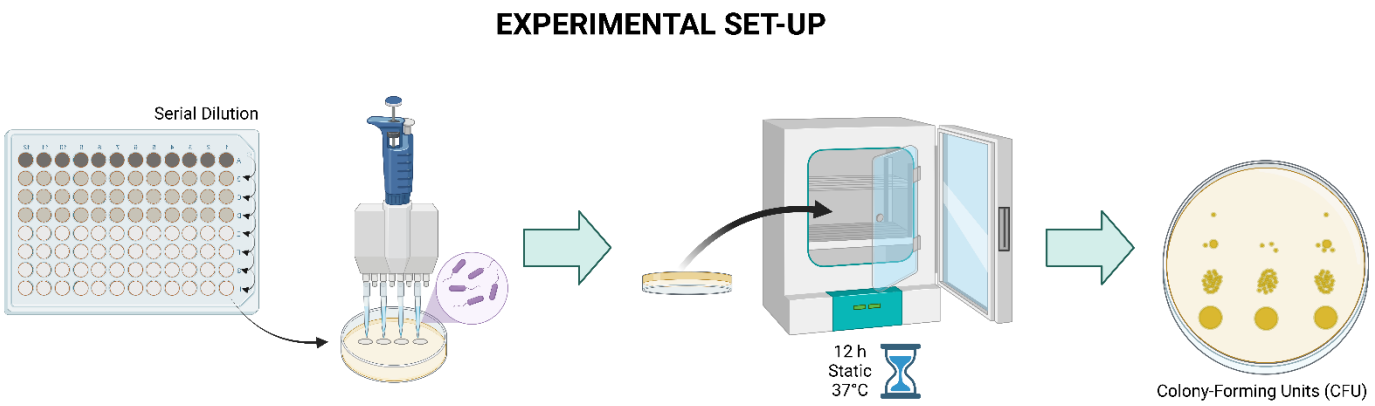
Colony Forming Units (CFU) Assay

This method is an extension of an additional method where the number of colony forming units is to be quantified

Graphical Schematic of Procedure:



Materials:

- DPBS -$CaCl_2$, -$MgCl_2$ (Gibco Cat. No. 14190-094)
- Pipette tips
- multichannel pipette
- 96-well plate with lid (round bottom)
- LB agar plates

Work under sterile conditions

Methods:

Day 1 → Fill a 96-well plate with 180µL PBS (-,-) buffer using multichannel pipette via a reservoir. Add 20µL of incubated compound-bacterial solution that grew from previous day, to row A, each well has a different replicate/compound, and mix thoroughly. Change tips between compounds.

Serially dilute 20µL of mixture from row A to H, changing tips between rows and mixing thoroughly by pipetting up and down at least 10 times per well. May use multichannel pipette. Can throw away last 20µL from row H, to ensure same volume in each of the wells. (See diagram below).

Meanwhile dry LB-agar plates thoroughly on clean bench.

Mark LB-agar plates with a line using marker to be able to orient the lid.

Plate 20µL drops onto LB-agar starting from H to E, can use the same tip for each compound when increasing the dilution. Place lid on top of plate and trace where the droplets were placed onto the lid and label each drop. Carefully allow the drops to fully dry in clean bench, the colonies will become invisible once fully dry. See potential layout of 20µL drops below (Figure 3). Once dry cover and place upside down to grow ON in the incubator at 37°C.

