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Microtiter plate microbial growth measurements

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

This method and accompanying software are intended for research use only.

Abstract

Automated microtiter plate growth kinetics measurements for microorganisms are known to be subject to significant bias due to several factors, including variation in initial optical density (OD) of growth curves, improper background subtraction, and variation in background OD for uninoculated media. We have devised a method with accompanying Matlab LiveScript software to help facilitate accurate measurements for microbial growth parameters. The method employs an automated microtiter plate reader with 96 well microtiter plates having a final volume of 200 uL of growth media per well. The method and software can be used for estimating maximum growth rate, lag time, and maximum OD readings. Serial dilutions of cells are prepared within the plate and multiple uninoculated background wells are used so various sources of error can be both estimated and minimized. The Matlab LiveScript software, ProcessMicroplate, generates output data including comma-delimited spreadsheet files and graphs showing maximum growth rate, doubling time, minimum and maximum OD for the growth curves, along with standard deviations for selected replicate data in rows or columns.

Overnight culture preparation

18h 5m

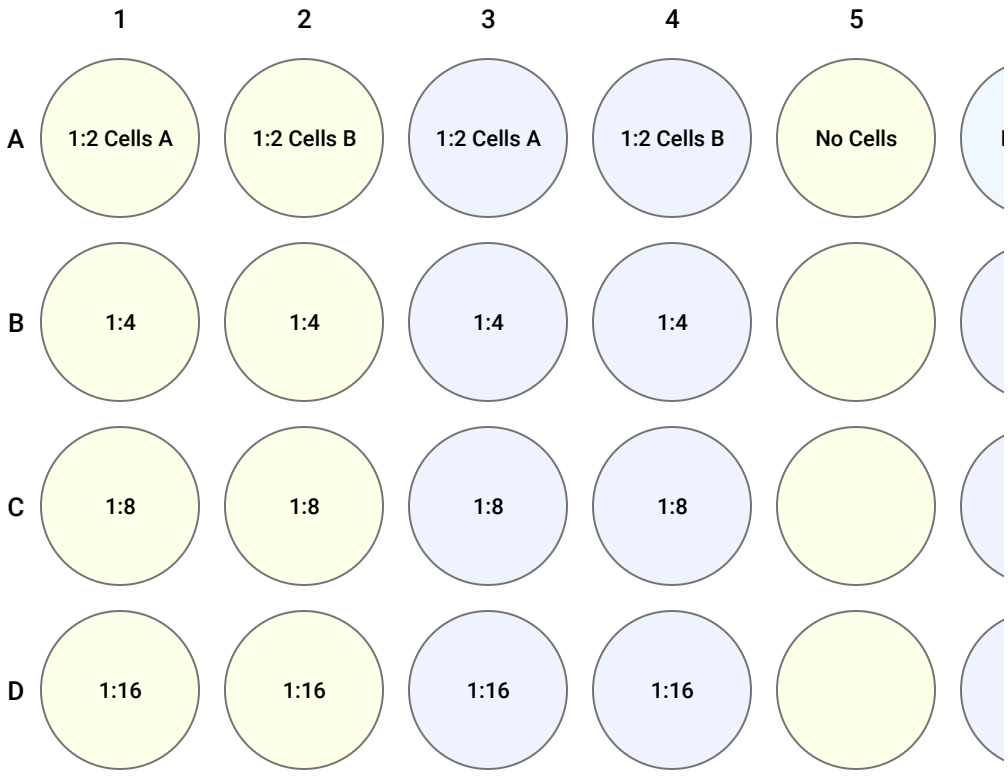
1 Introduction and objective:
The objective is to determine bacterial growth rates using optical density (OD) in a 200 µL microtiter plate (flat bottom). Because initial optical density can influence growth kinetics, serial dilutions may be done within the plate to help identify a minimum optical density to use for calculating the kinetic parameters. A Matlab Script (ProcessMicroplate.mlx, see below) can be used to compare growth kinetic parameters between treatments on a microtiter plate and view graphs of the results.

