Concentrating Viruses by Tangential Flow Filtration

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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.

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Guidelines

Diagnosing Issues and Troubleshooting:

If the pressure suddenly decreases without adjusting, then the presure 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 cannt increase if the pump speed is too low.

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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.

Protocol

Machine Preparation

Step 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.

Machine Preparation

Step 2.

Make 500 mL 0.1 N NaOH, 45ºC. Pour into the TFF reservoir.

Machine Preparation

Step 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.

Machine Preparation

Step 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!

DURATION

00:20:00

Machine Preparation

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Step 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.

Machine Preparation

Step 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).

Machine Preparation

Step 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.

DURATION
00:10:00

Machine Preparation

Step 8.

Circulate 300 mL of 50mM Tris-HCl, pH=7.8, 10 mM $MgCl_2$ 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

Step 9.

Fill the reservoir with the virus stock. Continue to run the system at the same psi (30-40 psi for the bottom pressue and 10 psi for the top pressure gauge). After the sample has concentrated, empty the remaining volume in the reservior 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 fourth with the plunger to dislodge viruses that are stuck to the surface.

Purification

Step 10.

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

PROTOCOL

. Virus Purification by Sucrose Density Gradients

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Step 10.1.

Centrifuge lysate in the Sorvall Lynx 4000 centrifuge at 5,000 rpm, 5 min, 4ºC. Discard the pellets.

Step 10.2.

Add Triton X-100 to the lysate supernatants for a final concentration of 1% (from a 10 or 20% stock).

Step 10.3.

Centrifuge the lysate in the Sorvall WX Ultra Series ultracentrifuge at 17,000 rpm, 50 min, 4ºC. Discard the supernatants.

Step 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

Step 10.5.

Layer the virus suspension onto 100-400 mg/mL (10-40%) linear sucrose gradients equilibriated 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.

Step 10.6.

Centrifuge the gradients in the ultracentrifuge at 20,000 rpm, 20 min, 4ºC. The virus will be the major band about 1/2 to 2/3 deep in the gradient.

Step 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, 4ºC. Discard the supernatants.

Step 10.8.

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Resuspend the virus pellets with a small volume of 50 mM Tris-HCl. Store the virus at 4ºC. Do not freeze. Filter sterilization using a 0.45 um cellulose acetate filter is recommended.

Titering

Step 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

Step 12.

Clean the TFF system with NaOH in the same process as in the preparation steps. Rinse with MilliQ.

Machine Cleaning

Step 13.

Using a syringe filter, fill the Pellicon filter with 0.1 N NaOH, 4°C until the next use.

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