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Backflush of Dead-end Ultrafilter

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The backflush procedure pushes out all the cells and particles that were captured during the pumping procedure. It is most easily carried out in the lab with established (pump holding equipment) or ad hoc installation of pump, flushing material, filter and a >500 sterile vessel to collect the backflush.

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protocol

Microbiota of the Hickey Run Tributary of the Anacostia River Robin Cagle ,
Padmini Ramachandran , Elizabeth Reed , Seth Commichaux , Mark K. Mammel ,
David W. Lacher , Daniela Miller , Sabina Lindley , Mihai Pop , Michael C. Bazaco ,
Beverly Wolpert , Errol A. Strain , Scott Aker , Joan Feely , and Andrea Ottesen
Julia A. Maresca , Editor

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Ultrafilter is backflushed with a  **500 mL** solution of 0.5% Tween 80

- Geotech Geopump Peristaltic Pump
- Kimwipes/paper towels
- Scissors
- Bench protector paper, to catch water from leaks (Fisher Scientific, Cat. 14-206-65, or similar)
- Faceshield (Fisher Scientific, Cat. 17-310, or similar)
- L/S 36 tubing (Cole Parmer EW-96410-36 or EW-06434-36)
- DIN adapter (Molded Products MPC855NS.375)
- SNP-8 hose clamps (Cole Parmer EW-06832-08)
- Nitrile or latex gloves
- 0.5% Tween 80
- 500 mL bottle, sterile
- 1 Liter bottle, sterile
- Sterile distilled water
- Pliers
- Sharpie
- Appropriate Personal protective equipment (PPE)

Sterile gloves should be worn during all steps of this procedure to prevent possible exposure to waterborne pathogens and prevent contamination of the ultrafilter. Since the system is under pressure, a face shield should be worn to protect eyes and mouth.

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Make a fresh backflush solution:

- Add **2.5 µL** of Tween 80 to **500 mL** sterile distilled water. Swirl slowly to dissolve the 0.5% Tween 80.

Prepare backflushing solution

- 1 A **500 mL** volume is required per ultrafilter to be backflushed. Make a fresh backflush solution as follows:
 - Add **2.5 µL** of Tween 80 to **500 mL** sterile distilled water. Swirl slowly to dissolve the 0.5% Tween 80.

- 1.1 Be patient, the Tween 80 can take a while to dissolve. Attempt to limit the amount of foam produced, but some foam is inevitable.

Backflush Station Set Up

- 2 Assemble the filtration system by coordinating the position of the peristaltic pump, ultrafilter and backflush solution.

Tubing will flow from backflushing solution, through peristaltic pump, into the "top" of the ultrafilter. Backflush will then flow from the ultrafilter to a sterile beaker or bottle.

- 3 Cut enough fresh tubing (L/S 36) to run from the **500 mL** vessel containing backflush solution, through the pump, into the side of the "top" of the ultrafilter.
- 4 Open the side port of the "top" of the ultrafilter by removing the cap. Push the freshly cut tubing on the side port all the way to where the port meets the ultrafilter. Feed the tubing through the peristaltic pump and place the end of the tubing into the backflush solution.

- 4.1 Secure all of the connection points with zip ties or clamps as needed.

- 5 Cut another piece of fresh tubing (L/S 36) to run from the colored "bottom" part of the

ultrafilter into a sterile vessel. Attach a DIN adapter and screw into the bottom of the ultrafilter which will feed into the sterile vessel.


- 6 Secure all of the connection points with zip ties or clamps as needed. Attach the tubing and feed the tube into the sterile vessel for collection of backflush solution that has flowed through the ultrafilter.

- 6.1 Ensure the 2 remaining caps on the ultrafilter are secured tightly so as to prevent leaks.

- 7 Once the tubing, pump, ultrafiltration cartridge, and sterile vessel have been set up, plug the appropriate power cord into the outlet in the back of the pump and the other end of the power cord into a battery source or outlet within the laboratory.


Backflush the filter

- 8 Determine the desired direction of water flow and toggle the switch for flow direction. Turn the pump on in the direction that pulls backflush solution towards the ultrafilter and then into the sterile vessel.

- 9 Continue pumping until no backflush solution remains in the  **500 mL** bottle and the flow out of the ultrafilter has slowed to a trickle.


- 9.1 It will be necessary to briefly pump air after the backflush solution is used up in order to get all the liquid out of the filter.

- 10 Turn off the pump and replace the lid on the backflush solution that has passed through the ultrafilter.

- 10.1 **This is your sample.** Place at  **4 °C** until further analysis. Ensure this vessel is properly labeled.

- 11 Disassemble backflush station and ensure all used materials are placed in the appropriate biohazard waste containers.

- 11.1 The ultrafilter may be stored at  **4 °C** if needed. Ensure all open ports (side



and end) are capped to prevent any possible leaks)