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Working

Calibration Protocol - Conversion of OD₆₀₀ to Colony Forming Units (CFUs) - v2

Forked from [Calibration Protocol - Conversion of OD₆₀₀ to Colony Forming Units \(CFUs\)](#)

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ABSTRACT

This procedure can be used to calibrate OD₆₀₀ to colony forming unit (CFU) counts, which are directly relatable to the cell concentration of the culture, i.e. viable cell counts per mL.

This protocol assumes that 1 bacterial cell will give rise to 1 colony.

For the CFU protocol, you will need to count colonies for your two Positive Control (BBa_I20270) cultures and your two Negative Control (BBa_R0040) cultures.

EXTERNAL LINK

<https://2019.igem.org/Measurement>

GUIDELINES

Disclaimer: The 2018 InterLab study found that this protocol gave very variable results. We therefore advise teams treat this protocol with some caution, and encourage them to find ways to improve it.

MATERIALS

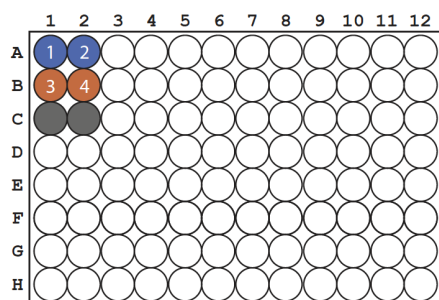
NAME ▾	CATALOG # ▾	VENDOR ▾	CAS NUMBER ▾	RRID ▾
1.5 mL Eppendorf tubes				
96 well plate				
Chloramphenicol (25 mg/ml in EtOH)	View			
LB Broth				
2.0 mL Eppendorf tubes				

BEFORE STARTING

Read through this entire protocol carefully before you start your experiment and prepare any materials you may need. See the "Results" section for an example of a completed data analysis spreadsheet. Please see disclaimer in guidelines section.

Sample Preparation

- 1 This protocol will result in CFU/mL for 0.1 OD₆₀₀. Your overnight cultures will have a much higher OD₆₀₀ and so this section of the protocol, called "Sample Preparation", will give you the "Starting Sample" with a 0.1 OD₆₀₀ measurement.
- 2 Measure the OD₆₀₀ of your cell cultures, making sure to dilute to the linear detection range of your plate reader.
e.g. Add 25 µL culture to 175 µL LB + Chloramphenicol (Cam) in a well in a black 96-well plate, with a clear, flat bottom
- 3 Recommended plate setup is below. Each well should have 200 µL



- A1 - A2: Positive Controls (cultures 1-2)
- B1 - B2: Negative Controls (cultures 3-4)
- C1 - C2: Blank media - 200 μ L of LB + Cam (in duplicate)

- 4 Dilute your overnight culture to $OD_{600} = 0.1$ in 1mL of LB + Cam media. Do this in triplicate for each culture.

Use $(C_1)(V_1) = (C_2)(V_2)$ to calculate your dilutions

C_1 is your starting OD_{600}

C_2 is your target OD_{600} (= 0.1)

V_1 is the unknown volume in μ L

V_2 is the final volume (= 1000 μ L)



Important:

When calculating C_1 , subtract the blank from your reading and multiple by the dilution factor you used.

Example: $C_1 = (1:8 \text{ } OD_{600} - \text{blank } OD_{600}) \times 8 = (0.195 - 0.042) \times 8 = 0.153 \times 8 = 1.224$

Example: $(C_1)(V_1) = (C_2)(V_2)$

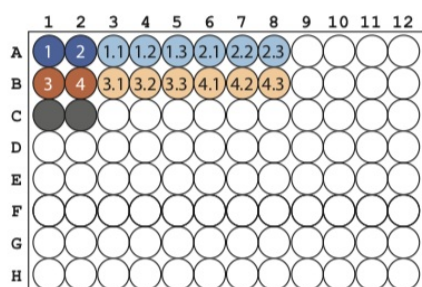
$(1.224)(x) = (0.1)(1000 \mu\text{L})$

$x = 100/1.224 = 82 \mu\text{L culture}$

Add 82 μ L of culture to 918 μ L media for a total volume of 1000 μ L

- 5 Check the OD_{600} and make sure it is 0.1.

