Titering phages

Titering phages is probably the most common phage protocol of all. There are many ways to count phages, but here we present the two most common methods, both of which involve serially diluting the phage sample and placing defined aliquots onto a bacterial lawn so that the resulting plaques can be counted. Spot titers are quick and easy, but not completely accurate. Whole plate titers are more cumbersome but give the most accurate results. Each will be discussed in more detail below.

The first step for both methods is to serially dilute your phage sample.

Serial dilution

Serial dilution involves diluting your sample in defined increments until a given aliquot of your dilution will yield countable plaques in your bacterial lawn. While theoretically any defined dilution factor can be used, it is most common and simplest to use 10-fold dilutions.

1. For each dilution required, label a sterile microcentrifuge tube with the phage name and the dilution factor that will be made in the tube.
    1. For example, if you are serially diluting from 10^{0} (undiluted) to 10^{-8} you will need to label 8 tubes, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}
    2. Label one extra tube as a control. This tube will contain buffer only, no phages, and will serve as a negative control to ensure that your buffer is not contaminated.
2. Into each tube, aseptically aliquot 900 µL of λ-dil buffer. Close the tubes.
3. Take 100 µL of your sample and add it to the 10^{-1} tube. Briefly vortex the tube.
4. Take 100 µL of your 10^{-1} dilution and add it to the 10^{-2} tube. Briefly vortex the tube.
5. Repeat for each dilution.

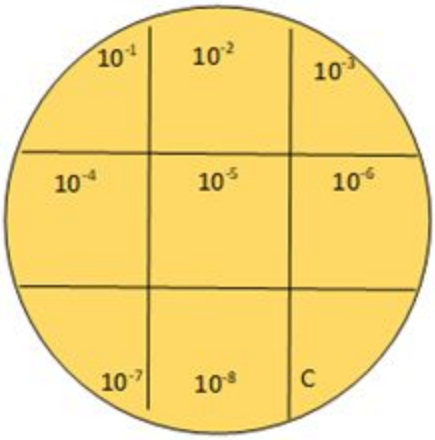
You will now have a series of dilutions, each in 10-fold increments, from undiluted to 10^{-8}. Use any suitable dilution range for the phage sample(s) you are working with.

Spot titer

Spot titers involve pouring a lawn of bacteria then placing aliquots of phage dilution onto the lawn. Typically, a small volume of dilution (5-20 µL) is spotted onto the lawn, so a single lawn can hold up to ~15 spots. We have found that 5 µL spots tend to not run into each other on 0.5% top agar, so we most commonly use this volume. However, a larger volume aliquot will give more accuracy as long as the spots remain separate and do not spread into each other.

Label your plate(s) and mark your plate(s) with the location that each dilution factor (or control) spot will be placed.. Make sure that each spot is as far from other spots as possible. The CPT

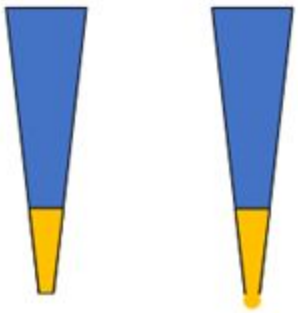
often use grids that allow for 9 spots per plate. Mark a location for each of your serial dilutions plus your negative control.



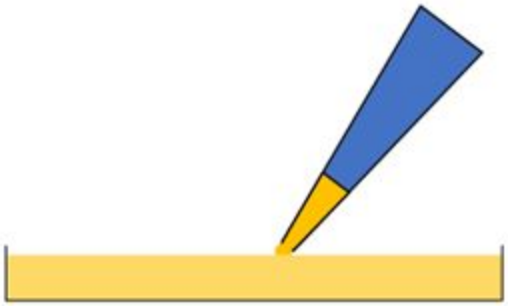
The grid pattern used here allows for 9 spots to be placed onto a single lawn. Spots should be placed centrally within the appropriate zone, avoiding the labels and markings. “C” is the control spot, note that it is closest to the most dilute spots to avoid possible contamination from the more concentrated phage spots.

Pour your lawn as described here then leave it to set.

Starting with your control tube (and working from 10^{-8} to 10^{-1}, not the other way around) aspirate 5 µL of sample with a pipettor then carefully place that aliquot oto the lawn. Before you place your spot onto the lawn, very gently depress the push button so that a tiny bleb of liquid can be seen protruding from the tip. A steady hand is required, so place your non-dominant hand on the bench so you can rest your dominant hand holding the pipettor on it for stability. Touch the bleb of sample against the agar surface so that you see the liquid make contact with the lawn, then gently eject the remaining liquid into the spot you have made. Make sure to fully depress the push button so that the entire aliquot is ejected.