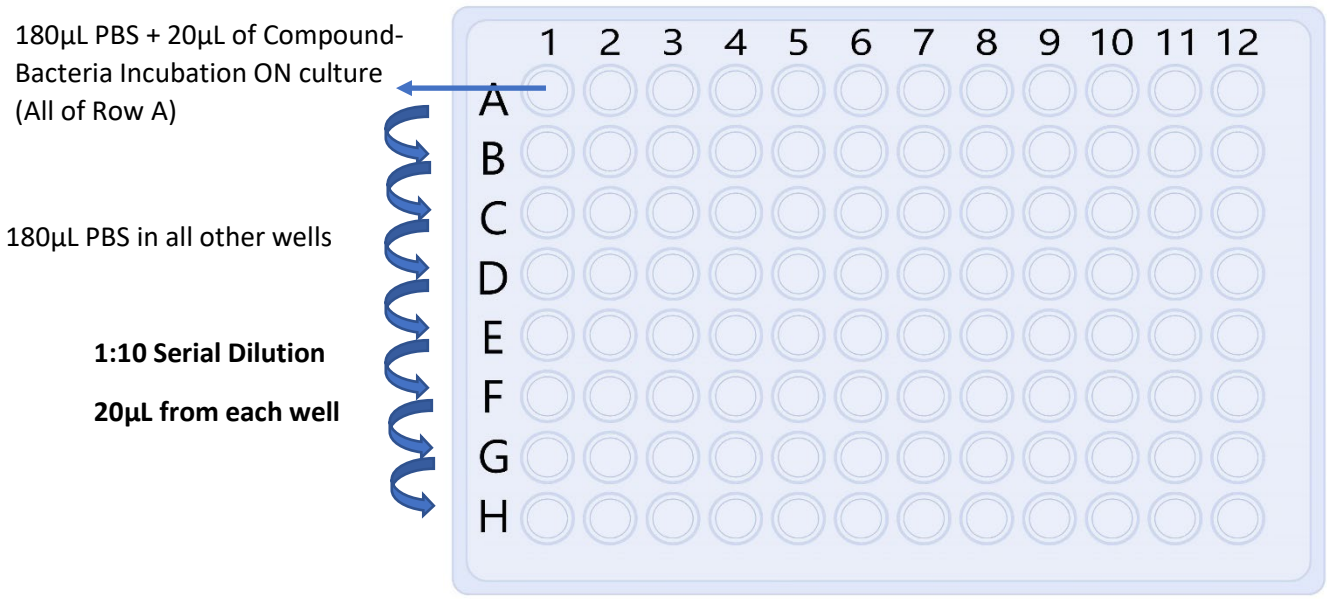


Figure 1: 96-Well Plate Set-Up of Serial Dilution

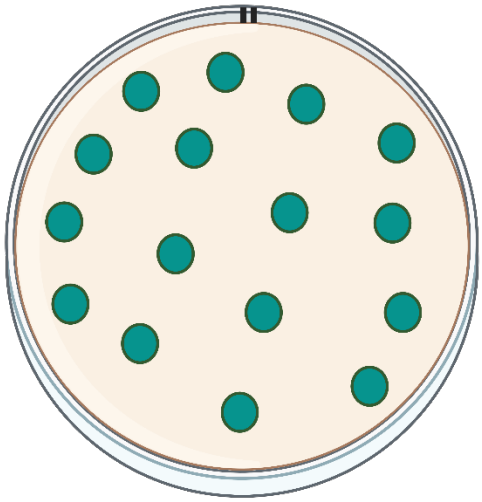


Figure 2: Drop Colony Method for CFU. Each colony is 20µL. A standard LB plate can have between 16-25 20µL colonies.

Day 2 → Using colony counter and marker, count the colonies in each drop and write the numbers down and the corresponding dilution. Record the numbers in lab notebook. Analyze values in excel applying the following formula for the relationship between number of colonies, volume plated (20µL) and dilution factor:

$$\frac{CFU}{mL} = \frac{(number\ of\ colonies\ x\ dilution)}{volume\ plated}$$

Then graph the resulting CFU/mL as bar graphs using standard deviation values as error bars values.

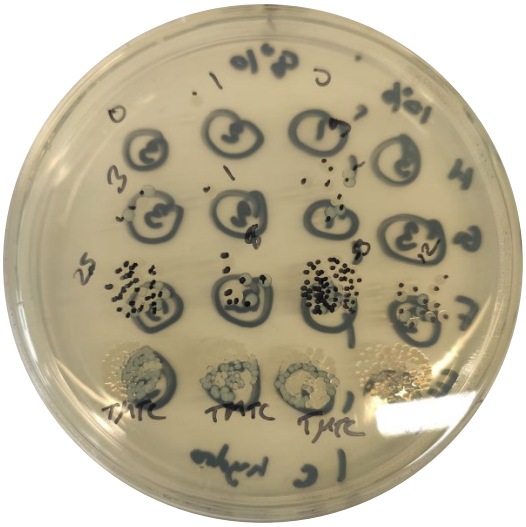


Figure 3: LB Agar plate after colonies have formed and counted with marker