OCT 20, 2019

Bacteria Staining

Guillermo Fernández Rodríguez¹

¹AEGIS - Madrid iGEM 2019

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

MATERIALS

⊗ Crystal violet **Gold Biotechnology Catalog #C-328**

⊗ Nuclease-free water or water filtered using a Milli-Q filtering system **Ambion Catalog #AM9932**

⊗ Sodium bicarbonate **Sigma – Aldrich Catalog #S6014**

⊗ PBST (PBS 1:1000 Tween-20) **Contributed by users**

⊗ Centrifuge **Eppendorf Centrifuge Catalog #5415D**

⊗ LB **Research Products International (rpi) Catalog #L24400-2000.0**

⊗ White 96-Well Immuno Plates, Maxisorp, Flat-Bottom, MaxiSorp, 350µL **Thermo Fisher Catalog #436110**

BEFORE START INSTRUCTIONS

Clean all the working surface with ethanol.

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.8gshtwe

Protocol Citation: Guillermo Fernández Rodríguez 2019. Bacteria Staining. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.8gshtwe>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working

Created: Oct 19, 2019

Last Modified: Oct 20, 2019

PROTOCOL integer ID:
28914

Keywords: Staining, Crystal violet, Coating, Maxisorp-96well

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 NaHCO₃-Na₂CO₃, 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 NaHCO₃-Na₂CO₃, 50mM, pH 9,6. Mix by inverting the tube.
- 4 Read the absorbance (600nm). Dilute the sample with NaHCO₃-Na₂CO₃, 50mM, pH 9,6. and adjust the absorbance to 1.
- 5 Make the following dilutions with -Na₂CO₃, 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 distilled water. Wait for 01:30:00 for air-drying before counting colonies in the microscope.