Jun 13, 2016

Chlorovirus Purification

DOI
dx.doi.org/10.17504/protocols.io.er2bd8e

David Dunigan and Irina Agarkova[1]

[1]The University of Nebraska-Lincoln

VERVE Net

Irina Agarkova
UNL

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DOI: dx.doi.org/10.17504/protocols.io.er2bd8e

Protocol Citation: David Dunigan and Irina Agarkova 2016. Chlorovirus Purification. **protocols.io https://dx.doi.org/10.17504/protocols.io.er2bd8e**

Protocol status: Working

Created: March 25, 2016

Last Modified: February 12, 2018

Protocol Integer ID: 2586

Keywords: chlorovirus purification, purification

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 1.5-2.5 X 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^{o}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^{o}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.