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## CviJI Purification From IL-3A Virus Infected NC64A Chlorella

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Protocol status: Working



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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 MqAc
- 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.



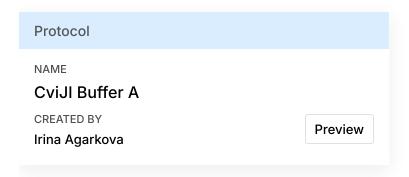
# Troubleshooting

## Before start

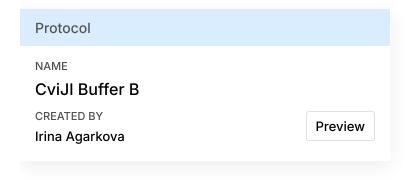
7 hour IL-3A virus infected NC64A, pellets frozen at -80°C



1 Prepare Buffer A:



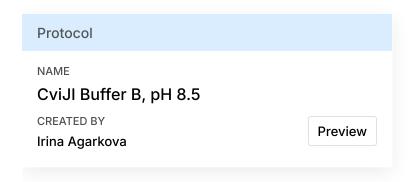
- 1.1 10 mM Tris-Acetate, pH 8.0
- 1.2 10 mM 2-ME
- 1.3 50 μg/mL PMSF
- 2 Prepare Buffer B:



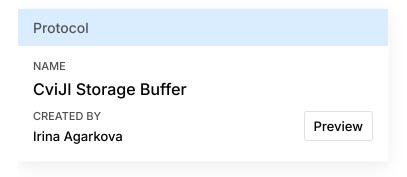
- 2.1 20 mM Tris-Acetate, pH 8.0
- 2.2 0.5 mM EDTA
- 2.3 7 mM 2-ME,10% Glycerol



3 Prepare Buffer B, pH 8.5:



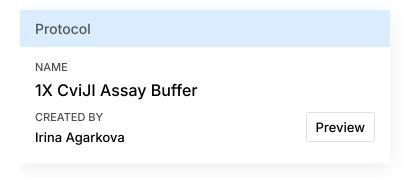
- 3.1 20 mM Tris-Acetate, pH 8.5
- 3.2 0.5 mM EDTA
- 3.3 7 mM 2-ME
- 4 Prepare Storage Buffer:



- 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:



- 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 Α.



### Note

Suspend with 20 mL per flask per 1.0-1.5 X 10<sup>11</sup> 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<sub>2</sub> 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.
  - **(5)** 00:20:00
- 11 Save the supernatant.
- 12 Adjust the homogenate supernatant to 70% saturation with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 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.
  - **(5)** 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.

**(5)** 00:10:00

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



20 Save the supernatant.

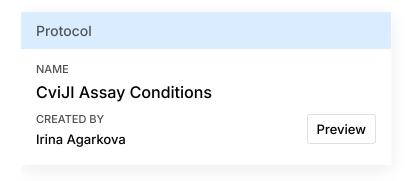
### Note

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

**(3)** 18:00:00

- 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.



24.1 All assays are carried out in 20.0  $\mu$ L volumes with 1  $\mu$ g of pUC19 DNA as substrate for 60 to 120 min at 25°C.

**3** 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.

00:00:00

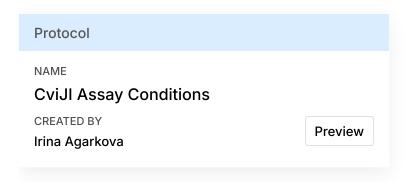
24.3 Gels are stained with 0.5  $\mu$ g/mL ethidium bromide for 30 min and photographed on a UV light box.

**©** 00:30:00

- Dilute the pooled fractions with 10-15 volumes of Buffer B to reduce the salt concentration.
- Load the material overnight onto a Blue-Sepharose column equilibrated with Buffer B in the cold room.

**(5)** 18:00:00

- 27 Elute the Blue-Sepharose column with Buffer B using a 0-2.0 M KOAc gradient.
- Assay the column fractions and pool the active fractions.



28.1 All assays are carried out in 20.0  $\mu$ L volumes with 1  $\mu$ g of pUC19 DNA as substrate for 60 to 120 min at 25°C.

**©** 02:00:00

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.

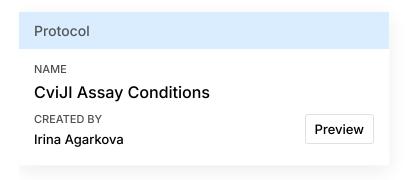
**6**) 01:00:00

28.3 Gels are stained with 0.5  $\mu$ g/mL ethidium bromide for 30 min and photographed on a UV light box.

**©** 00:30:00



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



32.1 All assays are carried out in 20.0  $\mu$ L volumes with 1  $\mu$ g 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.

**6**) 01:00:00

32.3 Gels are stained with 0.5  $\mu$ g/mL ethidium bromide for 30 min and photographed on a UV light box.

**©** 00:30:00

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

**(5)** 18:00:00

- 34 Add BSA (10 mg/mL) to a final concentration of 100  $\mu$ g/mL.
- 35 Store the enzyme at -20°C.

