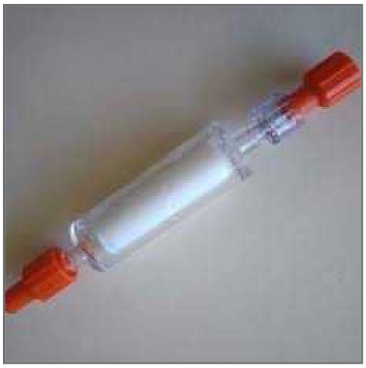

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eDNA extraction from water samples filtered through Sterivex filter units (NucleoMag DNA/RNA Water Kit - MACHEREY NAGEL).

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ABSTRACT

The objective of this protocol is the **environmental DNA (eDNA) extraction from water samples filtered through Sterivex filter units.** The Sterivex units can be prefilled with preservation buffer or not.

DNA extraction is performed using a **MagnetaPure 32 Nucleic Acid Purification System** (Dutscher) and with the **NucleoMagDNA/RNA Water Kit** (Macherey Nagel).

The procedure is based on **reversible adsorption of nucleic acids to paramagnetic beads** under appropriate buffer conditions.

The benefits of using the **MagnetaPure 32 Nucleic Acid Purification System** (Dutscher) are **increased productivity and repeatability, as well as eliminating human error and the pain of repetitive work**.

This protocol is used **prior to molecular biology analysis** (e.g. qPCR, metabarcoding, ddPCR) to specifically target **both macro- and micro-organisms eDNA extracted from water samples.**

This protocol is optimised for rare eDNA and the suggested **elution volumes are therefore low** (between 50 and 65 µL), but can be increased if targets are more abundant.

IMAGE ATTRIBUTION

Marine Vautier

Protocol status: Working
We use this protocol and it's working

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Keywords: eDNA, water, Sterivex, DNA, extraction, filter, rare DNA

GUIDELINES

The main steps of the protocol are:

- Material preparation
- Plate preparation
- Sample lysis
- Extraction with the MagnetaPure 32 System (Dutscher)
- DNA elution

MATERIALS

- **Materials:**

- 1000 µL pipette
- 100 µL pipette
- Vortex + benchtop centrifuge
- Horizontal vortex with holder for Sterivex (15 mL tube holders)
- Centrifuge for 2 mL tubes (relative centrifugal force needed: 11,000 x g)
- Incubator (temperature needed: 70°C)
- MagnetaPure 32 Nucleic Acid Purification System (Dutscher)
- Specific DNA-workstation (sterile area equipped with air filtration and UV systems)

- **Consumables:**

All tubes and tips must be sterile

- 1000 µL tips with filter
- 100 µL tips with filter
- 50 mL tubes: to prepare aliquots
- 5 mL tubes: 1 per 8 samples to prepare NucleoMag B-Beads and MWA2 mix
- 2 mL tubes: 1 per sample to transfer lysate + 2 to prepare aliquots
- 1.5 mL tubes: 1 per sample to transfer eluted DNA
- 5 mL syringes with Luer lock tip
- 96-well plate with 2 mL deep-wells, U-Bottom (Macherey Nagel - 746032.DEEP): 1 per 16 samples
- Magnetic rod coverfor MagnetaPure 32 (Macherey Nagel – 747032.TC): 1 per 8 samples
- Plastic film to protect the 96-well plate
- Gloves

For any manipulation in a rare DNA room, provide complete equipment (disposable coat, cap, mask, shoe covers & gloves).

- **Reagents:**

- NucleoMag DNA/RNA Water kit (Macherey Nagel)

Note: shelf life of reagents for 24 months from production

- Buffer C1 (Macherey Nagel)

Note: Buffer C1 for lysis of Sterivex filtered samples is not supplied in the kit and must be ordered separately from the supplier (900 µL per sample)

- **Samples to be extracted:**

- Sterivex filter units prefilled with preservation buffer (e.g. C1) or not

BEFORE START INSTRUCTIONS

- **Filtration and preservation of the water samples through Sterivex units**

This procedure can be performed with Sterivex filter units prefilled with preservation buffer (e.g. C1 buffer) or not. If the Sterivex is not prefilled with buffer, it should be frozen immediately after water filtration and until DNA extraction.

*Note: If Sterivex are preserved with C1 buffer o*r the preservation buffer from the protocol below*, the extraction can be performed directly, but for other preservation buffers such as Longmire and CTAB, the protocol* proposed here *will work, but upstream precipitation is recommended to increase the extraction efficiency.*

The following protocol can be used to filter water through the Sterivex and to preserve them with a preservation buffer:

Protocol

NAME

Fish eDNA: water sampling and filtration through Sterivex filter unit

CREATED BY

Marine Vautier

PREVIEW

- **The following precautions must be applied:**

- Wear gloves throughout the extraction process
- Clean the bench with a DNA-removing solution (e.g. DNA-off, DNA away).
- 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)

For any manipulation in a rare DNA room, provide complete equipment (disposable coat, cap, mask, shoe covers & gloves).

