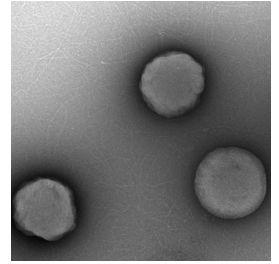


Feb 25, 2020

Virus purification protocol for Ehv-163 and other viruses.

DOI

dx.doi.org/10.17504/protocols.io.bcpvivn6



Elena Yakubovskaya¹, Tatiana Zaliznyak¹, Joaquin Martinez Martinez², Gordon T Taylor¹

¹School of Marine & Atmospheric Sciences, Stony Brook University; ²Bigelow Laboratory for Ocean Sciences

TaylorLab



Elena Yakubovskaya

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Protocol Citation: Elena Yakubovskaya, Tatiana Zaliznyak, Joaquin Martinez Martinez, Gordon T Taylor 2020. Virus purification protocol for Ehv-163 and other viruses.. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bcpvivr6>

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Protocol status: Working

We use this protocol and it's working

Created: February 18, 2020

Last Modified: February 25, 2020

Protocol Integer ID: 33237

Keywords: purification of virus, virus purification, virus purification protocol, purified virus, identification of individual virus particle, individual virus particle, reliable protocol for virus purification, physical properties of the virus, virus, undamaged by the purification procedure, purification procedure, purification, other virus, contaminating host, cryoem analysis, quality ehv sample

Abstract

Reliable protocol for virus purification is an essential step for characterization and identification of individual virus particles in order to obtain information on biochemical and physical properties of the virus and for studying interactions between host and virus at the single-cell level. The purified virus should be physically and chemically undamaged by the purification procedure and free from contaminating host-material. To obtain high-quality EhV sample (the virus that infects coccolithophorid, *Emiliana huxleyi*) suitable for AFM, Raman and CryoEM analysis, we modified the protocol published by Lawrence and Steward ("Purification of viruses by centrifugation" J. E. Lawrence and G. F. Steward; *MAVE Chapter 17, 2010, 166–181* © 2010, by the American Society of Limnology and Oceanography, Inc.).

Materials

MATERIALS

⊗ Optiprep (Iodixanol) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D1556-250ML**

⊗ F/2 medium **Catalog #MCK50L**

⊗ SW40 Ti (with Beckman ultracentrifuge) rotor with ultra-clear tubes (14×95 mm) **Beckman Coulter Catalog #344060**

⊗ Vivaflow 200 tangential flow filtration unit (0.2 µm, PES), including size 16 tubing for Masterflex peristaltic pump **Sartorius Catalog #VF20P7**

⊗ Spectra/Por Float-A-Lyzer G2 Dialysis Device, 100000 MWCO, 10mL **Thermo Fisher Catalog #CP0290646**

⊗ Glass microfibre filter (0.7 µm; GF/F ø=47 mm)

⊗ CaptoCore 700 **GE Healthcare Catalog #17-5481-01**



Troubleshooting

Before start

If working with a lytic virus, after host culture clear (in case of EhV-clearance of the 163 6 days), filter the lysate through a 0.8 μm nitrocellulose filter to remove cell debris and then filter supernatant through a 0.45 μm PES filter. We started with 1-liter of *Emiliana huxley* infected with EhV-163.

- 1 Concentrate lysate 50 times by tangential flow filtration with a Vivaflow 50 cartridge (100 KDa MWCO) to decrease volume. For EhV-163 virus, the final volume was 50 ml and the final concentration was $\sim 7 \times 10^9$ EhV/ml.
- 2 Prepare OptiPrep solutions using F/2-Si media or 150mM NaCl, 20mM HEPES pH 8.0 buffer. 1mM EDTA buffer as the diluent. For many viruses, a gradient from 20%- 50% OptiPrep will provide a good range for separation, but very dense or light viruses may require adjustment. For EhV-163 virus, we used 20%, 25%, 30%, 35%, 40% and 50% v/v OptiPrep solutions (OptiPrep is sold as a 60% solution).
- 3 Using the underlying technique with a syringe and pipetting needle, pour 6-step gradients into open-topped ultracentrifuge tubes, beginning with the least dense solution first. 1ml 20%, 2ml 25%, 2ml 30%, 2ml 35%, 2ml 40%, 1ml 50%. Allow blending for 2 h at room temperature.
- 4 Carefully overlay the gradient with virus concentrate. For the 10-mL gradient, we used 2-mL concentrated virus. For SW-41 ultracentrifuge rotor 4h at 26,000 rpm (100,000g) is sufficient h for good separation of Ehv-163 but overnight run also works. Allow the rotor to decelerate using a slow deceleration program below 4000 rpm or turn off the brake below 4000 rpm.
- 5 Virus bands are visible with a regular flashlight. For harvesting a single band in a density gradient use side or bottom puncture with a needle.



- 6 To remove iodixanol from the sample of Ehv-163 use 3x dialysis against 1L of 150mM NaCl, 20mM HEPES pH 8.0, 1mM EDTA buffer using Float-A-Lyzer G2 with 100kD cut off dialysis membrane.
- 7 Equilibrate 3 ml of CaptoCore 700 resin with 10ml of 150mM NaCl, 20mM HEPES pH 8.0, 1mM EDTA buffer. Purify EhV-163 on a Capto Core 700 resin using in batch or column method to remove traces of iodixanol.



Aliquot purified virus sample Store virus sample in al at 4°C in the dark.