M12 Tangential Flow Filtration

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Abstract

Please contact Dr. Steven Wilhelm (wilhelm@utk.edu) for additional information regarding this protocol.

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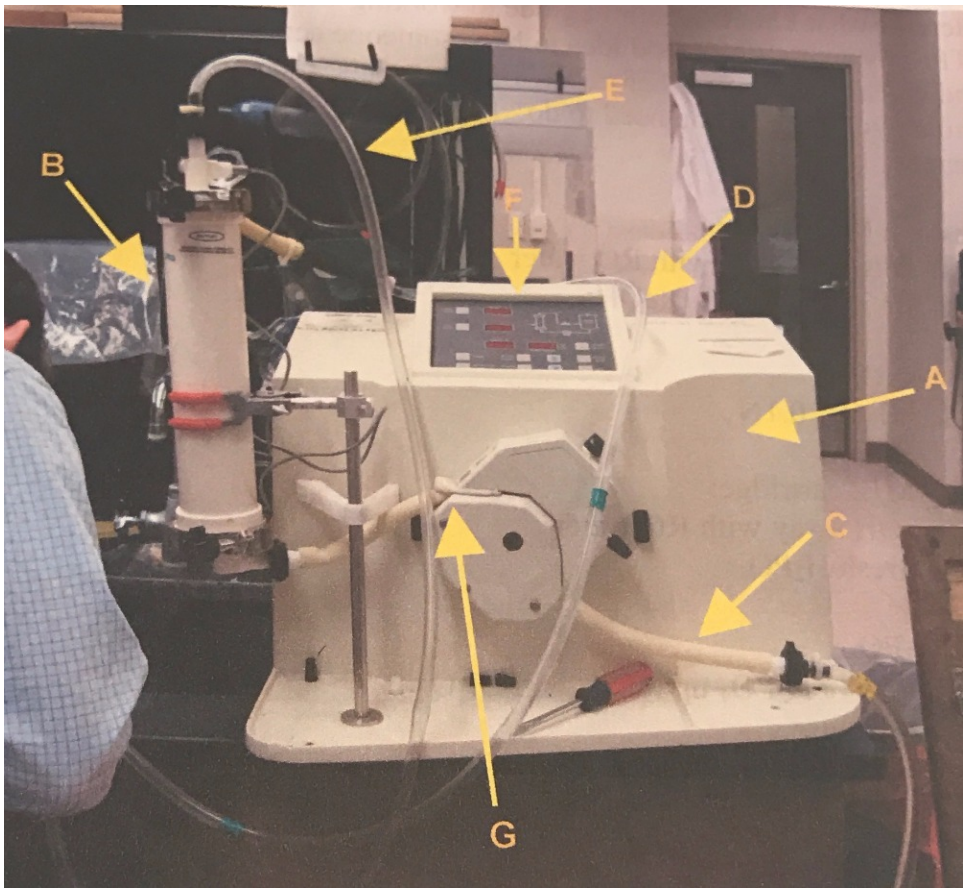
Guidelines



A: M12 System

B: Filter cartridge (refrigerated while stored and supported by a clamp during use)

C: Sample water

D: Retentate

E: Permeate

F: Control panel

G: Tygon tubing

Protocol

M12 Setup

Step 1.

Look at the guidelines for a key of each piece related to the use of the M12 system.

M12 Setup

Step 2.

Ensure that the M12 is bolted down (preferably near a sink/drain)

NOTES

Alyssa Alsante 07 Jun 2017

Make sure to put gloves on, because the cartridges are stored in acid, which can get on our hands if you're not careful.

Step 3.

Use an arm clamp to hold the cartridge up-side-down.

NOTES

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This end is indicated by having only one port for hosing to be connected, you cannot trust the writing on the cartridge as it depends on how the caps were placed on the cartridge, so you will have to visually determine this.

Step 4.

Carefully unwrap the parafilm

Step 5.

Connect the sample hosing to this port

NOTES

Alyssa Alsante 09 Jun 2017

Step 6.

Connect one of the pressure sensors (does not matter which one) to the pressure sensor port.

NOTES

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You may want to make sure that there is fresh Teflon tape around the threads for a secured fit. and wait until the cartridge is in its final position before connecting the other end of the pressure sensor to the M12.

Step 7.

