ABSTRACT
You will prepare a dilution series of monodisperse silica microspheres and measure the Abs\textsubscript{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

DOI
dx.doi.org/10.17504/protocols.io.5n5g5g6

EXTERNAL LINK
https://2019.igem.org/Measurement

PROTOCOL CITATION
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FORK FROM
Forked from Calibration Protocol - Particle Standard Curve with Microspheres, Paul Rutten

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CREATED
Jul 21, 2019
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 Contributed by users

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

   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).

3. Add 900 µL of ddH2O to the microspheres

   ddH2O Contributed by users

4. Vortex well. This is your Microsphere Stock Solution

Prepare the serial dilution of microspheres
Accurate pipetting is essential. Serial dilutions will be performed across columns 1-11. **Column 12 must contain ddH\textsubscript{2}O only.**

Initially you will setup the plate with the microsphere stock solution in column 1 and an equal volume of 1x ddH\textsubscript{2}O in columns 2 to 12.

You will perform a serial dilution by consecutively transferring 100 \( \mu \)l from column to column with good mixing.

Add 100 \( \mu \)l of ddH\textsubscript{2}O into wells A2, B2, C2, D2....A12, B12, C12, D12

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

Immediately add 200 \( \mu \)l of microspheres stock solution into A1

Transfer 100 \( \mu \)l of microsphere stock solution from A1 into A2

Mix A2 by pipetting up and down 3x and transfer 100 \( \mu \)l into A3

Mix A3 by pipetting up and down 3x and transfer 100 \( \mu \)l into A4

Mix A4 by pipetting up and down 3x and transfer 100 \( \mu \)l into A5

Mix A5 by pipetting up and down 3x and transfer 100 \( \mu \)l into A6
13

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

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 OD₆₀₀ of all samples in instrument

23 Record the data in your notebook

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Import data into this Excel sheet:

[IGEM Data Analysis Template - Particle Standard Curve - v1.xlsx]

Congratulations!

You have now completed this calibration protocol.