **Photometric Accuracy**: Three Cuvette Set sections that measure the accuracy or linearity of the optical density.

- **Photometric Precision**: Measures the precision or reproducibility of the optical density.
- Group sections perform calculations.

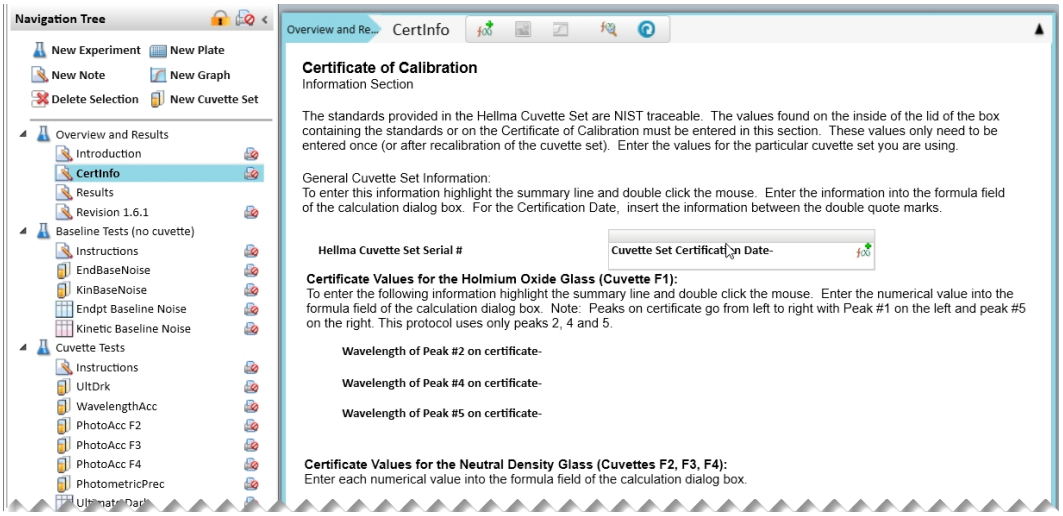
Enter Certificate Information

Before you run a validation protocol you must enter information from the Certificate of Calibration that came with the cuvette set validation package into the SoftMax Pro Software. Some of the following information may be printed inside the lid of the box that contains the standards.

Enter the information one time before the initial use of the protocol and then again each time Hellma recertifies the cuvette set validation package and sends you a new Certificate of Calibration.

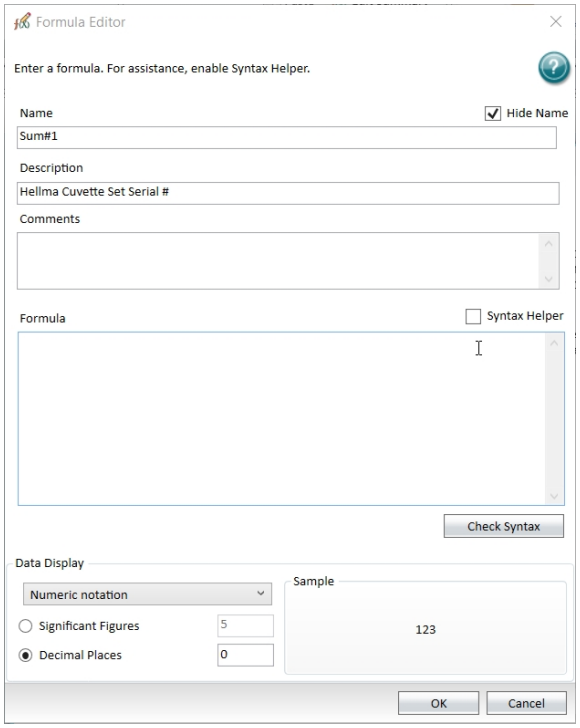
Note: If you correctly complete the entry of the certification information the Results Note section labels the results Acceptable or Out of Specification. If you use double quotes around the filter values or if do not use quotes around the Certification Date, the Results section displays error messages.

1. In the SoftMax Pro Software, select the Home tab and click **Protocol Manager** > **Protocol Library** > **Reader Validation-Cuvette Abs** and open the validation protocol.
2. In the Navigation Tree, expand the **Overview and Results** experiment and select the **CertInfo** Note section.



3. Double-click the **Hellma Cuvette Set Serial Number** field to display the Formula Editor dialog.

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



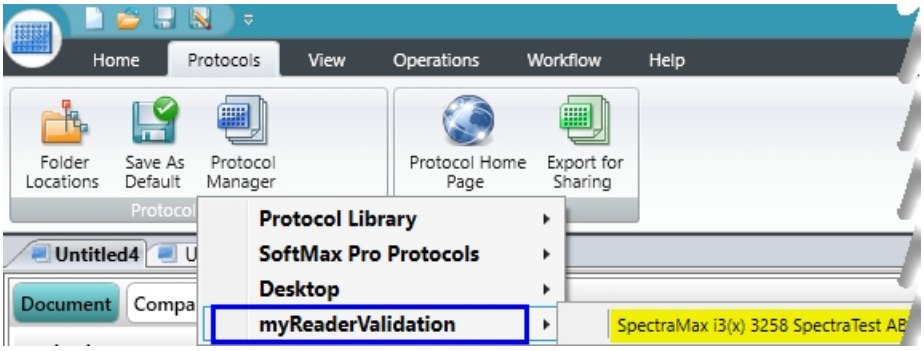
5. Double-click the **Cuvette Set Certification Date** field. In the **Formula** field, enter the certificate date in the double quotes.
6. Double-click each **Wavelength of Peak** field. In the **Formula** field, enter the values from the certificate and click **OK**.

