Version 3

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Calibration Protocol - Plate Reader Abs600 (OD) Calibration with Microsphere Particles V.3

Forked from Calibration Protocol - Particle Standard Curve with Microspheres

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1 Works for me dx.doi.org/10.17504/protocols.io.bgy6jxze

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ABSTRACT

You will prepare a dilution series of monodisperse silica microspheres and measure the Abs_{600} in your plate reader.

The size and optical characteristics of these microspheres are similar to cells, and there is a known amount of particles per volume. This measurement will allow you to construct a standard curve of particle concentration which can be used to convert 600 nm absorbance measurements into an estimated equivalent number of cells.

EXTERNAL LINK

https://2019.igem.org/Measurement

ATTACHMENTS

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

GUIDELINES

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

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

NAME	CATALOG #	VENDOR
96 well plate		
double distilled water (ddH2O)		
300µl Silica beads		

STEPS MATERIALS

NAME	CATALOG #	VENDOR

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NAME	CATALOG #	VENDOR
300µl Silica beads		
ddH20		

MATERIALS TEXT

The Silica beads are provided in the iGEM Measurement Kit or can bought online. The beads provided are the 550-1000nm Silica Nanoparticles produced by Nanocym (link). The 96-well plate should preferably be black with a clear flat bottom.

BEFORE STARTING

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

Prepare the Microsphere Stock Solution

1 Obtain the tube labeled “Silica Beads” from the Measurement Kit and vortex vigorously for 30 seconds.

300µl Silica beads

Microspheres should NOT be stored at 0°C or below, as freezing affects the properties of the microspheres. If you believe your microspheres may have been frozen, please contact the iGEM Measurement Committee for a replacement (measurement@igem.org).

2 Immediately pipet 100 µL microspheres into a 1.5 mL eppendorf tube

3 Add 900 µL of ddH_2O to the microspheres

ddH20

4 Vortex well. This is your Microsphere Stock Solution

Prepare the serial dilution of microspheres

5 Accurate pipetting is essential. Serial dilutions will be performed across columns 1-11. **Column 12 must contain ddH_2O only**.

Initially you will setup the plate with the microsphere stock solution in column 1 and an equal volume of 1x ddH_2O 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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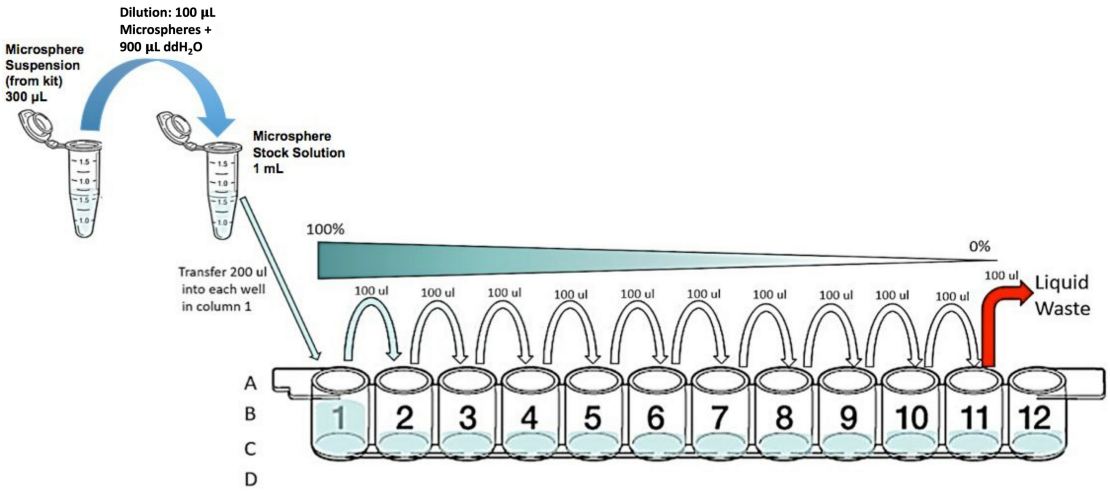

6 Add 100 µl of ddH_2O into wells A2, B2, C2, D2....A12, B12, C12, D12

7 Vortex the tube containing the stock solution of microspheres vigorously for 10 seconds

8 Immediately add 200 µl of microspheres stock solution into A1

9 Transfer 100 µl of microsphere stock solution from A1 into A2

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

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

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

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

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

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

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

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

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

19 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

20 Repeat dilution series for rows B, C, D

21 **IMPORTANT!**

Re-Mix (pipette up and down) each row of your plate **immediately before** putting in the plate reader! (This is important because the beads begin to settle to the bottom of the wells within about 10 minutes, which will affect the measurements.)

Take care to mix gently and avoid creating bubbles on the surface of the liquid

Measure OD

22 Measure OD_{600} of all samples in instrument. Disable any path length correction setting on your instrument, if it has one.

If you will be using your data in conjuction with measurements from the Fluorescence standard curve protocol, make sure you use the same instrument settings for both protocols.

23 Record the data in your notebook. Please note your standard curve should still work well even if a few of your measurements are saturing the instrument

24 Import data into this Excel sheet:

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Congratulations!

25 You have now completed this calibration protocol

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