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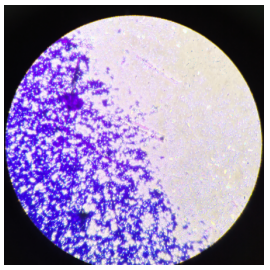
Bacteria Staining



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

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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 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S6014
- PBST (PBS 1:1000 Tween-20)
- Centrifuge **Eppendorf Catalog #**5415D
- LB **Research Products International Corp (RPI) Catalog #**L24400-2000.0
- White 96-Well Immuno Plates, Maxisorp, Flat-Bottom, MaxiSorp, 350μL **Thermo Fisher Catalog #**436110

Troubleshooting

Before start

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.