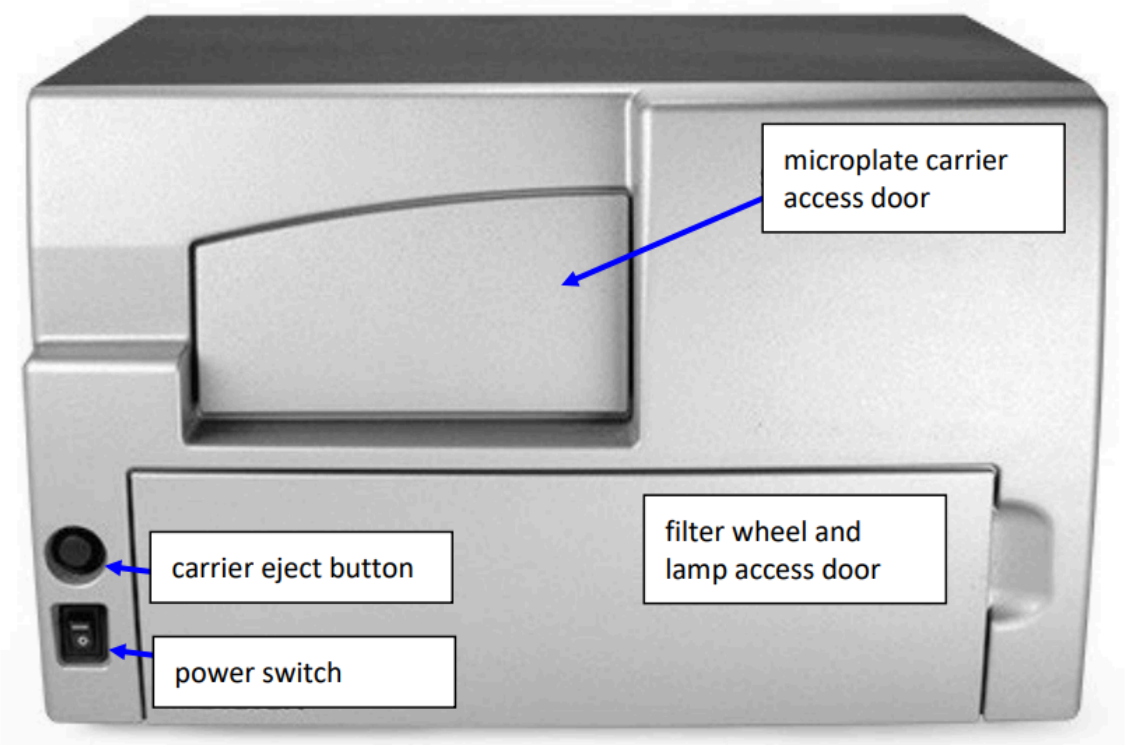
Standard Operating Procedure (SOP) for Agilent BioTek Synergy HTX Multi-Mode Microplate Reader



Prerequisites:

- Reader has been installed and passed system tests.
- Gen5 software is installed and configured on the host computer.
- Operator has the required training in lab safety and relevant assays.

1. Safety and Preparations

- Ensure the area is dry, clean, and within 18°C–40°C.
- Wear gloves and safety goggles, especially when working with biological samples or reagents.
- If using DMSO, keep concentration below 2% to avoid optical contamination.

2. Start-Up

1. **Turn on the Reader**
   Use the **power switch** on the front. The green LED will light up.
2. **Launch Gen5 Software**
   Open Gen5 and log in (default admin password: `admin`).
3. **Verify Connection**
   - Go to `System > Instrument Configuration > Add Reader`
   - Select “Synergy HTX” and test communication.
   - Ensure the system test passes on start-up.

3. Loading the Plate

1. Press the **carrier eject button** to extend the microplate tray.
2. Load your microplate with well A1 at the **left rear corner**.
3. Gently close the plate carrier or use the eject button again.

4. Running an Experiment

A. Using an Existing Protocol:

1. Go to `Experiments > Create using an existing protocol`.
2. Select the desired protocol (e.g., absorbance, fluorescence, luminescence).

3. Click the **Read Plate** icon or go to `Plate > Read Plate`.

B. Creating a New Protocol:

1. Go to `Protocols > Create New`.
2. Define **Plate Type**, **Read Step(s)** (absorbance, fluorescence, luminescence), and any **Shake/Incubation** steps.
3. (Optional) Add **data reduction**, **plate layout**, and **report/export options**.
4. Save the protocol.

5. Filter Selection (for fluorescence/luminescence)

- Confirm filters are correctly installed (Excitation = EX, Emission = EM).
- To update filter configurations:
    1. Go to `System > Instrument Configuration > View/Modify`.
    2. Click `Setup > Fluorescence/Luminescence tab`.
    3. Enter filter wavelength and bandwidth.
    4. Click `Send Values` to apply.

6. Shaking or Heating (if required)

- Add a **Shake step** in your protocol:
    - Modes: Linear or Orbital (Slow/Fast).
    - Select amplitude (1–6 mm) and frequency (Hz).
- To heat the plate:
  Go to `System > Instrument Control > Pre-Heating`, set to 37°C, and wait for equilibrium.

