

**Frederick National Laboratory  
for Cancer Research**

*sponsored by the National Cancer Institute*

Vaccine, Immunity and Cancer Directorate  
Standard Operating Procedure

**SOP Title:** Use and Maintenance of a Molecular Devices Plate Reader

**Document ID:** 26003

Version

3.0

**Page 1 of 12**

Supersedes

2.0

**Effective Date:** 03Sep21

**Written by:**

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**SOP Title:** Use and Maintenance of a Molecular Devices Plate Reader

**Document ID:** 26003

Version

3.0

**Page 2 of 12**

Supersedes

2.0

**Effective Date:** 03Sep21

## **1. PURPOSE**

- 1.1. The purpose of this procedure is to set instructions in the proper use and handling of the Molecular Devices Plate Reader.

## **2. SCOPE**

- 2.1. This procedure applies to all Plate Readers.

## **3. REFERENCES**

- 3.1. Molecular Devices M series Plate Reader User Manual
- 3.2. 10007: Non-Routine Equipment Maintenance
- 3.3. 10009: General Record Review
- 3.4. 15000: Waste Disposal at the Advanced Technology Research Facility

## **4. RESPONSIBILITIES**

- 4.1. The Research Associate, hereafter referred to as Analyst, is responsible for reviewing and following this procedure, and documenting performance of equipment maintenance.
- 4.2. The Quality Control Analyst is responsible for reviewing and following this procedure. Quality Control Analyst is responsible for maintaining monthly ABS plate verifications.
- 4.3. The Scientific Manager or designee is responsible for training personnel in this procedure and reviewing associated documentation.
- 4.4. The Quality Assurance Specialist is responsible for quality oversight and approval of this procedure.
- 4.5. Trained personnel perform equipment maintenance record review per "10009: General Record Review."

## **5. DEFINITIONS**

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**SOP Title:** Use and Maintenance of a Molecular Devices Plate Reader

**Document ID:** 26003

Version

3.0

**Page 3 of 12**

Supersedes

2.0

**Effective Date:** 03Sep21

- 5.1. As Needed Maintenance – maintenance that is performed outside of routine maintenance but is not performed in response to equipment malfunction.
- 5.2. Non-routine Maintenance – maintenance that is performed in response to equipment malfunction or failure.
- 5.3. Routine Maintenance – maintenance that is performed at planned intervals to identify and prevent problems before they result in equipment failure.

## **6. REAGENTS, MATERIALS, AND EQUIPMENT**

- 6.1. Absorbance Verification Plate (Molecular Devices, Cat # 0200-6117, or equivalent)
- 6.2. Plate Reader with Microplate Adapter, Molecular Devices SpectraMax M series Multi-mode
- 6.3. Primary Disinfectant (Cavicide, Warehouse, Cat # 79300360, or equivalent)
- 6.4. Softmax Pro GxP Software
- 6.5. Wipe, Low-Lint, Wypalls (Warehouse, Cat # 79300335 or equivalent)
- 6.6. Compressed Air (Rudolph Office Supply, Cat # FALDPSXL or equivalent)

## **7. HEALTH AND SAFETY CONSIDERATIONS**

- 7.1. Proper safety precautions should be taken while working in a laboratory setting. This includes, but is not limited to, proper protective equipment such as lab coats, safety glasses, closed-toe shoes, and non-latex gloves.
- 7.2. Refer to the respective Safety Data Sheet (SDS) when working with any chemicals.
- 7.3. Refer to “15000: Waste Disposal at the Advanced Technology Research Facility,” “EHS-WM-1: Disposal and Minimization of Chemical Waste,” and “EHS-WM-2: Biological Waste Handling and Disposal” for waste disposal processes.

## **8. OPERATION**

- 8.1. Reading a Microplate

**Note:** SoftMax Pro Software must be installed on a compatible computer connected to the instrument.

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**SOP Title:** Use and Maintenance of a Molecular Devices Plate Reader

**Document ID:** 26003

Version

3.0

**Page 4 of 12**

Supersedes

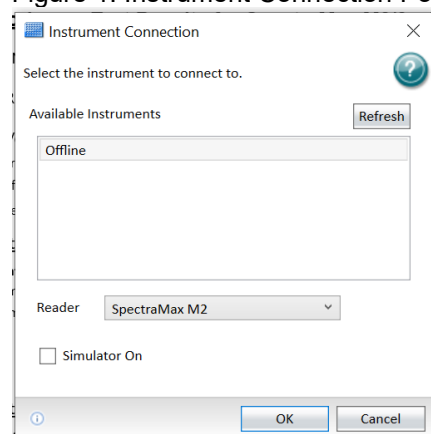
2.0

**Effective Date:** 03Sep21

**Note:** Confirm that Plate Reader and software are communicating properly. If not, a red X, or null sign appears over the instrument icon in the upper-left corner of the software window.

