Plaque Assay For Screening Viral Concentrates for Bacteriophage

Dr. Steven Wilhelm

Abstract

Please contact Dr. Steven Wilhelm (wilhelm@utk.edu) for additional information regarding this protocol.

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Protocol

Culture Preparation

Step 1.

Grow a 4 mL liquid culture of the bacterial isolate of interest in an appropriate growth medium overnight

AMOUNT
4 ml Additional info:

Step 2.

Dilute the culture 10-20% and let it grow for 4 hrs prior to plaque assay

Step 3.

Melt 0.6% top-agar in the microwave and let cool 10-15 min

Step 4.

Pipet 1.5 mL liquid culture into 1.5 mL Eppendorf tubes (or enough liquid culture for 500 µL for each plate sample that you want to make).

Step 5.

Centrifuge 2 min at 14,000 rpm

NOTES
Alyssa Alsante 15 Jun 2017

Some bacterial isolates do not require centrifugation. This is something that needs to be

empirically determined for each putative bacterial host.

Step 6.

Discard the supernatant

Step 7.

Resuspend the remaining cells in 1.5 mL BG-11 media and mix well with pipet

AMOUNT
1.5 ml Additional info:

Step 8.

Pipet 4 mL of molton top agar into sterile culture tubes

AMOUNT
4 ml Additional info:

Step 9.

Keep top agar in a 45ºC head block to keep in a liquid state

Step 10.

In 500 µL of resuspended cells, add 100 µL of 0.22 µm filtered viral concentrate

Step 11.

Incubate for 15 min at an appropriate growth temperature for the bacterium in use

Plaque Assay

Step 12.

Pipet infected cells into molton top agar

Plaque Assay

Step 13.

Vortex briefly avoiding bubbles

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Step 14.

Pour top agar onto the plate and spread

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Step 15.

Let agar solidify at room temperature 30-60 min

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Step 16.

Flip plates over and place in an incubator at an appropriate growth temperature and light conditions for the bacterium in use

Plaque Assay

Step 17.

Check plates daily until a confluent lawn appears on plates. Plaques will be obvious clearings.