Calibration Protocol - Fluorescence Standard Curve with Fluorescein

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ABSTRACT

Plate readers report fluorescence values in arbitrary units that vary widely from instrument to instrument. Therefore absolute fluorescence values cannot be directly compared from one instrument to another. In order to compare fluorescence output of test devices between teams, it is necessary for each team to create a standard fluorescence curve. Although distribution of a known concentration of GFP protein would be an ideal way to standardize the amount of GFP fluorescence in our E. coli cells, the stability of the protein and the high cost of its purification are problematic. We therefore use the small molecule fluorescein, which has similar excitation and emission properties to GFP, but is cost-effective and easy to prepare. (The version of GFP used in the devices, GFP mut3b, has an excitation maximum at 501 nm and an emission maximum at 511 nm; fluorescein has an excitation maximum at 494 nm and an emission maximum at 525nm).

You will prepare a dilution series of fluorescein in four replicates and measure the fluorescence in a 96 well plate in your plate reader. By measuring these in your plate reader, you will generate a standard curve of fluorescence for fluorescein concentration. You will be able to use this to convert your cell based readings to an equivalent fluorescein concentration. Before beginning this protocol, ensure that you are familiar with the GFP settings and measurement modes of your instrument. You will need to know what filters your instrument has for measuring GFP, including information about the bandpass width (530 nm / 30 nm bandpass, 25-30 nm width is recommended), excitation (485 nm is recommended) and emission (520-530 nm is recommended) of this filter.

Note: The iGEM Particle Standard Curve with Microspheres calibration method is a pre-requisite for carrying out this protocol. You will need data from that calibration to analyse the results of this protocol.

EXTERNAL LINK

https://2019.igem.org/Measurement

XLSX

iGEM Data Analysis Template - Fluorescence Standard Curve - v1.xlsx

MATERIALS

NAME	CATALOG #	VENDOR	CAS NUMBER	RRID
96 well plate				
PBS				
Fluorescein				

STEPS MATERIALS

NAME	CATALOG #	VENDOR	CAS NUMBER	RRID
Fluorescein				
PBS				

MATERIALS TEXT

Fluorescein is provided in the iGEM Measurement Kit. The 96-well plate should preferably be black with a clear flat bottom.

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BEFORE STARTING

Read through this entire protocol carefully before you start your experiment and prepare any materials you may need.

Note: The iGEM Particle Standard Curve with Microspheres calibration method is a pre-requisite for carrying out this protocol. You will need data from that calibration to analyse the results of this protocol.

Prepare the fluorescein stock solution

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

Fluorescein

2 Prepare 10x fluorescein stock solution (100 µM) by resuspending fluorescein in 1mL of 1X PBS

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.

PBS

3 Dilute the 10X fluorescein stock solution with 1X PBS 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

4 Accurate pipetting is essential. Serial dilutions will be performed across columns 1-11. **Column 12 must contain PBS buffer only.** Initially you will setup the plate with the fluorescein stock in column 1 and an equal volume of 1X PBS in columns 2 to 12.

You will perform a serial dilution by consecutively transferring 100 µl from column to column with good mixing.

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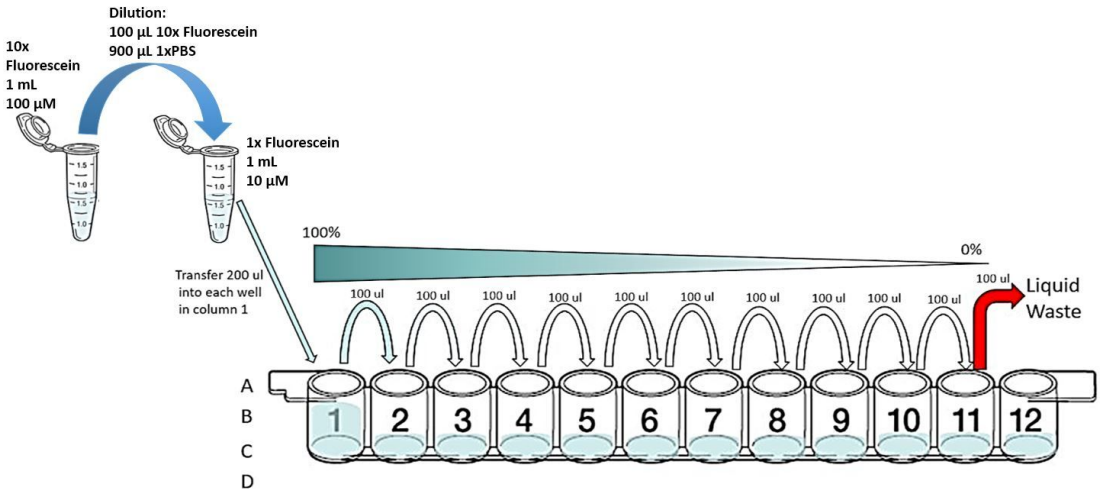

5 Add 100 µl of 1X PBS into wells A2, B2, C2, D2....A12, B12, C12, D12

6 Add 200 µl of fluorescein 1X stock solution into A1, B1, C1, D1

7 Transfer 100 µl of fluorescein stock solution from A1 into A2

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

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

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

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

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

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

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

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

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

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

Take care not to continue serial dilution into column 12

18 Repeat dilution series for rows B, C, D

Measure fluorescence

19 Measure fluorescence of all samples in instrument

20 Record the data in your notebook

21 Import data into this Excel sheet provided (fluorescein standard curve tab):

iGEM Data Analysis Template - Fluorescence Standard Curve - v1.xlsx

Congratulations!

22 You have now completed this calibration protocol

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