



2022 iGEM InterLab study

Experiment 3* - Cell measurement protocol USING PREVIOUS DISTRIBUTIONS

NOTE: In case your team does not receive the 2022 distribution (on time or at all) you can perform the Experiment 3 with devices from previous distributions.

This year we plan to test protocols that will eventually be automated. For this reason, we will use 96-well plates instead of test tubes for culturing. Consequently, we want to evaluate how the performance of our plate culturing protocol compares to culturing in test tubes (e.g. 50mL falcon tube) on a global scale.

At the end of the experiment, you will have two plates to be measured. You will measure both fluorescence and absorbance in each plate.

Before performing the cell measurements, you need to perform all the calibration measurements. Please do not proceed unless you have completed the calibration protocol. Completion of the calibrations will ensure that you understand the measurement process and that you can take the cell measurements under the same conditions. For consistency and reproducibility, we are requiring all teams to use E. coli K-12 DH5-alpha. If you do not have access to this strain, you can request streaks of the transformed devices from another team near you. If you are absolutely unable to obtain the DH5-alpha strain, you may still participate in the InterLab study by contacting the Engineering Committee ([engineering \[at\] igem \[dot\] org](mailto:engineering[at]igem[dot]org)) to discuss your situation.

For all below indicated cell measurements, you must use the same type of plates and the same volumes that you used in your calibration protocol. You must also use the same settings (e.g., filters or excitation and emission wavelengths) that you used in your calibration measurements. If you do not use the same type of plates, volumes, and settings, the measurements will not be valid.

Protocol summary: You will transform the eight devices listed in Table 1 into E. coli K-12 DH5-alpha cells. The next day you will pick two colonies from each transformation (16 total) and use them to inoculate 12mL overnight cultures (this step is still in tubes). Each of these 16 overnight cultures will be used to inoculate four wells in a 96-well plate (200uL each, 4 replicates) and one test tube (12mL). You will measure how fluorescence and optical density develops over 6 hours by taking measurements at time point 0 hour and at time point 6 hours. Follow the protocol below and the visual instructions in Figure 1 and Figure 2.

Protocol Outputs:

- baseline absorbance of culture (day 2) measurements of cultures (0 hr timepoint)
- 0 hr absorbance timepoint measurements of plate 1
- 0 hr fluorescence timepoint measurements of plate 1
- 6 hr absorbance timepoint measurements of plate 1
- 6 hr fluorescence timepoint measurements of plate 1
- 6 hr absorbance timepoint measurements of plate 2
- 6 hr fluorescence timepoint measurements of plate 2

Protocol Materials:

- *E. coli* DH5 alpha competent cells
- Negative control
- Positive control (I20270)
- Test Device 1 (J364000)
- Test Device 2 (J364001)
- Test Device 3 (J364002)
- Test Device 4 (J364007)
- Test Device 5 (J364008)
- Test Device 6 (J364009)
- LB Broth + Chloramphenicol (34 ug/mL)
- LB Agar + Chloramphenicol (34 ug/mL)
- Chloramphenicol stock solution (34 mg/mL)
- Ice
- Plate reader
- Shaking incubator
- Petri dish (x 8)
- culture tube (x 32)
- 1.5mL microfuge tube (x 32)
- 50mL conical tube (x 16)
- 96 well microplate black with flat bottom (x 2)
- microplate adhesive sealing film

Table 1: Part Locations in Distribution Kit

Device	Part Name	Coordinate
Negative Control	BBa_J428100	2021 Kit Plate 6 Well <u>21P</u>
Positive Control	BBa_I20270	2021 Kit Plate 6 Well 23P
Test Device 1	BBa_J364000	2021 Kit Plate 6 Well 21B
Test Device 2	BBa_J364001	2021 Kit Plate 6 Well 23D
Test Device 3	BBa_J364002	2021 Kit Plate 6 Well 23F
Test Device 4	BBa_J364007	2021 Kit Plate 6 Well 23H
Test Device 5	BBa_J364008	2021 Kit Plate 6 Well 23J
Test Device 6	BBa_J364009	2021 Kit Plate 6 Well 23L

Protocol Steps:

Day 1

1. Obtain 8 x Petri dish containing LB Agar + Chloramphenicol (34 ug/mL) growth medium for culturing transformant strains
2. Transform Negative control DNA into *E. coli* DH5 alpha competent cells. Repeat for the remaining transformant DNA: Positive control (I20270), Test Device 1 (J364000), Test Device 2 (J364001), Test Device 3 (J364002), Test Device 4 (J364007), Test Device 5 (J364008), and Test Device 6 (J364009). Plate transformants on LB Agar + Chloramphenicol (34 ug/mL) transformant strains plates. Incubate overnight (for 16 hour) at 37.0°C.

