Preparation of PBCV-1 Lysin

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Abstract

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Protocol

Step 1.
Centrifuge the NC64A chlorella (use actively growing cells approximately 1.0-2.0 X 10^7 cells/mL) in the Sorvall GSA rotor at 7,000 rpm, 5 min, 4°C.

DURATION
00:05:00

Step 2.
Use sterile bottles to harvest the cells.

Step 3.
Discard the supernatants.

Step 4.
Resuspend the cells with sterile BBM.

Step 5.
Adjust the final volume to 500 mL with sterile BBM.

Step 6.
Add filter-sterilized tetracycline (12.5 mg/Ml stock, add 0.1 mL per 50 mL of BBM solution) and PBCV-1 at an moi (multiplicity of infection) of 0.01.

Step 7.
Incubate overnight at 25°C with continuous light and shaking. This material is now termed “lysate”.

DURATION
18:00:00

Step 8.
Centrifuge the lysate in the Sorvall GSA rotor at 3,500 rpm, 10 min, 5°C.

DURATION
00:10:00

Step 9.
Decant the supernatants to clean bottles.

Step 10.
Centrifuge the supernatant on the Sorvall GSA rotor at 10,000 rpm, 60 min, 5°C.

DURATION
01:00:00

Step 11.

Decant the supernatant to clean flasks.

Step 12.
Add d-H_2O to a final volume of 1000 mL.

Step 13.
Place at 4°C.

Step 14.
Add, at 4°C: 2-ME to 2 mM EDTA to 5 mM NaN_3 to 200 mg/liter $(NH_4)_2SO_4$ to 40% saturation

Step 15.
Adjust the pH to 7.0 with NaOH.

Step 16.
Cover and incubate at 4°C overnight.

DURATION
18:00:00

Step 17.
Centrifuge the material in the Sorvall GSA rotor at 10,000 rpm, 60 min, 5°C.

DURATION
01:00:00

Step 18.
Decant the supernatant to clean flasks.

Step 19.
Discard the pellets.

Step 20.
Add, at 4°C, $(NH_4)_2SO_4$ to 65% saturation.

Step 21.
Adjust the pH to 7.0 with NaOH.

Step 22.
Cover and incubate at 4°C overnight.

DURATION
18:00:00

Step 23.
Centrifuge the material in the Sorvall GSA rotor at 10,000 rpm, 10 min, 5°C.

DURATION
00:10:00

Step 24.
Aspirate off the supernatant. Save the pellet.

Step 25.
Resuspend the pellet with 10.0 mL of 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 2 mM 2-ME + 2 mg NaN_3.

Step 26.
Centrifuge the material in the Sorvall SS34 rotor at 15,000 rpm, 60 min, 5°C.

DURATION
01:00:00

Step 27.
Save the supernatant.

Step 28.
Dialyze the supernatant for 48-72 hours at 4°C against 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 2 mM 2-

ME, 200 mg/liter NaN_3.

DURATION

12:00:00

Step 29.

Change the buffer once every 24 hours.

Step 30.

Remove the material from the dialysis.

Step 31.

Centrifuge the material in the Sorvall SS34 rotor at 13,000 rpm, 60 min, 5°C.

DURATION

01:00:00

Step 32.

Save the supernatant.

Step 33.

Aliquot the supernatant into microfuge tubes and store at -20°C.

Step 34.

Assay the supernatant fraction for lysin activity.