Plaque Assay Protocol for Pseudoalteromonas

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Abstract

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Before start

What you need before you start:
1. 20% nutrient Zobell plates
2. Top agar – 3.5 ml per plate
    a. 100% nutrient Zobell
    b. 6g agar/liter
3. Your host grown to exponential phase. You will need 0.4ml for each plate in the assay.
4. Phages to be tested. This could be
    a. a lysate (0.2 µm filtered)
    b. a plaque picked into buffer
    c. an environmental sample
    d. a sample from an experiment (e.g., a one-step growth curve assay)
5. A water bath set to 40°C.

Protocol

Step 1.
First, autoclave the top agar to melt it.

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Note: If your top agar was autoclaved previously, liquify it by putting it in a microwave (no stir bar!) and heating until it is completely melted.

Step 2.
While the autoclave is running:
a. Let your bottom agar plates warm to room temperature and label them
b. Make whatever dilutions of your phages you plan to plate

Note: it is wise to plate one dilution higher and one dilution lower than what you calculated as optimal

Step 3.
Aliquot the agar into tubes and place in 40°C water bath.

NOTES

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Before you use the agar, make sure the tube feels the same temperature as the water bath. If the agar is still too hot, you'll kill your host You'll need to wait about 5—15 minutes for it to cool down enough. If the agar is too cool, it might start getting chunky If your agar is starting to solidify, do NOT use it Make up a new tube if your big bottle is still hot or re-autoclave it If it will take you more than 30 minutes to do all your infections, you're doing too many at a time – your agar will start to solidify

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If you are preparing more than one plate with the same host, you can aliquot enough top agar for however many plates you are comfortable doing at one time (up to 12) into a sterile 50 ml conical tube. Be sure to leave room in your tubes to add host.

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Use 3.5 ml top agar per plate

Step 4.

Pipet 100 µl of your phage sample onto the plate

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If you made up agar aliquots for more than one plate, add phage to each plate now.

Step 5.

Remove the agar tube from the water bath and add the host to it using a 1 ml or 5 ml pipettor

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Pipet up and down or gently invert to mix. Try not to introduce a lot of bubbles (i.e., do not shake or vortex the tube).

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Use 0.4 ml of host per plate.

Step 6.

Add 3.5 ml of the agar/host mixture to each plate.

NOTES

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If you want your plates in stacks, stack them as you go. They cannot be moved after plating for at least an hour.

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A 5 ml pipettor works well for adding the agar/host to the plates. If only one plate is prepared, you can simply pour the contents of the tube onto the plate.

Step 7.

Swirl the plate to spread the agar making sure it covers the whole plate.

Step 8.

Leave the plates on the bench overnight

DURATION

18:00:00

Step 9.

Count or pick plaques the next day

NOTES

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If you’re calculating PFU, you will probably need to leave them out and count them for one more day.