JUL 01, 2018

🌐 iGEM 2018 Interlab Study Protocol: Calibration 2

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OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.qn6dvhe

Protocol Citation: Ylenia Longo 2018. iGEM 2018 Interlab Study Protocol: Calibration 2. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.qn6dvhe>

MANUSCRIPT CITATION:
<http://2018.igem.org/Measurement/InterLab>

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Protocol status: Working
 We use this protocol in our group for the interlab challenge and it is working

Created: Jun 02, 2018

Last Modified: Jul 01, 2018

PROTOCOL integer ID:
 12734

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 protocols. For the second calibration a dilution series of monodisperse microspheres is performed and absorbance at 600nm is measured.

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.

MATERIALS

MATERIALS

⊗ ddH2O **Contributed by users**

⊗ 96 well plate (black, flat bottom preferred) **Contributed by users**

⊗ 300µl Silica beads **Contributed by users**

BEFORE START INSTRUCTIONS

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.

Silica beads

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

Silica beads stock solution

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

ddH2O

- 3 Add 904µl ddH2O to the microspheres

Microsphere stock solution

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

Serial dilutions of microspheres--> ddH2O

- 5 Pipet 100µl ddH2O into wells A2-A12. Do the same for row B,C and D.

Serial dilutions of microspheres--> Microsphere stock solut...

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

Serial dilutions of microspheres

- 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

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

Measurement

- 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

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