**Note:** If not communicating, try to reconnect the instrument via the computer software. Click on Instrument icon, and then in the "Instrument Connection" pop up window, verify the correct reader is selected and then click on the "Refresh" toggle. After connection, click on "OK" toggle.

Figure 1. Instrument Connection Pop-Up Window



- 8.1.1. Insert microplate into drawer, matching well A1 with position A1, ensuring microplate is flat against drawer itself with the included microplate carrier adapter.
- 8.1.2. Open SoftMax Pro data file or protocol file that contains the appropriate experiment settings. Alternatively, create new settings by selecting Plate section in the SoftMax Pro program and configure file using **Plate > Settings** dialog box.
- 8.1.3. Select **Control > Read** command or press **Read** button in SoftMax Pro Software header (Green) to start plate read.
- 8.1.4. When reading is complete, drawer automatically opens, allowing for removal of microplate.

## 9. MAINTENANCE

- 9.1. As Needed Maintenance

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**SOP Title:** Use and Maintenance of a Molecular Devices Plate Reader

**Document ID:** 26003

Version

3.0

**Page 5 of 12**

Supersedes

2.0

**Effective Date:** 03Sep21

9.1.1. Spills

**Note:** Clean up all spills immediately.

**Note:** Ensure drawer slide is closed and Plate Reader is turned off before cleaning.

9.1.2. Spray Cavicide on a low-lint wipe and wipe the outside surface of the machine. DO NOT spray directly onto the Plate Reader.

9.1.3. Document As Needed Maintenance in its respective section on "26003-01: Molecular Devices Plate Reader Monthly Maintenance Form."

9.2. Monthly Plate Verification Check (Absorbance Validation Plate)

Note: Monthly Plate Verification Check is maintained by Quality Control.

9.2.1. Use form "26003-02: Molecular Devices Plate Reader Plate Calibration Form" to record material and equipment information.

9.2.2. Turn on Plate Reader and wait for it to complete its start-up routine.

9.2.3. Open and log into SoftMax Pro Software.

**Note:** DO NOT insert validation plate into the drawer. The first set of tests are performed with an empty drawer.

9.2.4. Open the current "DDMMYY thru DDMMYY ABS1 Validation.spr" file.

9.2.5. In **SpectraTest ABS1** experiment, open **CertInfo** section and confirm entry of the appropriate data as follows:

Note: The current annual certificate data sheet is maintained in the ABS1 Validation hard storage case, while previous certificate data sheets are archived.

9.2.5.1. The SpectraTest ABS1 Validation Plate Serial Number

9.2.5.2. The Validation Plate Certification Date

9.2.5.3. The Certificate of Calibration Number

9.2.5.4. Certificate Value for NG11 glass at 440 nm

9.2.5.5. Certificate Value for NG11 glass at 465 nm

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**SOP Title:** Use and Maintenance of a Molecular Devices Plate Reader

**Document ID:** 26003

Version

3.0

**Page 6 of 12**

Supersedes

2.0

**Effective Date:** 03Sep21

- 9.2.5.6. Certificate Value for NG11 glass at 546 nm
- 9.2.5.7. Certificate Value for NG11 glass at 590 nm
- 9.2.5.8. Certificate Value for NG11 glass at 635 nm
- 9.2.5.9. Certificate Value for NG5 glass at 440 nm
- 9.2.5.10. Certificate Value for NG5 glass at 465 nm
- 9.2.5.11. Certificate Value for NG5 glass at 546 nm
- 9.2.5.12. Certificate Value for NG5 glass at 590 nm
- 9.2.5.13. Certificate Value for NG5 glass at 635 nm
- 9.2.5.14. Certificate Value for NG4(2mm) glass at 440 nm
- 9.2.5.15. Certificate Value for NG4(2mm) glass at 465 nm
- 9.2.5.16. Certificate Value for NG4(2mm) glass at 546 nm
- 9.2.5.17. Certificate Value for NG4(2mm) glass at 590 nm
- 9.2.5.18. Certificate Value for NG4(2mm) glass at 635 nm
- 9.2.5.19. Certificate Value for NG4(3mm) glass at 440 nm
- 9.2.5.20. Certificate Value for NG4(3mm) glass at 465 nm
- 9.2.5.21. Certificate Value for NG4(3mm) glass at 546 nm
- 9.2.5.22. Certificate Value for NG4(3mm) glass at 590 nm
- 9.2.5.23. Certificate Value for NG4(3mm) glass at 635 nm
- 9.2.5.24. Certificate Value for Holmium Oxide Peak #1
- 9.2.5.25. Certificate Value for Holmium Oxide Peak #2
- 9.2.5.26. Certificate Value for Holmium Oxide Peak #3
- 9.2.5.27. Certificate Value for Didymium Peak #1
- 9.2.5.28. Certificate Value for Didymium Peak #2

