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Concentration of Phage Lysate using Vivaflow Tangential/Crossflow Filtration Cassette

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External link: https://www.sartorius.us/us/product-family/product-family-detail/m-vivaflow-50/VF05P9/49945/

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Abstract

Purpose: To concentrate harvested phage lysate up to 20X - 40X. The Vivaflow device uses a peristaltic pump setup to recirculate the input sample over a 100,000 MWCO PES membrane, which allows the media filtrate to be diverted and the phage to remain in the starting sample.

Materials

MATERIALS

- Phage lysate (0.2 um filtered into 1 L sterile bottles)
- Vivaflow 50 cassette, (100,000 MWCO, PES),which includes size 16 tubing for inlet and outlet sample flow, and interconnector tub **Catalog #**VF05P4
- Pressure Indicator, must order separately for Vivaflow 50 **Catalog #**VFA020
- Masterflex console drive pumps (Cole-Parmer): 1 per phage sample
- Masterflex Easy Load II Model 7702-50 pump head (tubing size LS 13.14.16.25): 1 per pump – ours have capacity to load two separa
- Masterflex tubing of size 25, 2 ft length (cat# 96410-25): (cleaned, acid washed, autoclaved - wrapped in foil), 1 per Vivaflow
- 2 Filtrate collection bottles or flasks (acid wash container if keeping phage filtrate samples): 1 per phage sample
- 250 ml polycarbonate bottles: 3 per 2 Vivaflow 50 cassettes (for each phage) – for sample recovery and rinsing steps
- 2 sterile 50 ml conical tubes
- Parafilm
- 3 L of Nanopure water, 0.2 um filtered into acid washed bottles and autoclave sterilized: use to pre-rinse new Vivaflow cassette
- 600 ml ASW salts media
- 1.5 ml Eppendorf tubes (autoclaved) – for SYBR slide sample collection

Before start

MODIFICATION NOTES:

To ensure full phage recovery from large sample starting volumes of up to 4-8 L of lysate, some steps were added to the manufacturer's instructions based on empirical trials of filtering capacity. The first trial was done following the Sartorius user manual steps explicitly for 8 L filtered on a Vivaflow 200 device, but resulted in about 25% phage recovery. The second trial incorporated modifications which enhanced recovery to nearly 100% from 4 L of lysate using a Vivaflow 200 device and 2 L of lysate using a Vivaflow 50 device, and is described in this protocol for the Vivaflow 50. This may not be an issue for sample volumes of less than 2 L being concentrated on the same device (Vivaflow 50), but this was not verified.

Filtration of 8 L was successful using two of the Vivaflow 50 cassettes in parallel, taking about the same amount of time as using one Vivaflow 200 cassette (~1 L per hour); since the Vivaflow 50 set of 2 cassettes are much less expensive, though not reusable, we chose to use these to avoid any potential cross contamination of samples from repeated use (reuse is specified for the 200 and 50R devices).

Materials Notes:

- *To use the Masterflex tubing and pump head (size 25) specified below with the Vivaflow 50 PVC tubing (size 16) provided, modify the larger diameter (size 25) tubing with an appropriate adapter fitting. Attach the adapter fitting to the Masterflex tubing first and autoclave sterilize this together in a foil wrap. When ready to use, connect the larger and smaller tubing via the adapter, using sterile technique.*

1 To filter 4-8 L of phage lysate, set up one Vivaflow50 cassette per 4 L of sample (this was the capacity successfully tested by our lab). If two cassettes are needed for a phage sample, set these up in parallel with separate tubing lines, using the double tubing pump head.

Refer to the Sartorius user manual and online videos for tubing attachment and flow set-up. For all tubing, keep the ends to be placed in the sample and filtrate as clean as possible once removing from the packaging.

