Before You Begin

Read through this entire protocol sheet carefully before you start your experiment and prepare any materials you may need.

Calibration Protocols

1. OD_{600} Reference point

You will use LUDOX-S30 as a single point reference to obtain a ratiometric conversion factor to transform your absorbance data into a standard OD_{600} measurement. This has two key objectives. With standard 1 cm pathlength spectrophotometers, the reading is still instrument dependent (see above). With plate readers the path length is less than 1 cm and is volume dependent. In this instance the ratiometric conversion can both transform Abs_{600} measurements (i.e. the basic output of the instrument and not standardised optical density with 1 cm pathlength) into OD_{600} measurements, whilst simultaneously accounting for instrument differences.

[**IMPORTANT NOTE**: many plate readers have an automatic path length correction, this is based on volume adjustment using a ratio of absorbance measurements at 900 and 950 nm. Because scattering increases with longer wavelengths, this adjustment is confounded by scattering solutions, such as dense cells. **YOU MUST THEREFORE TURN OFF PATHLENGTH CORRECTION**.]

To measure your standard LUDOX Abs_{600} you must use the same cuvettes, plates and volumes (**suggestion**: use 100 μl for plate reader measurement and 1 mL for spectrophotometer measurement) that you will use in your cell based assays. The LUDOX solution is only weakly scattering and so will give a low absorbance value.

If using plates prepare a column of 4 wells with 100 μl 100% LUDOX and 4 wells containing 100 μl H_2O. Repeat the measurement in all relevant modes used in your experiments (e.g. settings for orbital averaging).

If using a cuvette, you will only have enough material for a single measurement, but repeat the reading multiple times. Use the same cuvette to measure the reference with H_2O (this value will be subtracted by the instrument to give the OD_{600} reading).

Materials:

1ml LUDOX (provided in kit)
H_20 (provided by team)
96 well plate or cuvettes (provided by team)

Method

- ☐ Add 100 µl LUDOX into wells A1, B1, C1, D1 (or 1 mL LUDOX into cuvette)
- ☐ Add 100 µl of H_2O into wells A2, B2, C2, D2 (or 1 mL H_2O into cuvette)
- ☐ Measure absorbance 600 nm of all samples in all standard measurement modes in instrument
- ☐ Record the data in the table below or in your notebook
- ☐ Import data into Excel (**OD600 reference point tab**) Sheet_1 provided

	LUDOX 100%	H_2O
replicate 1		
replicate 2		
replicate 3		
replicate 4		

Example data:

	A	B	C	D	Average	Corrected Abs_{600}	Reference OD_{600}	Correction Factor
Spectrophotometer	0.016	0.014	0.014	0.015	0.01475	0.01475	0.01475	1
Plate reader H_2O	0.033	0.033	0.034	0.033	0.03375	0		
Plate reader LUDOX	0.041	0.041	0.041	0.041	0.041	0.00775	0.01475	1.903

Table shows the data for OD_{600} measured in the reference spectrophotometer. For the plate reader data we show the measured Abs_{600} for the H_2O and LUDOX. The corrected Abs_{600} is calculated by subtracting the H_2O reading. The reference OD_{600} is defined as that measured by the reference spectrophotometer (you should use this value too). The correction factor to convert measured Abs_{600} to OD_{600} is thus the Reference OD_{600} divided by Abs_{600}. All cell density readings using this instrument with the same settings and volume can be converted to OD_{600} by multiplying by (in this instance) 1.903.

2. Protocol FITC fluorescence standard curve

You will prepare a dilution series of FITC in 4 replicates and measure the fluorescence in a 96 well plate in your plate reader or individually in cuvettes in a fluorimeter. By measuring these in all standard modes in your plate reader or fluorimeter, you will generate a standard curve of fluorescence for FITC concentration. You will be able to use this to correct your cell based readings to an equivalent fluorescein concentration. You will then be able to convert this into a concentration of GFP.

