Chlorovirus Purification

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Abstract

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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 1.5-2.5 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.

Protocol

Step 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.

Step 2.

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

Step 3.

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

DURATION
12:00:00

NOTES
Irina Agarkova 25 Mar 2016
This material is now termed “lysate”.

Step 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.

DURATION
00:05:00

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

DURATION
00:05:00

Step 6.
Discard the pellets.

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

DURATION
00:50:00

NOTES
Irina Agarkova 14 Apr 2016
Alternatively, centrifuge the lysate in Beckman Ti 50.2 rotors at 20,000 rpm (24,000 rcf), 60 min, 4°C.

Step 8.
Discard the supernatants.

Step 9.

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

NOTES
Irina Agarkova 25 Mar 2016
Approximately 1.0 mL per 100 mL of original lysate.

Step 10.

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

DURATION
01:00:00

Step 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.

NOTES
Irina Agarkova 25 Mar 2016
Layer approximately 3-4 mL per gradient.

Step 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.

NOTES
Irina Agarkova 26 May 2016

Layer approximately 3-4 mL per gradient.

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

DURATION
00:20:00

NOTES
Irina Agarkova 25 Mar 2016
The virus will be the major band about 1/2 to 2/3 deep in the gradient.

Step 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.

Step 15.

Split the virus from 3 gradients between 2 tubes.

Step 16.

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

Step 17.

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

DURATION
03:00:00

Step 18.
Discard the supernatants.

Step 19.

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

Step 20.
Store the virus at 4°C. Do not freeze.

Step 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.

NOTES

Irina Agarkova 26 May 2016

Layer approximately 4.0 mL per gradient.

Step 22.

Centrifuge the gradients in Beckman SW28 rotors at 20,000 rpm, 4 hours, 25°C.

NOTES
Irina Agarkova 26 May 2016

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.

Step 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.

Step 24.

Split the virus from 3 gradients between 2 tubes.

Step 25.

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

Step 26.

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

DURATION
03:00:00
Step 27.

Discard the supernatants.