Virus Purification by Sucrose Density Gradients Version 2

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Abstract

Adapted from: Van Etten, J. (n.d.). Titering of *Chlorella* Viruses. Retrieved from http://ncv.unl.edu/vanettenlab/

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Guidelines

For critical work, a second purification may be necessary.

This protocol is used for Chloroviruses and may need to be optimized according to the system that you are working with. Make sure that the virus sample that you are using will pellet sufficiently at this RPM in the ultracentrifuge before using this protocol.

Protocol

Step 1.

Centrifuge lysate in the Sorvall Lynx 4000 centrifuge at 5,000 rpm, 5 min, 4ºC. Discard the pellets.

Step 2.

Add Triton X-100 to the lysate supernatants for a final concentration of 1% (from a 10 or 20% stock).

Step 3.

Centrifuge the lysate in the Sorvall WX Ultra Series ultracentrifuge at 17,000 rpm, 50 min, 4ºC. Discard the supernatants.

Step 4.

Resuspend the virus pellets with a small volume of 50 mM Tris-HCl, pH 7.8

*Approximately 1.0 mL per 100 mL lysate

Step 5.

Layer the virus suspension onto 100-400 mg/mL (10-40%) linear sucrose gradients equilibriated with 50 mM Tris-HCl made in polypropylene tubes (layer approximately 3-4 mL per gradient).

*To make sucrose stocks, add 10-40% sucrose to Tris-HCl and autoclave.

Step 6.

Centrifuge the gradients in the ultracentrifuge at 20,000 rpm, 20 min, 4ºC. The virus will be the major band about 1/2 to 2/3 deep in the gradient.

Step 7.

Remove the virus bands from the gradients with sterile needles and transfer to 30 mL polypropylene centrifuge tubes. Split the virus from 3 gradients between two tubes. Slowly dilute the virus to the tube volume with 50 mM Tris-HCl. Centrifuge the tubes in the ultracentrifuge at 27,000 rpm, 3 hours, 4ºC. Discard the supernatants.

Step 8.

Resuspend the virus pellets with a small volume of 50 mM Tris-HCl. Store the virus at 4ºC. Do not freeze. Filter sterilization using a 0.45 um cellulose acetate filter is recommended.