



May 12, 2021

Fish eDNA: water sampling and filtration through Sterivex filter unit

Marine Vautier¹, Cécile Chardon¹, Isabelle Domaizon¹¹INRAE - UMR CARRTEL - Pole R&D Ecla1 Works for me dx.doi.org/10.17504/protocols.io.br5rm856

CARRTEL



Marine Vautier

ABSTRACT

The objective of this protocol is the sampling and the filtration of water samples through 0.45 µm Sterivex™ filter units, and their buffer preservation prior to DNA extraction. This protocol is used upstream to molecular biology analysis (e.g. qPCR, metabarcoding, ddPCR) to specifically target fish eDNA.

The use of preservation buffer allows sample preservation for up to several hours at room temperature, several days at 4°C and several months at -20°C, and thus the possibility to filter samples on site.

This protocol allows to filter approximately between 0.7 L and 5 L through a Sterivex™ filter unit depending on the water sample characteristics and the chosen filtration method (syringe or vacuum pump).

DOI

dx.doi.org/10.17504/protocols.io.br5rm856

PROTOCOL CITATION

Marine Vautier, Cécile Chardon, Isabelle Domaizon 2021. Fish eDNA: water sampling and filtration through Sterivex filter unit. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.br5rm856>



KEYWORDS

eDNA, fish, sampling, filtration, sterivex, environmental DNA, preservation, water, cartridge

LICENSE

———— This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Feb 05, 2021

LAST MODIFIED

May 12, 2021

PROTOCOL INTEGER ID

46993

GUIDELINES

- Sampling
- Filtration : Two options are possible, one with a syringe and the other with a vacuum pump
- Preservation with buffer

MATERIALS TEXT

■ Reagents

- Preservation Buffer containing Tris, EDTA and sucrose : 2 mL per sample

■ Materials

- Pipette 1000 µL (It is possible to use a sterile 5 mL syringe instead of a 1000 µL pipette).
- Decontaminated collection bottle (capacity from 1 L to 5 L according to water sample)
- A cooler with ice packs
- Field sheet + pen
- Sterile bag (or tube) to store the Sterivex after filtration
- Marker pen OR pre-printed label for sample identification
- Trash can
- Vacuum pump (optional)

■ Consumables

- Tips with filter 1000µL or sterile 5 mL syringe : 1 per sample
- Gloves : 2 pairs per sample
- Sterivex cartridge (Sterivex™ GP pressure filter units, 0.45µm; Millipore) : 1 cartridge per sample
- Sterile Syringe with Luer lock (Syringes BD Plastipack Luer Lock Vol 50-60mL) : 1 syringe per sample
- Caps for Sterivex (Luer-Lock male & female : outlet & inlet) : 2 per sample (to close the Sterivex after filtration)

BEFORE STARTING

- Prior to sampling, the bottles dedicated to DNA have to be washed (H₂O₂ 10% or bleach 10%), rinsed at least 3X with distilled water and dried. On site, the bottle should also be rinsed with the sampled water.
- Store all materials inside clean bags or containers to reduce any contamination.
- Wear gloves during the whole procedure
- During the handling try to reduce exposure to sunlight if possible.
- Prepare the required amount of preservation buffer (2 mL per sample) :

- Combine EDTA, Tris, and sucrose (see table) in a beaker
- Add around 0.5 V of ultrapure water and shake to dissolve
- Transfer the solution in a volumetric flask, complete with ultrapure water to the final volume and shake
- Filter-sterilize using a 0.2 µm unit filter
- Split into 2100 µL aliquots and store in the fridge

A	B	C
Reagent	Final concentration	For 10 mL
EDTA	40 mM	800 µL of 0.5 M EDTA
Tris-HCl (pH 8)	50 mM	500 µL of 1 M Tris-HCl
Sucrose	0.75 M	2.55 g

Concentration of preservation buffer reagents and example of volumes to prepare 10 mL of lysis buffer.

WATER SAMPLING

1h

- Put on gloves and open the bottle without touching the inside of the cap or the neck of the bottle.
 - Collect by hand subsurface water (10-20 cm below the water surface) and close the bottle carefully. If water is collected deeper with a sampling tank, it must also be decontaminated prior to sampling.
 - After water collection, the bottle has to be stored in cleaned containers to avoid contamination.
 - Use a cooler if the samples have to be transported.