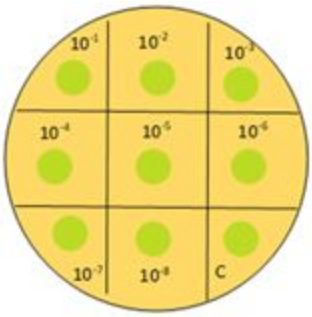


The tip on the left is loaded with sample and the push button fully released. The tip on the right shows a tiny bleb after the push button has been very slightly depressed. Touch the bleb gently to the surface of the lawn as shown below.



As long as you have no reason to think it might be contaminated by anything other than the previous sample, you can use the same tip for the 10^{-8} dilution, and so on through all of your serial dilutions from 10^{-8} to 10^{-1}, but NOT the other way around. This is because any residual

sample left in the tip from a more dilute phage sample will not significantly affect the results, whereas residual sample from a more concentrated sample will invalidate your results. Replace the tip each time if you prefer, or if you suspect the tip could be contaminated by anything other than one of the more dilute samples. Always eject the tip when you are finished with a given dilution series.



When you are done spotting, your plate should look something like this. Keep your spots well separated, if they run together then you will have to repeat the entire process. Before the plate can be incubated the spots need to absorb into the top agar. With the plate inside your working area around your flame, take the lid off of the plate and rest it on the side of the base so that the agar surface is fully exposed to the air. Leave the plate open for no more than 5 minutes, if the top agar dries out it will affect plaque formation.

Once the spots are sufficiently absorbed the lid can be closed and the plate inverted and incubated.

Whole plate titer

Whole plate titers are more accurate for a number of reasons. A larger aliquot of phage is used (typically 50-100 µL), which is itself more accurate than pipetting smaller volumes. The plaques from a single aliquot are spread over the surface of the entire Petri dish, so they are far less likely to overlap, meaning that more plaques can be counted more accurately. Finally, the phage and the cells are premixed, allowing the phage to adsorb to their host cells prior to lawn formation. This tends to lead to more plaques, and more uniform plaques than spot titers because all plaques are initiated at the start of lawn formation, whereas for spot titers the phage must diffuse through the top agar and adsorb to their host, which will take different amounts of time for each phage. If a phage takes too long to find a host then the lawn will already be growing, possibly approaching stationary phase, and the resulting plaque will be smaller or invisible as a result.

However, whole plate titers only allow for a single aliquot of phage sample per plate, so they are time-consuming and expensive to carry out.

To carry out a whole plate titer, first label your plate(s), including the dilution factor to be used for each plate.

In a sterile microcentrifuge tube, mix your cell culture aliquot (usually 10-100 µL of overnight or log phase cells) and your phage sample aliquot (usually 100 µL). Close the cap and gently tap the tube to mix the cells and phage, then leave for 5-10 minutes for the phages to fully adsorb. Some phages can produce progeny in as little as 15-20 minutes, so it is important not to let the mixture sit for too long.

Aspirate the entire mixture into a fresh pipette tip and pour a lawn with this entire reaction as described above. Allow the lawn to set before inverting and incubating the plate.

Results and calculations

After incubation, take a look at your plate(s). For spot titers, check to see if your control spots are devoid of plaques. If they are not then you may have to repeat the titer with fresh buffer, check with your instructor. Check to see if your serial dilutions are behaving as expected. As in, do you see the spots going from complete lysis to individual plaques (and, preferably, to extinction) in roughly 10-fold increments? Choose a spot to count, this will be the least most concentrated dilution to show entirely separate plaques. Note the concentration and count the plaques.

For whole plate titers, you also want to check your control plate (no plaques) and look to see if your dilutions performed roughly as expected. Identify the dilution that lead to the largest number of entirely separate plaques and count the plaques.

To calculate the titer in PFU/mL you need to know your plaque count (or mean count if n>1), the dilution factor and the aliquot volume of your phage sample. The calculation is as follows:

Phage titer (PFU/mL) = plaque count / (dilution factor x aliquot volume (mL))

So, if you counted 37 plaques (PFU) from a 100 µL aliquot of 10^{-6} dilution, the titer will be:

37 PFU/(1E-06 x 0.1mL) = 3.7E+08 PFU/mL

Notes:

It is possible to fit at least 15 5 µL spots on a single, thoroughly set lawn of a spot titer plate without them running into each other. This allows the user to spot 5 dilutions of a dilution series in triplicate, giving more reliable data if needed. This is especially suitable when an approximate titer is already known from experience or previous data.

Spot titers are often used to approximate sample titers and identify the most suitable dilution and aliquot volume to be used for whole plate titers. While this takes an extra day to generate results, the savings in materials and operator time are often desirable.