Invert the cartridge to where it is right-side-up (the upper end should have two real ports), and carefully unwrap the parafilm.

Step 8.

First, connect the retenate hosing to the port that faces horizontally.

Step 9.

Next, connect the permeate hosing to the vertically facing port.

NOTES

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The connector that screws into the top of the cartridge is essentially invaluable and cannot be

broken (be very careful). 

Step 10.

Connect the second pressure sensor in the same manner as the first and then to connect both of the sensors to the M12, the bottom on is inlet and the top is outlet.

NOTES

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The connection to the M12 can be tricky, make sure the sensors are securely in place.

Step 11.

Step 12.

Run the tygon section of the sample hosing through the pump.

Step 13.

Adjust the height and position of the cartridge to remove any kinks in the hosing.

Step 14.

When you close the clamp over the tubing, take care to ensure that the tubing is in the center-most position, using the tubing covered wire to help buffer the tygon tubing and keep it from moving toward either side.

NOTES
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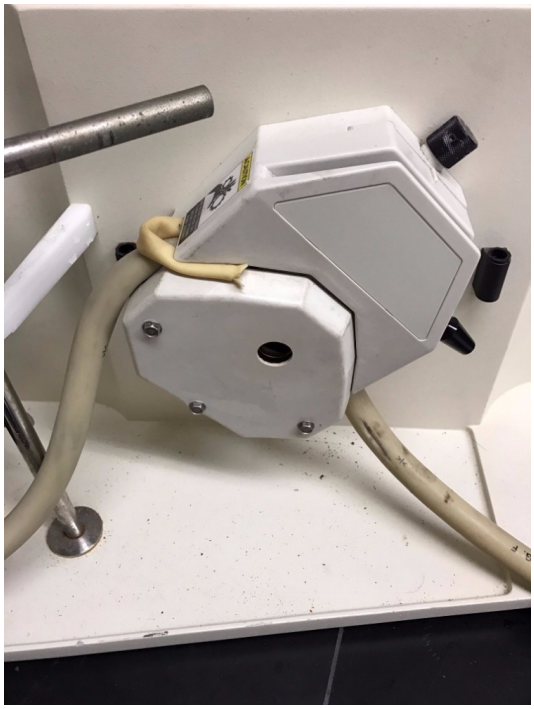
The round gears on either side chew through the tygon tubing. You will need to check often that the tubing is centered and that it is in usable condition.



Step 15.

Place the sample hosing in fresh Milli-Q H_2O and the retentate and permeate hoses into an appropriate waste container.

Washing M12 Cartridge

Step 16.

Turn the M12 power on

NOTES
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For this particular machine, the switch is located at the top of the machine.

Washing M12 Cartridge
Step 17.

Push the INTERLOCK OVERRIDE button

NOTES
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Make sure the valve to force back pressure on the retentate hosing is fully open (turn counter clockwise to open, clockwise to close, you can feel the tension change as to turn the knob).

Washing M12 Cartridge
Step 18.

Push CAL ZERO and push INTERLOCK OVERRIDE again

Washing M12 Cartridge
Step 19.

Push the START/STOP button

NOTES
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The pump will begin working at 10% speed. Check to make sure things are working properly and that no water is leaking.

Washing M12 Cartridge
Step 20.

Slowly adjust the speed using the up and down arrows. Take the speed up to 15% and then slowly adjust the retentate valve until the outlet and inlet pressure read 10 for each.

Washing M12 Cartridge
Step 21.

When you near the end of the Milli-Q H_2O, turn the pump off by pushing the START/STOP button again. Release the pressure on the retentate hosing.

NOTES
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Don't let the sample hosing run out of water. Allow 2-4 L of water to run through to flush out the

acid storage solution.

Virus Sample Concentration and Generation of Ultrafiltrate

Step 22.

Empty all three hoses of water the best that you can.

Virus Sample Concentration and Generation of Ultrafiltrate

Step 23.

Place the sample hosing in the container of sample water.

NOTES

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If you are filtering a natural whole water sample you should run the whole water through a GF/F and then 0.45 and 0.2 um filters prior to M12 use. Otherwise, the 30 kDa cartridge will become clogged very quickly.

Virus Sample Concentration and Generation of Ultrafiltrate

Step 24.

