iGEM 2018 Interlab Study Protocol: Calibration 3

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Abstract

This year´s interlab study allows participants to fulfil Bronze medal requirements.
For this purposes a set of experiments has to be performed, which in the end will be compared and validated with other team´s data.
Part of this Challenge are the Calibration protcols.
For the third calibration, a fluorescein standard curve is prepared and fluorescence (bandpass width: 520nm/30nm bandpass, 25-30nm width; excitation; 485nm; emission: 520-530nm) is measured.

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Guidelines

Make sure to always use the same plates, volumes and settings for the measurement as will be used for the other calibrations and the cell measurements.
Also take care to remain constant with your pipetting techniques.

Before start

Make sure to adjust the temperature of your plate reader to room temperature (22°C-25°C) before measurement.

Also prepare the fluorescein stock solution before starting. Follow the steps in this protocol.

Materials

- 96 well plate (black, flat bottom preferred) by Contributed by users
- Fluorescein by Contributed by users
- 1x PBS (pH 7.4-7.6) by Contributed by users

Protocol

Preparation of fluorescein stock solution

Step 1.

- Spin down fluorescein kit tube
- Resuspend fluorescein in 1mL of 1x PBS (this is the 10x fluorescein stock solution)
- Pipet up and down and make sure that no particulates are visible
- Dilute the 10x fluorescein stock solution to a 1x fluorescein stock solution by making a 1:10 dilution (e.g. 100µl 10x fluorescein stock solution and 900µl 1x PBS)

Preparation of serial dilutions: 1xPBS

Step 2.

Pipet 100µl 1xPBS into wells A2-A12. Do the same for row B,C and D.

Preparation of serial dilutions: 1x fluorescein stock solution

Step 3.

Pipet 200µl of 1x fluorescein stock solution into A1,B1,C1 and D1.

Serial dilutions of fluorescein stock solutions

Step 4.

Transfer 100µl of 1x fluorescein stock solution from A1 to A2.
Mix 3x by pipetting up and down. Then transfer 100µl from A2 to A3.
Mix again 3x by pipetting up and down and transfer again 100µl from A3 to A4.
Continue this procedure until arrived at well A11. Mix 3x by pipetting up and down and transfer 100µl into the **LIQUID WASTE**and **NOT** in A12.

As replicates are performed, the same procedure is performed for row B, C and D as well.

Serial dilutions of fluorescein stock solutions

Step 5.

Measurement

Step 6.

Measure fluorescence: (bandpass width: 520nm/30nm bandpass, 25-30nm width; excitation; 485nm; emission: 520-530nm)

Data transfer

Step 7.

- Import your data into the Excel sheet provided by iGEM (fluorescein standard curve tab)