Day 2

3. Obtain 16 x culture tubes to contain culture (day 1)
4. Pick 2 colonies from each transformant strains plate.
5. Inoculate 2 colonies of each transformant strains, for a total of 16 cultures. Inoculate each into 12.0mL of LB Broth + Chloramphenicol (34 ug/mL) in culture (day 1) and grow for 16.0 hour at 37.0°C and 220 rpm.

Day 3

6. Obtain 16 x culture tubes to contain culture (day 2)
7. Dilute each of 16 culture (day 1) samples with LB Broth + Chloramphenicol (34 ug/mL) into the culture tube at a 1:10 ratio and final volume of 12.0mL. Maintain at 4.0°C while performing dilutions. (This can be also performed on ice).
8. Obtain 16 x 1.5mL microfuge tubes to contain cultures (0 hr timepoint)
9. Hold cultures (0 hr timepoint) on ice. This will prevent cell growth while transferring samples.
10. Transfer 1.0mL of each of 16 culture (day 2) samples to 1.5mL microfuge tube containers to contain a total of 16 cultures (0 hr timepoint) samples. Maintain at 4.0°C during transfer. (This can be also performed on Ice).

11. Measure baseline absorbance of culture (day 2) of cultures (0 hr timepoint) at 600.0nm.
12. Obtain 16 x 50mL conical tubes to contain back-diluted culture. The conical tube should be opaque, amber-colored, or covered with foil.
13. Back-dilute each of 16 culture (day 2) samples to a target OD of 0.02 using LB Broth + Chloramphenicol (34 ug/mL) as diluent to a final volume of 12.0mL. Maintain at 4.0°C while performing dilutions.

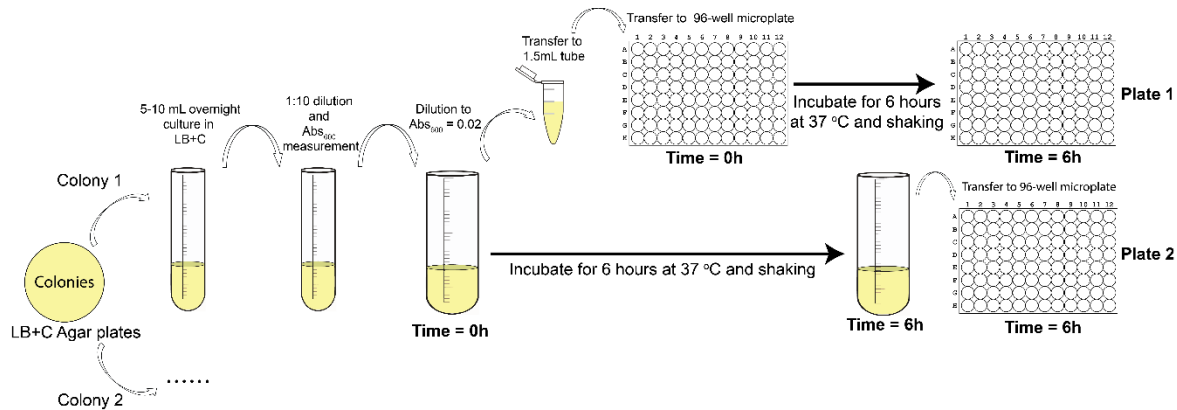


Fig 1: Visual representation of protocol

14. Obtain 16 x 1.5mL microfuge tubes to contain back-diluted culture aliquots
15. Hold back-diluted culture aliquots on ice. This will prevent cell growth while transferring samples.
16. Transfer 1.0mL of each of 16 back-diluted culture samples to 1.5mL microfuge tube containers to contain a total of 16 back-diluted culture aliquots samples. Maintain at 4.0°C during transfer. (This can be also performed on Ice).
17. Obtain a 96 well microplate to contain plate 1
18. Hold plate 1 on ice.
19. Transfer 200.0uL of each back-diluted culture aliquots sample to 96 well microplate plate 1 in the wells indicated in the plate layout. Maintain at 4.0°C during transfer.

