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CviJI Purification From IL-3A Virus Infected NC64A Chlorella

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David Dunigan and Irina Agarkova[1]

[1]The University of Nebraska-Lincoln

VERVE Net

Irina Agarkova
UNL

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Guidelines

MATERIALS AND ASSAY CONDITIONS:

1) 7 hour IL-3A virus infected NC64A, pellets frozen at -80°C
2) Buffer A:
- 10 mM Tris-Acetate, pH 8.0
- 10 mM 2-M
- 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 µg/mL casein (optional)

All assays are carried out in 20.0 µL volumes with 1 µg 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.

Troubleshooting

Before start

7 hour IL-3A virus infected NC64A, pellets frozen at -80°C

1 Prepare Buffer A:

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CviJI Buffer A

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1.1 10 mM Tris-Acetate, pH 8.0

1.2 10 mM 2-ME

1.3 50 µg/mL PMSF

2 Prepare Buffer B:

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CviJI Buffer B

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2.1 20 mM Tris-Acetate, pH 8.0

2.2 0.5 mM EDTA

2.3 7 mM 2-ME,10% Glycerol

3 Prepare Buffer B, pH 8.5:

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CviJI Buffer B, pH 8.5

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3.1 20 mM Tris-Acetate, pH 8.5

3.2 0.5 mM EDTA

3.3 7 mM 2-ME

4 Prepare Storage Buffer:

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CviJI Storage Buffer

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4.1 20 mM Tris-Acetate, pH 8.0

4.2 0.5 mM EDTA

4.3 0.1 mM DTT

4.4 50 mM KOAc

4.5 5 mM MgAc, 50% Glycerol

4.6 100 µg/mL BSA (the BSA is added after dialysis into the storage buffer from a 10 mg/mL stock)

5 Prepare 1X CviJI Assay Buffer:

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1X CviJI Assay Buffer

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5.1 20 mM GlycylGlycine, pH 8.5 (with KOH)

5.2 10 mM MgAc

5.3 0.1 mM DTT

5.4 50 mM KOAc

5.5 100 µg/mL casein (optional)

6 Thaw 7 hour IL-3A virus infected NC64A *chlorella* and suspend in MSK flasks with Buffer A.

Note

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

7 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.

00:01:30

8 Recover the homogenate to clean tubes.

9 Wash the glass beads 3X with 5 mL of Buffer A and combine with the homogenate.

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

00:20:00

11 Save the supernatant.

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

Note

Add the $(NH_4)_2SO_4$ gradually.

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

01:30:00

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

00:10:00

15 Save the pellet.

16 Suspend the pellets with Buffer A.

17 Per mL of suspension add: 0.45 mL of 4 M NaCl and 0.45 mL of 28% PEG 8000 (heated to 65°C).

18 Mix gently by inversion for 5-10 min.
00:10:00

19 Centrifuge the material in the Sorvall SS34 rotor at 10,000 rpm, 10 min, 4°C.
00:10:00

20 Save the supernatant.

Note

If there is small particulate matter in the supernatant, centrifuge a second time as before and save the supernatant.

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

22 Load the material overnight onto a Heparin-Sepharose column equilibrated with Buffer B in the cold room.
18:00:00

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

24 Assay the column fractions and pool the active fractions.

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24.1 All assays are carried out in 20.0 µL volumes with 1 µg of pUC19 DNA as substrate for 60 to 120 min at 25°C.
02:00:00

24.2 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.
01:00:00

24.3 Gels are stained with 0.5 µg/mL ethidium bromide for 30 min and photographed on a UV light box.
00:30:00

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

26 Load the material overnight onto a Blue-Sepharose column equilibrated with Buffer B in the cold room.
18:00:00

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

28 Assay the column fractions and pool the active fractions.

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CviJI Assay Conditions
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28.1 All assays are carried out in 20.0 µL volumes with 1 µg of pUC19 DNA as substrate for 60 to 120 min at 25°C.
02:00:00

28.2 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.
01:00:00

28.3 Gels are stained with 0.5 µg/mL ethidium bromide for 30 min and photographed on a UV light box.
00:30:00

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

30 Load the material overnight onto a Q-Sepharose column equilibrated with Buffer B, pH 8.5 in the cold room.
18:00:00

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

32 Assay the column fractions and pool the active fractions.

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CviJI Assay Conditions
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32.1 All assays are carried out in 20.0 µL volumes with 1 µg of pUC19 DNA as substrate for 60 to 120 min at 25°C.
02:00:00

32.2 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.
01:00:00

32.3 Gels are stained with 0.5 µg/mL ethidium bromide for 30 min and photographed on a UV light box.
00:30:00

33 Concentrate the pooled enzyme by dialysis overnight into storage buffer at 4°C.
18:00:00

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

35 Store the enzyme at -20°C.