The end of the sample hosing should be marked with a red flag, make sure that you can see that red flag at the bottom of your sample container to ensure that the full sample will be pumped through and to reduce the possibility of air getting into the hosing.

Virus Sample Concentration and Generation of Ultrafiltrate

Step 25.

Place the end of the retentate hosing into the sample container.

NOTES

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This water will have passed through the cartridge without having been forced through the filter. Essentially, it is the same as the sample water, which is why it is fed into that container.

Virus Sample Concentration and Generation of Ultrafiltrate

Step 26.

Place the end of the permeate hosing into a clean container, approximately the same size as the sample container.

NOTES

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It will contain the ultrafiltrate when you are finished. This water is what has been forced through the 30 kDa filter and is therefore virus "free".

Virus Sample Concentration and Generation of Ultrafiltrate

Step 27.

Turn on the pump, and check to make sure things are working properly.

Virus Sample Concentration and Generation of Ultrafiltrate

Step 28.

Slowly increase the speed to 25%

Virus Sample Concentration and Generation of Ultrafiltrate

Step 29.

Slowly adjust the backpressure (valve on the retentate hosing) until the outlet and inlet pressure read 15-16

NOTES

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The outlet pressure is of concern. Make sure that it does not exceed 16 or 17.

Virus Sample Concentration and Generation of Ultrafiltrate

Step 30.

When the level of water remaining in the sample container is slightly below what you want, turn off the pump and drain the hoses into the sample container.

Virus Sample Concentration and Generation of Ultrafiltrate

Step 31.

Remove the permeate hosing from the ultrafiltrate container and allow any remaining water to drain into the same container.

Virus Sample Concentration and Generation of Ultrafiltrate

Step 32.

Cover and store at 4ºC

M12 Cleaning

Step 33.

Remove the sample water from the M12.

NOTES

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This is especially important if you have been working with seawater.

M12 Cleaning

Step 34.

In a similar manner to when you rinsed out the acid storage solution, rinse out the sample water with Milli-Q or RO water (about 6 L).

M12 Cleaning

Step 35.

To wash the cartridge with 0.1 NaOH, dump the NaOH pellets into another 2 L beaker with 1 L RO water and make sure they dissolve before you feed to the M12.

M12 Cleaning

Step 36.

Place all three hoses into the beaker of NaOH to allow the NaOH to run through the cartridge.

M12 Cleaning

Step 37.

Let the washing last for 30-45 min

M12 Cleaning

Step 38.

If you will be using the M12 within a day or so, flush out the NaOH with 6 L Milli-Q water.

M12 Cleaning

Step 39.

Stop the M12, release the backpressure, and allow the three hoses to sit in clean water until the next use.

M12 Cleaning

Step 40.

If you are finished with the M12, first run 4-6 L of water through the system to remove the NaOH

M12 Cleaning

Step 41.

Next, run 1 L 0.05 M phosphoric acid through the system, for proper storage of the cartridge. When you are ready to store the cartridge, dilute the acid solution 10 fold.

M12 Cleaning

Step 42.

Run this solution through the cartridge in the same manner as you flushed water through it (i.e., sample hose in the acid solution with retentate and permeate leading to a waste container).

M12 Cleaning

Step 43.

When you are confident that the clean water has left the cartridge and the cartridge now contains the dilute acid solution, turn off the M12 without releasing the backpressure or removing the hoses from the containers.

NOTES
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This will be when there is very little acid left in the flask.

M12 Cleaning
Step 44.

Disconnect the pressure sensors from the M12, but not from the cartridge.

M12 Cleaning
Step 45.

Wearing gloves, carefully disconnect the tubing from the top of the cartridge and quickly replace the closures you removed during set up.

M12 Cleaning
Step 46.

Wrap these closures and the end of the cartridge with a generous amount of parafilm.

M12 Cleaning
Step 47.

Acting carefully to keep the end of the sample hose under the top of the acid solution, turn the cartridge up-side-down.

M12 Cleaning
Step 48.

Again, carefully remove the tubing and pressure sensor.

M12 Cleaning
Step 49.

Replace the closures that were removed at setup, and generously wrap closures and the end of the cartridge with parafilm.

M12 Cleaning
Step 50.

Store the cartridge at 4ºC

M12 Cleaning

Step 51.

Tubing/hoses should be rinsed with Milli-Q H_2O and dried as much as possible before being packed away.