Calibration Protocols

(1)OD 600 Reference point – LUDOX Protocol

Materials:

1ml LUDOX CL-X (provided in kit) ddH20 (provided by team) 96 well plate, black with clear flat bottom preferred (provided by team)

Method

- ❏ Add 100 µl LUDOX into wells A1, B1, C1, D1
- ❏ Add 100 µl of dd H2O into wells A2, B2, C2, D2
- ❏ Measure absorbance at 600 nm of all samples in the measurement mode you plan to use for cell measurements
- ❏ Record the data in the table below or in your notebook
- ❏ Import data into Excel sheet provided (OD600 reference point tab)

Use the LUDOX CL-X(45% colloidal silica suspension) as a single point reference to obtain a conversion factor to transform our absorbance data from our plater reader into a comparable OD600 measurement as would be obtained in a spectrophotometer.

	A	B	C	D
1		LUDOX CL-X	H2O	
2	Replicate 1	0.068	0.052	
3	Replicate 2	0.063	0.034	
4	Replicate 3	0.057	0.047	
5	Replicate 4	0.070	0.046	
6	Arith. Mean	0.065	0.045	
7	Corrected Abs600	0.020		
8	Reference OD600	0.063		
9	OD600/Abs600	3.190		

(2) Particle Standard Curve-Microsphere

Materials:

300 μL Silica beads - Microsphere suspension (provided in kit, 4.7 x 10^8 microspheres)

ddH20 (provided by team)

96 well plate, black with clear flat bottom preferred (provided by team)

Method:

Prepare the Microsphere Stock Solution:

❏ Obtain the tube labeled "Silica Beads" from the InterLab test kit and vortex

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vigorously for 30 seconds. NOTE: Microspheres should NOT be stored at 0° C or below, as freezing affects the properties of the microspheres. If you believe your microspheres may have been frozen, please contact the iGEM Measurement Committee for a replacement (measurement at igem dot org).

❏ Immediately pipet 96 μL microspheres into a 1.5 mL eppendorf tube .

❏ Add 904 μL of ddH2O to the microspheres .

❏ Vortex well. This is your Microsphere Stock Solution.

Prepare the serial dilution of Microspheres:

❏Add 100 μl of ddH2O into wells A2, B2, C2, D2....A12, B12, C12, D12

❏Vortex the tube containing the stock solution of microspheres vigorously for 10 seconds

❏Immediately add 200 μl of microspheres stock solution into A1

❏Transfer 100 μl of microsphere stock solution from A1 into A2.

❏Mix A2 by pipetting up and down 3x and transfer 100 μl into A3…

❏Mix A3 by pipetting up and down 3x and transfer 100 μl into A4...

❏Mix A4 by pipetting up and down 3x and transfer 100 μl into A5...

❏Mix A5 by pipetting up and down 3x and transfer 100 μl into A6...

❏Mix A6 by pipetting up and down 3x and transfer 100 μl into A7...

❏Mix A7 by pipetting up and down 3x and transfer 100 μl into A8...

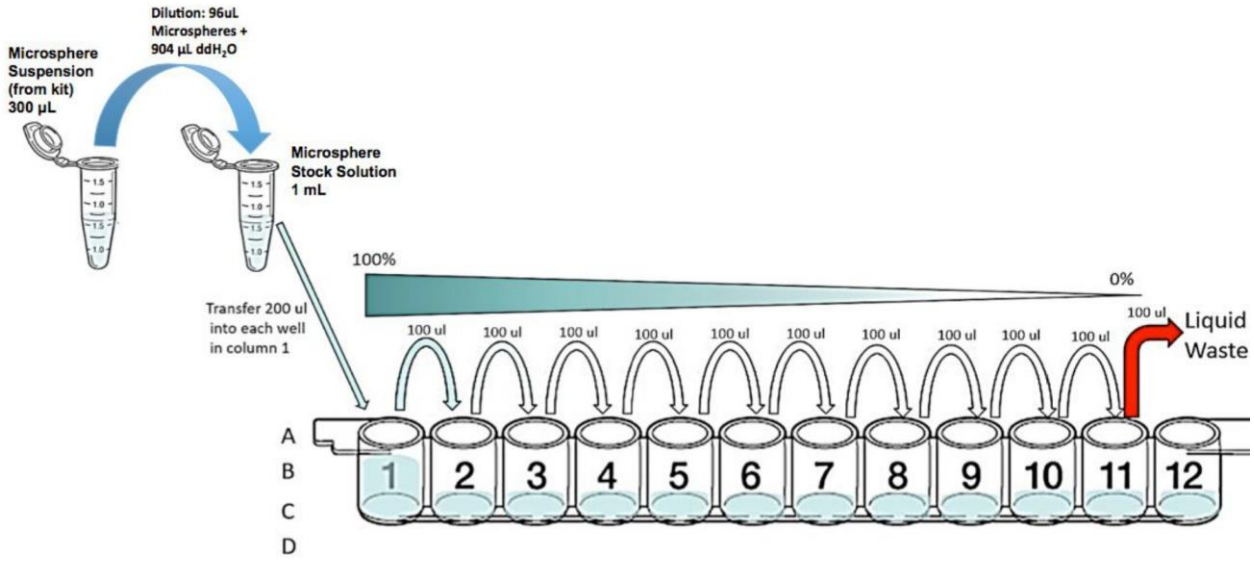
❏Mix A8 by pipetting up and down 3x and transfer 100 μl into A9...

