

Rig Filtration of SPOT 0m Water

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Abstract

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Protocol

Step 1.

Soak all small rig components in 10% acid for a minimum of 30 minutes

Step 2.

Rinse all components thoroughly using MilliQ (hold disks on edges). Place on rig once rinsed. Rinse red tubes and large plastic Erlenmeyer flask with MilliQ.

Step 3.

Assemble pump to rig using red tubes and large plastic Erlenmeyer flask. Attach tube to right side of pump for vacuum. Right knob on pump controls vacuum, loosen all the way

Step 4.

Remove filter top component and place upside down on bench

Step 5.

Remove micropore forceps from ethanol and rinse well with MilliQ. Hold in a new sterile Falcon tube during use

Step 6.

Using micropore forceps remove 0.8 μ m backing disk from between blue disks and place on each rig filter

Step 7.

Again using the micropore forceps remove 0.2 μ m filter disk from between blue disks and place carefully on top of each 0.8 μ m disk. Make sure they are centered and do not have holes or rips.

Step 8.

Place top component back on to each filter and twist on. Open each rig filter by turning orange knob to vertical position.

Step 9.

Using a serological pipet and sterile glass pipet tip remove desired amount from bucket and pipet directly onto disks. There should be 6 filtrations/filters of 10ml and 1 each of 20ml, 30ml, 40ml, 50ml, 60ml and 100ml.

Step 10.

Once desired amount of sample water is placed on filter and knobs are vertical, turn on pump. Adjust top right knob on pump so that the arrow on the top right gauge is slightly above 0.

Step 11.

Allow vacuum to work until most of the water has passed through the filters. Once all water has passed through a filter, turn orange knob closed to the horizontal position. Once all filtration has finished and all knobs are horizontal, turn off pump.

Step 12.

Twist off top component of rig filter and place upside down on bench. Using the micropore forceps carefully remove only the top 0.2 μ m filter disk and place on a labeled TCBS Agar plate. Use a sweeping or dragging motion to place filter flat on plate.

Step 13.

Repeat Step 12 for all 6 filters onto separate plates

Step 14.

Repeat Steps 7-13 for remaining volumes of water. The 0.8 μ m filter disks may remain on the filters, only new 0.2 μ m filter disks are needed each time.

Step 15.

Leave plates right side up at room temperature on bench for at least 10 minutes. Then place upside down in drawer to grow for at least 24 hours.