



Growth Curves: Synergy H1 Microplate Reader

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SECTION 1 INTRODUCTION

1.1 Purpose

The purpose of this document is to set forth *standard* guidelines for measuring bacterial growth over time in 96 well plates using the absorbance capabilities of the Synergy H1 Hybrid Mult-Mode Microplate Reader. Basic analysis is also described.

1.2 Scope

This SOP applies to activities within the Cornell Food Safety Lab (FSL), Laboratory for Food Microbiology and Pathogenesis of Foodborne Diseases, and the Milk Quality Improvement Program (MQIP)

1.3 Definitions

This protocol refers to components of the Synergy H1 plate reader using the terminology of the protocol author, generally informed by the user guides. Some potentially confusing terms are:

1.4 Safety

Manually loading the AutoPlate 4000 with samples requires working with open tubes of

SECTION 2 MATERIALS

- **Costar 3370 96-well Assay Plate** Sterile, clear plates, with lid
- **MicroAmp Optical Film** or other optical quality adhesive film to seal well
- **Multichannel Pipette (P200); P1000, P200, P20, as appropriate.**
- **Growth Media**
- **Bacterial Cultures**

SECTION 3 PROCEDURES

Valid growth curve information requires proper setup of the plate reader, inoculation of the 96-well plate in a way that minimizes biases, and appropriate data analysis. All three of these sections will be described below

3.1 Plate Reader Setup: Gen5 Software

The following operations are required BEFORE preparing a 96-well plate

1. **Develop a Gen5 Protocol to run the experiment.** A Protocol contains instruction on the Procedure the machine will execute and information about the samples required for data analysis. One the Procedure and Export are required, the Layout and Data Reduction are tools for data analysis.

Create a new protocol or open an existing and modify for your purposes

- a. **Procedure step:** Contains the plate reading instructions, written as a small program. The Kinetic step is a loop. Kinetic steps can be Discontinuous, meaning the plate is read, removed, then read again at a later time.
 - b. A basic Procedure:
 - i. *Temperature* – 37°C; Set to desired ranging 4C° above ambient to 45°C
 - ii. *Kinetic* [24:00:00, 00:20:00; overall time, time to repeat loop; hh:mm:ss]
 1. *Shake* [linear/orbital/double orbital, time 20s]
 2. *Read all A600*; specify wavelength and wells
 - iii. *End kinetic loop*
 - c. **Define the Plate Layout**, for use in data processing. Options are
 - i. Blanks – BLK; wells whose OD will be subtracted from other readings
 - ii. Samples – SPL#; wells with unknowns and/or conditions to be read
 1. Matt suggests only using SPL and BLK, other options are difficult
 - iii. Sample control – SPLC#; hard to use option for sample specific controls
 - iv. Standards – STD#; hard to use option for standard curves
 - v. Use the Layout screen to assign samples and blanks to well. At this stage only define where different samples will be, do not worry about their names
 - d. **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
 - i. Matt suggests defining a step to subtract the average blanks.
 - e. **Export** sets the options for exporting data. I prefer to export to Excel, grouping data by columns.
2. **Create a new experiment based on the desired protocol.** This step creates a file where data is stored about a specific plate that is handled according to the protocol.
 - a. Use Gen5 options to create a new experiment based on the chosen protocol
 - b. Enter the sample names for the specific plate, e.g. SPL1 contains X1-001

- c. Save the experiment with an appropriate name, e.g. BHI gth 37°C.
 - i. Be sure to save the experiment to the default, ‘All Users’ path. Doing so ensures access to the protocol and experiment file if another user is logged on to the computer. This is critical for allowing the machine to track discontinuous experiments properly.
- d. *Turn ON the temperature control, if needed.* Click in instrument control button, navigate to options, turn the temperature to ON and verify the setpoint.

3.2. 96-well Plate Setup: Benchwork

1. Pre-grow bacterial cultures to appropriate conditions, e.g. overnight in BHI at 37°C.
 - a. You can grow cultures in 96 deep-well plates for high throughput
2. Fill a 96-well Costar plate with appropriate growth media. A multichannel P200 in the biosafety hood saves time and ensures sterility, respectively.
3. Inoculate duplicate wells with bacterial culture, leaving room for a negative control and a sterile-media only well.
 - a. Basic example

	1	2	3	4	5	6	7	8	9	10	11	12
A	SPL1	SPL1	SPL2	SPL2	SPL3	SPL3	SPL4	SPL4	SPL5	SPL5	SPL6	SPL6
B	SPL7	SPL12
C	SPL13	SPL18
D	SPL19	SPL24
E	SPL25	SPL30
F	SPL31	SPL36
G	SPL37	SPL42
H	SPL43	SPL47 NegCont	SPL47 NegCont	BLK blank	BLK blank

- b. Consider inoculation on ice and randomization of the position of the strains in the plate.
4. Cover the plate with optical film. Take care to seal each well.
5. Load the plate onto the plate reader. Make sure to remove the plastic cover and to place the A1 corner in the indicated corner (back right, facing the machine)
6. Press the Green Arrow to start the experiment procedure. The experiment must still be loaded into the Gen5 software.
 - a. If the experiment is discontinuous, pressing the arrow will prompt to take an additional reading for a previous plate, or start a new plate. Take care to pick the appropriate option.

7. At the end of the run, manually export the data. Data may auto export if the protocol is appropriately configured.

3.3. Data Analysis: Gen5 Software

The following operations are for after a plate of data has been collected

1. Copy the well curves from the plate layout into another document, if desired.
2. Blank the data. Use the Data Reduction feature to subtract the average reading for the blank well from the readings of the other well. This is calculated for each time step.
3. Option possibly useful analyses
 - a. Calculate onset times, the time to reach a given OD, if desired.
 - b. Calculate the max and min readings, if desired.

3.4. Data Analysis: Excel and/or R

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.
 - a. Matt has a script to automatically plot the data in R and calculate the 4-fold increase times.
3. Plot the individual wells to visually interpret data.
4. Calculate the time for cells to quadruple, i.e. a 4-fold increase.
 - a. Roughly, this is a 4-fold increase in OD from the minimum value. It should be adjusted by the slope of the line relating $\log(\text{OD})$ to $\log(\text{CFU/ml})$. Matt has measured this slope for 20 strains, and found it to be, on average 0.9. Therefore, for a $\log(4\text{-fold})$ increase in CFU/ml, you need a $\sim 0.9 \cdot \log(4\text{-fold})$ increase in OD.
 - b. If you are using new strains, first develop your own calibration curve relating $\log(\text{OD})$ to $\log(\text{CFU/ml})$. Matt suggests filling a costar plate with 100 ul in rows B-H, all columns. Then add 300 ul of overnight culture to row A, duplicate for strains. Then make 2-fold serial dilutions through row H. Read A600 on spec. Plot and fit lines.

SECTION 4 TROUBLESHOOTING

This guide is meant to complement the User Guide with experience from resolving FSL specific problems, it is NOT a comprehensive troubleshooting guide. Always consult the User Guide for problems not listed here. Read the User Guide before attempting any maintenance. A comprehensive understanding of the system is necessary to fix the correct problem.

SECTION 5 REFERENCES

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

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