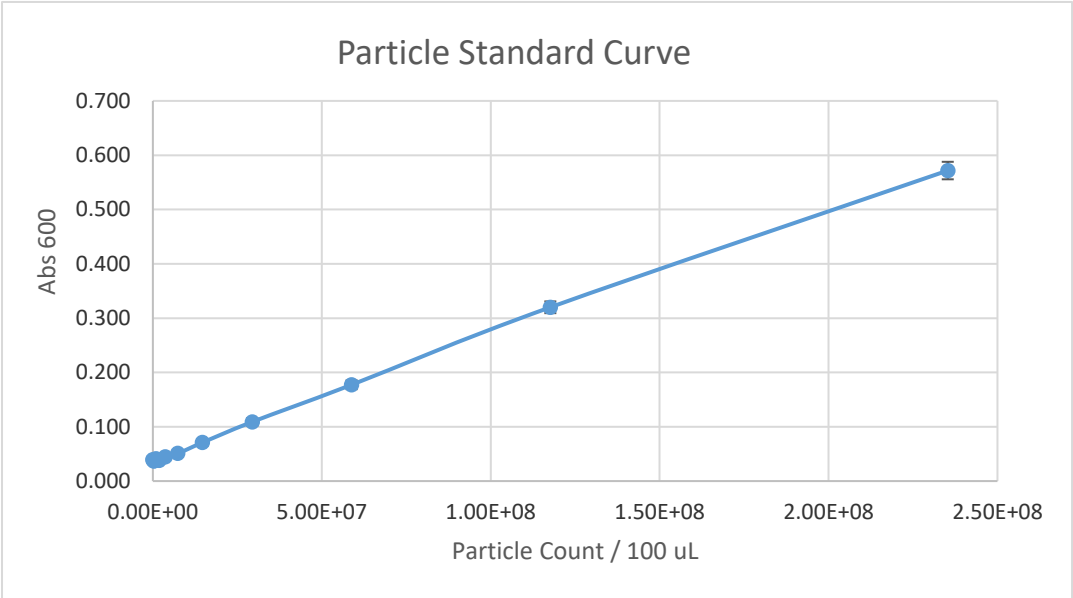
❏Mix A9 by pipetting up and down 3x and transfer 100 μl into A10...

