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Calibration Protocol - Particle Standard Curve with Microspheres

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Richard Tennant[1], Paul Rutten[1]

[1]iGEM Measurement Committee

iGEM Measurement
Tech. support email: **pauljrutten@gmail.com**

Paul Rutten
The University of Oxford

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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.

Attachments

XLSX

iGEM Data Analysis T...

25KB

Materials

MATERIALS

- 96 well plate
- double distilled water (ddH2O)
- 300µl Silica beads

STEP MATERIALS

- ddH20
- 300µl Silica beads

300 µL Silica beads are provided in the iGEM Measurement Kit. The 96-well plate should preferably be black with a clear flat bottom.

Protocol materials

ddH20

300µl Silica beads

96 well plate

double distilled water (ddH2O)

300µl Silica beads

300µl Silica beads

ddH20

Troubleshooting

Before start

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

Note

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

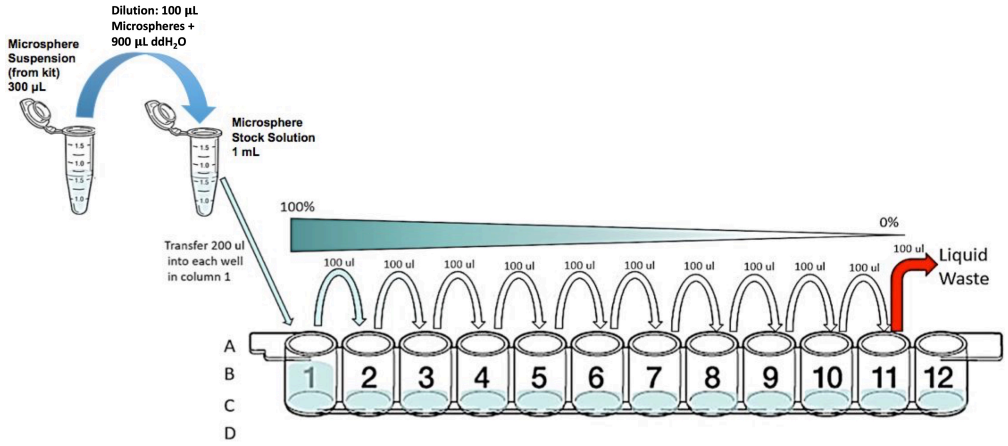
4 Vortex well. This is your Microsphere Stock Solution

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.



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

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

Note

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.)

Note

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

23 Record the data in your notebook

24 Import data into this Excel sheet:

iGEM Data Analysis Template - Part...

Congratulations!

25 You have now completed this calibration protocol