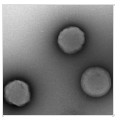

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Virus purification protocol for Ehv-163 and other viruses.

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1 Works for me dx.doi.org/10.17504/protocols.io.bcpvivn6

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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 infecta coccolithophorid, *Emiliania huxleyi)* sutable 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.).*

EXTERNAL LINK

http://www.soest.hawaii.edu/oceanography/faculty/steward/StewardLab/Projects_files/Lawrence%26Steward2010_MAVE.pdf

MATERIALS

NAME	CATALOG #	VENDOR
Optiprep (Iodixanol)	D1556-250ML	Sigma Aldrich
F/2 medium	MKK50L	
SW40 Ti (with Beckmanultracentrifuge) rotor with ultra-clear tubes (14x95 mm)	344060	Beckman Coulter
Vivaflow 200 tangential flow filtration unit (0.2 µm, PES), including size 16 tubing for Masterflex peristaltic pump	VF20P7	Sartorius
Spectra/Por Float-A-Lyzer G2 Dialysis Device, 100000 MWCO, 10mL	CP0290646	Thermo Fisher
Glass microfibre filter (0.7 µm; GF/F ø=47 mm)		
CaptoCore 700	17-5481-01	Ge Healthcare

BEFORE STARTING

If working with a lytic virus, after host culture clear (in case of EhV-clearance of the163 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 *Emiliania huxley i*nfected 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 ~7 x 10^9EhV/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-toped 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.