Before beginning this protocol ensure that you are familiar with the GFP settings and measurement modes of your instrument.

Materials:

194.7 μg FITC (provided in kit)
10ml 1xPBS (phosphate buffered saline; provided by team)
96 well plate or cuvettes (provided by team)

Method

Prepare the FITC stock solution:

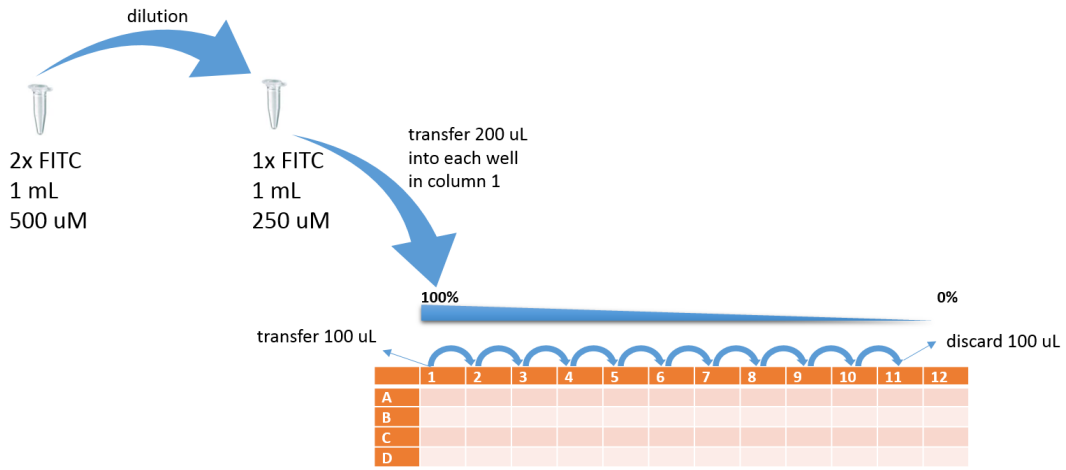
- ☐ Spin down FITC stock tube to make sure pellet is at the bottom of tube.
- ☐ Prepare 2x FITC stock solution (500 μM) by resuspending FITC in 1 mL of 1xPBS
- ☐ Incubate the solution at 42°C for 4 hours
- ☐ Dilute the 2x FITC stock solution in half with 1xPBS to make a 1x FITC solution and resulting concentration of FITC stock solution 250 μM.

[**Note**: it is important that the FITC is properly dissolved. To check this after the incubation period pipetted up and down – if any particulates are visible in the pipette tip continue to incubate overnight.]

Prepare the serial dilutions of FITC:

Accurate pipetting is essential. Serial dilutions will be performed across columns 1-11. COLUMN 12 MUST CONTAIN PBS BUFFER ONLY. Initially you will setup the plate with the FITC stock in column 1 and an equal volume of 1xPBS in columns 2 to 12. You will perform a serial dilution by consecutively transferring 100 μl from column to column with good mixing.

Overview samples in 96 well plate or cuvettes



- ☐ **Add 100 µl of PBS** into wells A2, B2, C2, D2....A12, B12, C12, D12
- ☐ **Add 200 µl of FITC 1x stock** solution into A1, B1, C1, D1
- ☐ Transfer 100 µl of FITC stock solution from A1 into A2.
- ☐ Mix A2 by pipetting up and down 3x and transfer 100 µl into A3…
- ☐ Mix A3 by pipetting up and down 3x and transfer 100 µl into A4...
- ☐ Mix A4 by pipetting up and down 3x and transfer 100 µl into A5...
- ☐ Mix A5 by pipetting up and down 3x and transfer 100 µl into A6...
- ☐ Mix A6 by pipetting up and down 3x and transfer 100 µl into A7...
- ☐ Mix A7 by pipetting up and down 3x and transfer 100 µl into A8...
- ☐ Mix A8 by pipetting up and down 3x and transfer 100 µl into A9...
- ☐ Mix A9 by pipetting up and down 3x and transfer 100 µl into A10...
- ☐ Mix A10 by pipetting up and down 3x and transfer 100 µl into A11...
- ☐ 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.

