

SOP Title: Use and Maintenance of a Molecular Devices M5 Plate Reader

Document ID: HSL_EQ_005

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1. PURPOSE

- 1.1. The purpose of this procedure is to set instructions in the proper use and handling of the Molecular Devices M5 Plate Reader.

2. SCOPE

- 2.1. This procedure applies to the Human Papillomavirus (HPV) Serology Laboratory located at the Advanced Technology Research Facility (ATRF), room C2007.

3. REFERENCES

- 3.1. Molecular Devices M5 Plate Reader User Manual
3.2. HSL_GL_001: Waste Disposal at the Advanced Technology Research Facility

4. RESPONSIBILITIES

- 4.1. The Research Associate, hereafter referred as analyst, is responsible for reviewing and following this procedure.
4.2. The Scientific Manager or designee is responsible for training personnel in this procedure and reviewing associated documentation.
4.3. The Quality Assurance Specialist is responsible for quality oversight and approval of this procedure.

5. DEFINITIONS

Term	Definition
ABS	Absorbance
SDS	Safety Data Sheets

6. REAGENTS, MATERIALS AND EQUIPMENT

- 6.1. Molecular Devices SpectraMax M5 Multi-mode Plate Reader
6.2. Softmax Pro GxP Software
6.3. Absorbance Verification Plate (Molecular Devices, Cat # 0200-6117)
6.4. Cavicide (Warehouse, Cat # 79300360)
6.5. Wypalls Paper Towel (Warehouse, Cat # 79300335 or equivalent)

7. HEALTH AND SAFETY CONSIDERAIONS

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- 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.

8. OPERATION

8.1. Reading a Microplate

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

- 8.1.1. Insert the filled microplate into the drawer, matching well A1 with position A1 in the drawer. Make sure the microplate is flat against the drawer bottom or against the adapter.
- 8.1.2. Open a SoftMax Pro data file or protocol file that contains the appropriate experiment settings for the plate read. Alternatively, create new settings by selecting the Plate section in the SoftMax Pro program and configuring the instrument using the **Plate >Settings** dialog box.
- 8.1.3. Select the **Control > Read** command or press the **Read** button in SoftMax Pro Software to start the plate read.
- 8.1.4. When reading is complete, the drawer of the instrument opens, allowing for removal of the microplate.
- 8.1.5. If the incubator is on, the drawer closes again after approximately 10 seconds. To open the drawer, press the **DRAWER** key.

9. MAINTENANCE

9.1. General Maintenance

- 9.1.1. Clean up all spills immediately. Spray Cavicide on a piece of low-lint wipe paper towel and wipe the outside surface of the machine. DO NOT spray directly onto the machine. Make sure the drawer is closed.
- 9.1.2. Document cleaning on "HSL_EQ_005.01: Molecular Devices M5 Plate Reader Use and Maintenance Form."

9.2. Monthly Plate Calibration Check (Absorbance Validation Plate)

- 9.2.1. Use form "HSL_EQ_005.02: Molecular Devices M5 Plate Reader Plate Calibration Form" to record material and equipment information.

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9.2.2. Prior to turning the instrument on, spray Cavicide on a piece of low-lint wipe paper towel and wipe the outside surface of the machine. DO NOT spray directly onto the machine. Make sure the drawer is closed.

9.2.3. Turn on the reader and wait for it to complete its start-up routine.

9.2.4. Start the SoftMax Pro Software.

9.2.5. Confirm that the instrument and the 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: DO NOT insert the validation plate in the drawer yet. The first set of tests are performed with an empty drawer.

