

Sampling Protocols: Water

Note: *Metadata should be taken prior to sample collection; these steps have been omitted from this protocol.*

Supplies

Gloves	DNA preservative	Whirlpack bags
50 mL syringe for water sampling	Sterivex filter	Sharpie
5 mL syringe for DNA preservative	Luer lock caps for Sterivex filters	Lab tape

Steps

1. With gloves on, open Sterivex filter and label with sample ID. Do not touch the filter inlet/ outlet nor completely remove from packaging until use.
2. Remove 50 mL syringe from package and prime by drawing up 50 mL of water at the location and rinsing the syringe. Repeat for a total of 100 mL of rinsing. Note: you will not sample from the rinsing location.
3. Obtain a 50 mL water sample from the target area using the 50 mL syringe. This location should be at least one meter upstream from the rinsing location.
4. Screw the Sterivex filter onto the end of the 50 mL syringe until firmly in place. Then, gently flush your water sample through the syringe, discarding the water. Remove the Sterivex filter from the 50 mL syringe.
5. Repeat Steps 3 and 4 above until filtering water becomes difficult and the filter has reached capacity. Record the total amount of water filtered directly on the syringe as well as in your metadata sheet.
6. Discard any remaining water in the 50 mL syringe, and draw 50 mL of air. Screw the Sterivex filter onto the 50 mL syringe to flush the filter of any remaining water. It is ok if a small amount of water remains in the filter after this step.
7. Using the 3 mL syringe, draw up 2.5 – 3 mL of Zymo solution. Screw the 3 mL syringe with Zymo solution onto the inlet end of the Sterivex filter and fill completely with Zymo solution. It may be helpful to hold the filter outlet upward to remove the air and add Zymo solution.
8. Add a Luer lock cap to the outlet end of the Sterivex filter containing Zymo solution. Remove the 3 mL syringe and add another Luer lock cap to the inlet end of the Sterivex filter.
9. Place the sealed Sterivex filter into the provided Whirl-Pac bag, and write the sample ID on the bag with a Sharpie marker. Store the filters at room temperature or in the fridge.
10. Repeat no less than one meter apart for biological replicates; samples should be in triplicate for highly active water and duplicate for slow moving or stagnant water.