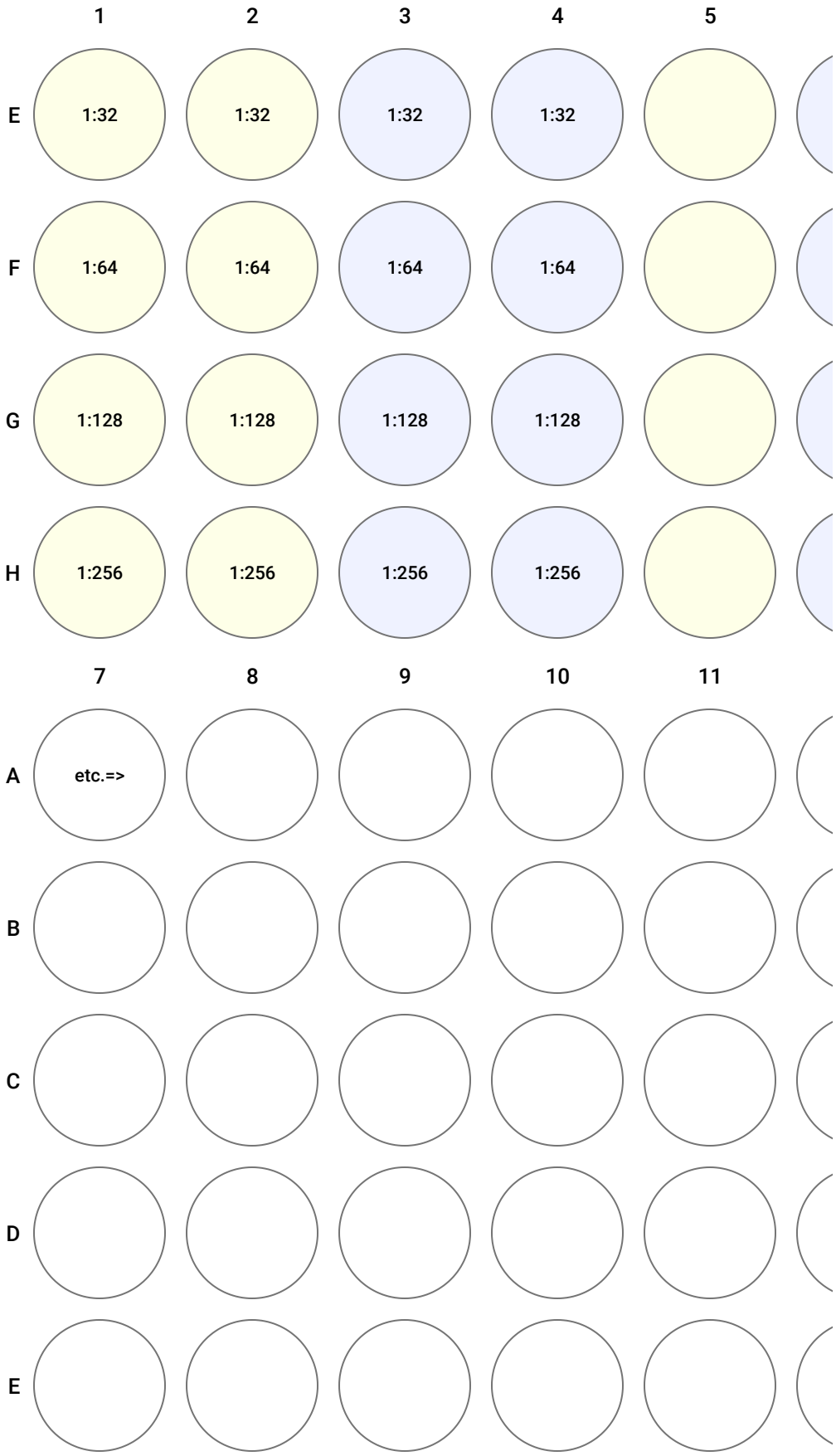
2 18h

Prepare microbial culture(s), typically 5 mL in a 15 mL plastic screw cap tube, one tube per biological replication (rep) of a culture. Use media that fully support culture growth. not necessarily the media to be used for measuring for growth rates. Typical growth conditions:
30 °C Lactic acid bacteria, yeasts 37 °C Pathogenic bacteria

Microtiter plate preparation in a sterile biological-containment hood (air-curtain hood)