7. Dispense Module Use (If Applicable)

1. Prime system using `System > Instrument Control > Prime` tab.

2. Load reagents, attach inlet tubes, and use **Dispenser 1 or 2** to prime or dispense.

8. Finishing Up

- Save your experiment results: `File > Save As`.
- Export data or generate reports as needed.
- Turn off the incubator and then power off the reader.
- Clean the plate carrier and any spills immediately.

9. Maintenance

- Wipe external surfaces daily with a damp cloth (not soaked).
- Clean filters quarterly.
- Prime and purge dispense system after each use (if injectors are used).
- Do not use lubricants or immerse any part of the reader.

Recommended Maintenance Schedule

Task	Daily	Quarterly	As Needed
All models:			
Clean exposed surfaces			√
Inspect/clean excitation and emission filters (if equipped)		√	
Decontaminate the instrument	*before shipment or storage*		
Models with injectors and an external dispense module:			
Flush/purge the fluid path	√		
(Optional) Run a Dispense protocol			√
Empty/clean tip prime trough	√		
Clean priming plate			√
Clean internal components		√	√
Clean tubing and injector heads		√	√
Clean optical probes		√	√
Clean internal surfaces		√	√

10. Instrument Testing

Routine performance testing ensures that the Synergy HTX is functioning within manufacturer's specifications. The following tests should be performed at recommended intervals or when troubleshooting suspected performance issues.

Recommended Qualification Schedule

The following schedule is recommended for a Synergy HTX used two to five times per week:

Tasks/Tests	IQ	OQ	PQ	
	Initially	Initially/ Annually	Monthly	Quarterly
All models:				
Installation, setup, and configuration of the reader, host computer, and Gen5	√			
System Test	√	√	√	
Models with absorbance capability:				
Absorbance Plate Test		√	√	
Absorbance Liquid Test 1 or Liquid Test 2*		√		√
(Optional) Absorbance Liquid Test 3 *or* 340 nm Absorbance Plate Test (using part number #7260551)		√		√
Models with fluorescence capability:				
Corners, Sensitivity, Linearity (FI) Tests		√	√	
Models with luminescence capability:				
Luminescence Test		√	√	
Models with injectors and an external dispense module:				
Installation and setup of external dispense module	√			
Injection System Test	√			
Dispense Accuracy and Precision Test		√		√

* If you have Absorbance Test Plate part number #7260522, perform Liquid Test 1. Otherwise, perform Liquid Test 2.

10.1 System Test (All Models)

Performed automatically at startup, or manually via Gen5.

Steps:

1. Turn on the reader and launch Gen5.
2. If needed, preheat using `System > Instrument Control > Pre-Heating`.
3. Navigate to `System > Diagnostics > Run System Test`.
4. Wait for test completion and ensure result = **"SYSTEM TEST PASS."**
5. If the test fails, press the **carrier eject** button to stop beeping, then contact technical support.

10.2 Absorbance Plate Test

Use **Test Plate #7260522** (visible) or **#7260551** (UV).

Steps:

1. In Gen5: `System > Diagnostics > Test Plates > Add/Modify Plates`
2. Enter the OD and peak wavelength values from the Test Plate's certificate.
3. Then: `System > Diagnostics > Test Plates > Run`
4. Insert the Test Plate (well A1 rear-left), run test, and confirm all values = PASS.
5. If any value fails: clean plate, check calibration certificate date, and re-run.

10.3 Fluorescence Test

Use the **Fluorescence Test Plate (Part #1400006)**.

Steps:

1. Load the test plate.
2. Run the provided Gen5 protocol.
3. View embedded Excel report for PASS/FAIL status.
4. PASS Criteria includes:
    - %CV for corner wells < 3.0%

- Sensitivity thresholds:
  - Top Optics: ≤53 pM
  - Bottom Optics: ≤30 pM
- Linearity: $R^2 \geq 0.95$

10.4 Luminescence Test

Use the **Harta Luminometer Reference Microplate (Part #8030015)** and adapter.

Steps:

1. Power on the Harta plate; check battery using the test button.
2. Load plate on adapter into the reader.
3. Run Gen5 protocol `Synergy HTX LumTest_Harta.prt`.
4. Evaluate:
   - A8 > 0.2 × A7 → battery OK
   - Detection limit:
     - ≤60 amol (low-noise PMT)
     - ≤500 amol (red-shifted PMT)
   - S/N ratio and attomoles conversion from calibration certificate

10.5 Dispense Accuracy and Precision Test

Required for models with injectors. Uses **green test dye** and absorbance reading.

Materials:

- New 96-well plate
- Green Test Dye (BTI #7773003)
- Balance (0.001 g readability)
- DI water

- Gen5 protocols: `Synergy HTX Disp 1 Test.prt` and `Disp 2 Test.prt`

Steps:

1. Prime dispensers with DI water, then dye.
2. Dispense 80, 20, and 5 µL to separate columns (with tip priming).
3. Weigh plate after each dispense.
4. Manually add 150 µL water to each well.
5. Run read steps (405/750 nm for 80 µL; 630/750 nm for 20/5 µL).

Pass Criteria:

Dispense Volume	To pass, %CV must be:	To pass, Accuracy % Error must be:
80 µL	≤ 2.0%	≤ 2.0%
20 µL	≤ 7.0%	≤ 5.0%
5 µL	≤ 10.0%	≤ 20.0%