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Using a Peristaltic Pump to Flow Buffer Through a Nanoporous Membrane in Filter Holder Assembly

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

Abstract

This protocol provides steps for seating and flowing liquid through a 50 µm, 13mm round membrane containing a hexagonal arrangement of 25nm pores with lattice constant 65nm using a peristaltic pump. Porous, alumina membranes are fragile and pressure is required to pass buffer through the 25nm pores.

A peristaltic pump at a low flow rate reproducibly hydrates membranes compared to syringe-based assemblies. Steps in this protocol minimize membrane breaks and damage during membrane placement in the filter adapter assembly, liquid flow through the membrane and membrane retrieval.

Materials

MATERIALS

- Graduated plastic transfer pipettes **Fisher Scientific Catalog #**13-711-9BM
- Swinnex Filter Holders, 13mm **Merck Millipore (EMD Millipore) Catalog #**SX0001300
- SmartPor25 **Catalog #**25nm

Troubleshooting

Safety warnings

To prevent membrane cracking or breaking use insulated, flat or paper forceps.

Before start

Assemble tubes and adaptors and check peristaltic pump flow rates.

Mount the Membrane in the Filter Assembly

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1. Insert a teflon ring into the luer-lock end half of the filter assembly. Without a membrane, connect both halves of filter assembly. A tight connection ensure the teflon ring is properly seated in the luer-lock end.
2. Connect assembly to a peristaltic pump so that the flow is towards the reinforced end. Use 'prime' or a fast flow setting on peristaltic pump to wash tubing and filter holder for several seconds using buffer of choice.
3. Unscrew filter holder assembly. Use a transfer pipette to dropwise add buffer to the luer-lock end half until a slight positive meniscus forms above the teflon ring . Use a gloved finger to prevent the buffer from leaking out.
4. Use padded or paper forceps to transfer the membrane into the reservoir created in the luer-lock end. The membrane rarely falls into place on the teflon ring so use the transfer pipette or an impermeable, padded membrane to gently nudge the membrane into place against ring.
5. Screw the reinforced side into the luer-lock end to assemble the filter assembly. Connection should be finger tight. Go slowly so that the reinforced end can properly seat the membrane against the ring in case it was not properly seated.
6. Use a transfer pipette to add fluid to each side of the filter assembly to create a positive meniscus.
7. Insert filter assembly into flow line.


Luer-lock end
Reinforced end
FLOW
Luer-lock end
Reinforced end

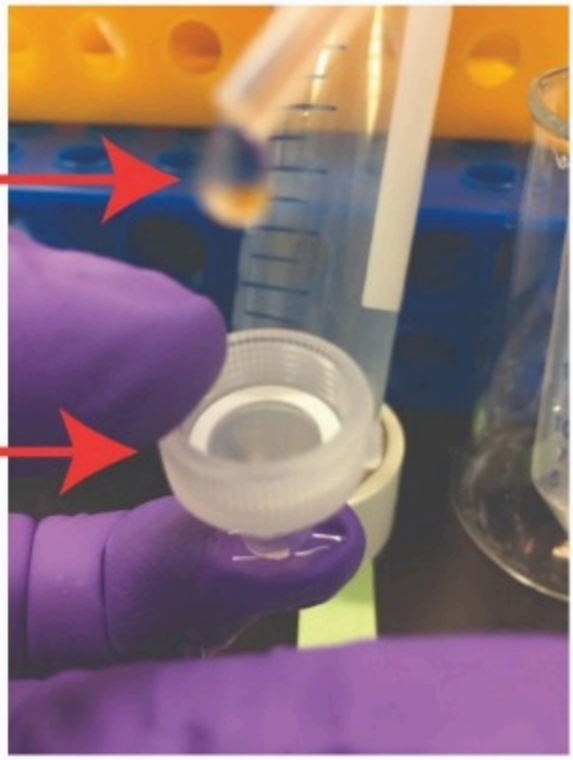
data sheet delivery note 13199.pdf

Flow Fluid Through the Membrane

2

1. SmartPor25 alumina membranes are 50µM thick (info attached) and are brittle. For these membranes we use a flow rate of 0.1mL. However, the flow rate for other membranes will need to be empircally determined. Watch that buffer exits the tip from the reinforced end of the filter assembly. A noticeable drop formation exiting the end of the reinforced end may take several minutes at a slow flow rate. Ensure that fluid does not escape between the interfaces of the filter assembly halves.
2. Continue flow for 1-2 hrs.

Use a finger to cover the luer-lock end and add a few drops to create a positive menis-cus before adding the membrane.



Once the mem-brane has been added, screw together both halves of the filter assembly. Use a transfer pipette to add fluid to both the luer-lock and reinforced ends.

With the positive meniscus, attach the luer-lock to the filter assembly.

