

PERFORM Operating Document

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

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1. Introduction

The Synergy™ HI is a hybrid multi-mode microplate reader with detection modes that 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.

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. Definition of Terms and Abbreviations

ELISA	Enzyme-Linked ImmunoSorbent Assay
EIA	Enzyme ImmunoAssays
CAS	Clinical Analysis Supervisor
Nm	Nanometer

3. Overview of Microplate Reader and Washer

Synergy 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 (230 – 999nm) with a 1 nm step increments. It is an ideal system for all the standard microplate applications found in life science

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research laboratories. All operations of the Synergy HI are computer-controlled by the Gen5 software, including data reduction and analysis.

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.

3.1 Training requirements

Prior to using the microplate reader and washer users should:

- Read and sign online 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.

3.2 General precautions

3.2.1 Microplate Reader

- Microplates should be clean and free from dust or bottom scratches. Wipe the bottom of the plate with a lint-free paper or cloth before placing it in the reader.
- Use new microplates from sealed packages.
- Filter solutions to remove particulates that could cause erroneous readings.
- 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.
When loading plates on the reader, make sure the AI position of the microplate is in the proper orientation
- Inaccuracy in pipetting has a large effect on measurements, especially if smaller volumes of liquid are used. For best results, use at least 100 µL per well in a 96-well plate and 25 µL in a 384-well plate.
- For best results, remove the air bubbles by degassing the plate in a vacuum chamber or spinning the plate in a centrifuge before reading
- Do not open the access door on the front of the instrument during operation, it may affect measurements.

3.2.2 Microplate Washer

- For proper operation, ambient temperatures should remain between 15°-30°C. Performance may be adversely affected if temperatures fluctuate above or below this range.

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- 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
- 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. Use of wash buffers containing acetic acid is limited to washers upgraded with Teflon valves (Biotek part number 68098).
- 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.
- Solutions containing proteins such as bovine serum albumin, will compromise the washer's performance over time unless a strict maintenance protocol is adhered to.

3.3 Relevant documents

- Synergy HI™ Operator's Manual
- ELx405™ Microplate Washer Operator's Guide
- ELx405™ Getting Started Guide

4. Procedure**4.1 Microplate Reader****4.1.1 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.

Follow the sequence below to turn on the microplate reader and run a system test:

1. Turn on the reader from a power button on the lower left side and then launch Gen5 software.
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 the 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.

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.

- **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.
- Data reduction automatically converts the data into Excel. Report builder function builds the report which can be customized.

4.1.2 Data acquisition software layout and instrument controls

BioTek Gen5 software controls 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. To read and analyze the plate, you must open the existing protocol in Experiments. Refer to user manual for detailed procedures.

4.1.3 Preventive maintenance

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

As needed

Clean exposed surfaces using a moistened cloth with water and/or water and mild detergent. Inspect/clean the emission and excitation filters and mirrors (if used), run dispense protocol, clean priming plate, and clean dispensing tubes and injectors

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To ensure accurate performance and a long life for the dispense module and injectors, flush and purge the fluid lines with deionized (DI) water every day or after completing an assay run (page 60 and 61).

Quarterly

Biotek recommends inspecting filters for dust and moisture every 3 months using isopropanol or methanol and a lens- cleaning tissue. Inspect mirrors especially if the filter cube has been changed. Using nitrogen gas under low pressure is the best method to remove excess of debris on mirror. Perform this cleaning step only if necessary because mirrors can be easily damaged.

4.1.4 Instrument Qualification

Proceed to an instrument qualification conducting absorbance (p.91-98) and fluorescence (p.99-111) liquid test, refer to Synergy™ HI Operator's Manual.

4.1.5 Filters and Mirror

Please refer to chapter 4 of the Synergy™ HI Operator's Manual. Synergy HI is equipped with excitation and emission filters for obtaining fluorescence and luminescence measurements. The excitation filter selects the band of light to which the sample will be exposed. To install a filter cube please refer to p.44-47 of the manual.

4.2 Microplate Washer

Before Running Any Program:

- Fill the wash/rinse bottles with sufficient fluid. Make sure the supply tube is in the liquid. Empty the waste bottles and firmly seat the waste bottle stoppers. To ensure that fluid does not backflow into the vacuum pump during operation, always operate the washer with the waste cable sensor activated.
- 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.
- Keeping the tubing wet between runs and regular maintenance **are vital** to keeping the washer performing as expected.
- Sometimes there are large air pockets in the tubing, run a Prime program before running any program. Do not rely on AutoPrime. See the recommended minimum prime volumes on page 32 of the ELx405 Getting Started Guide.

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- When placing a microplate 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 microplate spring holds the microplate 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 use 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.

4.2.1 Protocol Parameters

Set the dispense height low enough to ensure that excess fluid is removed by the aspirate tubes when the manifold is dispensing, set the plate clearance height, set the vacuum dissipation delay (10 seconds when using 10 Liter waste bottles or 20 seconds when using 20 liter bottles), be sure to specify the correct plate type (96- or 384-wells).

4.2.2 Basic Operation

Press On/Off switch in the rear panel.

The ELx405 performs a self-test. If the test passes, the main menu will appear. If the self-test fails, the ELx405 will beep and display an error code. If the error persists, look up the code in Appendix B Error Codes of the operator manual ELx405™ Getting started Guide.

4.2.3 ELx405 Keypad

Press a key to choose the matching option (Run, Define, Main, Util). Press Run to run a previously defined protocol. Press define to lead to protocol creation and editing mode. Press Maint to run a maintenance routine. Press Util to access to instrument setting.

4.2.4 Create or edit protocol.

Select define at the main menu. Select create or edit, and choose the type of protocol (wash, dispense, aspirate, prime, soak or link). Define the plate type and the wash component. Refer to ELx405™ Getting started Guide from page 28-36.

4.2.5 Maintenance of the Microplate Washer

The ELx405 is equipped with pre-defined preventive maintenance programs that will make it easier for most of the recommended tasks. (Refer to Chapter 4 of the Operator's Manual for detailed instructions).

As needed

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

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The following components should be periodically cleaned:

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

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. Never use alcohol to flush out BSA. Use distilled water to flush phosphate buffer from the system, and an enzyme-active detergent such Terg-A-zyme to remove proteins.

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 or run the P_DAY_RINSE prime program three times with deionized water if you plan to use the washer immediately. Clean 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.