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# Fish eDNA: DNA extraction from water samples filtered through Sterivex filter unit and preserved in buffer

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ABSTRACT

The objective of this protocol is DNA extraction from water samples filtered through 0.45 µm Sterivex™ filter units and preserved in 2 mL of buffer (dx.doi.org/10.17504/protocols.io.br5rm856).

Extraction is performed using NucleoSpin® Soil kit (MACHEREY-NAGEL) with specific modifications adapted to DNA extraction from Sterivex™ filter units.

This protocol is used upstream to molecular biology analysis (e.g. qPCR, metabarcoding, ddPCR) to specifically target fish eDNA.

DOI

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PROTOCOL CITATION

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KEYWORDS

fish, extraction, eDNA, environmental DNA, water, buffer preservation, Sterivex



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36224

GUIDELINES

- Sample preparation
- Sample lysis
- Contaminants elimination
- DNA fixation and washing
- DNA elution

MATERIALS TEXT

- Samples
  - Sterivex cartridge filled with 2mL of buffer and stored at -20°C or 4°C (dx.doi.org/10.17504/protocols.io.br5rm856)
- Reagents
  - NucleoSpin® Soil kit (MACHEREY-NAGEL)
  - Ethanol (96 - 100%), molecular grade to prepare buffer SW2
  - Lysozyme solution (add 2 mg lysozyme to 40 µL of Lysis Buffer): 40 µL per sample
  - Proteinase K solution (20 mg/mL): 50 µL per sample
  - 20% SDS: 100 µL per sample
- Materials
  - Specific DNA-work station (sterile area equipped with air filtration and UV systems)
  - Microcentrifuge for 1.5 to 2 mL tubes (relative centrifugal force needed :11,000 x g)
  - Incubator (37°C and 55°C)
  - Tube Rotator with holder for 15mL tubes or Sterivex
  - Pipettes : 1000 µL - 100 µL
  - 2 trash cans : 1 for liquid and 1 for solid
  - Vortex with holder for 15 mL tubes
- Consumables
  - Tips with filter : 1000µL - 100µL
  - Sterile 10 mL or 5 mL syringe (1 per sample)
  - 2 mL sterile microcentrifuge tubes (about 6 tubes are required per sample)
  - 1.5 sterile microcentrifuge tube to collect DNA at the end of extraction (1 per sample )
  - Gloves

SAFETY WARNINGS

- SDS 20%
  CAS number : CAS 151-21-3
  Signal word : Corrosive substance and Harmful
  Hazard phrases : 315, 318, 335
  Precaution phrases : 261, 280, 302+352, 304+340+312, 305+351+338+310
- Proteinase K solution
  CAS number : CAS 56-81-5 + CAS 39450-01-6
  Signal word : Health hazard
  Hazard phrases : 334
  Precaution phrases : 261, 284, 304+340, 342+311
- Absolute ethanol
  CAS number : 64-17-5
  Signal word : Harmul and Flammable
  Hazard phrases : 225, 319
  Precaution phrases : 210, 305+351+338



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The manufacturer advise to wear gloves and goggles and to flow the safety instructions for 2 reagents :

- SB coutains Guanidinium thiocyanate 45 - 60%,
  CAS number : CAS 593-84-0
  Signal word : Irritant
  Hazard phrases : 302, 412
  Precaution phrases : 264W, 273, 301+312, 330

- SW1 coutains Guanidine hydrochloride 36 - 50% and 2 - propanol 20 - 35%
  CAS number : CAS 50-01-1, 67-63-0
  Signal word : Irritant and Flammable
  Hazard phrases : 226, 302, 319, 336
  Precaution phrases : 210, 260D, 264W, 280sh, 301+312, 330

BEFORE STARTING

- The following precautions must be applied :

- Wear gloves throughout the extraction process

- Clean the bench with DNA-off solution

- Use tips with filters to avoid contaminations

- **All steps have to be performed under a specific DNA-work station (sterile area equipped with air filtration and UV systems).**

- Material preparation :

- Clean a specific DNA work station and apply UV for 15min

- Turn on the incubator at +37°C

- Solutions preparation :

- Check buffer SW2 - before the first utilisation, you need to add the indicate volume of ethanol (96 - 100%) to buffer SW2 concentrate and mark the label of the bottle to indicate that ethanol was added. This solution is stable at room temperature (18 - 25°C) for at least one year

- Check absence of precipitate in SDS 20%. Dissolve any precipitate by incubating the solution at 30-40°C for 5-10 min and shaking solution every 2 min.

## Prepare the sample 30m

1 30m

*Note : If the samples have been stored at RT or at 4°C, proceed directly to step 2. If the samples have been frozen, proceed to step 1.*

- Take the Sterivex™ filter unit stored at -20 °C after sampling, filtration and preservation with Buffer.

- Defrost the sample at **4 °C** (about **00:30:00** )

*Note : during this step heat the incubator at* **37 °C**

## Sample Lysis 2h 50m

2 2h 50m

- Fix the Sterivex cartriges on the vortex and vortex for **00:05:00** to remove particles from the filter



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- Remove the Inlet Cap (Figure 1) and with a pipette tip, add **40 µl** of **lysozyme solution** into the Sterivex already filled with 2mL of buffer. Insert pipette completely into the inlet to see the pipette tip inside the unit just above the membrane. Re-cap the Sterivex.

