

Working with the Synergy™ Microplate Reader Using Gen5™ Software

Version 3.15.15

Chapter 1 – What Is the Synergy™ Microplate Reader?

The **Synergy™ Microplate Reader** is a **multi-mode microplate reader** used to measure biological and chemical reactions performed in microplates.

Depending on the instrument configuration, the Synergy can perform several types of measurements, including:

- **ELISA**
- **MTT assays**
- **Thioflavin T (ThT)**
- **Bradford protein assay**
- **BCA protein assay**
- **DNA/RNA quantification**
- **Fluorescence measurements**
- **Luminescence measurements**
- **Kinetic measurements**
- **Spectral scans**

The instrument is operated using **Gen5™ software**.

Gen5 is used to:

- Select or create measurement protocols, Define measurement parameters
- Acquire experimental data, Analyse results, Save and export data.

Important: The measurement parameters must always match the experimental method being used. Do not change parameters unless you understand their purpose and have permission to do so.

Chapter 2 – Types of Measurements

The Synergy microplate reader can perform several different types of measurements. Each measurement mode is designed to detect a different type of signal.

Measurement Type	What Is Measured?	Examples
Absorbance	The amount of light absorbed by the sample. In an absorbance measurement, the instrument shines light through the sample and measures how much light is absorbed.	ELISA, MTT, Bradford, BCA
Fluorescence	Light emitted by a fluorescent molecule after excitation. The sample is illuminated at an excitation wavelength, and the emitted fluorescent light is measured at an emission wavelength.	Thioflavin T (ThT), GFP, other fluorescent dyes or molecules
Luminescence	Light produced by a chemical or biological reaction. Unlike fluorescence, luminescence does not require excitation light from the instrument.	Luciferase assays
Kinetic	Changes in a measured signal over time. The instrument records the signal repeatedly at defined time intervals to monitor the progress or rate of a reaction.	Enzyme activity, reaction kinetics; for example, measuring enzyme activity every 30 seconds for 30 minutes
Spectral Scan	Signal measured across a range of wavelengths. A spectral scan can be used to determine an optimal wavelength, characterize a fluorescent dye, examine an absorbance spectrum, or optimize experimental conditions.	Determining or optimizing wavelengths; characterizing absorbance or fluorescence spectra

Important Considerations

Selecting the correct measurement mode is essential for obtaining meaningful results. The measurement mode should match the type of signal produced by the assay.

- Absorbance measures light absorbed by the sample.
- Fluorescence measures light emitted by a fluorescent molecule following excitation.

- Luminescence measures light generated by a chemical or biological reaction.
- Kinetic measurements monitor changes in a signal over time.
- Spectral scans measure a signal across multiple wavelengths to characterize or optimize the measurement.

Important: Selecting the wrong measurement mode can produce incorrect or meaningless results.

Chapter 3 – Before Starting the Experiment

3.1 Preparing the Instrument

Before starting:

- ☐ The computer is switched on.
- ☐ The Synergy microplate reader is switched on.
- ☐ The instrument is connected to the computer.
- ☐ After making a reservation through **BOOKIT**, log into the computer using your username and password.
- ☐ Open the Windows search menu and search for **Gen5**.
- ☐ Click the **Gen5 icon** to open the software.
- ☐ Confirm that the Synergy instrument is recognized by Gen5.
- ☐ Confirm that the instrument is in **Ready** status.

Do not begin the experiment if the instrument reports an error or is not recognized by Gen5. Contact the laboratory supervisor or responsible staff member.

Chapter 4 – Preparing the Microplate

Before placing the microplate into the reader, check all of the following:

- ☐ The correct plate type is being used, and the plate is labelled appropriately for the experiment.
- ☐ Samples are in the correct wells.
- ☐ There are no spills or leaks.
- ☐ **There are no visible air bubbles in the wells.**
- ☐ The bottom of the plate is clean and there are no fingerprints on the bottom of the plate.

☐ **The plate is inserted in the correct orientation.**

Plate Orientation

Pay particular attention to the position of **well A1**.

The plate must be inserted according to the orientation indicated by the instrument.

Important: An incorrectly oriented plate can cause samples to be measured in the wrong wells.

Chapter 5 –Creation Working Protocol

A **Protocol** is a file containing the settings required to perform a measurement.

A protocol may include:

- Microplate type
- Measurement mode
- Wavelengths
- Gain
- Read height
- Shaking
- Temperature
- Kinetic settings
- Measurement timing
- Data analysis settings

Before every measurement, the protocol must be checked to make sure that it matches the experimental method.

