

## Plaque Assay of T4 Phage

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Plaque assays are used to titer viruses. To titer is to determine the concentration of the virus. Plaques are produced because the virus does not infect all of the host cells. These cells grow into a cloudy layer of cells on the agar plate. A plaque appears to be a clear area. The plaque is the result of virus lysing the bacterial cells. Since the cells are lysed, they no longer scatter light and therefore no longer look cloudy. Plaque assays are routinely done to determine how much virus is present in solutions.

### Materials

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Nutrient peptone broth [8g Nutrient broth, 5g peptone, 5 g NaCl, 1g dextrose, 1 liter H<sub>2</sub>O]

37°C incubator and 45°C water baths

Tryptone base-layer agar plates (5/group) [10g tryptone, 11g agar, 5g NaCl, 2 ml 1M CaCl<sub>2</sub>, 1 liter H<sub>2</sub>O]

Tryptone top agar (30 ml per group) [10g tryptone, 9g agar, 5g KCl, 1 liter H<sub>2</sub>O]

Sterile test tubes (7 per group)

*E.coli* B or K12 cells grown overnight

T4 stock

### Methods

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- 1) Melt a supply of top agar in the microwave. Let it cool to 45°C in a water bath.
- 2) Make serial dilutions of the T4 phage control stock as follows:
  - a) Add 990 µl of broth to each of 3 numbered sterile test tubes, and 900 µl of broth to each of 3 more numbered sterile test tubes (the fifth and sixth tubes).
  - b) Add 10 µl of your phage stock to the first tube. Mix thoroughly but **GENTLY!**
  - c) Remove 10 µl from the first tube and place it in your second tube. Mix gently.
  - d) Continue doing this until the first three tubes contain dilutions of phage.
  - e) Remove 100 µl from the third tube and place it in your fourth tube. Mix gently.
  - f) Remove 100 µl from the fourth tube and place it in your fifth tube. Mix gently.
  - g) Remove 100 µl from the fifth tube and place it in your sixth tube. Mix gently.

*Note:* This procedure dilutes the T4 up to 10<sup>9</sup> fold!
- 3) Prepare tube seven as a sample without phage by adding only 1 ml of media and no phage.
- 4) Add 2 *drops* of *E.coli* per tube of phage (this is about 60 µl). Note that we will **not** be plating the first two dilutions (tubes 1 and 2).
- 5) Using aseptic technique, add 4.0 ml of top agar to the no phage sample and to each of the eight prepared sample tubes containing dilutions of T4, and then **immediately** pour the mixture onto an agar plate. To reduce contamination problems, start with the most dilute phage samples, then proceed to the less dilute ones (i.e., start at tube 7 and go to tube 3).

- 6) After the plates set (gel), *invert* and place them in a 37°C incubator. Incubate them overnight.
- 7) **Day 2:** Count the plaques on the plates. If there are too many plaques, the plate will be “clear”. Pick the ones that have a countable number of plaques and count them. By back calculating, you should be able to calculate the titers of the phage stock.

## **Analysis**

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Determine the titer of the T4 stock as phage per ml. Assuming that you grew a 100 ml culture, how many T4 would be in the flask?