Chlorella NC64A and Mictractinium Pbi virus Purification

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Abstract

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Guidelines

Supplemental notes:

An A_{260} may be determined on a UV spectrophotometer (usually a 1:100 dilution works well).
1 A_{260} unit of PBCV-1 routinely yields 1.5-2.5 X 10^{10} PFU/ml of virus.

For critical work, a second purification through sucrose gradients or a set of iodixanol gradients may be necessary.

Protocol

Step 1.
Inoculate flasks with Chlorella NC64A in MBBM (or Micractinium Pbi in FES) and incubate at 25°C with continuous light and shaking until the cells are in the actively growing phase (about 1-2 X 10^7 cells/ml).

Step 2.
Infect the flasks of chlorella with virus at a multiplicity of infection (moi) of 0.01 to 0.001.

Step 3.
Incubate the flasks for 48-72 hours at 25°C with continuous light and shaking. This material is now termed “lysate”.

DURATION
12:00:00

Step 4.
Centrifuge the lysate in the Sorvall GSA rotor in 250 ml bottles at 5,000 rpm (4,000 rcf), 5 min, 4°C.

DURATION
00:05:00

Step 5.
Discard the pellets.

Step 6.
Add Triton X-100 to the lysate supernatants for a final concentration of 1% (from a 10 or 20% stock).

Step 7.
Centrifuge the lysate in the Beckman Type 19 225 ml ultracentrifuge rotor at 17,000 rpm (43,000 rcf),

50 min, at 4°C.

DURATION
00:50:00

NOTES
Irina Agarkova 31 Mar 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 (approximately 1.0 mL per 100 mL of original lysate).

Step 10.
Layer the virus suspension onto 100-400 mg/mL (10-40%) linear sucrose density gradients equilibrated with 50 mM Tris-HCl, pH 7.8, made up in Beckman SW28 rotor tubes (layer approximately 3-4 mL per gradient).

Step 11.
Centrifuge the gradients in a Beckman SW28 rotor at 20,000 rpm (72,000 rcf_{max}), 20 min, 4°C.

DURATION
00:20:00

NOTES
Irina Agarkova 26 Apr 2016
The virus will be the major band about 1/2 to 2/3 deep in the gradient.

Step 12.
Remove the virus bands from the gradients with sterile bent needles and transfer to oak ridge 30 mL polypropylene centrifuge tubes.

Step 13.
Split the virus from 3 gradients between 2 tubes.

Step 14.
Slowly dilute the virus to the tube volume with 50 mM Tris-HCl, pH 7.8.

Step 15.
Centrifuge the tubes in Beckman Ti 50.2 rotor at 27,000 rpm (44,000 rcf), 3 hours, 4°C.

DURATION
03:00:00

NOTES
Irina Agarkova 31 Mar 2016
Alternatively, dilute the virus from the gradients ~10-fold with Tris buffer and centrifuge in the Type 19 rotor for 1 hour, 17,000 rpm, 4°C. A GSA type high speed rotor can be used for at 2 hours, 12,000 rpm.

Step 16.
Discard the supernatants.

Step 17.
Gently wash the pellet and bottle with some 50 mM Tris, pH 7.8 buffer to wash residual sucrose away.

Step 18.
Then resuspend the virus pellets with a small volume of 50 mM Tris-HCl, pH 7.8.

Step 19.
Store the virus at 4°C. Do not freeze.

Step 20.
Filter sterilization using a 0.45 µm cellulose acetate or other low protein binding filter is recommended.