



FOOD SAFETY LAB / MILK QUALITY
IMPROVEMENT PROGRAM
Standard Operating Procedure



Title: Using the Synergy H1 Microplate Reader

SOP #: 2.6

Version: 02

Revision Date: 01-13-2021

Effective Date:

Author: Jordan Skeens and Matthew Stasiewicz

Approved by:

***Using the Synergy H1 Microplate Reader to Perform
Growth Curve and 'Snapshot' OD readings***

**FILE NAME: 2.6-Using the Synergy H1 Microplate
Reader SOP_04262021.docx**



TABLE OF CONTENTS

1.	INTRODUCTION	3
	Purpose	
	Scope	
	Safety	
2.	MATERIALS	4
3.	PROCEDURE	5
	Appendix 1	14
	Appendix 2	15
4.	TROUBLESHOOTING	16
5.	REFERENCES	17
6.	METHOD REVIEWS & CHANGES	18



SECTION 1 INTRODUCTION

1.1 Purpose

The purpose of this document is to set forth standard guidelines for measuring the OD of a bacterial culture in 96-well plates using the absorbance capabilities of the Synergy H1 Hybrid Multi-Mode Microplate Reader. Suggestions for basic analysis of the results is also described.

1.2 Scope

This SOP applies to activities within the Cornell Food Safety Lab (FSL) and the Milk Quality Improvement Program (MQIP), as well as associated lab personnel.

1.3 Safety

The Synergy H1 is as shared device used for analysis of BSL-1 and BSL-2 organisms. Take the appropriate precautions necessary to avoid any potential exposure to harmful organisms.



SECTION 2 MATERIALS

- Costar 3370 96-well Assay Plate Sterile, clear plates, with lid (note that other plates can also be used, including 6-well and 384-well plates)
- Multichannel Pipette P200; P1000, P200, P20, as appropriate.
- Liquid Growth Media
- Agar Growth Media
- 96 deep-well plates
- Neutral buffer (PBS, BPW, etc.)



SECTION 3 PROCEDURES

Additional information regarding the Synergy H1 plate reader and Gen 5 v2.09 software can be found in their respective documentation (see References).

3.1 Plate Reader Setup:

The following operations are required *BEFORE* setting up a plate. Protocols do not need to be created new if a previously prepared Gen5 Protocol can be used or modified for the current experiment. Skip to Section 3.2 if this is the case.

- 1) Turn on the **Synergy H1 Microplate Reader**. The power switch is on the front of the reader at the bottom left. A green light should illuminate when the device is turned on. The plate tray may open when startup is complete. If the tray stays open, press the button next to the power switch to close the tray (this button can be used to open the tray as well)
- 2) Log in to the attached computer with the provided credentials (not your netID/password).
- 3) Open the **Gen 5** software.
- 4) In **Gen5**, create a new **Protocol**.
 - a) From the Task Manager window, select **Protocols > Create New...**
 - b) Select **Standard Protocol** then click **OK**
 - c) If prompted to select a reader, select the **Synergy H1** and click **OK**.
 - d) Setup the **Procedure**:
 - i) From the top menu, select **Protocol > Procedure**
 - ii) In the Procedure window, select “96 WELL PLATE” from the **Plate Type** dropdown menu
 - iii) Check the box labeled “Use Lid” next to the **Plate Type** dropdown menu.
 1. **Note! If you do not select this box and insert the plate with the lid still on, the plate can get stuck in the machine.**
 - iv) (OPTIONAL) In the left side menu, click **Set Temperature** to add a temperature to the procedure:
 - (1) Enter the desired temperature then click **OK**
 - (2) The Synergy H1 can only heat, not cool. Range can be set at 4°C above room temperature up to 45°C.
 - v) (OPTIONAL) Click **Shake** in the left side panel – This step will shake the plate before the read to mix the wells. *Note! If you are going to use the shaking option it is recommended that you use a maximum of 200 µL volume to avoid cross contamination of cultures.*
 - (1) Set the **Shake Type, Duration, and Intensity** (ex. linear/orbital, 00:10 seconds, gentle) then click **OK**
 - vi) Click **Read** in the left side panel
 - (1) Select **Absorbance** under **Detection Method** and **Endpoint / Kinetic** under **Read Type**, then click **OK**
 - (2) Set Wavelength 1 to 600nm., then click **OK**



- e) (OPTIONAL) Setup the **Plate Layout** for later data analysis within Gen5. See the Gen5 software user manual for details on plate layout setup and use. This setup can be skipped if analysis will be done manually outside the Gen5 software. *Note: There is anecdotal evidence to suggest that potential issues with alignment of the light source and detector may yield differing OD readings for these wells, therefore it is advised to not use the edge wells (i.e., wells in the exterior-most rows and columns) of the plate.*
- f) (OPTIONAL) **Data Reduction** should be set up in Gen5 if **Plate Layout** is setup. **Data Reduction** can be used to subtract blanks, calculate time to reach certain ODs, and fit curves to data. Each step requires an input data set and the name for an output data set. See the Gen5 User Manual for details on using Data Reduction
- g) Save the **Protocol** by clicking **File > Save As**. The saved protocol can be used again for future growth curve experiments, making changes where needed for the specific experiment.