Never assume that the previously used protocol is correct for your experiment. Always verify the settings before pressing Read.

Creating and checking the Protocol

Before clicking **Read**, carefully check the following settings.

5.1 Plate Settings

Verify:

- ☐ Correct plate type/ plate manufacturer
- ☐ Correct number of wells
- ☐ Correct plate color

☐ Correct bottom type

Example

A protocol may specify:

Greiner Black Flat Bottom Fluotrac

The plate used in the experiment must match the protocol requirements.

Important: Plate properties can affect optical measurements, particularly fluorescence measurements. Always use the plate specified by the experimental method.

Chapter 7 – Measurement Mode

Check that the correct measurement mode has been selected.

Possible measurement modes include:

- ☐ **Absorbance**
- ☐ **Fluorescence**
- ☐ **Luminescence**
- ☐ **Kinetic**
- ☐ **Endpoint**
- ☐ **Spectral Scan**

7.1 Choosing Endpoint: Normal or Extended

When selecting an **Endpoint** measurement in Gen5, the software may provide a choice between **Normal Endpoint** and **Extended Endpoint**. an **Endpoint** settings can affect fluorescence results

Normal Endpoint

Use **Normal Endpoint** when the experiment requires a single measurement at the defined endpoint of the protocol.

For example:

- Measure absorbance once after an MTT reaction.
- Measure fluorescence once after a reaction has reached its defined endpoint.
- Measure luminescence once after the assay has been prepared according to the protocol.

Extended Endpoint

Use **Extended Endpoint** only when the experimental protocol specifically requires an extended endpoint measurement or additional measurement time.

An extended endpoint may be useful when the assay requires additional time or repeated acquisition associated with the endpoint measurement.

Important: Do not select **Extended Endpoint** simply because it appears to provide a longer or more detailed measurement. The choice between **Normal Endpoint** and **Extended Endpoint** must follow the validated experimental protocol or the instructions provided by the laboratory supervisor.

Student Check

Before proceeding, confirm:

- ☐ **Endpoint** is selected as the measurement mode.
- ☐ The correct **Normal** or **Extended** option is selected.
- ☐ The selection matches the experimental protocol.
- ☐ All other measurement settings are correct.

Chapter 8 – Optical Settings

Depending on the measurement type, check the appropriate optical settings.

- ☐ **Top Read / Bottom Read**
- ☐ **Excitation wavelength**
- ☐ **Emission wavelength**
- ☐ **Gain**
- ☐ **Read Height**
- ☐ **Integration Time**
- ☐ **Shaking**
- ☐ **Temperature**

Top Read vs. Bottom Read

The instrument can measure some samples from the **Top** or **Bottom** of the microplate.

The correct setting depends on the assay and plate configuration.

Top Read

The optical system measures the sample from above the plate.

This is commonly used for many fluorescence and luminescence applications.

Bottom Read

The optical system measures through the bottom of the plate.

This may be appropriate for certain assays and plate types.

Always follow the experimental protocol when selecting Top Read or Bottom Read.

Chapter 9 – Fluorescence Settings

For fluorescence measurements, two wavelengths are particularly important:

Excitation wavelength (Ex)

The wavelength of light used to excite the fluorescent molecule.

Emission wavelength (Em)

The wavelength of fluorescent light measured by the instrument.

For example:

Excitation → Fluorescent molecule → Emission

The correct excitation and emission wavelengths depend on the fluorescent compound being used.

Do not select wavelengths randomly. Use the wavelengths specified by the experimental protocol or established method.

Chapter 10 – Gain

Gain controls the sensitivity of the fluorescence detection system.

An inappropriate gain can cause:

- Signal that is too weak.
- Signal that is too strong.
- Saturation.
- Poor comparison between samples.

Therefore:

- ☐ Check the gain setting.
- ☐ Use the **gain specified by the protocol**.
- ☐ If automatic gain is used, verify that it is appropriate for the experiment.

Important: Gain settings can affect fluorescence results. Do not change the gain between samples within an experiment unless the method specifically requires it.

Chapter 11 – Read Height, Shaking, and Temperature

Read Height, shaking, and temperature are additional measurement parameters that may affect the quality and reproducibility of an experiment. These settings should always be checked before starting the measurement.

11.1 Read Height

Read Height refers to the vertical position at which the instrument measures the sample.

The appropriate Read Height depends on:

- Plate type
- Sample volume
- Assay
- Measurement mode

Check that the Read Height matches the experimental protocol.