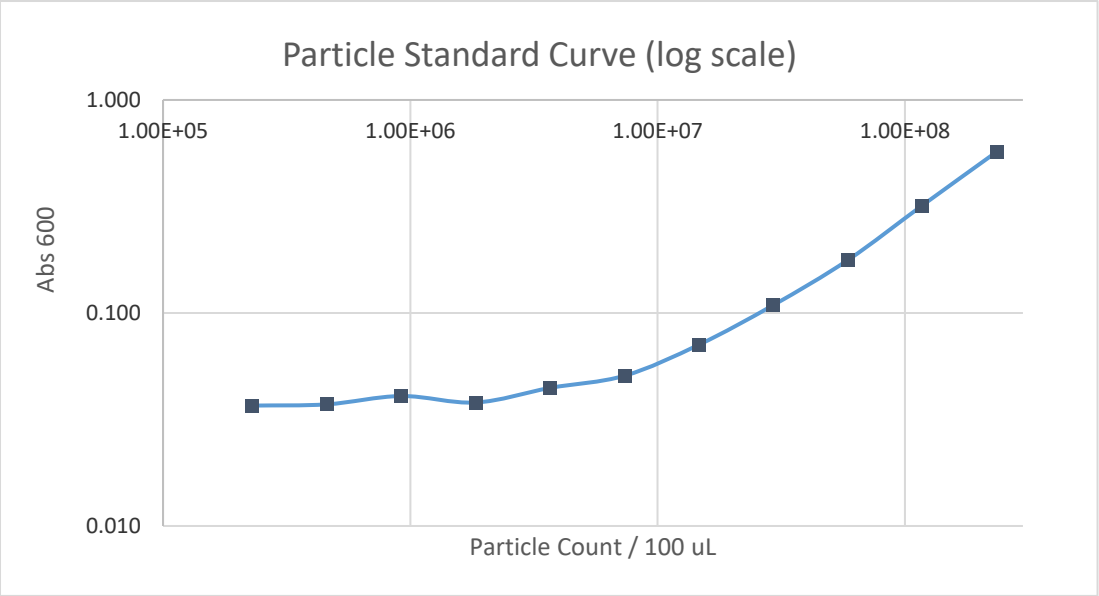
❑Mix A10 by pipetting up and down 3x and transfer 100 µl into A11...

❑Mix A11 by pipetting up and down 3x and transfer 100 µl into liquid waste

❑ Measure Abs 600 of all samples in instrument .







(3)Flurescence standard curve

Materials:

Fluorescein (provided in kit)

10ml 1xPBS pH 7.4-7.6 (phosphate buffered saline; provided by team)

96 well plate, black with clear flat bottom (provided by team)

Method :

Prepare the fluorescein stock solution:

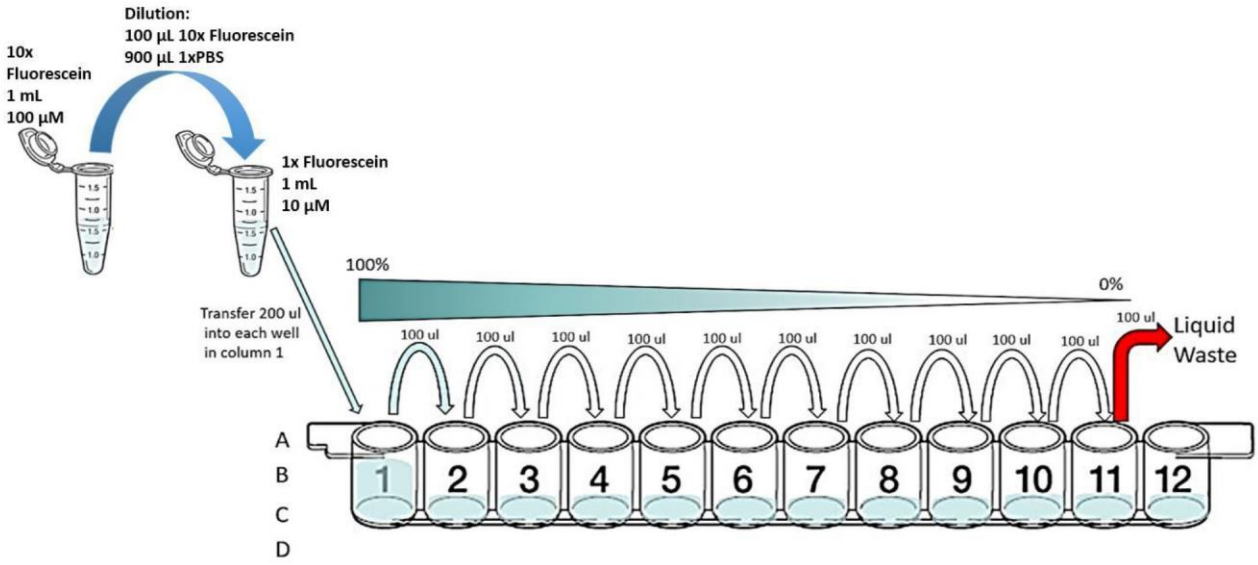
❏ Spin down fluorescein kit tube to make sure pellet is at the bottom of tube.