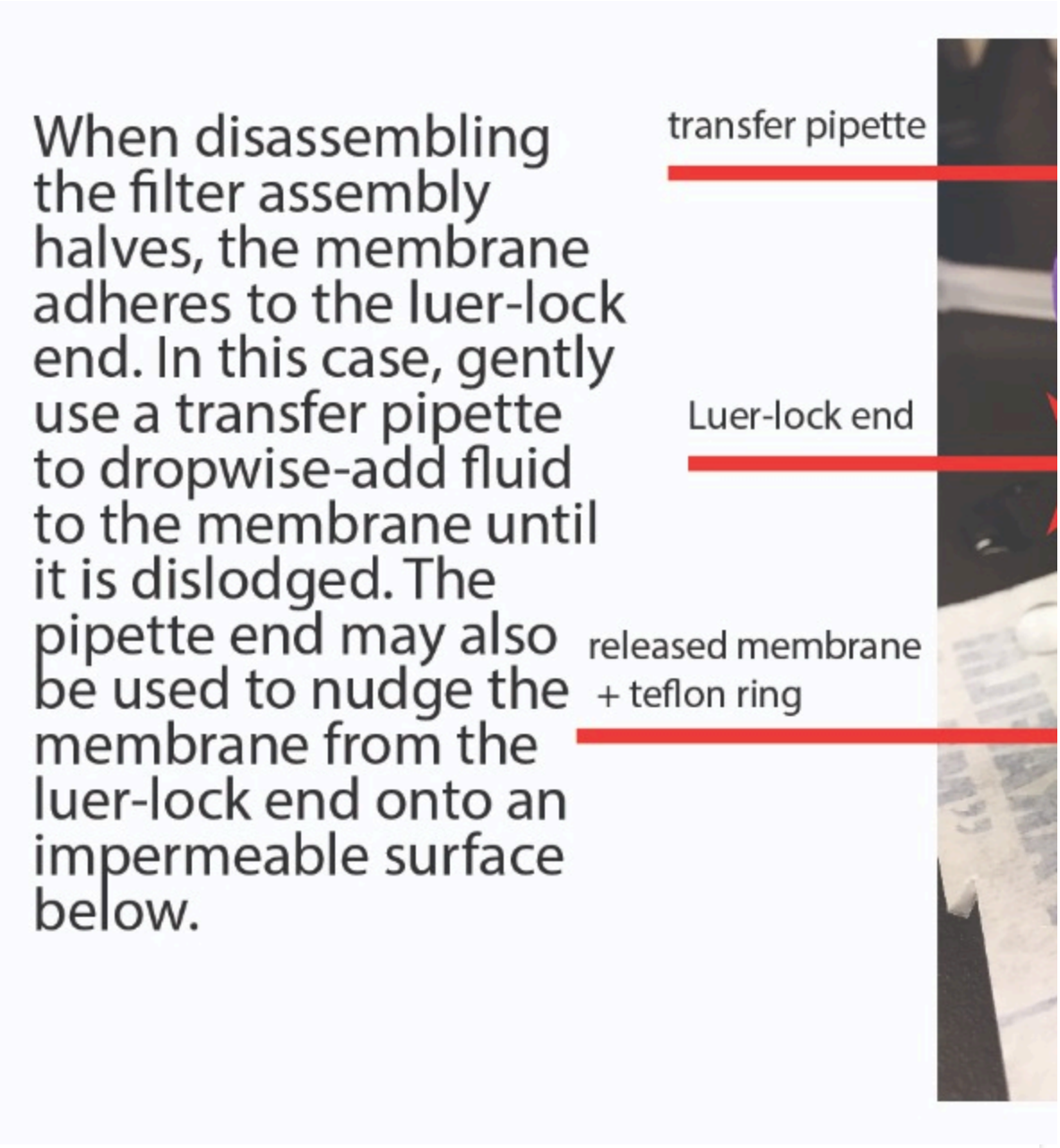
Note

1) A syringe pump instead of a peristaltic pump may also be used.
2) In our experience, a flow rate of 0.5 ml/min is too high. Pressure from this speed cracks the membrane. This is evident as a soft cracking sound from the filter assembly.
3) If flow escapes through the interface between the filter assembly halves then the halves may be tightened carefully. Pressure may also be reduced.
Cracking around the outside of the membrane, just inside the teflon ring contact is indicative of too-high pressure.
4) A rough estimate of the flow rate can be calculated by observing the volume the flow rate will fill a microcentrifuge tube in 1 min.

Remove Membrane from Filter Assembly

3

1. Detach the filter assembly from the flow line.
2. Unscrew in counter-clockwise manner the two halves of the filter assembly.
3. If membrane becomes stuck on the luer-lock side, invert membrane onto parafilm or other impermeable surface.
4. Use a transfer pipet to place a few drops through the luer-lock side onto the stuck membrane. Use the transfer pipette tip to nudge the membrane away from the luer-lock end.
5. Sometimes the teflon rings sticks to the membrane. Use the transfer pipette and padded forceps to push the membrane down but also tug at the ring to separate the membrane.
6. Remove excess fluid using a transfer pipette. Do not blot with a wipe.
7. Mount membrane for study onto material choice.


When disassembling the filter assembly halves, the membrane adheres to the luer-lock end. In this case, gently use a transfer pipette to dropwise-add fluid to the membrane until it is dislodged. The pipette end may also be used to nudge the membrane from the luer-lock end onto an impermeable surface below.
transfer pipette
Luer-lock end
released membrane + teflon ring