Restriction Endonuclease Purification From Virus Infected Chlorella

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Abstract

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Guidelines

MATERIALS AND ASSAY CONDITIONS:

1) Virus infected NC64A *chlorella* or Pbi *chlorella*, pellets frozen at -80°C

2) Buffer A: 10 mM Tris-Acetate, pH 8.0, 10 mM 2-ME, 50 µg/mL PMSF

3) Buffer B: 20 mM Tris-Acetate, pH 8.0, 0.5 mM EDTA, 7 mM 2-ME, 10% Glycerol

4) Buffer B, pH 8.5: 20 mM Tris-Acetate, pH 8.5, 0.5 mM EDTA, 7 mM 2-ME

5) Storage buffer: 20 mM Tris-Acetate, pH 8.0, 0.5 mM EDTA, 0.1 mM DTT, 50 mM KOAc, 5 mM MgAc, 50% Glycerol, 100 µg/mL BSA (the BSA is added after dialysis into the storage buffer from a 10 mg/mL stock)

6) 4 M NaCl

7) 28% Polyethyleneglycol (PEG) 8000

8) Assay buffer and conditions:

1X CviJI assay buffer:

20 mM GlycylGlycine, pH 8.5 (with KOH)

10 mM MgAc

0.1 mM DTT

50 mM KOAc

100 ug/ml casein (optional)

All assays are carried out in 20.0 ul volumes with 1 ug of pUC19 DNA as substrate for 60 to 120 min at 25°C. The assays are electrophoresed on 2.0% agarose gels (100 ml gels with double 20 lane combs) for 1 hour at 100 volts in 1X TPE buffer. Gels are stained with 0.5 µg/ml ethidium bromide for 30 min and photographed on a UV light box.

Protocol

Step 1.
Thaw the virus infected *chlorella* and suspend in MSK flasks with Buffer A.

Step 2.
Suspend with 20 ml per flask per 1.0-1.5 X 10^{11} infected cells.

Step 3.
Homogenize the cells in the MSK mechanical homogenizer with 15 gm of 0.3 mm glass beads at 4,000 rpm for 90 sec (2 X 45 sec) with CO_2 cooling.

DURATION
00:01:30

Step 4.
Recover the homogenate to clean tubes.

Step 5.
Wash the glass beads 3X with 5 ml of Buffer A and combine with the homogenate.

Step 6.
Centrifuge the homogenate in the Sorvall SS34 rotor at 10,000 rpm, 20 min, 4°C.

DURATION
00:20:00

Step 7.
Save the supernatant.

Step 8.
Adjust the homogenate supernatant to 70% saturation with $(NH_4)_2SO_4$ at 4°C with gentle stirring.

Step 9.
Add the $(NH_4)_2SO_4$ gradually.

Step 10.
Incubate at 4°C for 60-90 min without stirring.

DURATION
01:30:00

Step 11.
Centrifuge the material in the Sorvall SS34 rotor at 10,000 rpm, 10 min, 4°C.

DURATION
00:10:00

Step 12.
Save the pellet.

Step 13.
Suspend the pellets with Buffer A. Per mL of suspension add: 0.45 mL of 4 M NaCl and 0.45 mL of 28% PEG 8000 (heated to 65°C).

Step 14.
Mix gently by inversion for 5-10 min.

DURATION
00:10:00

Step 15.
Centrifuge the material in the Sorvall SS34 rotor at 10,000 rpm, 10 min, 4°C.

DURATION
00:10:00

Step 16.
Save the supernatant.

NOTES
Irina Agarkova 19 Apr 2016
If there is small particulate matter in the supernatant, centrifuge a second time as before and save the supernatant.

Step 17.
Dilute the supernatant with 10-15 volumes of Buffer B to reduce the NaCl concentration.

Step 18.
Load the material overnight onto a Heparin-Sepharose column equilibrated with Buffer B in the cold room.

DURATION
18:00:00

Step 19.
Elute the Heparin-Sepharose column with Buffer B using a 0-2.0 M KOAc gradient.

Step 20.
Assay the column fractions and pool the active fractions.

Step 21.
Dilute the pooled fractions with 10-15 volumes of Buffer B to reduce the salt concentration.

Step 22.
Load the material overnight onto a Blue-Sepharose column equilibrated with Buffer B in the cold room.

DURATION
18:00:00

Step 23.
Elute the Blue-Sepharose column with Buffer B using a 0-2.0 M KOAc gradient.

Step 24.
Assay the column fractions and pool the active fractions.

Step 25.
Dilute the pooled fractions with 10-15 volumes of Buffer B, pH 8.5 to reduce the salt concentration.

Step 26.
Load the material overnight onto a Q-Sepharose column equilibrated with Buffer B, pH 8.5 in the cold room.

DURATION
18:00:00

Step 27.
Elute the Q-Sepharose column with Buffer B, pH 8.5 using a 0-2.0 M KOAc gradient.

Step 28.
Assay the column fractions and pool the active fractions.

Step 29.
Concentrate the pooled enzyme by dialysis overnight into storage buffer at 4°C.

DURATION
18:00:00

Step 30.
Add BSA (10 mg/mL) to a final concentration of 100 µg/mL.

Step 31.
Store the enzyme at -20°C.