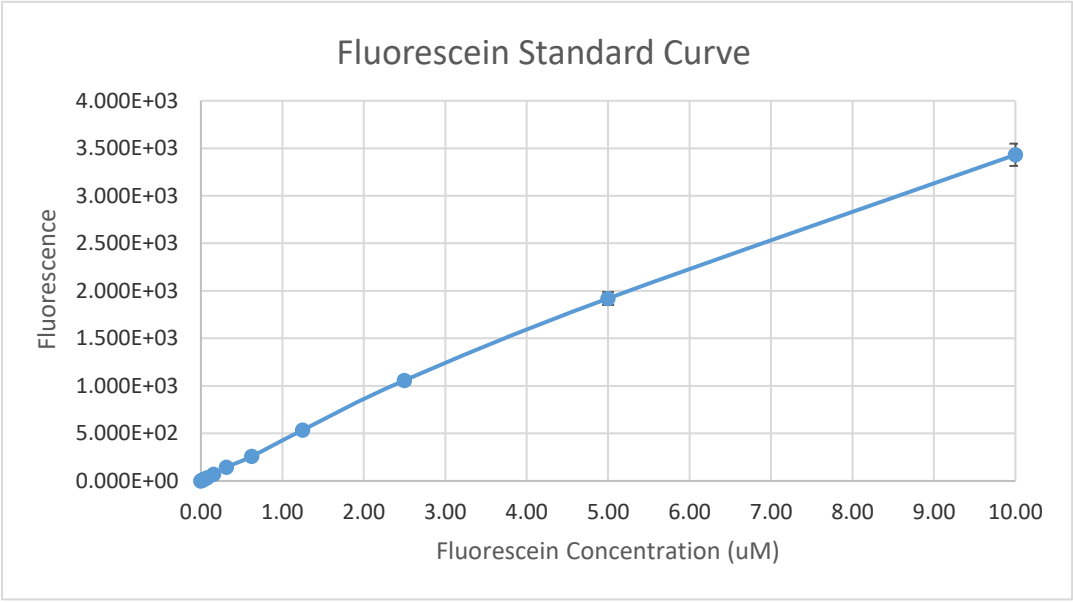
❏ Prepare 10x fluorescein stock solution (100 µM) by resuspending fluorescein in 1 mL of 1xPBS. [Note: it is important that the fluorescein is properly dissolved. To check this, after the resuspension you should pipette up and down and examine the solution in the pipette tip – if any particulates are visible in the pipette tip continue to mix the solution until they disappear.]

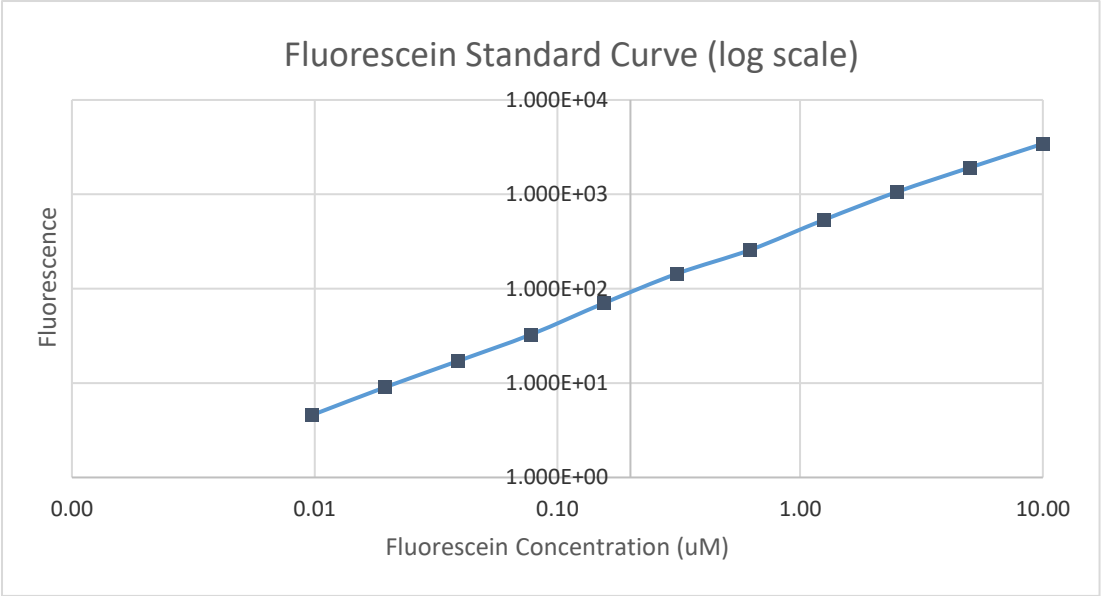
❏ Dilute the 10x fluorescein stock solution with 1xPBS to make a 1x fluorescein solution with concentration 10 µM: 100 µL of 10x fluorescein stock into 900 µL 1x PBS