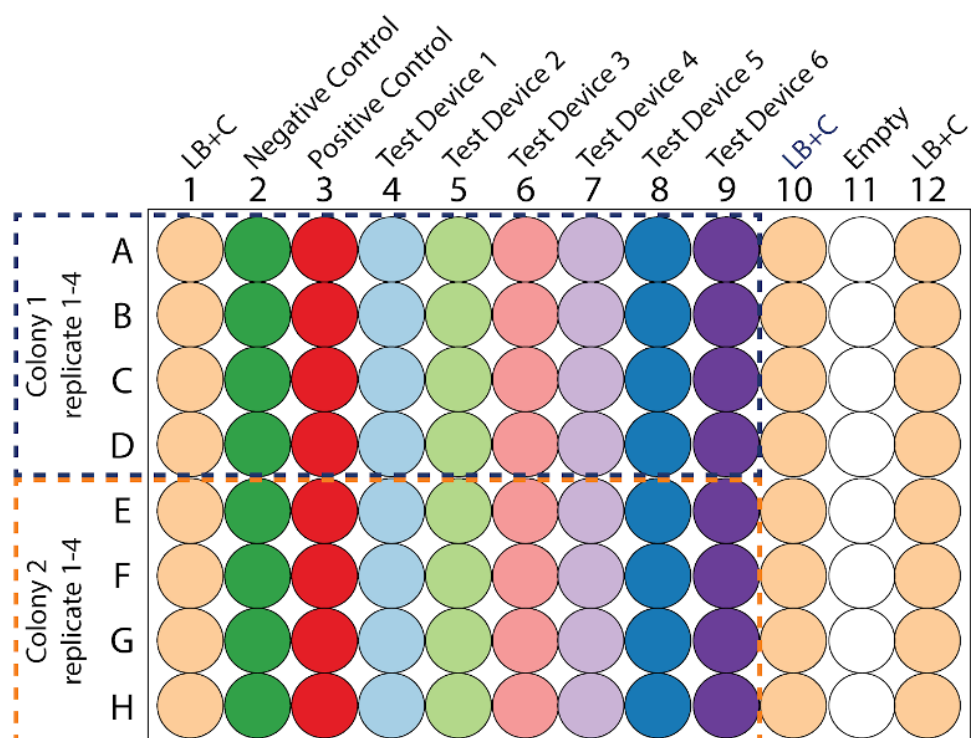


Fig 2: Plate layout

20. Transfer 200.0uL of LB Broth + Chloramphenicol (34 ug/mL) sample to wells A1:H1, A10:H10, A12:H12 of 96 well microplate plate 1. Maintain at 4.0°C during transfer. These samples are blanks.
21. Measure 0 hr absorbance timepoint of plate 1 at 600.0nm.
22. Measure 0 hr fluorescence timepoint of plate 1 with excitation wavelength of 488.0nm and emission filter of 530.0nm and 30.0nm bandpass.
23. Cover plate 1 samples in 96 well microplate with your choice of material to prevent evaporation.
24. Incubate all back-diluted culture samples for 6.0 hour at 37.0°C at 220 rpm.
25. Incubate all plate 1 samples for 6.0 hour at 37.0°C at 220 rpm.
26. Hold all back-diluted culture samples on ice. This will inhibit cell growth during the subsequent pipetting steps.
27. Hold all plate 1 samples on ice. This will inhibit cell growth during the subsequent pipetting steps.
28. Obtain a 96 well microplate to contain plate 2
29. Hold plate 2 on ice.
30. Transfer 200.0uL of each back-diluted culture sample to 96 well microplate plate 2 in the wells indicated in the plate layout. Maintain at 4.0°C during transfer.
31. Transfer 200.0uL of LB Broth + Chloramphenicol (34 ug/mL) sample to wells A1:H1, A10:H10, A12:H12 of 96 well microplate plate 2. Maintain at 4.0°C during transfer. These are the blanks.
32. Measure 6 hr absorbance timepoint of plate 1 at 600.0nm.

33. Measure 6 hr fluorescence timepoint of plate 1 with excitation wavelength of 485.0nm and emission filter of 530.0nm and 30.0nm bandpass.
34. Measure 6 hr absorbance timepoint of plate 2 at 600.0nm.
35. Measure 6 hr fluorescence timepoint of plate 2 with excitation wavelength of 485.0nm and emission filter of 530.0nm and 30.0nm bandpass.
36. Import data for baseline absorbance of culture (day 2) measurements of cultures (0 hr timepoint), 0 hr absorbance timepoint measurements of plate 1, 0 hr fluorescence timepoint measurements of plate 1, 6 hr absorbance timepoint measurements of plate 1, 6 hr fluorescence timepoint measurements of plate 1, 6 hr absorbance timepoint measurements of plate 2, 6 hr fluorescence timepoint measurements of plate 2 into provided Excel file.

Protocol version: 1.2.2