

PERFORM Operating Document

Use and Maintenance of Hybrid Multi-Mode Microplate Reader and Washer

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01	New POD	20-July-2013
02	Updated procedures	6-September-2016

Summary

The content of this PERFORM Operating Document (POD) provides guidelines for the use and maintenance of Microplate Reader and Washer.

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I . Definition of Terms and abbreviations

PERFORM operating document (POD)	Operating documents that are specific to an instrument or technique that require approval by area managers.
ELISA	Enzyme-Linked ImmunoSorbent Assay
EIA	Enzyme ImmunoAssays
CAS	Clinical Analysis Supervisor
Nm	Nanometer

2. Introduction

The Synergy HI is a hybrid multi-mode microplate reader, detection modes include fluorescence intensity (FI), fluorescence polarization (FP), time-resolved fluorescence (TRF), luminescence, and UV-visible absorbance. The monochromator-based system, which has both top and bottom probes, is used for absorbance, fluorescence and luminescence. Absorbance measurements are made using the reader's monochromator optics.

The ELx405 Select Microplate Washer uses a patented manifold design which provides independent control of aspirate and dispense tube location and height enabling bubble free fluid dispense and overflow protection in 96- and 384-well plates.

2.1 Overview of Microplate Reader and Washer

SynergyTM HI can be turned into a high-performance patented Hybrid system with the addition of a filter-based optical module. The monochromator optics uses a third generation quadruple grating design that allows working at any excitation or emission wavelength with a 1 nm step. It is ideal system for all the standard microplate applications found in life science research laboratories.

The availability of methods to measure cytokine and other inflammatory mediators or markers with high sensitivity Enzyme-linked immunosorbent assay (ELISA), also known as an enzyme immunoassay (EIA), is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample. The ELISA has been used as a diagnostic tool in medicine and plant pathology, ELISA tests are widely utilized to detect substances that have antigenic properties primarily proteins (as opposed to small molecules and ions such as glucose and potassium). The substances detected by ELISAs test include hormones, antigens and antibodies.

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The Elx405™ Microplate Washer has set the standard in microplate washing based on its superior performance and reliability. All models can be configured with biomagnetic separation and vacuum filtration modules for full plate washing of magnetic and polystyrene bead-based assays such as Luminex® xMAP®. The ELx405™ is a standard 96-well microplate and provides microplate priming, washing, aspiration and dispenses into 96 and 384-well microplates.

2.2 Training requirements

Prior to using the ELISA Plate reader and washer users should:

- Read and sign this POD.
- Undergo appropriate Microplate reader and washer training and/or provide a proof of an external training to the Clinical Analysis Supervisor or delegate prior to use.

2.3 Booking and operation

Reserve the Hybrid Multi-Mode Microplate Reader using online scheduler available at: <http://perform.concordia.ca/booking>

In order to access to PERFORM Centre online Booking system for facilities and equipment, an account on the system will be required. Accounts are only created for people who are approved to do research at the PERFORM Centre. The Principal Investigator can request access and accounts for themselves and their research assistants online at: <https://perform.concordia.ca/GettingStarted/forms.asp>

The Principal Investigator (PI) can also request a PDF version or paper copy of the account request form from the PERFORM Centre's Research Operations Coordinator or System's Administrator.

2.4 General precautions

2.4.1 Microplate Reader

- Microplates should be clean and free from dust or bottom scratches. Inspect emission and excitation filters and clean with Isopropyl or ethanol.
- Use new microplates from sealed packages.
- Filter solutions to remove particulates that could cause erroneous readings.

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- Although the Synergy HI supports standard flat, U-bottom, and V-bottom microplates, the reader achieves optimum performance with flat-bottomed wells when running in Absorbance mode.
- Non-uniformity in the optical density of the well bottoms can cause loss of accuracy, especially with U- and V-bottom polyvinyl microplates. Flat bottomed wells provide optimum performance when running the absorbance mode, although the Synergy HI support standard flat, U-bottom, and V-bottom microplates.
- Inaccuracy in pipetting has a large effect on measurements, especially if smaller volumes of liquid are used. For best results, use at least 100 uL per well in a 96-well plate and 25 uL in a 384-well plate.