Prepare the serial dilutions of fluorescein:

- ❑ Add 100 µl of PBS into wells A2, B2, C2, D2....A12, B12, C12, D12
- ❑ Add 200 µl of fluorescein 1x stock solution into A1, B1, C1, D1
- ❑Transfer 100 µl of fluorescein stock solution from A1 into A2.
- ❑ Mix A2 by pipetting up and down 3x and transfer 100 µl into A3⋯
- ❑ Mix A3 by pipetting up and down 3x and transfer 100 µl into A4...
- ❑ Mix A4 by pipetting up and down 3x and transfer 100 µl into A5...
- ❑ Mix A5 by pipetting up and down 3x and transfer 100 µl into A6...
- ❑ Mix A6 by pipetting up and down 3x and transfer 100 µl into A7...
- ❑ Mix A7 by pipetting up and down 3x and transfer 100 µl into A8...
- ❑ Mix A8 by pipetting up and down 3x and transfer 100 µl into A9...
- ❑ Mix A9 by pipetting up and down 3x and transfer 100 µl into A10...
- ❑ Mix A10 by pipetting up and down 3x and transfer 100 µl into A11...
- ❑ Mix A11 by pipetting up and down 3x and transfer 100 µl into liquid waste
- ❑ Repeat dilution series for rows B, C, D
- ❑ Measure fluorescence of all samples in instrument
- ❑ Record the data in your notebook
- ❑ Import data into Excel sheet provided (fluorescein standard curve tab)

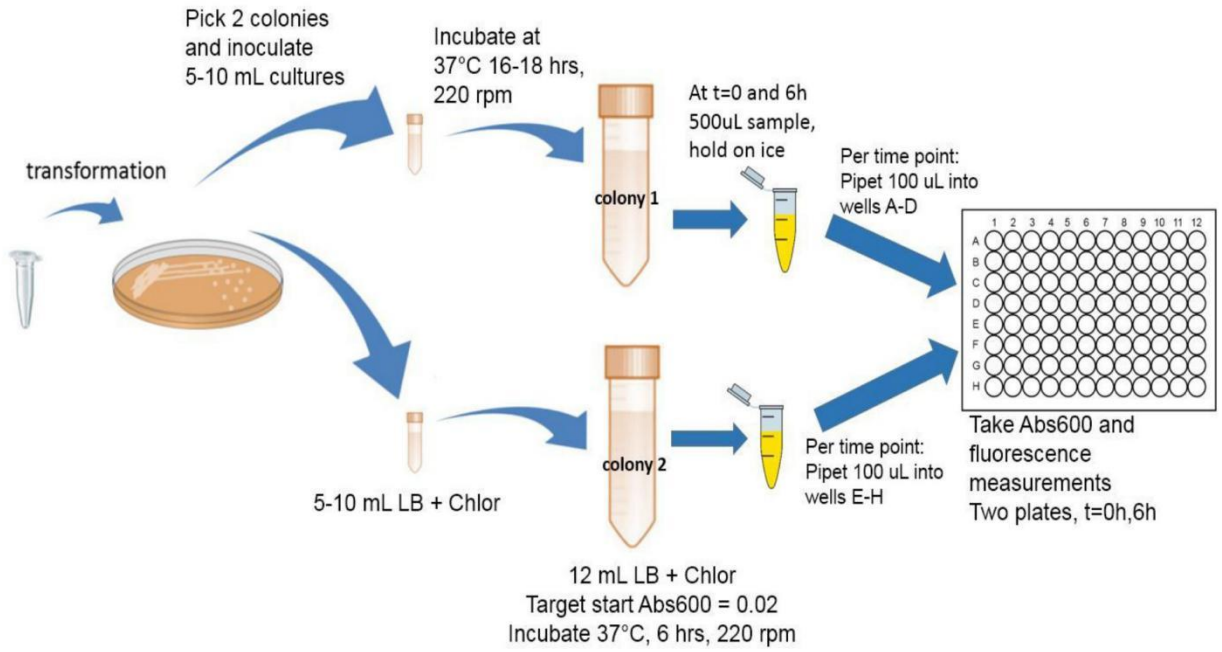

Dilution:
100 µL 10x Fluorescein
900 µL 1xPBS
10x
Fluorescein
1 mL
100 µM
1x Fluorescein
1 mL
10 µM
100%
0%
Transfer 200 ul
into each well
in column 1
100 ul
Liquid
Waste
A
B
C
D
1
2
3
4
5
6
7
8
9
10
11
12