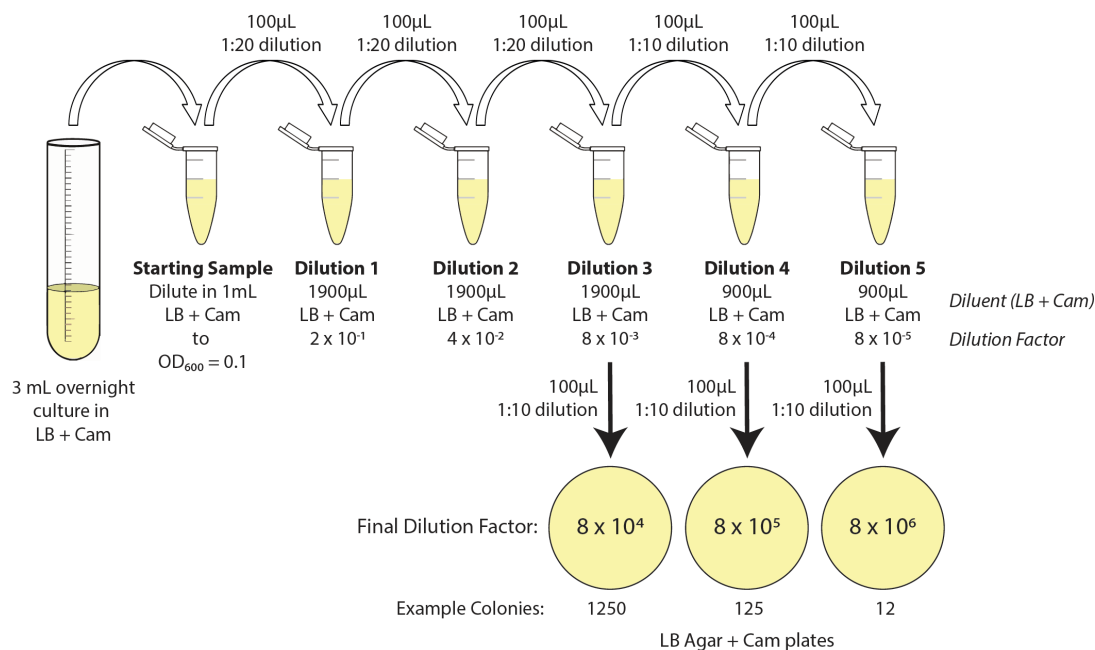
Recommended plate setup is below. Each well should have 200 μ L.



- A1 - A2: Positive Controls (cultures 1-2)
- B1 - B2: Negative Controls (cultures 3-4)
- C1 - C2: Blank media - 200 μ L of LB + Cam (in duplicate)
- A3 - A8: 0.1 Starting Sample Dilutions for Positive Controls (in triplicate per culture, 6 total dilutions)
- B3 - B8: 0.1 Starting Samples Dilutions for Negative Controls (in triplicate per culture, 6 total dilutions)

Dilution Series

- 6 Do the following serial dilutions for your triplicate Starting Samples you prepared in Step 5. You should have 12 total Starting Samples - 6 for your Positive Controls and 6 for your Negative Controls.



- 7 You will need 3 LB Agar + Cam plates (36 total)
- 8 Prepare three 2.0 mL tubes (36 total) with 1900 µL of LB + Cam media for Dilutions 1, 2, and 3
- 9 Prepare two 1.5 mL tubes (24 total) with 900 µL of LB + Cam media for Dilutions 4 and 5
- 10 Label each tube according to the figure above (Dilution 1, etc.) for each Starting Sample
- 11 Pipet 100 µL of Starting Culture into Dilution 1. Discard tip. Do NOT pipette up and down. Vortex tube for 5-10 secs
- 12 Repeat Step 11 for each dilution through to Dilution 5 as shown above
- 13 Aseptically spread plate 100 µL on LB + Cam plates for Dilutions 3, 4, and 5
- 14 Incubate at 37 °C overnight and count colonies after 18-20 hours of growth

CFU/mL/OD Calculation

- 15 Based on the assumption that 1 bacterial cell gives rise to 1 colony, colony forming units (CFU) per 1 mL of an $OD_{600} = 0.1$ culture can be calculated
- 16 First, count the colonies on each plate with fewer than 300 colonies

17 Next, multiply the colony count by the Final Dilution Factor on each plate, or use this Excel spreadsheet:

 [iGEM Data Analysis Template - Plate Reader CFU Calibration - v1.xlsx](#)



Example using Dilution 4 from above:

colonies x Final Dilution Factor = CFU/mL

125 x (8×10^5) = 1×10^8 CFU / mL in Starting Sample ($OD_{600} = 0.1$)

Congratulations!

18 You have now completed this calibration protocol



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