2.5 Relevant documents

- Synergy H1™ Operator's Manual
- ELx405™ Microplate Washer Operator's Guide
- ELx405™ Getting started Guide

3. Procedure**3.1 Microplate Reader****3.1.1 ELISA Reader Operation**

Each time the Synergy HI is turned on, it automatically performs a series of tests on the reader's motors, lamp, the PMT, and various subsystems. The duration of this System test depends on the reader model, and can take a few minutes to complete. If all tests pass, the microplate carrier is ejected and the LED on the power switch remains on. If any test results do not meet the internally coded Failure Mode Effects Analysis (FMEA) criteria established by BioTek, the reader beeps repeatedly and the LED on the power switch flashes.

If the system is not turned on, follow the sequence below to turn it on:

1. Turn on the reader from a power button on the lower left side and launch Gen5.
2. If the assay uses incubation, turn on the Temperature Control and allow the incubator to reach its set point before running the System Test. To access this feature, select System > Reader Control > Synergy HI > Pre-Heating tab. Enter a requested temperature and click on. Return to Gen5's main view. Wait until the incubator temperature reaches the set point before continuing.

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3. Select System > Diagnostics > Run System Test.

If the test fails during execution, a message box appears in the software. Close the box; the test report contains the error code that was generated by the failure.

4. When the test is complete, a dialog appears, requesting additional information. Enter your user name and other information (if required) and then click OK.

5. The results report appears. Scroll down toward the bottom of the report; it shows either "SYSTEM TEST PASS" or "SYSTEM TEST FAIL" ***ERROR (error code) DETECTED

6. BioTek Gen5 stores the results in a database, so the results can be retrieved at any time. It is recommended that you print and save the reports to document that the test was performed.

7. If the test fails, contact BioTek's Technical Assistance Centre.

8. The configuration of the plates should be defined in the method build up in the protocol.

3.1.2 Data acquisition software layout and instrument controls

BioTek Gen5 software control the reader and the dispense module, perform data reduction and analysis on the measurement values, print or export results, and more. Open the Gen 5 software, create a new protocol by selecting the protocol type. Set up the procedure for temperature, shake, dispense, kinetic, pause, process mode etc. Select plate layout and well type. Under data reduction, select tools such as transformation, well analysis, curve analysis and qualitative analysis. Edit report/export builder. Once the protocol is set up save it, read the plate and analyze the data. Refer to user manual for detailed procedures.

3.1.3 General recommendations for optimum performance

- The lifetime guarantee for the Xenon lamp is 3 years but if the performance is optimum there is no need to replace it.
- For bigger wells or areas, it is possible to take multiple readings and it will be averaged by the system. It is useful for the cells when they are scattered in a well.
- High PMT voltage gives higher sensitivity. Sensitivity can be changed for each plate. There is an automatic sensitivity adjustment option for optimization.
- Blank is automatically subtracted from the value
- Data reduction automatically converts the data in to excel. Report builder function builds the report which can be customized.

3.1.4 Preventive maintenance

For the detailed procedure refer to Synergy™ HI Operator's Manual.

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Clean exposed surfaces and inspect/clean the emission and excitation filters and mirrors (if used), run dispense protocol, clean priming plate, and clean dispensing tubes and injectors

Daily

Flush/purge the fluid lines with deionized (DI) water every day or after completing an assay run, whichever is more frequent.

Quarterly

Inspect clean emission and excitation filters (do not touch the filters with bare fingers), clean dispense tubes and injectors.

3.2 Microplate Washer

The ELx405 Microplate Washer provides automation of dispense and aspiration steps.