Fluorescein Standard Curve
Fluorescence
4.000E+03
3.500E+03
3.000E+03
2.500E+03
2.000E+03
1.500E+03
1.000E+03
5.000E+02
0.000E+00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
Fluorescein Concentration (uM)



cell measurements

Workflow



Materials:

Competent cells (Escherichia coli strain DH5 α)

LB (Luria Bertani) media

Chloramphenicol (stock concentration 25 mg/mL dissolved in EtOH)

50 ml Falcon tube (or equivalent, preferably amber or covered in foil to block light)

Incubator at 37° C

1.5 ml eppendorf tubes for sample storage

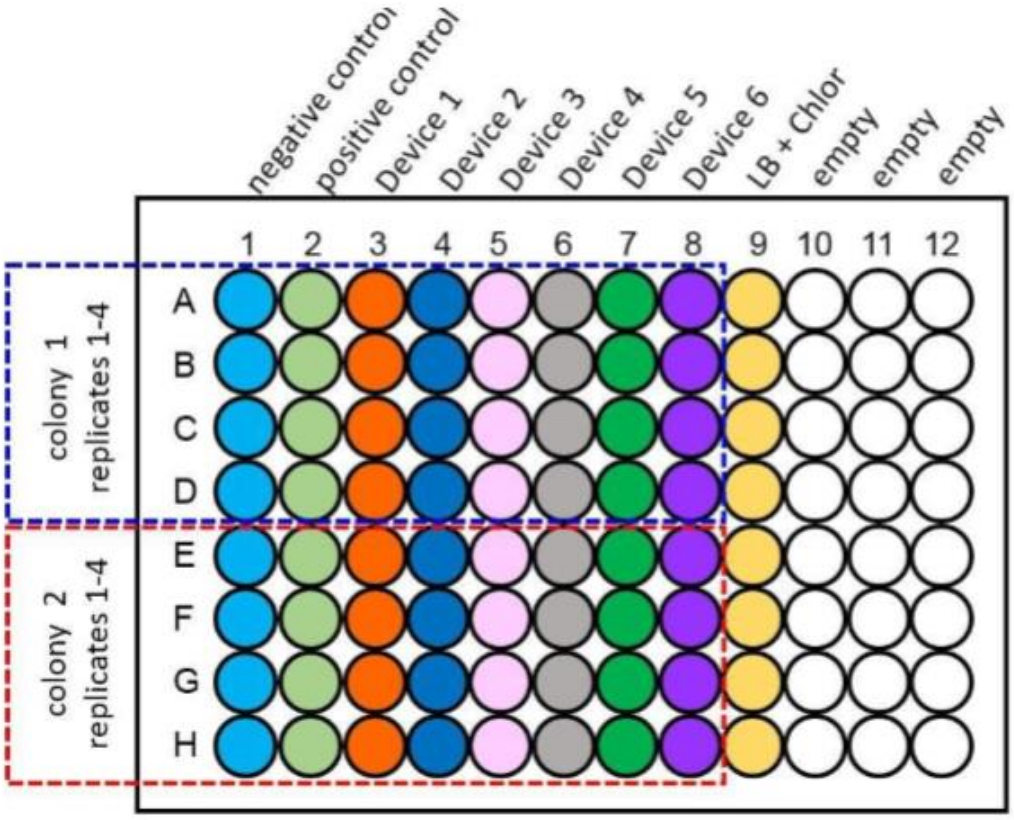
Ice bucket with ice

Micropipettes and tips

96 well plate, black with clear flat bottom preferred (provided by team)

Devices (from Distribution Kit, all in pSB1C3 backbone):

Device	Part Number	Plate	Location
Negative control	BBa_R0040	Kit Plate 7	Well 2D
Positive control	BBa_I20270	Kit Plate 7	Well 2B
Test Device 1	BBa_J364000	Kit Plate 7	Well 2F
Test Device 2	BBa_J364001	Kit Plate 7	Well 2H
Test Device 3	BBa_J364002	Kit Plate 7	Well 2J
Test Device 4	BBa_J364007	Kit Plate 7	Well 2L
Test Device 5	BBa_J364008	Kit Plate 7	Well 2N
Test Device 6	BBa_J364009	Kit Plate 7	Well 2P