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**SOP Title:** Use and Maintenance of a Molecular Devices Plate Reader

**Document ID:** 26003

Version

3.0

**Page 7 of 12**

Supersedes

2.0

**Effective Date:** 03Sep21

9.2.5.29. Certificate Value for Didymium Peak #3

9.2.6. In **SpectraTest ABS1** experiment, open **Results** section and confirm entry of the appropriate data as follows:

9.2.6.1. Serial number of Plate Reader tested.

9.2.6.2. Analyst's initials running test and date.

9.2.7. Save data file in Equipment Verification folder (O:\HSL\Equipment Verification\Plate reader), as ABScheck\_DDMMYY\_Analyst Initials where DDMMYY refers to date.

9.2.8. In **Expt#2** section, select plate section **Endpoint#1**, and then click **Read**. All plate sections in experiment are read automatically.

9.2.9. Once plate read is completed, place SpectraTest ABS1 Validation Plate with adapter plate in drawer of Plate Reader with well A1 in position A1 of the drawer.

9.2.10. In **Expt#3** section, select plate section titled **OptiAlign&UltDrk**, and then click **Read**. All plate sections in experiment are read automatically.

9.2.11. When all plate sections have been read, remove validation plate from drawer, and save file.

9.2.12. Verify that each section Passed. See Attachment 1. If any section Failed, verify the SpectraTest ABS1 Validation Plate is free of dust by lightly spraying compressed air on plate, then repeat monthly plate calibration check.

9.2.13. Once completed, return validation plate to its protective plastic sleeve and store in its hard storage case.

9.2.14. Print out SoftMax result data file, initial and date page and attach to 26003-02. Submit 26003-02 for review and file.

9.2.15. Document performance on 26003-01.

9.3. Annual Certification

9.3.1. Facilities, Maintenance, and Engineering (FME) or a contracted vendor calibrate Plate Reader every year as required, for routine use.

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**SOP Title:** Use and Maintenance of a Molecular Devices Plate Reader

**Document ID:** 26003

Version

3.0

**Page 8 of 12**

Supersedes

2.0

**Effective Date:** 03Sep21

9.3.2. Plate Readers are assessed for recalibration after repair, damage, or if physical, or electronic changes occur that could impact the operation, range, accuracy, or tolerance of the equipment. This is determined by the Scientific Manager or designee.

9.3.3. Print Certification report and file.

**9.4. Non-Routine Maintenance**

9.4.1. In the case that the Plate Reader is not operating correctly, transition processes being performed to another unit (when applicable), post a sign stating the equipment is out of service and initiate non-routine maintenance documentation per "10007: Non-Routine Equipment Maintenance."

9.4.2. Document the nature of any failure or malfunction, how and when it was discovered, and the personnel involved on "10007-01: Non-Routine Equipment Maintenance Form."

9.4.3. Initiate a service request and complete the non-routine maintenance process following 10007.

**10. ATTACHMENTS**

10.1. Attachment 1: Plate Calibration Check Sample Results

10.2. Attachment 2: 26003-01: Molecular Devices Plate Reader Monthly Maintenance Form

10.3. Attachment 3: 26003-02: Molecular Devices Plate Reader Plate Calibration Form

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**SOP Title:** Use and Maintenance of a Molecular Devices Plate Reader

