MEASURING FLUORESCENCE ON A PLATE READER

If you have a lot of measurements to make, it can be very useful to use a plate reader, as you can measure up to 96 wells at once. You can also program the machine to take measurements regularly so that you can do other experiments in the meanwhile.

Make sure to add around 20µl of oil/wax on top of your sample to avoid evaporation.

MATERIALS:

- Cells
- LB medium with apropriate antibiotic
- 10X MOPS buffer (at pH 5.5 and 6.5)
- 10X HEPES buffer (at pH 8.5)

PROCEDURE:

□ Incubate Cells in different media for at least 2h:

LB + Chloramphenicol buffered with 10X MOPS + HCl, adjusted to a pH of 5.5

LB + Chloramphenicol buffered with 10X MOPS, at pH 6.5

LB + Chloramphenicol buffered with Water (as a control)

LB + Chloramphenicol buffered with 10X HEPES, at pH 8.5

□ Measure the OD value

□ Centrifuge the cell for 3min at2000rpm at 24°C for pellet formation

□ Discard supernatant

□ Wash cells with 1ml 1X PBS

□ Centrifuge the cell for 3min at2000rpm at 24°C for pellet formation

□ Remove PBS and suspend cells in 5-10ml LB

□ Dilute the suspended cells in fresh LB-Chloramphenicol medium in order to obtain an OD of 0.1-0.5

□ Pipette 100µl of bacteria + 100µl buffer onto the plate reader, making at least three replicates for each buffer and at least three blanks for each buffer

□ Add 20µl of oil or wax on top of each sample

□ Make Absorbance and Fluorescence measurements every 30 minutes for 18h.

□ Calculate the average absorbance and fluorescence for each buffer as well as the blanks.

□ Subtract the average blanks from the average Absorbance and Fluorescence

□ Normalize the fluorescence by dividing it by the absorbance and plot the resulting values.