11.2 Shaking

Some experiments require the plate to be shaken before or between measurements.

Check:

- ☐ Whether shaking is required.
- ☐ Shaking speed.
- ☐ Shaking duration.
- ☐ When shaking occurs relative to the measurement.

For example:

Shake for 10 seconds before each measurement.

Shaking may be used to mix samples, maintain a uniform suspension, or improve reaction consistency, depending on the assay.

Do not introduce shaking if the experimental protocol does not require it.

11.3 Temperature

Some experiments require temperature control.

Check:

- ☐ Required temperature.

☐ Whether temperature control is enabled.

☐ Equilibration time, if required.

Temperature can affect biological reactions and therefore may significantly affect the measured signal.

For temperature-sensitive experiments, allow the instrument and samples to reach the required temperature according to the experimental protocol.

Chapter 12 – Kinetic Measurements

For a kinetic experiment, check:

☐ Number of measurement cycles

☐ Time between measurements

☐ Total experiment duration

☐ Initial delay, if required

☐ Shaking between measurements, if required

Example

A kinetic protocol might specify:

Parameter	Example
Number of cycles	60
Interval	30 seconds
Total time	30 minutes
Initial delay	0 minutes

These values are examples only. Always use the values specified in your experimental protocol.

Chapter 15 – Absorbance Measurements

For absorbance measurements, check:

☐ Primary wavelength

☐ Reference wavelength, if required

☐ Pathlength correction, if required

Primary wavelength

This is the wavelength used to measure the absorbance of the analyte or reaction product.

Reference wavelength

Some assays use a reference wavelength to correct for background effects or nonspecific absorbance.

Pathlength Correction

Pathlength correction may be used when the sample volume varies between wells and the method requires normalization to a standard optical path length.

Only use pathlength correction when required by the assay or validated protocol.

Chapter 19 – Saving the Data

After completing the measurement:

- ☐ Save the experiment file.
- ☐ Use an appropriate file name.

For example:

StudentName_MTT_2026-08-05

or:

StudentName_ThT_2026-08-05

A good file name should include:

Student/Researcher + Experiment + Date

If required:

- ☐ Save the file in the designated laboratory folder.
- ☐ Export the data to Excel.
- ☐ Confirm that the exported file opens correctly.
- ☐ Do not delete or overwrite the original experiment file.

Important: The original Gen5 experiment/data file should be preserved according to your laboratory's data-management requirements.

Chapter 20 – Exporting Data

If numerical data are required for additional analysis:

1. Open the results/data section in Gen5.
2. Select the appropriate data table.

3. Choose the export option.
4. Select the required format, such as Excel, if available.
5. Save the exported file using an appropriate file name.
6. Verify that the exported values are correct.

Keep the original Gen5 data file even after exporting to Excel.

Chapter 21 – After the Experiment

When the measurement is finished:

- ☐ Remove the microplate.
- ☐ Leave the work area clean.
- ☐ Save your data.
- ☐ Close the experiment appropriately.
- ☐ Report any instrument problem to the laboratory supervisor.

Chapter 22 – Common Errors

✗ Incorrect plate type

The protocol specifies one type of plate but a different plate is used.

Why it matters: Optical properties can affect the measurement.

✗ Incorrect plate orientation

The plate is inserted incorrectly.

Why it matters: Samples may be assigned to the wrong wells.

✗ Incorrect wavelength

The wrong excitation, emission, or absorbance wavelength is selected.

Why it matters: The analyte may not be detected correctly.

✗ Incorrect Gain

The fluorescence gain is inappropriate.

Possible result: Signal is too weak or saturated.

✗ Incorrect Top/Bottom Read

The wrong reading direction is selected.

Possible result: Reduced or incorrect signal.

✗ Air bubbles in wells

Bubbles are present in the sample.

Possible result: Light scattering and incorrect measurements.

Solution

Remove bubbles according to the laboratory's approved procedure before placing the plate in the reader.

✗ Dirty plate bottom

Fingerprints, dust, or liquid are present on the bottom of the plate.

Possible result: Optical interference.

Solution

Clean the outside/bottom of the plate with an appropriate lint-free material according to laboratory procedures.

✗ Data not saved

The experiment is completed but the data file is not saved correctly.

Solution: Always verify that the Gen5 experiment file has been saved before leaving the instrument.

✗ Opening the drawer during measurement

Do not open the plate drawer while the instrument is measuring.

This can interrupt the measurement and may affect the experiment.