3

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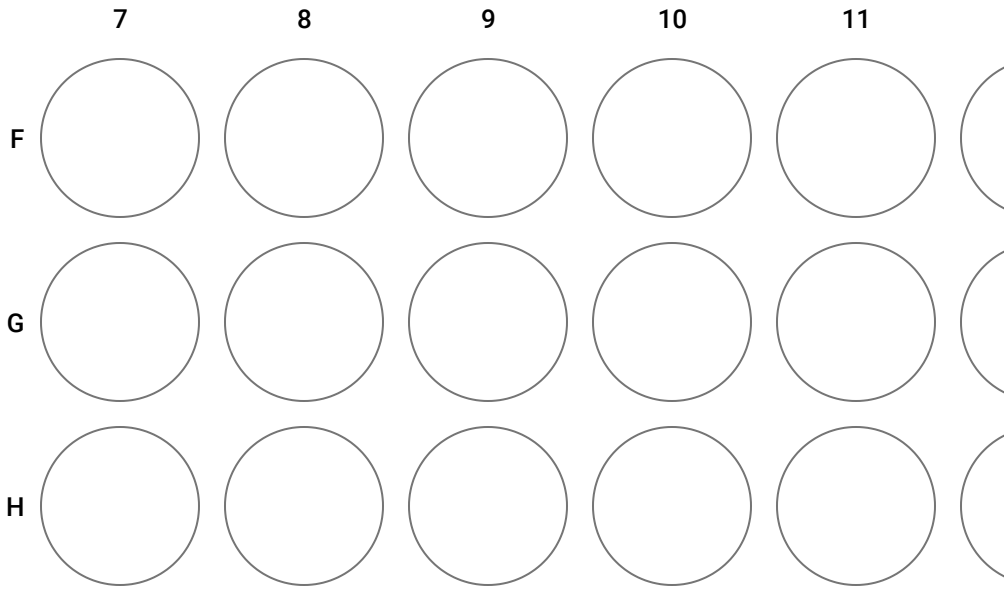

4 Prepare microtiter plate according to the example above (Step 2) with growth medium (colors) as shown above. Add additional columns as needed for multiple treatments or replicates. Pipet 100 µL of appropriate media into all wells according to the chosen setup prior to the addition of cells (Step 6). Include one column for each biological rep and growth medium type combination, as well as one an additional control column for each growth medium type. Multiple control wells will be used to determine a mean value for the background optical density (with no cells) for each type of medium. The columns with cells will be used for serial 1:2 dilutions (as indicated by the numbers for rows A - H) that can be done simultaneously with a multichannel pipettor. 30m

Inoculum preparation 5m

5 Vortex or mix the overnight cell cultures to assure cells are evenly suspended, then remove 0.5 mL of each culture and transfer to a microcentrifuge tube. Prepare one microfuge tube for each biological replication (A and B in example plate above) and media type (colors in example above) combination. In the example plate above, this would mean 4 microcentrifuge tubes (two for the yellow medium/reps A and B; blue medium/reps A and B). Centrifuge to pellet cells and decant the supernatant without disturbing the pellet. 5m

13000 rpm, 22°C , Room temperature centrifugation, microcentrifuge at full speed.

6 Resuspend cells by vortexing, using 1 mL (an initial 1:2 dilution of the cells) of the medium to be used for measuring growth rates for that culture. Cells should be immediately used by adding to a pre-prepared microtiter plate (Step 2). The cell culture dilution series depends on cell concentration, and further dilution may be necessary. The objective is to have 10m

the optical density of the final 1:2 serial dilution approximately equal to the optical density of the media with no cells.