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

7. Double-click each **Optical Density** field. In the **Formula** field, enter the values from the certificate and click **OK**.
8. After you enter all certificate information, click **Save As** to save the file as a protocol file with a new name in order to save the certificate information and to prevent over writing the original protocol.

9. In the Save As dialog, click the **Save As Type** drop-down and select **Protocol Files** to have the software append the applicable .spr or .ppr file extension to the file name.

Tip: Name the file with the validation expiration date and instrument type, for example, Cuvette 2019-Feb-28 SpectraMax M5e and save the new protocol file. You can save the file to the folder of your choice.



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

Run Baseline Noise Tests

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

1. Power on the instrument and wait for the instrument to complete its start-up routine.
2. Start the SoftMax Pro Software.
3. Confirm that the instrument and the software are connected and communicating properly. If not, a red X displays over the instrument icon in the upper-left corner of the software window.
4. Open the protocol file that contains the certification data you entered.
5. Make sure that there is no cuvette in the cuvette port and close the cuvette port door.
6. Expand the **Overview and Results** experiment and select the **Results** Note section.
7. Enter the following information:
   - **Instrument Serial Number**: Enter the instrument serial number.
   - **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.
8. Click  **Save As** to save the file as a data file with a name of your choice.
9. Expand the **Baseline Tests (no cuvette)** experiment and select the **EndBaseNoise** Cuvette Set section.
10. Select the Home tab and click  **Ref**.
11. One by one, select each of the cuvettes in the Cuvette Set section, and then click  **Read** to read each cuvette.
12. In the Navigation Tree, select the **KinBaseNoise** Cuvette Set section.
13. Select the Home tab and click  **Ref**.
14. After the reference read completes, click  **Read**.
15. Click  **Save** to save the data file.

Run Cuvette Tests

You can run the four tests in the Cuvette Tests experiment in any order. You must complete all four tests to validate the cuvette port. The Wavelength Accuracy test uses a spectral scan that can last several minutes, you can 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.

Run Ultra Dark Test

Now that you have entered the certification data and run the Baseline Tests, you are ready to run the Cuvette Tests.

During the validation tests, the beam travels from left to right in the SpectraMax Plus 384, M2, M3, M4, M5, and M5e and rear to front in the SpectraMax ABS Plus. You must insert cuvette F0 so that the writing on the top of the standard is readable and oriented so that opaque walls are side-to-side (blocking the beam) in the SpectraMax Plus 384, M2, M3, M4, M5, and M5e. The opaque walls are rear-to-front (blocking the beam) in the SpectraMax ABS Plus.

1. Power on the instrument and wait for the instrument to complete its start-up routine.
2. Start the SoftMax Pro Software.
3. Confirm that the instrument and the software are connected and communicating properly. If not, a red X displays over the instrument icon in the upper-left corner of the software window.
4. Open the data file that contains the certification data and the results of the BaselineTests.
5. In the Navigation Tree, expand the **Cuvette Tests** experiment and select the **UltDrk** Cuvette Set section.
6. Make sure there is no cuvette in the cuvette port and close the cuvette port door.
7. Select the Home tab and click **Ref**.
8. Place cuvette standard F0 in the cuvette port so that the writing on the top of the standard is readable and oriented so that opaque walls are side-to-side (blocking the beam) in the SpectraMax Plus 384, M2, M3, M4, M5, and M5e. The opaque walls are rear-to-front (blocking the beam) in the SpectraMax ABS Plus.
9. One by one, select each cuvette in the **UltDrk** Cuvette Set section and then click **Read** to read each cuvette.
10. Click **Save** to save the data file.
11. Remove cuvette standard F0 from the cuvette port and place it in the storage case.

Run Wavelength Accuracy Test

During the validation tests, the beam travels from left to right in the SpectraMax Plus 384, M2, M3, M4, M5, and M5e, and rear to front in the SpectraMax ABS Plus. You must insert cuvette F1 so that the writing on top of the standard, F1 label is readable and the red dot is facing the right in the SpectraMax Plus 384, M2, M3, M4, M5. The red dot should face the rear of the instrument in the SpectraMax ABS Plus.

Note: This test uses a spectral scan and takes several minutes to complete.