- ☐ Repeat dilution series for rows B, C, D
- ☐ Measure fluorescence of all samples in all standard measurement modes in instrument
- ☐ Record the data in your notebook
- ☐ Import data into Excel (**FITC standard curve tab**) Sheet_1 provided

For cuvette usage:

Use 2.0mL tubes for cuvette dilutions, and then transfer 1.0mL into your cuvettes.

- ☐ **Add 1 mL of PBS** into tubes 2-11.
- ☐ **Add 2.0 mL of FITC 1x stock** solution tube 1
- ☐ Transfer 1.0 ml of FITC stock solution tube 1 into tube 2
- ☐ Mix tube 2 by pipetting up and down 3x and transfer 1 mL into tube 3…

- ☐ Mix tube 3 by pipetting up and down 3x and transfer 1 mL into tube 4…
- ☐ Mix tube 4 by pipetting up and down 3x and transfer 1 mL into tube 5…
- ☐ Mix tube 5 by pipetting up and down 3x and transfer 1 mL into tube 6…
- ☐ Mix tube 6 by pipetting up and down 3x and transfer 1 mL into tube 7…
- ☐ Mix tube 7 by pipetting up and down 3x and transfer 1 mL into tube 8…
- ☐ Mix tube 8 by pipetting up and down 3x and transfer 1 mL into tube 9…
- ☐ Mix tube 9 by pipetting up and down 3x and transfer 1 mL into tube 10…
- ☐ Mix tube 10 by pipetting up and down 3x and transfer 1 mL into tube 11…
- ☐ Mix tube 11 by pipetting up and down 3x and and transfer 1 mL into **liquid waste**

Measurement Notes

You must now measure the plate (or cuvettes) in your plate reader (or fluorimeter). The machine must be setup with the standard GFP settings (filters or excitation and emission wavelengths) that you will use when measuring your cells (if you change them you will not be able to use this standard curve). It is therefore a good idea to repeat the measurement a number of times with different settings. You will then have a series of standard curves to choose from. Most important it is necessary to use a number of settings that affect the sensitivity (principally gain and/or slit width). Be sure to also consider other options (orbital averaging, top/bottom optics). As before, TURN OFF path length correction if available.

Make sure to record all information about your instrument (wavelengths, etc.) as these will be required in the Plate Reader Form.

Cell measurement protocol

Prior to performing the measurement on the cells you should perform the **calibration measurements**. This will ensure that you understand the measurement process and that you can take the cell measurements under the same conditions.