The following precautions are provided to help avoid damage to the instrument:

- Do not expose the instrument to temperature extremes. For proper operation, ambient temperatures should remain between 15°-30°C. Performance may be adversely affected if temperatures fluctuate above or below this range.
- Do not expose any part of the instrument to the recommended diluted sodium hypochlorite solution (bleach) for more than 20 minutes. Prolonged contact may damage the instrument surfaces. Be certain to rinse and thoroughly wipe all surfaces.
- Do not use the device in close proximity to sources of strong electromagnetic radiation as this may interfere with the proper operation.
- Some chemicals may cause irreparable damage to washers. The following chemicals have been deemed safe for use in washers: buffer solutions (such as PBS), saline, surfactants, deionized water, 70% ethyl, isopropyl, or methyl alcohol, 40% formaldehyde, and 20% sodium hydroxide. Never use DMSO or other organic solvents. These chemicals may cause severe damage to the instrument.
- The washer is not sealed and liquids can seep into sensitive components. Make sure that any spilled wash buffer solution is wiped off the washer. Prolonged exposure to salt solution may corrode parts of the microplate carrier, movement rail, springs, and other hardware.
- Bovine Serum Albumin. Solutions containing proteins, will compromise the washer's performance over time unless a strict maintenance protocol is adhered to.

3.2.1 Before Running Any Program

- Always prime the ELx405 before running a wash or dispense program. Do not rely on AutoPrime. See the recommended minimum prime volumes on page 32 of the ELx405 Getting Started Guide. Fill the wash/rinses bottles with sufficient fluid. Make sure the supply tube is in the liquid. Empty the waste bottles and firmly seat the waste bottles stoppers. To ensure that fluid does not back up into the vacuum pump during operation, always operate the washer with the waste sensor cable sensor activated.

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- Keeping the tubing wet between runs and regular maintenance are vital to keeping the washer performing as expected.

As needed

Clean exposed surfaces, run dispense protocol, clean priming plate, and clean dispensing tubes and injectors.

Daily Maintenance

Flush the washer with an appropriate reagent or deionized water throughout the day. Routinely rinsing the washer helps to prevent the aspirate and dispense tubes from clogging between washes.

Monthly

Flush the system with 0.1-0.5 N NaOH, followed by neutralization with an equivalent normality (0.1 - 0.5 N) of HCl. Rinse well with deionized water to remove HCl by running the overnight loop Maintenance program. Clean the debris and bottles, clean manifold and mist shields, aspirate and dispense tubes, fluid inlet filter, run decontamination and decontaminate external surface.

Annually

Replace O-rings, and channel-end seals.

- Check the external tubing connections for kinks and clogs.
- Make sure the bottles, solutions, and tubing are clean and do not contain any particles or mold. Water and dye solutions that are recycled over several days will grow algae, bacteria, mold, or other undesirable organisms.
- Sometimes there are large air pockets in the tubing, run a Prime program before running any program. When placing a micro plate on the carrier, make sure that well A1 is in the left rear corner as you face the front of the washer, and that the plate is firmly seated in the carrier. The micro plate spring holds the micro plate against the front edge of the carrier.
- There are two different types of plate carriers. Vacuum Filter plate manifold is required only for Luminex magnetic bead assays and standard manifold is used for ELISA kits.
- To select the manifold, go to Menu, More Carrier selection, select standard or vacuum.
- Adjust the needle height for wash program, test it with a blank plate and "adjust". The needle should not hit the bottom of the plate. Adjust the manifold for proper cleaning. Use wash program and save it with a name.

3.2.2 Run wash program

Refer to manual for ELX 405 washer manual for setting up a wash program.

Rinse the washer after completion and run a rinse and soak program at the end to avoid any blockages.

3.2.3 Preventive Maintenance

The following components should be periodically cleaned:

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- Bottles
- Plate carrier system
- Manifold
- Aspirate/dispense tubes
- Mist shield
- Inlet filter

APPENDIX I

POD Training Record Form

Centre PERFORM Centre**POD Title**

Use and Maintenance of Hybrid Multi-Mode Microplate Reader and Washer

Ownership	Document Type	Area	SOP Number	Version
PC	POD	CA	004	V02

Training Record

Full Name	
Institution/PI	
Contact (e-mail or phone number)	

Signature

Sign here

Date