3.2 Snapshot OD Reading

Skip to section 3.3 if performing a growth curve experiment.

- 1) 96-well Plate Setup
 - a) For each isolate, take a single colony from a fresh agar media plate and inoculate into 5ml fresh BHI broth (or appropriate liquid media for your organisms).
 - i) 96 deep-well plates can be used for high throughput overnight cultures.
 - b) Transfer 200uL of each culture to a 96-well plate in duplicate. Extra wells should be reserved for any positive or negative controls and sterile-media only (Blank) wells, all of which should be included in duplicate. *Note: see above 3.4.e*
 - i) Do not write anything on the 96-well growth plate or lid, as it will interfere with the readings.
 - ii) Keep a record of the sample layout in the growth plate. If a **Plate Layout** was setup in the **Protocol**, ensure the samples are inoculated according to the template defined there.
 - iii) To minimize positional bias, consider randomization of the position of the samples in the plate, especially if any subsequent biological replicate experiments will be performed.
 - c) To verify the original inoculum culture concentration, keep the 96 deep-well plates containing the diluted cultures on ice until after the growth curve has been started, then spread plate or spiral plate 50uL of each 10^3 CFU/mL culture dilution in duplicate on the appropriate enumeration plating media for the sample organisms. Incubate and enumerate to verify expected original culture CFU/mL. (See FSL SOP [5.3.5-Eddy Jet 2 Spiral Plater and SphereFlash Colony Counter](#))
 - d) Cover the growth plate with the lid and bring it to the plate reader.
- 2) Taking the snapshot reading
 - a) At the Synergy H1 computer, select **Read Now > Existing Protocol** in the task manager window of Gen5 and find the protocol prepared in section 3.1.
 - i) If a temp was set, the plate reader will begin to warm. To start the procedure before reaching the target temp, click **Override**.



- b) Click the Green Arrow on the top bar to start the experiment procedure. A message to “Load plate” should appear on screen and the plate tray should extend if it is not already.
- c) Load the plate onto the plate reader tray. Make sure to place the A1 plate corner in the indicated tray corner with the notch (back right when facing the machine, also labeled A1). If the tray is not open, press the button next to the power switch on the Synergy H1. Then press OK on the prompt.
- d) The plate tray should retract and close. If the internal temperature has not reached a defined setpoint, a prompt may appear asking if the readings should wait until the setpoint has been reached. This can be accepted or declined based on the user preference.
- e) The read will then initiate. In the Plate 1 window, the well matrix charts should populate with the OD data point as each well is read. After the end of the reading, carefully check these charts to ensure that each well has been read as expected.
- f) When the read has stopped, manually export the data and well curves by selecting **Plate > Export** from the top menu. An Excel file containing the raw read data will open and should be saved.
- g) Data may also automatically export if the protocol is appropriately configured, but data should always be saved manually.

3.3 Preliminary Growth Curve Benchwork

Skip to Section 3.5 if not performing a growth curve experiment. Preliminary work is needed to determine the appropriate setup for the growth curve. Skip to Section 3.4 if prior work has already been done to determine this information for your organisms. Consult a senior lab member if unsure.

- 1) For each isolate, take a single colony from a fresh agar media plate and inoculate into 5ml fresh BHI broth (or appropriate liquid media for your organisms).
 - a) A sub-set of the isolates can be tested for the preliminary work; select a few representative isolates for each “group” within the overall sample isolates. Ex: a few isolates from each *Listeria* lineage and serotype. Consult a senior lab member for assistance making this decision.
- 2) Incubate the liquid cultures overnight at desired temperature (with gentle agitation if appropriate).
- 3) In a 96 deep-well plate, serially dilute the overnight cultures 1:10 in growth media up to a 10^{-7} dilution and keep on ice. The final volume of all dilutions should be at least 900uL.
- 4) To determine the average CFU/mL of the inoculum cultures:
 - a) Spread plate or spiral plate each 10^{-7} , 10^{-5} , and 10^{-3} dilution on the appropriate growth media in duplicate using 50 μ L aliquots, then incubate at the appropriate conditions.
 - b) Enumerate the grown colonies to determine an expected original CFU/mL for the overnight cultures. (See FSL SOP [5.3.5-Eddy Jet 2 Spiral Plater and SphereFlash Colony Counter](#))
- 5) To determine the Synergy H1 OD limit of detection (LOD) for the cultures (snapshot plate):



- a) For each isolate, 200 μ L of each of the 7 dilutions prepared (10^{-1} , 10^{-2} , etc.) should be transferred to individual wells in a 96-well clear flat-bottom assay plate, including 2 wells filled with 200 μ L uninoculated growth media as blanks. (see Appendix 1 for an example snapshot plate template)
- b) Follow the steps described in Section 3.2 to take a snapshot OD reading of the plate
- c) Export the data and subtract the average media blank OD value from the sample ODs to generate reduced ODs.
- d) For each sample, record the dilution level that resulted in a reduced OD value close to, but not more than zero. This is the “<LOD” dilution level to be used during the growth curve experiments.