**Document ID:** 26003

Version

3.0

**Page** 9 of 12

Supersedes

2.0

**Effective Date:** 03Sep21

## 11. REVISION HISTORY

Version	Change	Reason
1.0	Create new SOP for the use of the Molecular Devices M5 Plate Reader	Currently No SOP
2.0	<ol style="list-style-type: none"> <li>Updated to new template; forms now separate.</li> <li>Minor formatting and grammar revisions throughout the procedure.</li> <li>Removed HSL_GL_002, HSL_GL_003, HSL_GL_006, HSL_GL_007, HSL_GL_008, HSL_GL_009, HSL_GL_010 from References section.</li> <li>Moved softmax software to materials section.</li> <li>Removed HPV, ATRF, FL, LUM, HSL, PM, UV from Definitions section.</li> <li>Added step to wipe down instrument at monthly maintenance with cavicide.</li> <li>Added step for verifying results and subsequent action.</li> <li>Revised HSL_EQ_005.01 to track performance of maintenance only.</li> <li>New form HSL_EQ_005.02 to document equipment, materials and results of monthly maintenance.</li> </ol>	<ol style="list-style-type: none"> <li>Consistency between procedures; ease of use.</li> <li>Clarification.</li> <li>Not referenced in body of procedure.</li> <li>Clarification.</li> <li>HPV and ATRF used earlier in procedure, other acronyms not used in procedure.</li> <li>Clarification; reflect current practice.</li> <li>Clarification; reflect current practice.</li> <li>Ease of use.</li> <li>Ease of use.</li> </ol>
3.0	<ol style="list-style-type: none"> <li>Add responsibilities of Quality Control Analyst.</li> <li>Corrected "Considerations" in section 7.</li> <li>Added Non-Routine Maintenance section.</li> <li>Updated reference SOP title numbers.</li> </ol>	<ol style="list-style-type: none"> <li>Added clarification of responsibilities.</li> <li>Clarification. Misspelled.</li> <li>SOP did not previously have section for non-routine maintenance.</li> <li>Reflect changes from previous "HSL" SOP titles.</li> </ol>

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**SOP Title: Use and Maintenance of a Molecular Devices Plate Reader**

