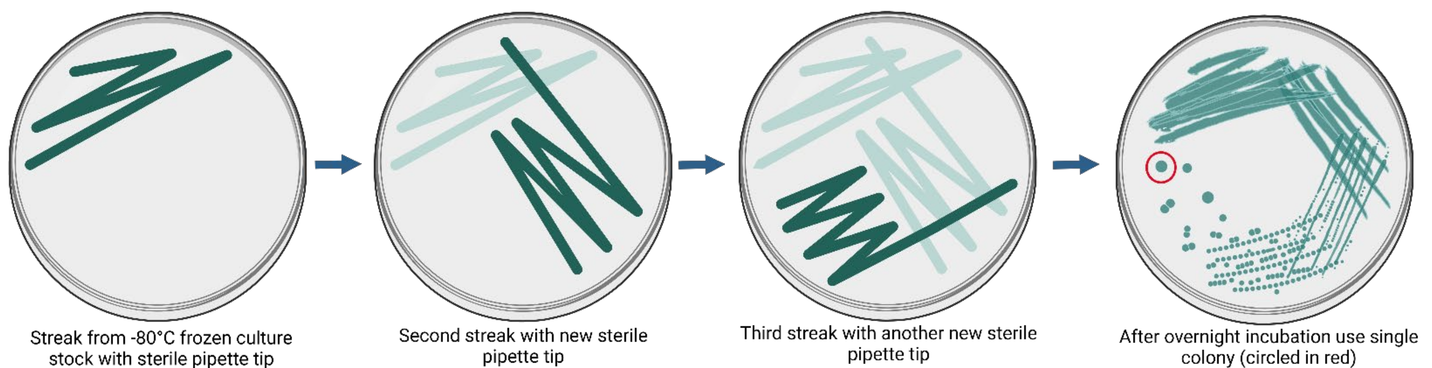
Calibration Curve Protocol for Flow Clear

Materials:

- Sterile Double LB
- Sterile MilliQ water/Evian water
- Sterile LB
- EPOCH Plate Reader
- 96-well plate with lid (round bottom)
- Anti-Fog Solution (Recipe Below)
- LB agar plates

Methods:

Day 1 → Streak out (single-colony method) bacterial strain (*E. coli*) on LB Agar plate as shown below (can use tip instead of toothpick) and allow to incubate for at least 6-8 hours until colonies form at 37°C.



Prepare bacterial culture by inoculating 5mL LB with single colony from plate. Prepare negative control of only LB (3mL). Grow shaking 180-250 rpm at 37°C overnight (ON).

Day 2 → Confirm that the negative control LB is still clear and sterile. Measure OD_{600} of overnight culture in 1:10 dilution (900µL LB + 100µL ON culture), blank with 1mL of LB (can use LB from negative control if clean). Remember to multiply measured value by 10 to get actual OD value.

Dilute to an OD_{600} of 1.0 with a final volume of 1mL using $C_1V_1=C_2V_2$ formula.

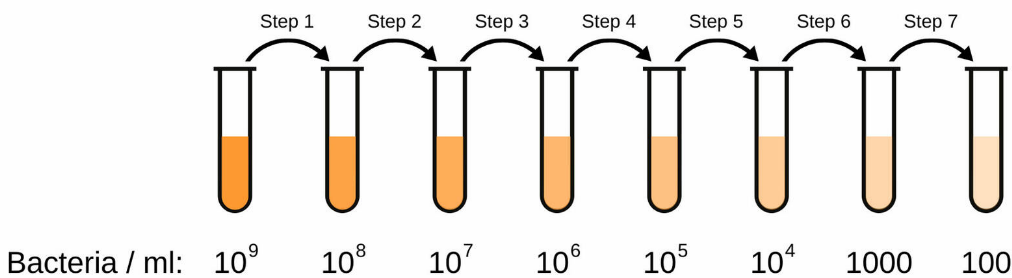
Note: 1.0 $OD_{600} = 2.66 \times 10^9$ *cells/mL of E. coli*

For example, if overnight culture OD_{600} is 2.13 then: $V_1 = \frac{1}{2.13}$ = 0.469mL = 469µL. So, add 469µL overnight culture to 531µL of sterile MilliQ water to get a final volume of 1mL.

In the clean bench, apply antifog solution (0.05% TritonX-100, 20% Ethanol in water) to the inside of 96 well lid, fully cover with solution and pour back the excess solution. Allow the lid to fully dry (or alternatively dry with kimwipes™.

Wash the bacteria by centrifuging the 1mL Eppi at 10,000 xg for 5 min to pellet the bacteria. Discard the supernatant and resuspend in sterile MilliQ water.

Serially dilute in 1.5mL Eppendorf tubes 1:10 in MilliQ water. Prepare eight 1.5mL Eppendorf tubes with 900µL of sterile MilliQ water in them. Label each tube 10^8-10^1. Add 100µL of 1.0 OD_{600} culture (that was just created and label it 10^9) to the tube labeled 10^8 900µL of water, mix and then add 100µL of the mixed solution to the tube labeled 10^7, continue as diagramed below until tube labeled 10^1 is reached. Discard 100µL from the last tube in the dilution series.



In a 96-well plate, apply 100µL of double LB in each well that is to be used for analysis. To the double LB filled wells add 100µL of sample, 3 replicates for each concentration being tested. Don't forget the blank control in triplicate (100µL double LB and 100µL water). For easier analysis use the set-up below in a 96-well plate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	3	1	2	3	1	2	3	1	2	3
B												
C	1	2	3	1	2	3	1	2	3	1	2	3
D												
E	1	2	3	1	2	3						
F												
G												
H												

10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1

Blank
filled with LB to prevent evaporation

Cover plate and place in EPOCH 2 plate reader with continuous shaking (double orbital) at 37°C measuring at OD_{600} in 15 min intervals for 20 hours.

Optional CFU Controls of Dilution Series: Plate the dilution series in 20µL spots on to dry LB agar plates and allow to dry fully before closing lids and storing upside down in the 37°C incubator.

Day 3 → Export data into excel, save and analyze.

Optional: Count, record and analyze the colony number and transform into CFU values.