3.4 Growth Curve Readings

Growth curves can take multiple days to complete, sign up for the plate reader a few days ahead of your experiment to ensure its availability. This procedure requires that pre-grown bacterial cultures in liquid media broth are prepared in advance, and that prior work has been done to determine the typical CFU/mL of these overnight cultures, and the limit of detection for the cultures in the Synergy H1 reader. This preliminary work is described in Section 3.3. Additional information regarding the Synergy H1 plate reader and Gen 5 v2.09 software can be found in their respective documentation (see References).

- 1) Follow the steps to prepare a protocol as described in Section 3.1 with the following modifications for growth curve setup:
 - a) After setting the temperature (optional) as described in Section 3.1.4.d.iv:
 - i) In the left side panel, click **Start Kinetic** > **OK** to open the Kinetic step controls.
 - (1) Set the timeline for the growth curve (**Total Time** – the total time of the growth curve, and **Interval** – the frequency of reads), then click OK
 - (2) An example procedure is taking reads every 10 minutes (**Interval:** 00:10:00) for 24 hours (**Run Time:** 24:00:00). Set these parameters appropriately for the planned experiment. Preliminary growth information for the organism will help determine the time settings required.
 - (3) The **Run Time** should be set for longer than is necessary, it can be stopped early if needed.
 - ii) Then add the **Shake** (optional) and **Read** parameters as described in Sections 3.1.4.d.v-3.1.4.d.vi.
 - (1) The shake and read parameters must appear in between the “Start Kinetic” and “End Kinetic” in the protocol timeline shown. If they are not, they can be dragged there with the mouse.
- 2) 96-well Plate Setup
 - a) For each isolate, take a single colony from a fresh agar media plate and inoculate into 5ml fresh BHI broth (or appropriate liquid media for your organisms).
 - i) 96 deep-well plates can be used for high throughput overnight cultures.



- b) In a 96 deep-well plate, serially dilute each overnight culture 1:10 in growth media to the <LOD dilution level previously determined (see Section 3.3). Then further dilute the cultures in neutral buffer to an expected 10^3 CFU/mL as determined by the preliminary work. The final volume of all dilutions should be at least 900 μ L.
 - c) Transfer 200 μ L of each <LOD dilution culture to the 96-well growth plate in duplicate. Extra wells should be reserved for any positive or negative controls and sterile-media only (Blank) wells, all of which should be included in duplicate.
 - d) Do not write anything on the 96-well growth plate or lid, as it will interfere with the readings.
 - e) Keep a record of the sample layout in the growth plate. If a **Plate Layout** was setup in the **Protocol**, ensure the samples are inoculated according to the template defined there.
 - f) To minimize positional bias, consider randomization of the position of the samples in the plate, especially if any subsequent biological replicate experiments will be performed.
 - g) To verify the original inoculum culture concentration, keep the 96 deep-well plates containing the diluted cultures on ice until after the growth curve has been started, then spread plate or spiral plate 50 μ L of each 10^3 CFU/mL culture dilution in duplicate on the appropriate enumeration plating media for the sample organisms. Incubate and enumerate to verify expected original culture CFU/mL. (See FSL SOP [5.3.5-Eddy Jet 2 Spiral Plater and SphereFlash Colony Counter](#))
 - h) Cover the growth plate with the lid and bring it to the plate reader.
- 3) Starting the Growth Curve
- a) At the Synergy H1 computer, select **Read Now > Existing Protocol** in the task manager window of Gen5 and find the protocol prepared in section 3.1.
 - i) If a temperature was set, the plate reader will begin to warm. To start the procedure before reaching the target temp, click **Override**.
 - b) Click the Green Arrow on the top bar to start the experiment procedure. A message to “Load plate” should appear on screen and the plate tray should extend if it is not already.
 - c) Load the plate onto the plate reader tray. Make sure to place the A1 plate corner in the indicated tray corner with the notch (back right when facing the machine, also labeled A1). If the tray is not open, press the button next to the power switch on the Synergy H1. Then press OK on the prompt.
 - d) The plate tray should retract and close. If the internal temperature has not reached a defined setpoint, a prompt may appear asking if the readings should wait until the setpoint has been reached. This can be accepted or declined based on the user preference.
 - e) The run will then initiate. In the Plate 1 window, the well matrix charts should populate with the first OD data points as each well is read. After the end of the reading, carefully check these charts to ensure that each well has been read as expected. The first OD readings for each well may appear to vary widely, but will even out as additional readings are taken.
 - f) Leave a note at the plate reader indicating your netID, organism info, and the time/date of growth curve start and end.
 - g) Plan to be present for the final read timepoint, and periodically check the curves during the run to monitor progress and look for any potential issues.