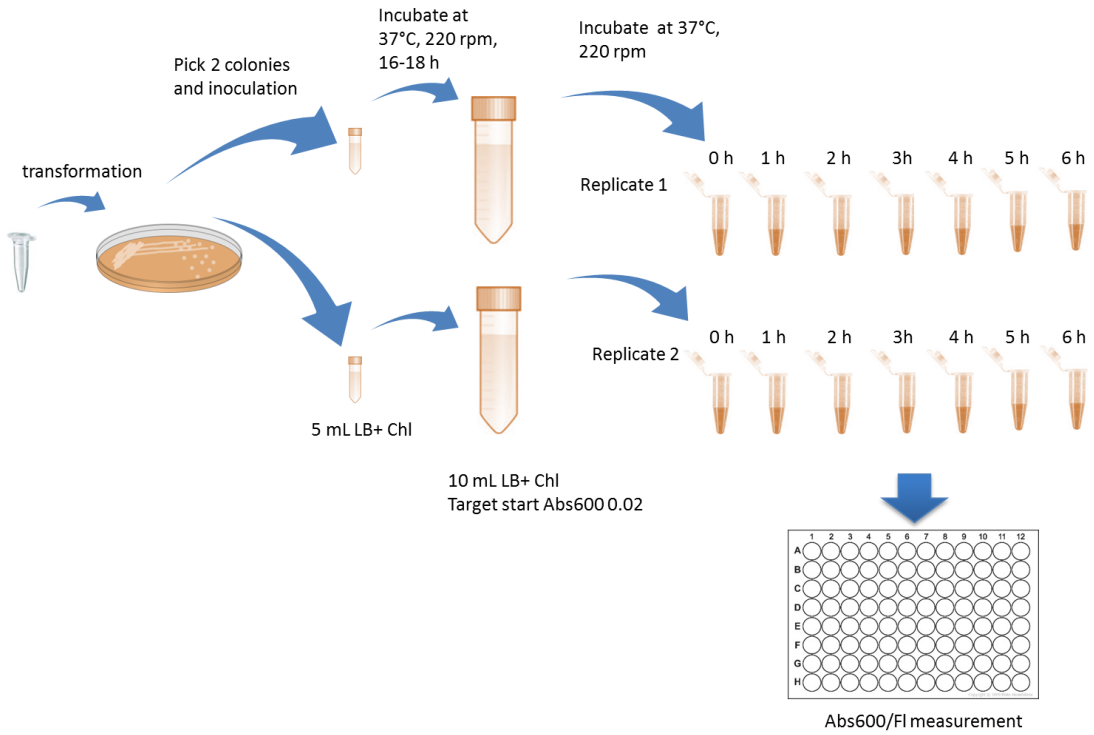
Materials:

Competent cells (ideally *Escherichia coli* strain DH5α or TOP10)
Terrific broth (at half strength: 0.5x TB) or can use LB (Luria Bertani) media as an alternative
Chloramphenicol (stock concentration 25 mg/mL dissolved in EtOH)
50 ml Falcon tube (or equivalent) or 250 ml shake flask for cell growth
Incubator at 37°C
1.5 ml eppendorf tubes for sample storage
Ice bucket with ice
Pipettes

Devices (from InterLab Measurement Kit):

- Positive control
- Negative control
- Device 1: J23101+I13504
- Device 2: J23106+I13504
- Device 3: J23117+I13504

Workflow



Method

Day 1: transform *Escherichia coli* DH5α or TOP10 with these following plasmids:

- Positive control
- Negative control
- Device 1: J23101+I13504
- Device 2: J23106+I13504
- Device 3: J23117+I13504

Day 2: Pick 2 colonies from each of plate and inoculate it on 5-10 mL LB medium + Chloramphenicol. Grow the cells overnight (16-18 hours) at 37°C and 220 rpm.

Day 3: Cell growth, sampling, and assay

Note the differences, depending on whether you are making your readings in a plate reader or 1 ml cuvettes:

- ☐ Set your instrument to read OD600 (as OD calibration setting)
- ☐ Measure OD600 of the overnight cultures
- ☐ Record data in your notebook
- ☐ Import data into Excel (**normalisation tab**) Sheet_1 provided
- ☐ Dilute the cultures to a target OD_{600} of 0.02 (see the volume of preloading culture and media in Excel (**normalisation tab**) Sheet_1) in 10 ml 0.5x TB medium +

Chloramphenicol in 50 mL falcon tube (if using cuvettes, you can use 100 ml in a 500 ml shake flask).

- ☐ Incubate the cultures at 37°C and 220 rpm.
- ☐ Take 100 µL (1% of total volume) samples of the cultures at 0, 1, 2, 3, 4, 5, and 6 hours of incubation (if using cuvettes, remove 1 ml from 100 ml culture).
- ☐ Place samples on ice.
- ☐ At the end of sampling point you need to measure your samples (OD and Fl measurement), see the below for details.
- ☐ Record data in your notebook
- ☐ Import data into Excel (**cell measurement tab**) Sheet_1 provided