9.2.6. Open the "DDMMYY thru DDMMYY ABS1 Validation.spr" file.

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

9.2.7.1. The SpectraTest ABS1 Validation Plate Serial Number

9.2.7.2. The Validation Plate Certification Date

9.2.7.3. The Certificate of Calibration Number

9.2.7.4. Certificate Value for NG11 glass at 440 nm

9.2.7.5. Certificate Value for NG11 glass at 465 nm

9.2.7.6. Certificate Value for NG11 glass at 546 nm

9.2.7.7. Certificate Value for NG11 glass at 590 nm

9.2.7.8. Certificate Value for NG11 glass at 635 nm

9.2.7.9. Certificate Value for NG5 glass at 440 nm

9.2.7.10. Certificate Value for NG5 glass at 465 nm

9.2.7.11. Certificate Value for NG5 glass at 546 nm

9.2.7.12. Certificate Value for NG5 glass at 590 nm

9.2.7.13. Certificate Value for NG5 glass at 635 nm

9.2.7.14. Certificate Value for NG4(2mm) glass at 440 nm

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- 9.2.7.15. Certificate Value for NG4(2mm) glass at 465 nm
- 9.2.7.16. Certificate Value for NG4(2mm) glass at 546 nm
- 9.2.7.17. Certificate Value for NG4(2mm) glass at 590 nm
- 9.2.7.18. Certificate Value for NG4(2mm) glass at 635 nm
- 9.2.7.19. Certificate Value for NG4(3mm) glass at 440 nm
- 9.2.7.20. Certificate Value for NG4(3mm) glass at 465 nm
- 9.2.7.21. Certificate Value for NG4(3mm) glass at 546 nm
- 9.2.7.22. Certificate Value for NG4(3mm) glass at 590 nm
- 9.2.7.23. Certificate Value for NG4(3mm) glass at 635 nm
- 9.2.7.24. Certificate Value for Holmium Oxide Peak #1
- 9.2.7.25. Certificate Value for Holmium Oxide Peak #2
- 9.2.7.26. Certificate Value for Holmium Oxide Peak #3
- 9.2.7.27. Certificate Value for Didymium Peak #1
- 9.2.7.28. Certificate Value for Didymium Peak #2
- 9.2.7.29. Certificate Value for Didymium Peak #3
- 9.2.8. In the **SpectraTest ABS1** experiment, open the **Results** section and confirm the entry of the appropriate data as follows:
 - 9.2.8.1. The serial number of the instrument being tested.
 - 9.2.8.2. The name of the analyst running the test and the date of the test.
- 9.2.9. Save the data file in the applicable Equipment Verification folder [REDACTED] as ABScheck_DDMMYY_Analyst Initials where DDMMYY refers to the current date.
- 9.2.10. In **Expt#2** section, select the plate section titled **Endpoint#1**, and then click **Read**. All plate sections in the experiment are read automatically.

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- 9.2.11. After the plate is done reading, place the SpectraTest ABS1 Validation Plate with adapter plate in the drawer of the instrument with well A1 in position A1 of the drawer.
 - 9.2.12. In **Expt#3** section, select the plate section titled **OptiAlign&UltDrk**, and then click **Read**. All plate sections in the experiment are read automatically.
 - 9.2.13. When all plate sections have been read, remove the validation plate from the drawer, and save the file.
 - 9.2.14. Verify that each section Passed. See Attachment 1. If any section Failed, repeat the monthly plate calibration check. Prior to repeating the process, verify the SpectraTest ABS1 Validation Plate is free of dust by lightly spraying compressed air on plate.
 - 9.2.15. Return the validation plate to its protective plastic sleeve and place the validation plate and sleeve in the storage case.
 - 9.2.16. Print out SoftMax result data file, initial and date page and attach to HSL_EQ_005.02. Submit HSL_EQ_005.02 for review.
 - 9.2.17. Document that the monthly absorbance validation plate was performed on HSL_EQ_005.01 when passing results obtained.
- 9.3. Annual calibration is performed by Facilities, Maintenance and Engineering (FME) or a contracted vendor.

10. ATTACHMENTS

- 10.1. Attachment 1: Plate Calibration Check Sample Results
- 10.2. Attachment 2: HSL_EQ_005.01: Molecular Devices M5 Plate Reader Monthly Maintenance Form
- 10.3. Attachment 3: HSL_EQ_005.02: Molecular Devices M5 Plate Reader Plate Calibration Form

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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- [REDACTED]

Tests Run By (initials and date) [REDACTED] 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- [REDACTED] Date SpectraTest ABS1 Validation Plate was calibrated- [REDACTED]

SpectraTest ABS1 Validation Plate Certificate of Calibration Number- 2

Results of Baseline Noise Tests:

<p>Endpoint Acceptable</p> <p>Minimum OD (≥ -0.003) = -0.002</p> <p>Maximum OD (≤ 0.003) = 0.001</p>	<p>Kinetic Acceptable</p> <p>Data points within reduction limits (20 is within): 20.000</p> <p>Maximum Kinetic Rate (≤ 0.2): 0.037</p> <p>Minimum Kinetic Rate (≥ -0.2): -0.039</p> <p>Maximum OD (≤ 0.003): 0.000</p> <p>Minimum OD (≥ -0.003): -0.002</p>
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Results of Tests with SpectraTest ABS1 Validation Plate:

Optical Alignment Acceptable

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

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

Ultimate Dark Acceptable

Minimum ODs at 200, 405 and 850 nm (≥ 3.3): 4, 4, 4

Photometric (absorbance) Accuracy Acceptable

PANG11@440 (≥ 0.26328 and ≤ 0.28072) = 0.2693625	PANG5@440 (≥ 0.52068 and ≤ 0.54332) = 0.529
PANG11@546 (≥ 0.24645 and ≤ 0.26355) = 0.25425	PANG5@546 (≥ 0.49692 and ≤ 0.51908) = 0.5063
PANG11@590 (≥ 0.28605 and ≤ 0.30395) = 0.293125	PANG5@590 (≥ 0.55632 and ≤ 0.57968) = 0.5653
PANG11@635 (≥ 0.28803 and ≤ 0.30597) = 0.2951875	PANG5@635 (≥ 0.55236 and ≤ 0.57564) = 0.562375
PANG42mm@440 (≥ 1.06716 and ≤ 1.10084) = 1.078125	PANG43mm@440 (≥ 1.5285 and ≤ 1.5715) = 1.5418625
PANG42mm@546 (≥ 1.01568 and ≤ 1.04832) = 1.029575	PANG43mm@546 (≥ 1.45425 and ≤ 1.49575) = 1.4723125
PANG42mm@590 (≥ 1.0929 and ≤ 1.1271) = 1.1055375	PANG43mm@590 (≥ 1.56612 and ≤ 1.60988) = 1.5821625
PANG42mm@635 (≥ 1.05924 and ≤ 1.09276) = 1.0731625	PANG43mm@635 (≥ 1.51761 and ≤ 1.56039) = 1.535375

Intrawell Photometric (absorbance) Precision- Acceptable

PPNG11 (> 0.1 and < 0.5) = Min: 0.2541 Max: 0.2545	PPNG42mm (> 0.5 and < 2) = Min: 1.0294 Max: 1.03
PPNG5 (> 0.3 and < 1.2) = Min: 0.5061 Max: 0.5065	PPNG43mm (> 0.8 and < 3) = Min: 1.4719 Max: 1.4726

Stray Light Test Acceptable

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

Wavelength Precision Acceptable

Maximum Difference (≤ 0.042): 0.014 Minimum OD (≥ 0.1): 0.496 Maximum OD (≤ 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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Attachment 2: HSL_EQ_005.01: Molecular Devices M5 Plate Reader Monthly Maintenance Form

<p>Frederick National Laboratory for Cancer Research <i>sponsored by the National Cancer Institute</i></p>		<p>HPV Serology Laboratory Standard Operating Procedure Form</p>	
<p>Form Title: Molecular Devices M5 Plate Reader Monthly Maintenance Form</p>			
<p>Document ID: HSL_EQ_005.01</p>		<p>Version:</p>	<p>2.0</p>
<p>Associated SOP: HSL_EQ_005</p>		<p>Effective Date:</p>	
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Maintenance Year:	
Equipment ID:	HSL_

Monthly Maintenance, HSL_EQ_005.02

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:						

Unscheduled Maintenance

Date	QE Number	Activity Performed	Recorded by/date	Reviewed by/date

QA Reviewed by/date: _____

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Attachment 3: HSL_EQ_005.02: Molecular Devices M5 Plate Reader Plate Calibration Form

Frederick National Laboratory for Cancer Research <i>sponsored by the National Cancer Institute</i>		HPV Serology Laboratory Standard Operating Procedure Form	
Form Title: Molecular Devices M5 Plate Reader Plate Calibration Form			
Document ID: HSL_EQ_005.02		Version:	DRAFT
Associated SOP: HSL_EQ_005		Effective Date:	TBD
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Equipment

Description	Identification Number	Calibration Due Date	Certificate Information
M5 Plate Reader	HSL_		
Validation Absorbance Plate	HSL_		<input type="checkbox"/> Verified

Reagents

Description	Lot Number	Expiration Date
Cavicide		

Results

Data File Name:	
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Comments: <input type="checkbox"/> Passed <input type="checkbox"/> Fail, retest	<input type="checkbox"/> N/A
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Performed by/date:	
Reviewed by/date:	
QA Reviewed by/date:	

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