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Concentrating Viruses by Tangential Flow Filtration

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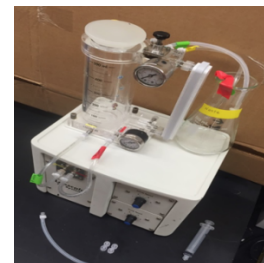
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Protocol status: Working

We use this protocol and it's working

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Abstract

Ultracentrifugation in the WX Ultra Series can support only 30 mL samples at a time, and therefore do not significantly concentrate viruses. Tangential flow filtration enables viral concentration of large volumes that can then be purified using sucrose density gradients.

Contact Dr. Steven Wilhelm (wilhelm@utk.edu) or Samantha Coy (srose16@vols.utk.edu) for additional information regarding this protocol.

Attachments



[TFF for Viruses.pdf](#)

305KB

Guidelines

Diagnosing Issues and Troubleshooting:

If the pressure suddenly decreases without adjusting, then the pressure may have been too high and caused a tear in the filter. This would allow the fluid to flow freely through the filter and into the waste vial much more quickly than normal. Prepare a sample from the waste vial for epifluorescent microscopy to confirm.

If your actual concentration is much lower than the theoretical concentration, then one of a few things may have happened:

- A. There may be a tear in the filter.
- B. The viruses may be sticking to the filter--use a syringe to force fluid into a Falcon tube.
- C. The viruses may be getting inactivated from residual NaOH on the filter or in the TFF system. This is unlikely if you have flushed with sufficient Milli-Q and buffer prior to adding the sample.

If your sample is concentrating very slowly, then you may need to check the pressure. If the back pressure is not ~10 psi, it will concentrate more slowly because fluid is circulating more than it is passing through the filter. The back pressure also cannot increase if the pump speed is too low.

Before start

It is imperative that the system be cleaned properly between uses to prevent cross-contamination. It may also be necessary to determine that the cleaning solution used will indeed inactivate/degrade/kill the sample being concentrated.

Machine Preparation

- 1 Set up the tangential flow filtration (TFF) apparatus so that the Pellicon XL 30 kDa filter is attached. Both the retenate and waste tubes should empty into your waste flask.

*Catalog number of Pellicon XL 30 kDa filter: PXC030C50


*Refer to Figure 1 in the attached PDF.

- 2 Make 500 mL 0.1 N NaOH, 45°C. Pour into the TFF reservoir.

- 3 Turn on the pump speed to 1 or 2, and gradually turn the back pressure valve clockwise to increase the pressure. The bottom pressure gauge should be 30-40 psi, and the top pressure gauge should be ~10 psi.

- 4 Circulate the fluids until ~350 mL is left. Re-attach the retenate tube to circulate the remaining cleaning solution that does not pass through the filter. Run the NaOH solution through system for ~20 minutes.

* Do NOT let the system run dry!


 00:20:00

- 5 When 50 mL is left, turn the pump speed to off and tighten a plastic clamp onto the tubing that flows to the pump inlet. Detach the tube, and undo the clamp to empty the remaining NaOH solution into the waste vial.

*Refer to Figure 2 in the attached PDF.

- 6 Attach a syringe to the tube connecting the pump outlet, and pull back the plunger to draw out any remaining NaOH inside of the machine. Re-attach the tubing, and use the syringe to drain any other tubing in the same manner (e.g. over the surface of the filter).

- 7 Wash the TFF system with at least 1L of MilliQ water, allowing the first 500 mL to empty into the flask through both the retenate and waste tubes. Re-attach the retenate tube to circulate the last 500 mL and run it through the apparatus for ~10 min. Empty the remaining volume in the same process outlined in step 5 and 6.

 00:10:00

- 8 Circulate 300 mL of 50mM Tris-HCl, pH=7.8, 10 mM MgCl₂ until ~25 mL is left.

*This step primes the TFF system with buffer to protect the viruses against any residual effects of the NaOH.

*Use any buffer that is appropriate for the viruses that you are concentrating.

Concentration

- 9 Fill the reservoir with the virus stock. Continue to run the system at the same psi (30-40 psi for the bottom pressure and ~10 psi for the top pressure gauge). After the sample has concentrated, empty the remaining volume in the reservoir into a Falcon tube. Virus may get stuck to the filter so use a syringe attached to the pump outlet tube, and draw out the remaining fluid from the filter. It may be necessary to go back and forth with the plunger to dislodge viruses that are stuck to the surface.

Purification

- 10 Purify the samples (e.g. filter sterilize if axenic cultures, perform sucrose density gradient if not).

Protocol



NAME

Virus Purification by Sucrose Density Gradients

CREATED BY

Steven W Wilhelm

PREVIEW

- 10.1 Centrifuge lysate in the Sorvall Lynx 4000 centrifuge at 5,000 rpm, 5 min, 4°C. Discard the pellets.
- 10.2 Add Triton X-100 to the lysate supernatants for a final concentration of 1% (from a 10 or 20% stock).
- 10.3 Centrifuge the lysate in the Sorvall WX Ultra Series ultracentrifuge at 17,000 rpm, 50 min, 4°C. Discard the supernatants.
- 10.4 Resuspend the virus pellets with a small volume of 50 mM Tris-HCl, pH 7.8
*Approximately 1.0 mL per 100 mL lysate
- 10.5 Layer the virus suspension onto 100-400 mg/mL (10-40%) linear sucrose gradients equilibrated with 50 mM Tris-HCl made in polypropylene tubes (layer approximately 3-4 mL per gradient).

*To make sucrose stocks, add 10-40% sucrose to Tris-HCl and autoclave.



- 10.6 Centrifuge the gradients in the ultracentrifuge at 20,000 rpm, 20 min, 4°C. The virus will be the major band about 1/2 to 2/3 deep in the gradient.
- 10.7 Remove the virus bands from the gradients with sterile needles and transfer to 30 mL polypropylene centrifuge tubes. Split the virus from 3 gradients between two tubes. Slowly dilute the virus to the tube volume with 50 mM Tris-HCl. Centrifuge the tubes in the ultracentrifuge at 27,000 rpm, 3 hours, 4°C. Discard the supernatants.
- 10.8 Resuspend the virus pellets with a small volume of 50 mM Tris-HCl. Store the virus at 4°C. Do not freeze. Filter sterilization using a 0.45 um cellulose acetate filter is recommended.

Titering

- 11 Titer the sample by plaque assay and determine the actual concentration compared to the theoretical.
*Theoretical concentration = $(V_1 * C_1) = (V_2 * C_2)$

Machine Cleaning

- 12 Clean the TFF system with NaOH in the same process as in the preparation steps. Rinse with MilliQ.
- 13 Using a syringe filter, fill the Pellicon filter with 0.1 N NaOH, 4°C until the next use.