

Feb 08, 2016

🌐 Obtaining pure cyanophage stocks (liquid assay)

📁 In 1 collection

DOI

dx.doi.org/10.17504/protocols.io.dqm5u5

Mathias Middelboe¹, Amy M. Chan¹, and Sif K. Bertelsen¹

¹Manual of Aquatic Viral Ecology

VERVE Net

Suttle Laboratory of Mar...



Amy Chan

OPEN  ACCESS



DOI: dx.doi.org/10.17504/protocols.io.dqm5u5

External link: http://www.aslo.org/books/mave/MAVE_118.pdf

Protocol Citation: Mathias Middelboe, Amy M. Chan, and Sif K. Bertelsen 2016. Obtaining pure cyanophage stocks (liquid assay). [protocols.io](https://dx.doi.org/10.17504/protocols.io.dqm5u5) <https://dx.doi.org/10.17504/protocols.io.dqm5u5>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working


Created: September 02, 2015

Last Modified: March 28, 2018

Protocol Integer ID: 1517

- 1 Determine the titer of the lysate using the 96-well assay as described earlier.

Protocol


 NAME
Lysate titer

CREATED BY
Amy Chan

PREVIEW

- 1.1 Prepare end-point dilution series (10-fold serial dilutions, 5 to 6 levels).
 - 1.2 Monitor plates for lysis every few days, recording the number and position of clear wells on the plate.
 - 1.3 When clear or nearly clear wells no longer appear for 1 week, record the final "score" for each dilution level.
 - 1.4 Use the MPN Assay Analyser program (Passmore et al. 2000) to determine the most-probable-number (Taylor 1962) of infective phages in the lysate.
- 2 Once the cyanophage titer is determined for the stock tube, proceed to purify a clonal virus.

Protocol

 NAME
Clonal virus purification

CREATED BY
Amy Chan

PREVIEW

- 2.1 Use 13-x-100-mm culture tubes (or 24-well plates).

- 2.2 Prepare exponentially growing target cells (e.g., >100 mL).
- 2.3 Dilute some of the titered lysate to 1 infective virus/mL.
- 2.4 Add 0.2 mL (0.2 infectious units) to each of 20 tubes of susceptible host cells.
- 2.5 Monitor tubes for 2 to 3 weeks.
- 2.6 Cultures in which lysis occurs are assumed to be the result of a single-virus infection.

Note

The probability that more than 1 infective unit occurred in a given culture is 0.0176.

- 2.7 If lysis occurs in 4 tubes or less of 20, it is assumed that lysis in each tube was caused by one infectious unit, therefore each tube would contain a separate phage clone.
- 2.8 Propagate an aliquot from all the tubes to confirm the results.
- 2.9 If lysis occurs in more than 4 tubes, repeat the clone out procedure by reducing the volume of diluted lysate added to the 20 tubes.

Note

Add 0.1 mL instead of 0.2 mL.

- 2.10 Scale up each phage clone to make primary phage stocks.

Note

e.g., add 5 μ L of the lysate to 40 mL of cells.

- 2.11 Centrifuge, filter, and titer the stock, store at 4°C in the dark.