Purification of viruses from culture lysates

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Abstract

Ultracentrifugation provides a means to concentrate, analyze, and purify viruses in solution, and therefore represents an invaluable tool for aquatic virologists. This protocol provides a method for purification of viruses from culture lysates from natural water samples.

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Guidelines

Materials and reagents

• OptiPrep (60% iodixanol solution)—Axis-Shield, Accurate Chemical and Scientific (Westbury), Progen Biotechnik, or Sigma Aldrich
• Open-topped ultracentrifuge tubes—i.e., Beckman Coulter Ultra-Clear
• Ultracentrifuge.
• Swing-out Ultracentrifuge Rotor—i.e., Beckman Coulter SW41, SW28, or MLS50
• 30 kDa cutoff disposable centrifugal ultrafiltration devices—i.e., Millipore
• 3-mL syringe with Luer-Lok or Luer-Slip
• Pipetting needle—i.e., Cadence Science, stainless-steel 14- or 16-guage 4-inch cannula with Luer hub or Slip hub
• Sterile 1.5 mL microcentrifuge tubes for collecting gradient fractions
• Sterile disposable transfer pipettes
• Sterile virus-free media for resuspending and diluting virus
• Polyethylene glycol, average molecular weight 6000- 8000—i.e., Fisher Scientific Carbowax PEG 8000, or Sigma Aldrich Biochemika Ultra 8000

Discussion

OptiPrep must be removed from samples before examination of virus particles by negative staining and TEM. This can be achieved using disposable Millipore centrifugal ultrafiltration devices with a 30 kDa cutoff. For most other applications OptiPrep does not need to be removed prior to further analysis, although it should be assayed to determine effects on the growth of specific viral-hosts when re-infection assays are used to confirm purification of the infectious agent.

Protocol

Clarify lysate

Step 1.
Centrifuge the lysate at 4000g for 30 min.

DURATION
00:30:00

Clarify lysate

Step 2.
Carefully decant and retain the supernatant.

Concentrate virus by PEG precipitation

Step 3.
Dissolve 8% PEG (w/v) in clarified lysate and allow to precipitate overnight at 4°C.

DURATION
18:00:00

Concentrate virus by PEG precipitation

Step 4.
Centrifuge the PEG solution at 10,000g for 20 min.

DURATION
00:20:00

Concentrate virus by PEG precipitation

Step 5.
Carefully decant the supernatant, retaining the pellet.

Concentrate virus by PEG precipitation

Step 6.
Resuspend pellet in a small volume of residual PEG solution and pool all pelleted material.

Concentrate virus by PEG precipitation

Step 7.
Repeat steps 5-6 as needed to concentrate virus to < 1 mL.

Concentrate virus by PEG precipitation

Step 8.
Resuspend virus in 10-50 volumes of culture media to dilute PEG and allow virus pellet to disaggregate overnight at 4°C.

DURATION
18:00:00

Concentrate virus by PEG precipitation

Step 9.
Concentrate sample to 1 mL through a 30 kDa cutoff disposable centrifugal ultrafiltration device.

Prepare continuous, isopycnic, purifying gradients

Step 10.
Prepare OptiPrep solutions using culture media as the diluent.

ANNOTATIONS

Janice Lawrence 19 Oct 2015
For many viruses a gradient from 25%- 40% OptiPrep will provide a good range for separation, but very dense or light viruses may require adjustment. To achieve this range, prepare 25%, 30%, 35%, and 40% v/v final-OptiPrep-concentration solutions, remembering that OptiPrep is sold as a 60% solution. The actual densities these concentrations achieve are dependent on the density of the culture media used as a diluent, and must be determined for each system.

Prepare continuous, isopycnic, purifying gradients

Step 11.
Using the underlayering technique with syringe and pipetting needle, pour 4-step gradients into open-

toped ultracentrifuge tubes, beginning with the least dense solution first.

ANNOTATIONS

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Be sure to leave enough room at the top of the centrifuge tube to load the sample, with 2-3 mm of space at the top.

Prepare continuous, isopycnic, purifying gradients

Step 12.

Allow to blend for 2 h at room temperature.

DURATION

02:00:00

ANNOTATIONS

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Make sure to prepare gradients to serve as balance tubes where appropriate.

Prepare continuous, isopycnic, purifying gradients

Step 13.

Mark the top of the gradients with a fine-tipped marker, and carefully overlay virus concentrate using a transfer pipette.

Prepare continuous, isopycnic, purifying gradients

Step 14.

Overlay culture media on balance gradients to create balance tubes.

Prepare continuous, isopycnic, purifying gradients

Step 15.

Balance the tubes by adding media to underweight tubes.

Prepare continuous, isopycnic, purifying gradients

Step 16.

Load tubes into rotor and ultracentrifuge at maximum permissible speed until density equilibrium is reached.

ANNOTATIONS

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As a guideline, a 4-mL gradient with 1-mL virus sample in a Beckman Coulter MLS-50 should be centrifuged for at least 4 h 15 min at 200,620g (50,000 rpm); an 11-mL gradient with 1-mL virus sample in a Beckman Coulter SW-41 should be centrifuged for at least 7 h 20 min at 207,570g (41,000 rpm). These conditions should be determined empirically for different systems.

Collect viral fraction

Step 17.

Using any fraction collection apparatus/technique, carefully extract purified viral concentrate from each tube.

Collect viral fraction

Step 18.

If bands are not visible, a starting point is to fractionate the gradient into 4 + fractions, and use a couple of techniques to identify the virus-containing fraction (i.e., TEM, bioassay, absorbance at 260 nm, nucleic acid analysis, epifluorescence microscopy or flow cytometry).

ANNOTATIONS

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Note that each of these techniques has drawbacks and may lead to false results. For example,

more than one virus-containing fraction may be detected by TEM when analyzing non-axenic cultures, because contaminating bacteria are usually host to phage. Likewise, no virus-containing fractions may be detected by epifluorescence microscopy if the virus in question contains a small ssRNA genome, since the fluorescence yields of dyes are currently too low for visual detection of small ssRNA genomes. A combination of approaches for identifying virus fractions may therefore be required when working with novel viruses.