CFU

Based on the assumption that 1 bacterial cell gives rise to 1 colony, colony forming units (CFU) per 1mL of an OD600 = 0.1 culture can be calculated as follows:

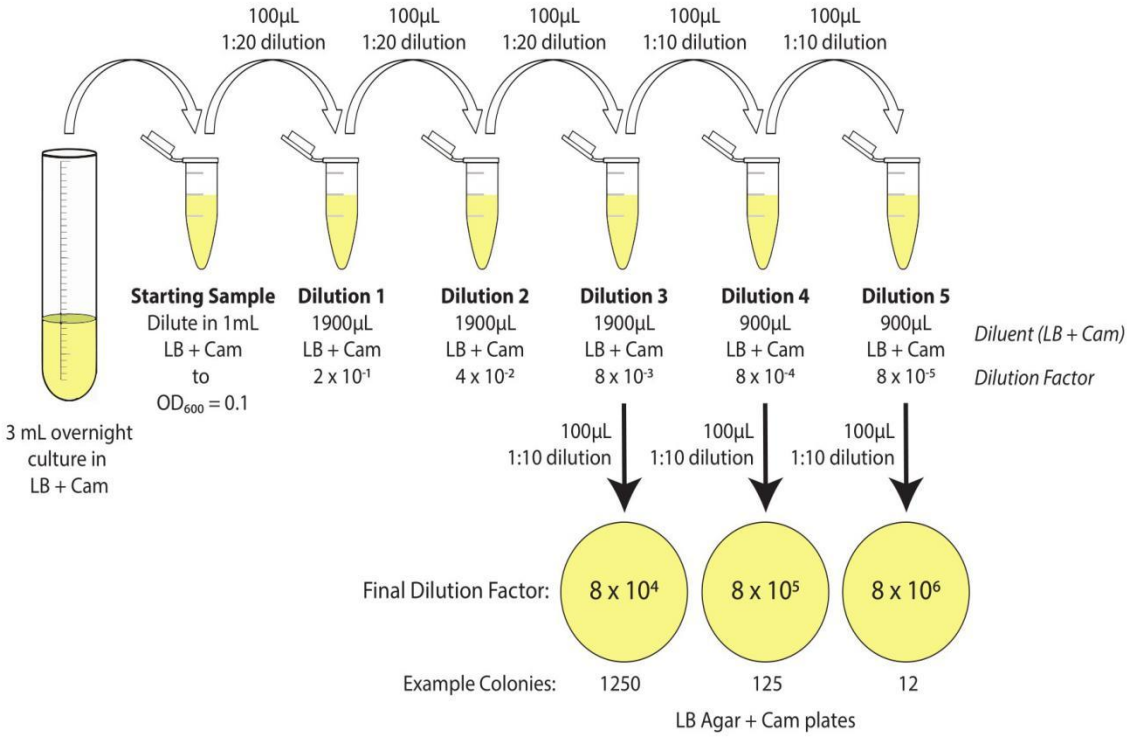
1. Count the colonies on each plate with fewer than 300 colonies.

2.Multiple the colony count by the Final Dilution Factor on each plate.

Example using Dilution 4 from above

colonies x Final Dilution Factor = CFU/mL

125 x (8 x 105) = 1 x 108 CFU ／ mL in Starting Sample (OD600 = 0.1)


100µL
1:20 dilution
100µL
1:20 dilution
100µL
1:20 dilution
100µL
1:10 dilution
100µL
1:10 dilution
3 mL overnight culture in LB + Cam
Starting Sample
Dilute in 1mL LB + Cam to OD_{600} = 0.1
Dilution 1
1900µL LB + Cam
2×10^{-1}
Dilution 2
1900µL LB + Cam
4×10^{-2}
Dilution 3
1900µL LB + Cam
8×10^{-3}
Dilution 4
900µL LB + Cam
8×10^{-4}
Dilution 5
900µL LB + Cam
8×10^{-5}
Diluent (LB + Cam)
Dilution Factor
100µL
1:10 dilution
100µL
1:10 dilution
100µL
1:10 dilution
Final Dilution Factor:
8×10^{4}
8×10^{5}
8×10^{6}
Example Colonies:
1250
125
12
LB Agar + Cam plates