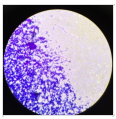

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Bacteria Staining

Guillermo Fernández Rodríguez[1]

[1]AEGIS - Madrid iGEM 2019

1 Works for me dx.doi.org/10.17504/protocols.io.8gshtwe

AEGIS - Madrid iGEM 2019

Guillermo Fernández Rodríguez

ABSTRACT

A bacteria staining protocol has been automated by OT-2. It allows to check the amount of target we had coated on the 96 well plate. (We used 5 replicates per dilution)

MATERIALS

NAME	CATALOG #	VENDOR
Crystal violet	C-328	Gold Biotechnology
Nuclease-free water or water filtered using a Milli-Q filtering system	AM9932	Ambion
Sodium bicarbonate	S6014	Sigma – Aldrich
PBST (PBS 1:1000 Tween-20)	View	
Centrifuge	5415D	Eppendorf Centrifuge
LB	L24400-2000.0	Research Products International (rpi)
White 96-Well Immuno Plates, Maxisorp, Flat-Bottom, MaxiSorp, 350µL	436110	Thermo Fisher

BEFORE STARTING

Clean all the working surface with ethanol.

Staining bacteria

1 Inoculate a single colony of E.Coli DH5α from LB agar plate in **10 ml** of LB. Use a sterile pipette tip, selecting a single colony from LB agar plate. The liquid culture is incubated overnight **37 °C** .

2 Spin at **4000 rpm** for **00:05:00** . Discard the supernatant, collect pellet and re-suspend in **10 ml** of $NaHCO_3$-Na_2CO_3, 50mM, pH 9,6. Mix by inverting the tube.

3 Spin at 4000 rpm for **00:05:00** Discard the supernatant, collect pellet and re-suspend in **8 ml** of $NaHCO_3$-Na_2CO_3, 50mM, pH 9,6. Mix by inverting the tube.

4 Read the absorbance (600nm). Dilute the sample with $NaHCO_3$-Na_2CO_3, 50mM, pH 9,6. and adjust the absorbance to 1.

5 Make the following dilutions with -Na_2CO_3, 50mM, pH 9,6:1:5, 1:10, 1:30, 1:50 and 1:100.

6 Add **200 µl** the sample into 96 well-plate Nunc MaxiSorp. Incubate overnight at **4 °C** .

7 Wash 3x **200 µl** PBS Tween 0,1%, pH 7,4. Remove the drops after the last wash.

8 Add **150 µl** of crystal violet/well. Incubate for **00:15:00** at **Room temperature**

9 Wash 4x **250 µl** destilled water. Wait for **01:30:00** for air-drying before counting colonies in the microscope.