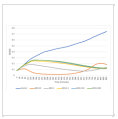

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Growth curve analysis

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1 Works for me dx.doi.org/10.17504/protocols.io.77jhrkn

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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

NAME	CATALOG #	VENDOR
96-well plate, flat bottom, tissue culture treated, black wall with clear bottom	3904	Fisher Scientific
Microplate Reader Synergy Mx	View	

STEPS MATERIALS

NAME	CATALOG #	VENDOR
96-well plate, flat bottom, tissue culture treated, black wall with clear bottom	3904	Fisher Scientific

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
by 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 x 10^{10} PFU ml^{-1}
MOI 10^{0} : 4.0 x 10^{9} PFU ml^{-1}
MOI 10^{-1} : 8.0 x 10^{8} PFU ml^{-1}
MOI 10^{-2} : 8.0 x 10^{7} PFU ml^{-1}
MOI 10^{-3} : 8.0 x 10^{6} PFU ml^{-1}

An OD600 of 0.10 correlates to 8.0 x 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.