



Jul 29, 2022

PLATERO - Green Fluorescence Calibration in Plate Readers

Forked from [iGEM Calibration Protocol - Red Fluorescent Proteins in Plate Readers](#)

Alejandro Vignoni¹, Yadira Boada¹

¹Universitat Politècnica de Valencia

1 Works for me

Share

This protocol is published without a DOI.

iGEM Measurement

Tech. support email: pauljrutten@gmail.com



Alejandro Vignoni

Universitat Politècnica de Valencia

ABSTRACT

One of the most common sources of information in Synthetic Biology is the data coming from plate reader fluorescence measurements. These experiments provide a measure of the light emitted by certain fluorescent molecules, such as the Green Fluorescent Protein (GFP). However, these measurements are generally expressed in arbitrary units, and are affected by the measurement device gain. This limits the range of measurements in a single experiment and hampers the comparison of results among experiments. In this work, we provide a calibration protocol to express fluorescence measures in concentration units of a reference fluorophore. The protocol removes the gain effect of the measurement device on the acquired data. In addition, the fluorescence intensity values are transformed into units of concentration using a Fluorescein calibration model. Both steps are expressed in a single mathematical expression which returns normalized, gain independent, and comparable data, even if the acquisition was done at different device gain levels. The protocol embeds a Linearity and Bias Analysis that provides an assessment of the uncertainty of the model estimations, and a Reproducibility and Repeatability analysis that evaluates the sources of variability originating from the measurements and the equipment. All the functions used to build the model, exploit it with new data, and perform the uncertainty and variability assessment are available in an open access repository.

PROTOCOL CITATION

Alejandro Vignoni, Yadira Boada 2022. PLATERO - Green Fluorescence Calibration in Plate Readers. **protocols.io**

<https://protocols.io/view/platero-green-fluorescence-calibration-in-plate-re-cbkyskxw>



FUNDERS ACKNOWLEDGEMENT

MCIN/AEI/10.13039/501100011033

Grant ID: PID2020-117271RB-C21

FORK NOTE

FORK FROM

Forked from iGEM Calibration Protocol - Red Fluorescent Proteins in Plate Readers,
Paul Rutten

LICENSE

————— This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Jun 18, 2022

LAST MODIFIED

Jul 29, 2022

PROTOCOL INTEGER ID

64888

GUIDELINES

For a full set of calibrations, you should run two protocols: this fluorescence calibration curve with Texas Red, and the Abs600 (OD) calibration with microspheres.

Before beginning these protocols, please ensure that you are familiar with the measurement modes and settings of your instrument. For all of these calibration measurements, you must use the same plates and volumes that you will use in your cell-based assays. You must also use the same settings (e.g., filters or excitation and emission wavelengths) that you will use in your cell-based assays. If you do not use the same plates, volumes, and settings, the calibration will not be valid.

Make sure to record all information about your instrument to document your experiment. If your instrument has variable temperature settings, the instrument temperature should be set to room temperature (approximately 20-25 C) for all measurements.

MATERIALS TEXT

MATERIALS

🔗 96 well plate **Contributed by users**

🔗 PBS **Contributed by users**

Stock Reference Fluorescein solution

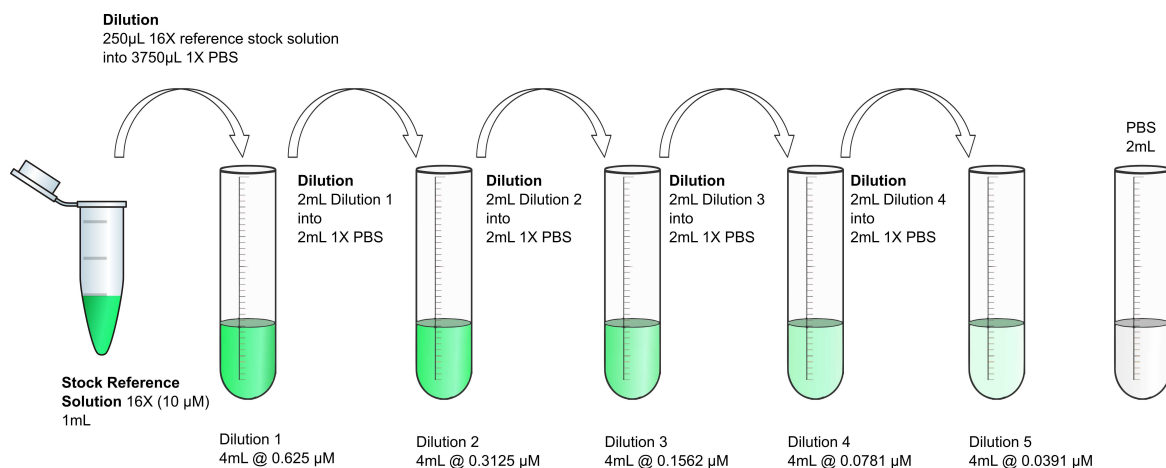
- 1 Start from at least 📏 1 mL of [M]10 micromolar (μM)
🔗 Fluorescein Sodium Salt **Contributed by users** solution in
🔗 1X PBS (Phosphate-buffered saline) .
- 2 **[OPTIONAL]** If you have access to a spectrophotometer, you can calculate the concentration of your Fluorescein reference stock solution even more accurately using the Beer-Lambert law.
 - 2.1 Measure the solution's absorbance at 492 nm and calculate concentration using an extinction coefficient of $68.029 \text{ mM}^{-1} \text{ cm}^{-1}$
 - 2.2 If the concentration of your stock reference solutions is different than [M]10 micromolar (μM) then you need to recalculate the dilution of the next step to obtain a Dilution 1 solution of [M]0.625 micromolar (μM)