Microtiter plate inoculation

20m

7

Note

While any configuration of treatments may be used for processing microbial growth kinetics, the suggested methods below have advantages:
1. Multiple control wells (media + no added cells) are used to generate mean values for subtracting background OD from cell count growth curve data points. To make these values relevant, there should be a control column for each media type being used.
2. The serial dilutions of initial OD for cells in the microtiter plate wells can allow users to determine how the initial optical density value for a growth curve influences other growth parameters, including: growth rates, lag time, and maximum OD.
3. Contiguous groups of microplate wells with replicate data can be easily grouped for displaying the mean values and standard deviations when analyzing the data

For each biological replication, the cultures are resuspended in growth medium, adding 100 µL to the appropriate wells in *ROW A ONLY* of the microtiter plate (already containing 100 µL of the corresponding growth medium). This results in a 1:2 dilution of the cells as indicated in Step 2 and a final volume for Row A with cells of 200 µL. For the control columns, 200 µL of the appropriate medium are added to ALL ROWS, giving 200 µL final volume. No dilution series is needed for control wells.

8 Using a multichannel pipettor, insert the tips into the wells of ROW A containing cells (with 200 µL total volume), and surge up and down two times to mix the cells and media. Do not completely expel the liquid so as not to introduce any air bubbles. Then remove 100 µL and transfer that to ROW B. Repeat the mixing and transfers for all rows. Finally remove the last 100 µL from ROW H. The result is that all wells with cells should have 100 µL of cell suspension with a series of 2-fold dilutions from ROW A to ROW H.

5m

9 With a multichannel pipettor add an additional 100 µL of the appropriate medium to each of the cell-containing wells. This makes another 1:2 dilution in every cell-containing well. Alternate formats for setting up microtiter plates with serial dilutions may be used, however, ***the objective is to dilute the cells so the last row (ROW H) has an optical density that is the same as a corresponding background media well (control, no cells).***

5m

10 Overlay all wells with 75 µL of sterile mineral oil using a multichannel pipettor. Mineral oil is essentially optically clear and prevents evaporation of the media during growth of the cells. Inspect plates for air bubbles and remove any if necessary with a microliter pipettor. 5m

Note

Air bubbles are the enemy of this procedure. They will interfere with optical density when the plate is incubated. Carefully examine the plate (lid on!) to determine if there are any air bubbles visible. Holding the plate over a dark surface may be helpful. If bubbles are observed, they may be removed using an inoculating needle or sterile syringe needle. With a flamed needle or a sterile syringe needle, lightly touch the bubble to pop it. Be careful not to contaminate the plate.

Cell growth and automated optical density data 2d

11 Place the microtiter plate into an automated microtiter plate reader (e.g., we have a BioTeK Epoch 2, BioTek Instruments, Inc., Winooski, VT). Set the reader to record optical density, not absorbance. Optical density is typically recorded at 600 nm for bacterial cells. Most commercial automated microtiter plates can record data at selected time intervals (typically every 15, 30, or 60 min for bacterial growth) while incubating the plate at an optimal growth temperature of the cells that are the subject of the experiment 30 °C or 37 0 °C . To resuspend cells prior to reading, a 5 or 10 sec. shake of the plate can be included in the automated reader program. To obtain kinetic data a complete growth curve through stationary phase is needed. Typically this is 24 to 48 hours for bacteria. 2d

12 Export the optical density data for the 96 growth curves to a comma delimited spreadsheet (.csv) file which can be directly opened in Excel. Data should be formatted in columns with rows corresponding to N time-points, and 97 columns with the first column listing the elapsed time (in hours), and the remaining 96 columns being optical density readings for wells A1-A12, B1-B12, C1-C12 ... H1-H12. The spreadsheet therefore should be Nx97 (N rows x 97 columns), where N is the total number of optical density readings for the experiment. Most microtiter plate readers can export data in this format, or a .csv file can be manually constructed in this format using Excel. An example data file can be found at the link in step 13 for the software. 5m

Data analysis with ProcessMicroplate, Matlab LiveScript software 10m

13 ProcessMicroplate software may be used to conveniently process the data. This program requires Matlab to run. It was built on MATLAB Version: 24.1.0.2578822 (R2024a) Update 2, on 10m

an MS Windows 11 computer. The program runs as a Matlab Live-Script file which includes detailed instructions for data entry and output, and automatically generates tables and graphs of the results. The software and sample data file and results files are available at this link: **ProcessMicroplate Software** . The user should be familiar with Matlab and running Live-Script files. The output data include comma-delimited spreadsheet files and graphs showing maximum growth rate, doubling time, minimum and maximum OD for the growth curves, along with standard deviations for selected replicate data in rows or columns.