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 We use this protocol and it's working

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Growth curve analysis

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ABSTRACT

To observe the potential of defense mechanisms of either native or synthetic systems in *Escherichia coli* (and more) when incubated with a bacteriophage stock.

MATERIALS

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96-well plate, flat bottom, tissue culture treated, black wall with clear bottom **Fisher Scientific Catalog #3904**

Microplate Reader Synergy Mx **Contributed by users**

STEP MATERIALS

96-well plate, flat bottom, tissue culture treated, black wall with clear bottom **Fisher Scientific Catalog #3904**

Preparations

- 1 Media and bacteriophage stock solutions :
 - 1L Luria-Bertani (LB) media (with antibiotics)
 - Desired Bacteriophage stock solution in LB media (with known Plaque Forming Units (PFU) ml⁻¹)
- 2 **Fill in plate reader protocol as follows:**
 - Set temperature: 37°C
preheat before moving to next step
 - Start kinetics:
Runtime 15:00:00 (HH:MM:SS), Interval 0:04:00
 - Shake:
medium, 0:30 (MM:SS)
 - Read:
Absorbance Endpoint, Full Plate
Wavelengths: 600
Read Speed: Normal, Delay: 100 msec
 - End kinetics
- 3 Prepare overnight cultures of desired samples (with associated antibiotics).

Plate reader

- 4 Measure OD600 of overnight cultures and dilute cultures to an OD600 of 0.02
- 5 Load 180 µL of diluted overnight culture into a 96-well plate, flat bottom, tissue culture treated, black wall with clear bottom **Fisher Scientific Catalog #3904**
Include a serie of LB (without bacteria) as a control and as zero point for the OD600 measurements!

6 Start plate reader protocol go to step #2 and let the bacteria grow to an OD600 of 0.11.

7 Prepare Bacteriophage PFU dilutions (with associated antibiotics) for;

MOI 10^1 : 4.0×10^{10} PFU ml⁻¹

MOI 10^0 : 4.0×10^9 PFU ml⁻¹

MOI 10^{-1} : 8.0×10^8 PFU ml⁻¹

MOI 10^{-2} : 8.0×10^7 PFU ml⁻¹

MOI 10^{-3} : 8.0×10^6 PFU ml⁻¹

An OD600 of 0.10 correlates to 8.0×10^8 cells per ml.

The above concentrations are required when 20 µl of bacteriophage dilution is added into 180 µl of cell culture with an OD600 of 0.11 (1:10 dilution).

8 At the moment an OD600 of 0.11 is reached, the plate reader must be stopped and 20 µL of bacteriophage dilution* must be added to a final volume of 200 µL to both the samples and the LB controls.

* include as a control, a serie without bacteriophages and only LB media (with antibiotics)

9 Restart the plate reader protocol and measure over 15 hours the growth of the samples.