Prepare the starting dilution of Fluorescein solution

- 3 Dilute 📏 250 μL of the stock reference solution into 📏 3750 μL of PBS to obtain 📏 4 mL of Dilution 1 @ [M]0.625 micromolar (μM)


Prepare the serial dilutions of Fluorescein

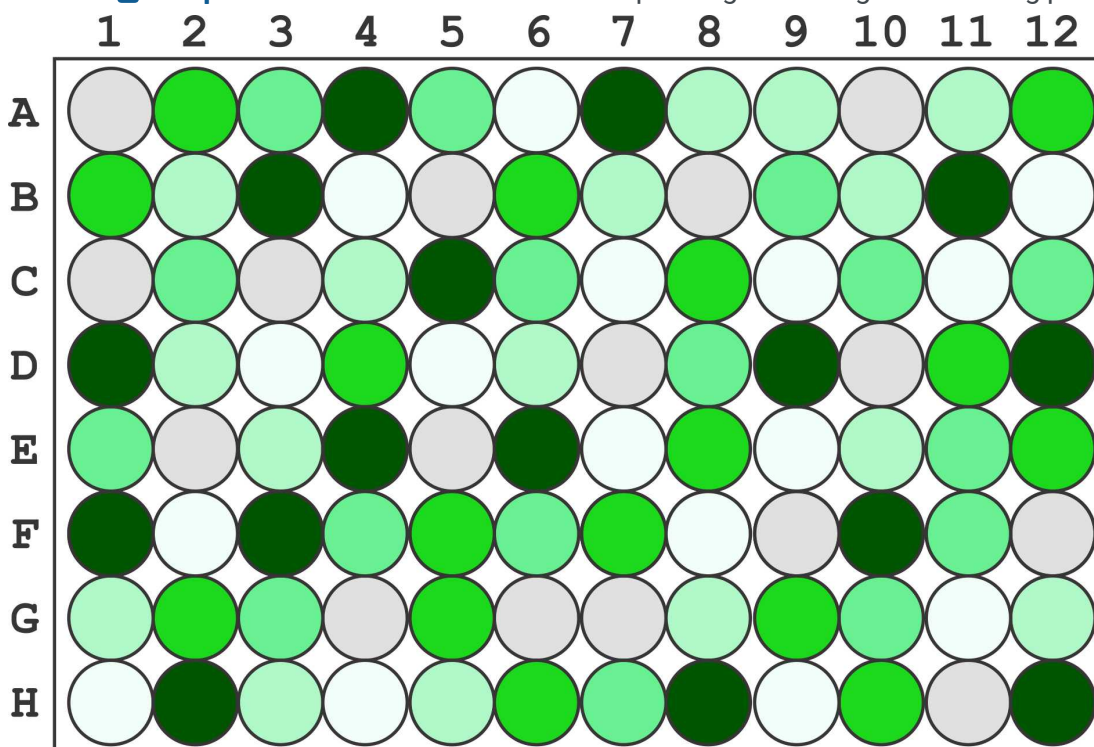
- 4 Accurate pipetting is essential. Serial dilutions will be performed in 5 tubes. There will be a sixth tube that **must contain PBS buffer only**. Initially, you will setup 5 tubes labeled Dilution 2 to 5 and PBS, with 📏 2 mL of PBS.
Then you will perform a serial dilution by consecutively transferring 📏 2 mL from Dilution 1 to Dilution 2 and so on, always with good mixing (pipetting up and down three times or more).



- 4.1 Label four tubes with the names Dilution 2 to 5, and one tube with the name PBS.
 - 4.2 Add **2 mL** of PBS **1X PBS (Phosphate-buffered saline)** to all the tubes.
 - 4.3 Transfer **2 mL** of Dilution 1 into Dilution 2 tube. Mix thoroughly by pipetting up and down three times or more.
 - 4.4 Transfer **2 mL** of Dilution 2 into Dilution 3 tube. Mix thoroughly by pipetting up and down three times or more.
 - 4.5 Transfer **2 mL** of Dilution 3 into Dilution 4 tube. Mix thoroughly by pipetting up and down three times or more.
 - 4.6 Transfer **2 mL** of Dilution 4 into Dilution 5 tube. Mix thoroughly by pipetting up and down three times or more.
- 5 Transfer **2 mL** of Dilution 5 into the liquid waste.

Micro-plate set up

- 6 Transfer  100 µL of each dilution into the corresponding well using the following plate map:



A	B
PBS	A1, A10, B5, B8, C1, C3, D10, D7, E2, E5, F12, F9, G4, G6, G7, H11
Dilution 1	A4, A7, B11, B3, C5, D1, D12, D9, E4, E6, F1, F10, F3, H12, H2, H8
Dilution 2	A12, A2, B1, B6, C8, D11, D4, E12, E8, F5, F7, G2, G5, G9, H10, H6
Dilution 3	A3, A5, B9, C10, C12, C2, C6, D8, E1, E11, F11, F4, F6, G10, G3, H7
Dilution 4	A11, A8, A9, B10, B2, B7, C4, D2, D6, E10, E3, G1, G12, G8, H3, H5
Dilution 5	A6, B12, B4, C11, C7, C9, D3, D5, E7, E9, F2, F8, G11, H1, H4, H9

You can use any random distribution of well you like, this one is just the distribution we used as an example.

Measure Fluorescence

- 7 Measure the fluorescence of all samples in your plate reader, repeat measurements using 4 different gain settings, and then repeat everything 8 times.

Remember to set your plate reader's excitation and emission settings correctly 488nm excitation and 530nm emission,

Record data

- 8 Save your measurements into an excel file with each repetition on a different sheet.