Calibration Protocol - Particle Standard Curve with Microspheres

Paul
Richard Tennant1, Rutten1
1iGEM Measurement Committee

iGEM Measurement
Tech. support email: paulrutten@gmail.com

Paul Rutten
The University of Oxford

ABSTRACT
You will prepare a dilution series of monodisperse silica microspheres and measure the Abs600 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
iGEM Data Analysis Template - Particle Standard Curve - v1.xlsx

MATERIALS

96 well plate Contributed by users
double distilled water (ddH2O) Contributed by users
300µl Silica beads Contributed by users

STEP MATERIALS

ddH2O Contributed by users
300µl Silica beads Contributed by users

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

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.
**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](Materials, Step 3)

**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 ddH₂O to the microspheres

   ![ddH₂O Contributed by users](Materials, Step 1)

4. Vortex well. This is your Microsphere Stock Solution
Accurate pipetting is essential. Serial dilutions will be performed across columns 1-11. Column 12 must contain ddH₂O only.

Initially you will setup the plate with the microsphere stock solution in column 1 and an equal volume of 1x ddH₂O in columns 2 to 12.

You will perform a serial dilution by consecutively transferring 100 µl from column to column with good mixing.

Add 100 µl of ddH₂O 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 µl of microspheres stock solution into A1

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**

22  Measure OD$_{600}$ of all samples in instrument

23  Record the data in your notebook
Import data into this Excel sheet:

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

Congratulations!

You have now completed this calibration protocol.