**Document ID: 26003**

Version

3.0

**Page 10 of 12**

Supersedes

2.0

**Effective Date: 03Sep21**

**Attachment 1: Plate Calibration Check Sample Results**

SpectraTest ABS1
Results

**Absorbance Test Results for SpectraMax M2/2e, M3, M4, M5/5e**

Serial Number of the instrument tested-MV02158

Tests Run By (initials and date) CA 31Jan19

Instrument Serial =

Tests Verified By (initials and date) \_\_\_\_\_

Tests were started at- 10:24 AM 1/31/2019

Tests were completed at- 1:50 PM 1/31/2019

Serial# of the SpectraTest ABS1 Validation Plate Used- 10397

Date SpectraTest ABS1 Validation Plate was calibrated- 25Sep17

SpectraTest ABS1 Validation Plate Certificate of Calibration Number- 2

**Results of Baseline Noise Tests:**

**Endpoint Acceptable**

Minimum OD ( $\geq -0.003$ ) = -0.002

Maximum OD ( $\leq 0.003$ ) = 0.001

**Kinetic Acceptable**

Data points within reduction limits (20 is within): 20.000

Maximum Kinetic Rate ( $\leq 0.2$ ): 0.037

Minimum Kinetic Rate ( $\geq -0.2$ ): -0.039

Maximum OD ( $\leq 0.003$ ): 0.000

Minimum OD ( $\geq -0.003$ ): -0.002

**Results of Tests with SpectraTest ABS1 Validation Plate:**

**Optical Alignment Acceptable**

Minimum ODs at 200, 405, and 850 nm ( $\geq -0.003$ ): -0.0003, -0.0002, -0.0021

Maximum ODs at 200, 405, and 850 nm ( $\leq 0.015$ ): 0, 0, -0.0007

**Ultimate Dark Acceptable**

Minimum ODs at 200, 405 and 850 nm ( $\geq 3.3$ ): 4, 4, 4

**Photometric (absorbance) Accuracy Acceptable**

PANG11@440 ( $\geq 0.26328$  and  $\leq 0.28072$ ) = 0.2693625

PANG11@546 ( $\geq 0.24645$  and  $\leq 0.26355$ ) = 0.25425

PANG11@590 ( $\geq 0.28605$  and  $\leq 0.30395$ ) = 0.293125

PANG11@635 ( $\geq 0.28803$  and  $\leq 0.30597$ ) = 0.2951875

PANG42mm@440 ( $\geq 1.06716$  and  $\leq 1.10084$ ) = 1.078125

PANG42mm@546 ( $\geq 1.01568$  and  $\leq 1.04832$ ) = 1.029575

PANG42mm@590 ( $\geq 1.0929$  and  $\leq 1.1271$ ) = 1.1055375

PANG42mm@635 ( $\geq 1.05924$  and  $\leq 1.09276$ ) = 1.0731625

PANG5@440 ( $\geq 0.52068$  and  $\leq 0.54332$ ) = 0.529

PANG5@546 ( $\geq 0.49692$  and  $\leq 0.51908$ ) = 0.5063

PANG5@590 ( $\geq 0.55632$  and  $\leq 0.57968$ ) = 0.5653

PANG5@635 ( $\geq 0.55236$  and  $\leq 0.57564$ ) = 0.562375

PANG43mm@440 ( $\geq 1.5285$  and  $\leq 1.5715$ ) = 1.5418625

PANG43mm@546 ( $\geq 1.45425$  and  $\leq 1.49575$ ) = 1.4723125

PANG43mm@590 ( $\geq 1.56612$  and  $\leq 1.60988$ ) = 1.5821625

PANG43mm@635 ( $\geq 1.51761$  and  $\leq 1.56039$ ) = 1.535375

**Intrawell Photometric (absorbance) Precision- Acceptable**

PPNG11 ( $>0.1$  and  $<0.5$ ) = Min: 0.2541 Max: 0.2545

PPNG5 ( $>0.3$  and  $<1.2$ ) = Min: 0.5061 Max: 0.5065

PPNG42mm ( $>0.5$  and  $<2$ ) = Min: 1.0294 Max: 1.03

PPNG43mm ( $>0.8$  and  $<3$ ) = Min: 1.4719 Max: 1.4726

**Stray Light Test Acceptable**

Minimum ODs at 250, 340, 405 and 650 nm ( $\geq 2.2$ ): 3.2346, 2.4319, 2.3323, 3.6893

**Wavelength Precision Acceptable**

Maximum Difference ( $\leq 0.042$ ): 0.014

Minimum OD ( $\geq 0.1$ ): 0.496

Maximum OD ( $\leq 1.5$ ): 0.522

**Wavelength Accuracy, Holmium Oxide Acceptable**

Peak 4 (537 +/- 3) = 537

**Wavelength Accuracy, Didymium V30 Acceptable**

Peak 1 (330.1 +/- 3) = 331

Peak 2 (430.7 +/- 3) = 432

Peak 3 (681.6 +/- 3) = 680

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**SOP Title:** Use and Maintenance of a Molecular Devices Plate Reader

**Document ID:** 26003

Version

3.0

**Page 11 of 12**

Supersedes

2.0

**Effective Date:** 03Sep21

**Attachment 2: 26003-01: Molecular Devices Plate Reader Monthly Maintenance Form**

<b>Frederick National Laboratory for Cancer Research</b> <small>sponsored by the National Cancer Institute</small>		Vaccine, Immunity and Cancer Directorate Standard Operating Procedure Form	
<b>Form Title:</b> Molecular Devices Plate Reader Monthly Maintenance Form			
<b>Document ID:</b> 26003-01		<b>Version:</b>	3.0
Associated SOP: 26003		<b>Effective Date:</b>	03Sep21
Supersedes Version:	2.0	<b>Page 1 of 1</b>	

Maintenance Year:	
Equipment ID:	HSL_

Monthly Maintenance:

Month	January	February	March	April	May	June
Recorded by/date:						
Reviewed by/date:						
Month	July	August	September	October	November	December
Recorded by/date:						
Reviewed by/date:						

As Needed Maintenance: ☐ N/A

Date	Activity Performed	Recorded by/date	Reviewed by/date
<input type="checkbox"/> N/A			
<input type="checkbox"/> N/A			

QA Reviewed by/date: \_\_\_\_\_

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**SOP Title:** Use and Maintenance of a Molecular Devices Plate Reader

**Document ID:** 26003

Version

3.0

**Page 12 of 12**

Supersedes

2.0

**Effective Date:** 03Sep21

### Attachment 2: 26003-02: Molecular Devices Plate Reader Plate Calibration Form

<b>Frederick National Laboratory for Cancer Research</b> <small>sponsored by the National Cancer Institute</small>		Vaccine, Immunity and Cancer Directorate Standard Operating Procedure Form	
<b>Form Title:</b> Molecular Devices Plate Reader Plate Calibration Form			
<b>Document ID:</b> 26003-02		Version:	3.0
Associated SOP: 26003		Effective Date:	03Sep21
Supersedes:	2.0	<b>Page 1 of 1</b>	

#### Equipment

Description	Identification Number	Calibration Due Date	Certificate Information
Plate Reader	HSL_		<input type="checkbox"/> N/A
Validation Absorbance Plate	HSL_		<input type="checkbox"/> Verified

#### Reagents

Description	Lot Number	Expiration Date
Cavicide		

#### Results

<b>Data File Name:</b>	
------------------------	--

<b>Comments:</b> <input type="checkbox"/> Passed <input type="checkbox"/> Fail, retest
<input type="checkbox"/> N/A

<b>Performed by/date:</b>	
<b>Reviewed by/date:</b>	
<b>QA Reviewed by/date:</b>	

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