iGEM 2018 Interlab Study Protocol: Calibration 2

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Abstract

This year´s interlab study allows participants to fulfil Bronze medal requirements.
For this purposes a set of experiments has to be performed, which in the end will be compared and validated with other team´s data.
Part of this Challenge are the Calibration protcols.
For the second calibration a dilution series of monodisperse microspheres is performed and absorbance at 600nm is measured.

Citation: Ylenia Longo iGEM 2018 Interlab Study Protocol: Calibration 2. **protocols.io**
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Guidelines

Make sure to always use the same plates, volumes and settings for the measurement as will be used for the other calibrations and the cell measurements.
Also take care to remain constant with your pipetting techniques.

Before start

Make sure to adjust the temperature of your plate reader to room temperature (22°C-25°C) before measurement.

Also prepare the silica beads stock solution before starting. Follow the steps in this protocol.

Materials

- ddH20 by Contributed by users
- 96 well plate (black, flat bottom preferred) by Contributed by users
- 300µl Silica beads by Contributed by users

Protocol

Silica beads

Step 1.

Vortex the tube "Silica beads" of the Interlab Measurement kit for 30 seconds vigorously.

Silica beads stock solution

Step 2.

Immediately pipet 96µl of microspheres into a 1.5ml eppendorf tube.

ddH20

Step 3.

Add 904µl ddH20 to the microspheres

Microsphere stock solution

Step 4.

Vortex the tube very well. This is the microsphere stock solution

Serial dilutions of microspheres--> ddH20

Step 5.

Pipet 100µl ddH20 into wells A2-A12. Do the same for row B,C and D.

Serial dilutions of microspheres--> Microsphere stock solution

Step 6.

Pipet 200µl of microsphere stock solution into A1,B1,C1 and D1.

Serial dilutions of microspheres

Step 7.

Transfer 100µl of microsphere stock solution from A1 to A2.
Mix 3x by pipetting up and down. Then transfer 100µl from A2 to A3.
Mix again 3x by pipetting up and down and transfer again 100µl from A3 to A4.
Continue this procedure until arrived at well A11. Mix 3x by pipetting up and down and transfer 100µl into the **LIQUID WASTE** and **NOT** in A12.

As replicates are performed, the same procedure is performed for row B, C and D as well.

Before measurement

Step 8.

It is important, that before measurements are performed, each row is mixed again, since silica particles start to settle down and inlfuence the final experimental results.

Measurement

Step 9.

- Measure absorbance at 600nm of all samples. Make sure to use the same measurement modes that will be used for the cell measurements.

Data transfer

Step 10.

- Import your data into the Excel sheet provided by iGEM (particle standard curve tab)