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Chlorovirus Purification

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Guidelines


Supplemental notes:

- 1) Concentration (A_{260}/mL) is determined on a UV spectrophotometer (not for iodixanol-purified isolates).
- 2) Titer (PFU/mL) is determined by plaque assay.
- 3) 1 A_{260} unit of PBCV-1 routinely yields $1.5\text{-}2.5 \times 10^{10}$ PFU.
- 4) For critical work, a second purification through sucrose gradients or a set of iodixanol gradients may be necessary.
- 5) SAG 3.83 virus purification procedure optimized by Irina Agarkova.

1 Inoculate flasks with NC64A chlorella in MBBM (or Pbi in FES, SAG 241-80 in MBBM) and incubate for several days at 25°C with continuous light and shaking.

2 Infect the flasks of chlorella with virus at an moi of 0.01 to 0.001.


3 Incubate the flasks for 48-72 hours at 25°C with continuous light and shaking.

 72:00:00


Note

This material is now termed “lysate”.

4 Add Triton X-100 to the lysate supernatants to a final concentration of 1%. This solubilizes the green pigment in the supernatant. Stir this solution at room temperature for at least one hour.


 00:05:00

5 Centrifuge the lysate in the Sorvall GSA rotor at 5,000 rpm (4,000 rcf), 5 min, 4°C.

 00:05:00

6 Discard the pellets.

7 Centrifuge the lysate in the Beckman Type 19 ultracentrifuge rotor at 17,000 rpm (43,000 rcf), 50 min, at 4°C.

 00:50:00

Note

Alternatively, centrifuge the lysate in Beckman Ti 50.2 rotors at 20,000 rpm (24,000 rcf), 60 min, 4°C.


8 Discard the supernatants.

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

Note

Approximately 1.0 mL per 100 mL of original lysate.

- 10 Adjust the resuspended virus material with Protease K to 0.02 mg/mL and incubate at 45°C for at least one hours.

 01:00:00

- 11 **For NC64A and Pbi virus lysates:** Layer the virus suspension onto 100-400 mg/mL (10-40%, w/v) linear sucrose density gradients equilibrated with 50 mM Tris-HCl, pH 7.8, made up in Beckman SW28 rotor tubes.

Note


Layer approximately 3-4 mL per gradient.

- 12 **For SAG 3.83 virus lysates:** Layer the virus suspension onto ~100-400 mg/mL linear iodixanol gradients equilibrated with 50 mM Tris-HCl, pH 7.8, made up in Beckman SW28 rotor tubes.

Note

Layer approximately 3-4 mL per gradient.

- 13 Centrifuge the gradients in a Beckman SW28 or SW32 rotor at 20,000 rpm (72,000 rcf_{max}), 20 min, 4°C.

 00:20:00

Note


The virus will be the major band about 1/2 to 2/3 deep in the gradient.

- 14 Remove the virus bands from the gradients with sterile bent needles via top (or via side puncture with sterile needle and syringe) to oak ridge 30 mL polypropylene tubes.

- 15 Split the virus from 3 gradients between 2 tubes.

- 16 Slowly dilute the virus to the tube volume with 50 mM Tris-HCl, pH 7.8.

- 17 Centrifuge the tubes in Beckman Ti 50.2 rotor at 27,000 rpm (~44,000 rcf), 3 hours, 4°C.

 03:00:00


- 18 Discard the supernatants.
- 19 Resuspend the virus pellets with a small volume of 50 mM Tris-HCl, pH 7.8.
- 20 Store the virus at 4°C. Do not freeze.
- 21 Layer the virus suspension onto 10-40%, w/v linear iodixanol or sucrose density gradients equilibrated with 50mM Tris-HCl, pH 7.8 made up in Beckman SW28 rotor tubes.

Note

Layer approximately 4.0 mL per gradient.
- 22 Centrifuge the gradients in Beckman SW28 rotors at 20,000 rpm, 4 hours, 25°C.

Note

The virus should be the major band about 1/2 to 2/3 deep in the gradient at a density of approximately 1.18 g/mL.
- 23 Remove the virus bands from the gradients with sterile bent needles via top (or via side puncture with sterile needle and syringe) to oak ridge 30 mL polypropylene tubes.
- 24 Split the virus from 3 gradients between 2 tubes.
- 25 Slowly dilute the virus to the tube volume with 50 mM Tris-HCl, pH 7.8.
- 26 Centrifuge the tubes in Beckman Ti 50.2 rotor at 27,000 rpm (~44,000 rcf), 3 hours, 4°C.

 03:00:00
- 27 Discard the supernatants.