To adapt the smaller cassette tubing to our lab's pump head size, make sure to attach the size 25 Masterflex tubing first. Keep clean/sterile the end to be placed in the phage sample.
(https://www.sartorius.us/us/products/laboratory/ultrafiltration/ultrafiltration-overview/)

2 If running more than one phage sample simultaneously, make sure to label all input and output tubing, cassettes and bottles or flasks with sample ID's, and keep separate to prevent any phage cross contamination.

3 Per the Sartorius manual, prepare the cassette (aka: module) before use by pre-rinsing the system at full pressure to remove trace amounts of glycerine and sodium azide and check for any leaks at the tubing connections:

- Place the input tubing into the 1 L bottle of 0.2 um filtered, sterile nanopure water. Place the output return tubing in the same bottle and pump liquid through the system to purge any air pockets. You will need ~500 ml of water per cassette, and you can set up two parallel cassettes to use the same bottle.
- The recirculation rate should be in the range of 200-400 ml/min and suitable flow should exit the filtrate line. If used, the pressure indicator should read approximately 2.5 bar. This corresponds to a pump setting of around 60.
- Allow 400 ml per cassette to pass into the filtrate collection. Check for any leaks at connections. Finally, drain the system and empty the filtrate flask.

4 To concentrate the phage sample, place the input and output lines into the initial lysate container (usually a 1L bottle). If the container lid is off, place a piece of parafilm over the opening to minimize contamination and secure the tubing in place.

5 Pump the liquid through the system at the same rate and pressure as specified for the rinsing step above. For two cassettes filtering one sample, a pump setting of around 60 corresponds to a filtrate production of about 250 ml per 14 min initially, but which may slow some as more concentrated sample is added to the recirculation.

6 Monitor the sample level and stop the pumping when volume reaches 100-200 ml to refill the bottle with more sample. Also monitor the filtrate flask to prevent overflowing and discard (or keep, if desired) the filtrate down the sink (this should be free of phage!).

7 Stop adding phage sample after you have concentrated 4 L to between 175 and 200 ml. Note that about 15-20 ml are left in the system. When the desired volume has been reached, reduce the recirculation rate to 20-40 ml/min (pump setting around 10) and recirculate the concentrated sample for 1-2 minutes to maximize recovery.

8 Stop adding phage sample after you have concentrated 4 L to between 175 and 200 ml. Note that about 15-20 ml are left in the system. When the desired volume has been reached, reduce the recirculation rate to 20-40 ml/min (pump setting around 10) and recirculate the concentrated sample for 1-2 minutes to maximize recovery.

9 If there are > 4 L of sample to concentrate, begin the next fraction: place the input and return tubes into the next lysate sample bottle, parafilm cover, and repeat the concentration process (steps 5-8). Then transfer the concentrated sample into a second 250 ml bottle and drain the remaining sample completely from the system.

10 To collect a final rinse from the filters for a more complete sample recovery, place 100 ml ASW salts into a clean 250 ml bottle. Place the input and return tubing into the bottle, cover the top with parafilm and recirculate on lower speed until the volume is reduced to about 20 ml. Then drain the system as before and collect the remaining rinse fraction into the bottle.

11 Even though the Vivaflow 50 cassettes are not reusable, they were kept until the concentration of phage sample was determined. To store the cassettes, load them with ASW salts and keep at 4 °C.

- Place 50 ml ASW salts into a sterile 50 ml tube and recirculate this through the filter and tubing to remove air bubbles. Then either leave the open tubing ends in the media tube and cover with parafilm, or seal the tubing ends with tape.

12 1. For phage concentration analysis via SYBR slide preparation, collect 0.5 ml of each of the following samples.

- Sample Fractions #1, #2 and Rinse[1]
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- Filtrate from beginning of the first 4 L concentration[2]
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- Filtrate from end of the first 4 L concentration[2]
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Footnotes:

1) In Feb 2016 experiments, sample fractions concentrations were determined before combining the two fractions for the next step. The second fraction was more

concentrated for both phage samples. The rinse sample was not counted or used.

2) In all previous Vivaflow concentration trials, no particles were seen on SYBR slides for the filtrate samples, as should be expected.