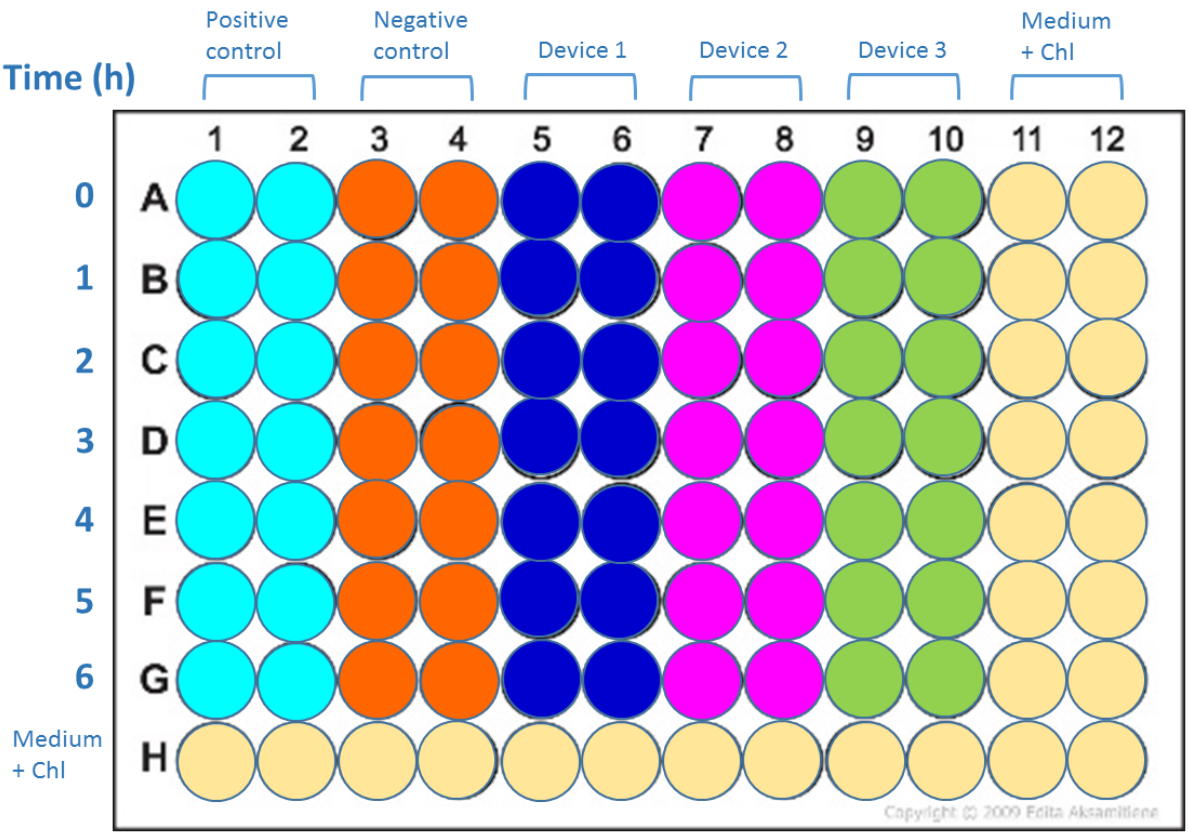
Measurement

It is important that you use the same instrument settings that you used when measuring the FITC standard curve. This includes using the sample volume (100 ul) or 1 mL sample for measurement using spectrophotometer.

Samples should be laid out according to Fig. 2. Pipette 100 µl of each sample into each well. Set the instrument settings as those that gave the best results in your calibration curves (no measurements off scale). If necessary you can test more than one of the previously calibrated settings to get the best data (no measurements off scale).

Hint:
No measurement off scale means the data you get does not out of range of your calibration curve.

Layout for Abs600 and Fluorescence measurement


Time (h)
Positive control
Negative control
Device 1
Device 2
Device 3
Medium + Chl
1 2 3 4 5 6 7 8 9 10 11 12
0
1
2
3
4
5
6
Medium + Chl
A
B
C
D
E
F
G
H
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