CviJI Purification From IL-3A Virus Infected NC64A Chlorella

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Abstract

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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.

Before start

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

Protocol

Step 1.
Prepare Buffer A:

PROTOCOL

. CviJI Buffer A

CONTACT: Irina Agarkova
Step 1.1.
10 mM Tris-Acetate, pH 8.0

Step 1.2.
10 mM 2-ME

Step 1.3.
50 µg/mL PMSF

Step 2.
Prepare Buffer B:

PROTOCOL

. CviJI Buffer B

CONTACT: Irina Agarkova
Step 2.1.
20 mM Tris-Acetate, pH 8.0

Step 2.2.
0.5 mM EDTA

Step 2.3.
7 mM 2-ME,10% Glycerol

Step 3.
Prepare Buffer B, pH 8.5:

PROTOCOL

. CviJI Buffer B, pH 8.5

CONTACT: Irina Agarkova

Step 3.1.
20 mM Tris-Acetate, pH 8.5

Step 3.2.
0.5 mM EDTA

Step 3.3.
7 mM 2-ME

Step 4.
Prepare Storage Buffer:

PROTOCOL

. CviJI Storage Buffer

CONTACT: Irina Agarkova

Step 4.1.
20 mM Tris-Acetate, pH 8.0

Step 4.2.
0.5 mM EDTA

Step 4.3.
0.1 mM DTT

Step 4.4.
50 mM KOAc

Step 4.5.
5 mM MgAc, 50% Glycerol

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

Step 5.
Prepare 1X CviJI Assay Buffer:

PROTOCOL

. 1X CviJI Assay Buffer

CONTACT: Irina Agarkova

Step 5.1.
20 mM GlycylGlycine, pH 8.5 (with KOH)

Step 5.2.
10 mM MgAc

Step 5.3.
0.1 mM DTT

Step 5.4.
50 mM KOAc

Step 5.5.
100 µg/mL casein (optional)

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

NOTES
Irina Agarkova 29 Mar 2016
Suspend with 20 mL per flask per 1.0-1.5 X 10^{11} infected cells.

Step 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.

DURATION
00:01:30

Step 8.
Recover the homogenate to clean tubes.

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

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

DURATION
00:20:00

Step 11.
Save the supernatant.

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

NOTES
Irina Agarkova 29 Mar 2016
Add the $(NH_4)_2SO_4$ gradually.

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

DURATION
01:30:00

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

DURATION
00:10:00

Step 15.
Save the pellet.

Step 16.
Suspend the pellets with Buffer A.

Step 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).

Step 18.
Mix gently by inversion for 5-10 min.

DURATION
00:10:00

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

DURATION
00:10:00

Step 20.
Save the supernatant.

NOTES
Irina Agarkova 29 Mar 2016
If there is small particulate matter in the supernatant, centrifuge a second time as before and save the supernatant.

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

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

DURATION
18:00:00

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

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

PROTOCOL

. CviJI Assay Conditions

CONTACT: Irina Agarkova

Step 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.

DURATION
02:00:00

Step 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.

DURATION
01:00:00

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

DURATION
00:30:00

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

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

DURATION
18:00:00

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

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

PROTOCOL

. CviJI Assay Conditions

CONTACT: Irina Agarkova

Step 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.

DURATION
02:00:00

Step 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.

DURATION
01:00:00

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

DURATION
00:30:00

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

Step 30.
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 31.
Elute the Q-Sepharose column with Buffer B, pH 8.5 using a 0-2.0 M KOAc gradient.

Step 32.
Assay the column fractions and pool the active fractions.

PROTOCOL

. CviJI Assay Conditions

CONTACT: Irina Agarkova

Step 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.

DURATION
02:00:00

Step 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.

DURATION

01:00:00

Step 32.3.

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

DURATION

00:30:00

Step 33.

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

DURATION

18:00:00

Step 34.

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

Step 35.

Store the enzyme at -20°C.