

## Filtration of pool-samples using PES 0.45 µm filter under vacuum.

**Vs 1.1**

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### **Materials:**

- vacuüm pump
- Merck™ Express™ PLUS-membrane filters (HPWP04700 ; Merck)
- VacTrap, 4L (305-4001-FLS; Witeg)
- 3-Place Stainless Steel Manifold (18060-01; Witeg)
- 500ml Funnel (long stem with stopper) (200500-01; Witeg)
- filter containers\* (20000655, Flaschenland.de)
- forceps (62110, Merkala)

- **Important:** Ensure that the sampling bottle and the filter containers for transport have the same code to ensure the samples can be traced back.

If the filtering is done at a lab, and not immediately after sampling, make sure the samples are refrigerated at 4 °C until filtration. It is also advised to keep cool the full sample bottles after sampling and transferring to filtration location

### **Filtering:**

1. Place the sampling bottle and the corresponding filter transport container in front of the same unit of the filtration station.
2. Use clean forceps to place a 0.45 µm PES-filter on the units
  - Ensure that the orientation in which the filter is placed on the unit is the same as it is in the container, dull side up. The blue discs are for protection of the white filter and need to be removed.
3. Place the upper part of the funnel on top of the part with the filter
4. Ensure the vacuum buffer vat is empty
5. Turn on the vacuum pump
6. Shake the bottle, and carefully empty 250 ml in the funnel. Turn the lever to the left (quarter turn) to put the unit under vacuum
7. Do this for every sample
8. Once the filter is visibly dry, remove vacuum by turning back the lever to the right, remove the upper part
  - Put this in a place where it can be cleaned
9. With a clean disposable forceps, place the filter with the “dirty” side down in the buffer containing transport container. Shake the containers with filter until “foamy” and send to the lab.

10. If filtration is done at the lab follow the steps described in eDNA isolation from PES 0.45  $\mu\text{m}$  filters with enhanced CTAB lysis

### Cleaning

- Thoroughly rinse the sampling bottles 3x with tap water, and let dry
- Thoroughly rinse the upper part of the filtration unit, and if necessary, use a dishwashing brush, to remove dirt
- Rinse the lower part of the unit thoroughly with water
- Empty vacuüm buffer vat

funnels



Filtering station



Vacuum buffer -vat



Vacuum pump