- h) If the growth curve plots for all wells appear to be in death phase, the read can be manually stopped without loss of data by clicking abort.
- i) When the read has stopped, manually export the data and well curves by selecting **Plate > Export** from the top menu. An Excel file containing the raw read data will open and should be saved.
- j) Data may also automatically export if the protocol is appropriately configured, but data should always be saved manually.
- k) The curve plot matrix can be optionally saved by clicking the button next to “Edit Matrix”. This will open an excel file of the plots, which can be saved for analysis.

3.5 Data Analysis: Excel and/or R

Data analysis will vary based on the goals of the experimenter. The following are suggested options for basic analysis.

- 1) Open the exported data to find the raw data for the run. There should be a matrix near the top of the file with the raw readings for each well, organized A1 to H12. Blanked data and additional transformations are included, if they were calculated.
- 2) Copy the raw data into an Excel file for further analysis, or export wherever else is desired.
- 3) For growth curves determine the information needed from the data based on the experiment. Typical analysis may include determination of:
 - a) Lag-phase duration
 - b) Maximum (intrinsic) growth rate
 - c) Carrying capacity (max cell density)
 - d) Formula for cell density at a given time-point in the growth curve
- 4) Plot the data for the individual wells in Excel or R to visually interpret data.
- 5) The R-package “growthcurver” is available for use for plotting and analysis of the growth curve data, with an example analysis described in this 2020 FSL publication:
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7054086/>
 - a) Other analysis tools are available and may have been developed since the preparation of this SOP. A search of recent publications can assist in finding these resources.



SECTION 4 TROUBLESHOOTING

Refer to the Synergy H1 Instructions document and the Gen5 User's Guide for assistance troubleshooting issues. Read these documents before attempting any maintenance. A comprehensive understanding of the system is necessary to fix the correct problem.

- 4.1 **Issues determining the preliminary data:** If preliminary enumeration plates were not able to be counted (too many or too few colonies) and/or if the snapshot plate does not provide a definitive dilution level below the Synergy H1 limit of detection, the initial CFU/mL of the inoculum cultures may have been higher or lower than typical. Regrow the inoculum cultures and use lower dilution levels or do additional dilutions to be used for the plating and snapshot. Additionally, some bacteria tend to form aggregates at the air-liquid interface, which may also influence the results. Be sure to take note if you see obvious clumping of bacterial cultures following growth in the synergy plate reader.

Furthermore, if the reduced ODs in the preliminary determination of the <LOD dilution are not below zero, even at the most diluted level, you will need to perform an additional 1:10 dilution for that sample.

- 4.2 **Experiment duration ended before all samples had reached death phase.** If the set duration of the growth curve was not long enough to capture the full curve for all isolates and the reader ended the procedure, the data for the plate should be exported and saved and then the protocol should be immediately restarted to continue the reads. Record the time between the last read of the first run and the first read of the second run so the curve data can be aligned during data analysis.



SECTION 5 REFERENCES

Gen5™ & Gen5 Secure User's Guide: Microplate Data Collection & Analysis Software. BioTek® Instruments, Inc. March 2007. © 2006-2007. PN 5321001. Revision D

IQ/OQ/PQ Qualification & Maintenance Procedures: Synergy H1™ Hybrid Multi-Mode Microplate Reader All Models. BioTek® Instruments, Inc.

Synergy™ H1: Hybrid Multi-Mode Microplate Reader Instructions for Use. BioTek® Instruments, Inc. June 2012. © 2012. PN 8041005. Revision B



SECTION 6 METHOD VERSION & CHANGES

VERSION	DATE	EDITOR	COMMENTS
Version 1	02/21/2014	Matthew Stasiewicz	Original SOP
Version 2	04/26/2021	Jordan Skeens	Updated to 04/2020 SOP template Complete overhaul of SOP to make a general Synergy H1 user protocol, with additional information for growth curves. Substantial changes made to update and expand content throughout.
Version 3	01/13/2022	Sophia Harrand and Rachel Cheng	Updated the volume from 300 μ L to 200 μ L throughout the SOP. Added comments about the 'edge effect' and recommendation to not use the outer wells of the plate when setting up growth experiments.