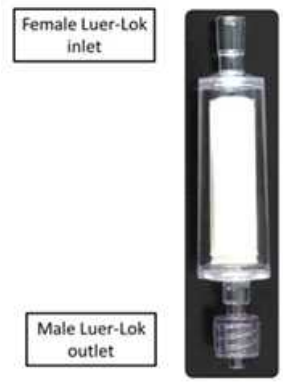


Figure 1: Position of the two tips of the Sterivex™ filter unit

- Rotate for **00:45:00** at **37 °C** (during this step, place the SDS solution in the incubator for the next step)
- Remove the Inlet Cap and and with a pipette tip, add **50 µl** of **Proteinase K solution** and **100 µl** of **SDS 20%**. Re-cap the Sterivex.

*Note : during this step heat the incubator at* **55 °C**

- Incubate for **02:00:00** at **55 °C** with agitation (or shake manually every 15 min).
- Remove the Inlet Cap and fix a sterile syringe to the Sterivex.
- Aspirate the liquid contained in the Sterivex with the syringe by moving back and forth to collect all the liquid
- Transfer the lysate into two 2 mL tubes (about 1.1 mL per tube).

*Note : after this step, place the **Buffer SE** in the incubator heated at* **55 °C** *to anticipate the elution step*

## Precipitate contaminants 6m

7m 2s

3
- In both 2mL tubes, add **200 µl** of **buffer SL3**.
- Vortex for **00:00:02**
- Incubate for **00:05:00** at **4 °C in a fridge**
- Centrifuge at **11000 x g, 00:02:00**

## Filter lysate 5m

4
- Place a NucleoSpin® Inhibitor Removal Column (red ring) in a collection Tube (2 mL, lid).
- Load up to **650 µl** of clear supernatant (obtained at the step 3) onto the filter.
- Centrifuge at **11000 x g, 00:01:00**



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- Remove the column and close the tube containing the flow through.
- Repeat these steps as many time as there is still some supernatant from step 3 to be filtered. For each centrifugation, collect the supernatant in a clean tube (about 4 tubes are required, but only 1 column for the filtration of all lysate from step 3).
- Discard the NucleoSpin® Inhibitor Removal Column.

*Note: If a pellet is visible after the centrifugation, transfer the clear supernatant to a new collection tube (not provided in the kit) to get rid of this pellet, and continue with the clear supernatant.*

## Adjust binding conditions 1m

5
- Add **250 µl** of **Buffer SB** per 2mL tube.
- *Note : 0.38V of buffer SB for 1V of flow through from step 4.*
- Close the lid.
- Vortex for **00:00:05** and centrifuge briefly.

## Bind DNA 6m

6
- Place a NucleoSpin® Soil Column (green ring) in a Collection tube (2 mL).
- Load **650 µl** of sample from step 5 onto the column.
- Centrifuge at **11000 x g, 00:01:00**
- Discard the flow through and place the column back into the collection tube.
- Repeat the three previous steps with all the tubes from step 5 and using the same column.
- Discard the flow through and place the column back into the collection tube.

## Wash silica membrane 5m

7 *Note : the same collection tube is used throughout the entire washing procedure to reduce plastic waste.*

7.1 1st wash :
- Add **500 µl** of **Buffer SB** to the NucleoSpin® Soil Column.
- Centrifuge at **11000 x g, 00:00:30**
- Discard the flow through and place the column back into the collection tube.



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7.2 2nd Wash :

- Add **550 µl** of **Buffer SW1** to the NucleoSpin® Soil Column.
- Centrifuge at **11000 x g, 00:00:30**
- Discard the flow through and place the column back into the collection tube.

7.3 3rd Wash :

- Add **650 µl** of **Buffer SW2** to the NucleoSpin® Soil Column.
- Vortex **00:00:02** and centrifuge at **11000 x g, 00:00:30** .
- Discard the flow through and place the column back into the collection tube.

7.4 4th Wash :

- Add **650 µl** of **Buffer SW2** to the NucleoSpin® Soil Column.
- Vortex **00:00:02** and centrifuge at **11000 x g, 00:00:30** .
- Discard the flow through and place the column back into the collection tube.

**Dry silica membrane** 2m

8
- Centrifuge at **11000 x g, 00:02:00** .

*Note: if for any reason, the liquid in the collection tube touched the NucleoSpin® Soil Column after the drying step, discard flow through and centrifuge again.*

**Elute DNA**

9
- Place the NucleoSpin® Soil Column into a new 1.5mL microcentrifuge tube.
- Add **30 µl** of **Buffer SE** previously heated to 55°C into the column.

*Note: a larger volume of SE buffer can be used but the DNA concentration will be lower*

- Do not close the lid and incubate for **00:01:30** at **Room temperature** (18-25°C).
- Close the lid and centrifuge at **11000 x g, 00:00:30**
- Throw the column and keep the tube containing the DNA.



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*Note: A second elution can be performed with the same column* to collect more DNA (the concentration will be lower for the second elution)

- We recommend storing DNA frozen at -20°C until the use of the DNA sample (or at -40°C to -80°C for longer storage).



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