1. In the Navigation Tree, expand the **Cuvette Tests** experiment and select the **WavelengthAcc** Cuvette Set section.
2. Make sure there is no cuvette in the cuvette port and close the cuvette port door.
3. Select the Home tab and click **Ref**.
4. Place cuvette standard F1 in the cuvette port so that the writing on top of the standard is readable and the red dot is facing the right in the SpectraMax Plus 384, M2, M3, M4, M5, and M5e. The red dot should face the rear of the instrument in the SpectraMax ABS Plus.
5. Click **Read** to read the cuvette.
6. Click **Save** to save the data file.
7. When the scan completes, remove cuvette standard F1 from the cuvette port and place it in the storage case.

Run Photometric Accuracy Test

During the validation tests, the beam travels from left to right in the SpectraMax Plus 384, M2, M3, M4, M5, and M5e, and rear to front in the SpectraMax ABS Plus. You must insert cuvettes F2 through F4 so that the writing on top of the standard is readable and the red dot faces to the right in the SpectraMax Plus 384, M2, M3, M4, M5, and M5e. The red dot should face the rear of the instrument in the SpectraMax ABS Plus.

1. In the Navigation Tree, expand the **Cuvette Tests** experiment and select the **PhotoAcc F2** Cuvette Set section.
2. Make sure there is no cuvette in the cuvette port and close the cuvette port door.
3. Select the Home tab and click  **Ref**.
4. Place cuvette standard F2 in the cuvette port. Align the standard so that the writing on top of the standard is readable and the red dot faces to the right in the SpectraMax Plus 384, M2, M3, M4, M5, and M5e. The red dot should face the rear of the instrument in the SpectraMax ABS Plus.
5. One by one, select each cuvette in the **PhotoAcc F2** Cuvette Set section and then click  **Read** 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 for the **PhotoAcc F3** Cuvette Set section using cuvette standard F3.
8. Repeat the previous steps for the **PhotoAcc F4** Cuvette Set section using cuvette standard F4.
9. Click  **Save** to save the data file.

Run Photometric Precision Test

During the validation tests, the beam travels from left to right in the SpectraMax Plus 384, M2, M3, M4, M5, and M5e, and rear to front in the SpectraMax ABS Plus. You must insert cuvettes F2 through F4 so that the writing on top of the standard is readable and the red dot faces to the right in the SpectraMax Plus 384, M2, M3, M4, M5, and M5e. The red dot should face the rear of the instrument in the SpectraMax ABS Plus.

1. In the Navigation Tree, expand the **Cuvette Tests** experiment and select the **PhotometricPrec** Cuvette Set section.
2. Make sure there is no cuvette in the cuvette port and close the cuvette port door.
3. Select the Home tab and click  **Ref**.
4. Place cuvette standard F2 in the cuvette port. Align the cuvette standard so that the writing on top of the standard is readable and the red dot faces to the right in the SpectraMax Plus 384, M2, M3, M4, M5, and M5e. The red dot should face the rear of the instrument in the SpectraMax ABS Plus.
5. Select the first cuvette in the **PhotometricPrec** Cuvette Set section and click  **Read**.
6. Remove cuvette standard F2 from the cuvette port and place it in the storage case.
7. Repeat the previous steps for the second cuvette in the **PhotometricPrec** Cuvette Set section using standard F3.
8. Repeat the previous steps for the third cuvette in the **PhotometricPrec** Cuvette Set section using standard F4.
9. Click  **Save** to save the data file.

Chapter 3: Interpret Test Results

The software enters the test results in the Overview and Results experiment's Results Note section.

Note: If you correctly complete the entry of the certification information the Results Note section labels the results Acceptable or Out of Specification. If you use double quotes around the filter values or if do not use quotes around the Certification Date, the Results section displays error messages. Add or remove the double quotes in the CertInfo Note section and the SoftMax Pro Software recalculates the test result and corrects the Results Note section display.

The Acceptable and Out of Specification limits for the tests are based on the instrument specifications plus any other applicable tolerances such as the filter tolerances specified in the Hellma Certificate of Calibration.

Note: If the 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, contact Molecular Devices technical support. See Obtaining Support on page 19.

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).

Acceptability Criteria for 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 and ABS Plus: Certificate Value – 2 ≤ Average Peak Value ≤ Certificate Value + 2 SpectraMax M2, M2e, M3, M4, M5, and M5e: 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))

Chapter 4: Maintenance and Troubleshooting

At the time of delivery, the Hellma Cuvette Set Validation Package meets the manufacturing specifications of Hellma. You are responsible for maintaining the cuvette standards in a clean, dry, and covered environment. Cuvette standard maintenance requires the same care that you would give to all optical components.

CAUTION! Treat the cuvette standards with care to retain their validity. When not in use, 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.

- Keep the standards in the original protective case whenever the standard is not in the instrument.
- Inspect the standard before all reads. Look for dust and dirt.

Recertification

Factory certification of the cuvette standards is done using a reference instrument that is reserved exclusively for Hellma Cuvette Set Validation Package calibration and is checked for accuracy at fixed intervals of time. Contact Hellma directly to make arrangements for recertification.

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.

Contact Us

Phone: +1-800-635-5577
Web: moleculardevices.com
Email: info@moldev.com

Visit our website for a current listing of worldwide distributors.

0112-0044 C

MOLECULAR DEVICES