Virus Purification by Sucrose Density Gradients

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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.

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).

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*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.

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