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Working

# Calibration Protocol - $OD_{600}$ Inter-equipment Conversion with LUDOX - v2

Version 2

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iGEM Measurement

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ABSTRACT

With this protocol you will use LUDOX CL-X (a 45% colloidal silica suspension) as a single point reference to obtain a conversion factor to transform absorbance ($OD_{600}$) data from your plate reader into a comparable $OD_{600}$ measurement as would be obtained in a spectrophotometer. This conversion is necessary because plate reader measurements of absorbance are volume dependent; the depth of the fluid in the well defines the path length of the light passing through the sample, which can vary slightly from well to well. In a standard spectrophotometer, the path length is fixed and is defined by the width of the cuvette, which is constant. Therefore this conversion calculation can transform $OD_{600}$ measurements from a plate reader (i.e. absorbance at 600 nm, the basic output of most instruments) into comparable $OD_{600}$ measurements. The LUDOX solution is only weakly scattering and so will give a low absorbance value.

EXTERNAL LINK

https://2019.igem.org/Measurement

GUIDELINES

**Disclaimer:** The 2018 InterLab study found that this protocol gave very variable results. We therefore advise teams treat this protocol with some caution, and encourage them to find ways to improve it.

- Many plate readers have an automatic path length correction feature. This adjustment compromises the accuracy of measurement in highly light scattering solutions, such as dense cultures of cells. **You must therefore turn off pathlength correction if it can be disabled on your instrument.**
- This calibration should be completed before your team takes $OD_{600}$ cell measurements.
- Optical density (OD) measures the amount of light that is absorbed, scattered and reflected as it passes from source to detector. The $OD_{600}$ (i.e. OD at 600 nm) of a bacterial culture that has been blanked against growth media serves as an approximation for the number of bacteria in the culture. Spectrometers should be calibrated if numbers of bacteria are important to the experiment. A detailed description of optical density can be found here.
- General information about calibrating microbial growth in microplate readers can be found here.

MATERIALS

| NAME | CATALOG # | VENDOR | CAS NUMBER | RRID |
|---|---|---|---|---|
| Agar | | | | |
| 96 well plate | | | | |
| ddH20 | | | | |
| 1ml LUDOX CL-X | | | | |
| iGEM Measurement Kit 2019 | View | | | |

STEPS MATERIALS

| NAME | CATALOG # | VENDOR | CAS NUMBER | RRID |
|---|---|---|---|---|
| 1ml LUDOX CL-X | | | | |
| ddH20 | | | | |

MATERIALS TEXT

LUDOX-CL is no longer provided in the iGEM Measurement Kit. The 96-well plate should preferably be black with a clear flat bottom.

BEFORE STARTING

Read through this entire protocol carefully before you start your experiment and prepare any materials you may need. Please see disclaimer in guidelines section. See the "Results" section for an example of a completed data analysis spreadsheet.

**Data collection:** OD 600 Reference point - LUDOX Protocol

1 Add 100 µl LUDOX CL-X into wells A1, B1, C1, D1

1ml LUDOX CL-X

2 Add 100 µl of ddH20 into wells A2, B2, C2, D2

ddH20

3 Measure absorbance at 600 nm of all samples in the measurement mode you plan to use for cell measurements

Plate reader

Generic .

4 Record the data in your notebook or in the table below:

| | LUDOX CL-X | ddH2O |
|---|---|---|
| Replicate 1 | | |
| Replicate 2 | | |
| Replicate 3 | | |
| Replicate 4 | | |

Table for recording $OD_{600}$ values from your plate.

5 Once you've collected your results, open this spreadsheet to process them.

iGEM 2019 Plate Reader LUDOX Calibration.xlsx

## Data processing and analysis

6 Input your raw values from Step 5 into the blue cells in the table. go to step #4

7 Yellow cells will automatically populate with values calculated from your raw data.

Here is an example of a completed table:

| | A | B | C | D |
|---|---|---|---|---|
| 1 | | LUDOX CL-X H2O | | |
| 2 | Replicate 1 | 0.078 | 0.038 | |
| 3 | Replicate 2 | 0.077 | 0.038 | |
| 4 | Replicate 3 | 0.078 | 0.038 | |
| 5 | Replicate 4 | 0.078 | 0.038 | |
| 6 | Arith. Mean | 0.078 | 0.038 | |
| 7 | Corrected Abs600 | 0.040 | | |
| 8 | Reference OD600 | 0.063 | | |
| 9 | OD600/Abs600 | 1.585 | | |
| 10 | | | | |
| 11 | | | | |

Example of a completed data analysis table for this experiment. Blue cells contain raw data, yellow cells are the result of automatic calculations based on that data. The corrected Abs600 is calculated by subtracting the H2O reading. The reference OD600 is defined as that measured by the reference spectrophotometer (as provided to you in the Excel sheet). The correction factor to convert measured Abs600 to OD600 is thus the Reference OD600 divided by Abs600. All cell density readings using this instrument with the same settings and volume can be converted to OD600 by multiplying by (in this example) 1.585.

## Congratulations!

8 You have now completed this calibration protocol