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Luminol Calibration

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Calibration curve using luminol in order to standardize luminescence data

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Preparation of the Buffers

1 Preparation of 100ml luminol stock solution (1 mM luminol sodium salt; 50 mM sodium carbonate; 300 mM sodium bicarbonate; 5 mM ammonium carbonate) :
dissolve the following components in ddH_2O

19,94mg of Luminol sodium salt
529,94mg of sodium carbonate
2,52g of sodium bicarbonate
48mg of ammonium carbonate

Preparation of the coppersulfate solution (1,5 mM $CuSO_4$):

dissolve 23,94mg of $CuSO_4$ in ddH_2O

Preparation of dilution series

2 Perform a dilution series in a 384 well plate similar to the protocol provided by the iGEM

foundation on Fluorescein calibration:

3 Add 100µl of the $CuSO_4$ solution in the first well

4 Fill every second well in that row with 50µl of water (8 rows in total)

5 Transfer 50µl of the first well into the next and carefully pipette up and down to ensure proper mixing of the solutions

6 Repeat this until you reach the second last well and then dispose the last 50µl

Perform the measurement

7 Premix the luminol buffer with hydrogen peroxide in a ratio of 4:1

8 Transfer 10µl of this solution into empty wells on the plate

9 Use a multichannel pipette to add the inducing copper sulfate solution to the luminol solution

10 Transfer the well plate into the plate reader and shake the plate for 30 seconds until you start the measurement

11 Measure the luminescence with an exposure time of 250ms

12 Plot the data in a linear correlation and use this calibration curve in order to normalize your luminescence data

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