WATER FILTRATION

1h

- Proceed rapidly to water filtration in order to minimize any potential DNA damage during transport. Place the water at **4 °C** in the dark for max. 6 hours until filtration.
 - There are **two filtration protocols**, one by hand **with a syringe**, and the other **with a vacuum pump**. Both protocols are presented below:

2.1 Filtration procedure with a syringe :

The set-up is easy and does not necessarily require countertop space (can be done on the field). Get the filtration material ready to use: gloves, Sterivex cartridge, caps and syringe.

- Put on gloves and remove the syringe and the Sterivex from their blister pack (handle carefully avoiding to touch either end of the filter to prevent contamination)
- Homogenize gently the water sample & fill the syringe (60mL) with water sample (verify that the neck of the bottle is larger than the syringe's diameter)
- Connect the syringe and the Sterivex together (Figure 1)



Figure 1: Sterivex cartridge attachment to the syringe

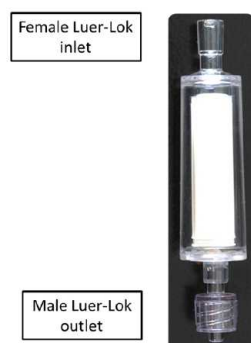


Figure 2: Sterivex cartridge Luer-Lock tips identification

- Filter (slowly) the water sample through the Sterivex unit
- Detach the syringe from the Sterivex to refill the syringe with water and filter it through the same Sterivex.
- Continue the filtration (with the same syringe and sterivex) to filter a maximum water volume and note the final water volume filtered (by subtracting the remaining volume from the initial volume of water, for example). The filtration process should not take longer than 1 hour. Typical volumes filtered are from 0.5L to 5L : the volume of water filtered will depend on the microbial load and turbidity of the water sample.
- At the end of the filtration, detach the Sterivex from the syringe, and remove as much of the remaining water as possible by inserting the plunger of the syringe and passing air through the Sterivex
- Proceed to sample preservation (step 3)

2.2 Filtration procedure with a vacuum pump :

The set-up does not necessarily require countertop space, but is easier to set up in a laboratory. Get the filtration material ready to use: vacuum pump, tubing, filter ramp adapted to the size of the Sterivex, gloves, Sterivex cartridge, caps and syringe

- Put on gloves and remove the Sterivex from their blister pack (handle carefully avoiding to touch either end of the filter to prevent contamination)
- Insert the Male Luer-lock Outlet tip of the Sterivex (Figure 2) into the clean silicone filter support holder (Figure 3).



Figure 3 : Sterivex filtration system with vacuum pump

- Insert the clean silicone filter support holder onto the filtration ramp (Figure 3)
- Remove the 50 mL syringe from its blister pack and remove the plunger from the syringe to use the body of the syringe as a funnel (keep the plunger in its blister pack to use it at the end of the filtration process).
- Connect the body of the syringe to the Female Luer-lock Inlet tip of the Sterivex (Figure 2 & 3).
- Homogenize gently the water sample
- Fill slowly the syringe with the water directly from the bottle as if it were a funnel (Figure 3)
- Switch on the pump and open the valves (the pressure must not exceed 400mBa (40kPa)
- Fill the syringe body (funnel) to ensure that there is always water in it
- Continue the filtration (with the same syringe and sterivex) to filter a maximum water volume and note the final volume of filtered water (by subtracting the remaining volume from the initial volume of water, for example). The filtration process should not take longer than 1 hour. Typical volumes filtered are from 0.5L to 5L : the volume of water filtered will depend on the microbial load and turbidity of the water sample.
- At the end of the filtration, disconnect the Sterivex from silicone filter support holder
- Remove as much of the remaining water as possible by inserting the plunger of the syringe and passing air through the Sterivex
- Proceed to sample preservation (step 3)

Sample preservation

3 Identical preservation method for both filtration systems :

Get the preservation material ready to use: buffer, 1000µL pipette or 5 mL syringe, indelible marker or pre-printed label, sterile bag (or tube) to store the Sterivex after filtration, field sheet and pen.

- Place the outlet cap on the Sterivex (Figure 2).
- Add immediately 2mL of buffer using a pipette (tip 1000µL) or a 5 mL syringe. Be careful to well insert the tip into the Sterivex to ensure the buffer does not overflow the cartridge (Figure 4). **Caution**: it is important **not to put more than 2 mL of buffer into the Sterivex** because reagents will be added during the DNA extraction step



Figure 4 : Well insert the tip into the Sterivex to ensure the buffer does not overflow the cartridge.

- Place the Inlet cap on the Sterivex (Figure 2).
- Place the labelled Sterivex in a clean bag/tube.
- Sterivex buffered can be stored at room temperature for several hours , or in a fridge or a cooler for few days. If a longer storage is needed, store it at -20°C.
- Complete the field sheet