Resource Summary Report

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Calibration Protocol - Plate Reader Fluorescence Calibration with Fluorescein

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Protocol Information

URL: https://dx.doi.org/10.17504/protocols.io.548g8zw

Authors: Geoff Baldwin, Traci Haddock-Angelli, Jacob Beal, Ari Dwijayanti, Marko Storch, Natalie Farny, Richard Tennant, Paul Rutten

Group: iGEM Measurement

Summary: 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

Abs600 (OD) calibration protocol 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.

Affiliations: Imperial College London, iGEM, iGEM Measurement Committee, Imperial College London, Imperial College London, iGEM Measurement Committee, iGEM Measurement Committee, iGEM Measurement Committee

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