

TITERING OF BACTERIAL VIRUSES

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http://biology.clc.uc.edu/fankhauser/Labs/Microbiology/Phage/Phage_Titer.htm

Related protocols: *Preparation of Phage Stocks*
 Commonly Used Media for Phage Growth
 Agar Overlay Technique

When an individual bacterial virus grows in a sensitive host suspended in a top agar lawn, its progeny infect and lyse the surrounding host cells. This causes the appearance of a hole or plaque in the otherwise homogeneous bacterial lawn. Since a plaque represents a single virus, the number of viruses in the aliquot is equal to the number of plaques which appear.

EQUIPMENT:

sterile capped 16x150 mm test tubes
sterile capped 13x100 mm tubes
sterile pipetters, 0.01 and 0.1 mL
hot block, 45°C
vortex
Bunsen Burner
receptacle for used tips, used tubes & tops
37°C incubator

SUPPLIES:

melted top agar, about 60°C
phage culture to be titered
4 mL sensitive host bacteria grown ON in TSB
(such as *E. coli* B)
sterile dH₂O in 10.0 mL repipet
pre-warmed to 37°C tryptone soy agar plates
(or LB agar plates for lambda phage)

PROTOCOL:

THE PREVIOUS NIGHT:

1. Inoculate about 4 mL (for thirty plates) of nutrient broth or tryptone soy broth with a sensitive host (*E.g. E. coli* B). Grow ON at 37°C in hot block.

THE DAY OF ASSAY:

2. **Prepare a dilution of the phage** such that there are about 10³ particles per mL (usually a dilution of 10⁶: 10 µL into 10.0 mL, repeat a second time).
3. **Set up seeded top agar:**
 - a. Pipet about 2 mL of melted agar into sterile capped 13x100 mm tubes in 45°C hot block.
 - b. Pipet about 0.1 mL host bacteria (*E. coli* B) using a 1.0 mL pipet into melted agar (down the side of the tube is OK).
4. **Add phage:** Pipet 10 or 100 µL of 10⁻⁶ phage into the host-inoculated tube (deliver with care, just below surface of agar).
5. **Vortex** to mix.
6. **Pour out and distribute over a pre-warmed agar plate**, immediately tilt back and forth to evenly distribute before it begins to gel. Let sit undisturbed until gelled.
6. When gelled (1-2 minutes), **invert plates, incubate ON** at 37°C.

THE NEXT DAY:

7. **Count all plaques, record data, calculate phage particles/mL** in the original culture. Apply the following formula:

$$\text{phage/mL} = (\text{number plaques/plate}) \times (1/\text{mL plated}) \times \text{dilution factor}$$

For example, if you got 347 plaques when you plated out 0.1 mL of a 10⁶ diluted phage suspension, the titer is 3.47 x 10⁹/mL.