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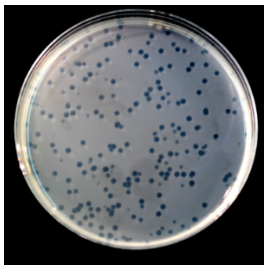
Plaque Assay For Screening Viral Concentrates for Bacteriophage



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Dr. Steven Wilhelm

The Aquatic Microbial E...

Steven W Wilhelm
The University of Tennessee, Knoxville

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Abstract

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

Culture Preparation

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

2 Dilute the culture 10-20% and let it grow for ~4 hrs prior to plaque assay
04:00:00

3 Melt 0.6% top-agar in the microwave and let cool ~10-15 min
00:15:00

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).

5 Centrifuge 2 min at 14,000 rpm
00:02:00

Note

Some bacterial isolates do not require centrifugation. This is something that needs to be empirically determined for each putative bacterial host.

6 Discard the supernatant

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

8 Pipet 4 mL of molton top agar into sterile culture tubes
4 mL

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

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

11 Incubate for 15 min at an appropriate growth temperature for the bacterium in use
00:15:00

Plaque Assay

12 Pipet infected cells into molton top agar

13 Vortex briefly avoiding bubbles

14 Pour top agar onto the plate and spread

15 Let agar solidify at room temperature ~30-60 min
01:00:00

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

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