- **Pre and post extraction equipment decontamination:**

- Clean a specific DNA work station and apply UV
- MagnetaPure 32 System (Dutscher): Visual check for residues to be removed and UV decontamination – *see instrument manual*

Material preparation

1

- *Preheat the incubator at* 70 °C

- *To limit contamination of the kit buffers, it is recommended to aliquote them:*

- Into 50 mL tubes for C1, MWA2, MWA3 and MWA4
- Into 2 mL tubes for NucleoMag B-Beads solution and DNase-free H_2O

- *Tubes annotation*

- one 2 mL tube per sample for lysate collection
- one 1.5 mL tube per sample for DNA collection
- one 5 mL tube per 8 samples for the NucleoMag B-Beads and MWA2 mix preparation

Plate preparation 1/2

2 30m

In this step, the buffers provided by the kit are distributed in a 96-well plate.
For DNA extraction from filtered water samples, the 12 columns of the plate are divided into 2 sections of 6 columns each, allowing up to 16 samples to be extracted per plate.

- Annotate the 96-well plate as recommended below:

	1	2	3	4	5	6	7	8	9	10	11	12
Solution	NucleoMag B-Beads and MWA2 mix + Lysate	MWA3	MWA3	MWA4	x	DNase-free H_2O	NucleoMag B-Beads and MWA2 mix + Lysate	MWA3	MWA3	MWA4	x	DNase-free H_2O
Column name	L	3	3	4	x	E	L	3	3	4	x	E
	Samples between 1 to 8						Samples between 9 to 12					

Table 1: Recommended plaque annotation

Note: It is useful to mark the dividing line between columns 6 and 7 with a marker pen to provide a visual cue for filling the plate.

- Add the appropriate buffers into the appropriate wells of the plate.

1st column / 7th column: *Will be filled during plate preparation 2/2*
2nd column / 8th column: 850 µL of **MWA3** *(1st wash)*
3rd column / 9th column: 850 µL of **MWA3** *(2nd wash)*
4th column /10th column: 850 µL of **MWA4** *(3rd wash and bead drying)*
5th column / 11th column: *not used*
6th column / 12th column: 50 µL or 65 µL of **DNase-free H_2O** *(DNA elution)*

Note: The choice of elution volume is based on the expected eDNA amount. The smaller the amount, the smaller the elution volume in order to obtain more concentrated DNA.

	1	2	3	4	5	6	7	8	9	10	11	12
Solution	Plate preparation 2/2	MWA3	MWA3	MWA4	x	DNase-free H_2O	Plate preparation 2/2	MWA3	MWA3	MWA4	x	DNase-free H_2O
Volume (µL)		850	850	850	x	50 or 65		850	850	850	x	50 or 65
Column name		3	3	4	x	E		3	3	4	x	E
		Samples between 1 to 8						Samples between 9 to 12				

Table 2: Plate preparation 1/2

- Film and reserve the plate at Room temperature

3 - Preparation of the NucleoMag B-Beads and MWA2 mix: 5m

- Prepare one 5 mL tube / maximum 8 samples
- Add **MWA2** only, the NucleoMag B-beads will be added during plate preparation 2/2 *(allow a margin of one sample for the mix preparation. For example: plan a mix for 9 samples if 8 samples are to be extracted)*

Sample Number	Volume NucleoMag B-Beads (µL)	Volume MWA2 (µL)
1	25	475
2	50	950
3	75	1425
4	100	1900
5	125	2375
6	150	2850
7	175	3325
8	200	3800
9	225	4275

Table 3: Volume required to prepare NucleoMag B-beads and MWA2 mix (*no margin*)

Sample Lysis

4 *During this step, a mechanical and chemical lysis of the sample is performed.* 20m 10s

- **For samples prefilled with buffer:**

- Collect the Sterivex cartridges

Note: If the Sterivex cartridges are frozen, defrost them 00:10:00 *at* Room temperature

- Place the Sterivex on the horizontal vortex 00:00:05 at maximum speed

- Place the Sterivex into the incubator at 70 °C - 00:05:00 , and if possible with automatic or manual agitation

- Aspirate the lysate from the Sterivex with a 5 mL syringue and transfer the lysate into a 2 mL tube *(volumes recovered may vary and it may therefore be relevant to note them)*

- Centrifuge at 11000 x g, 00:00:30

- Replace the tubes into the rack and reserve them at Room temperature until their distribution into the 96-well plate

- **For samples without buffer:**

- Collect the Sterivex cartridges from the freezer and place On ice

- Add immediately 900 µL of **C1** buffer into each Sterivex and shake manually

- Place the Sterivex on the horizontal vortex 00:00:05 at maximum speed

- Place the Sterivex into the incubator at 70 °C - 00:05:00 , and if possible with automatic or manual agitation

- Aspirate the lysate from the Sterivex with a 5 mL syringue and transfer the lysate into a 2 mL tube *(volumes recovered may vary and it may therefore be relevant to note them)*
- Centrifuge at 11000 x g, 00:00:30
- Replace the tubes into the rack and reserve them at Room temperature until their distribution into the 96-well plate

Plate preparation 2/2

5 15m

▪ Preparation of the NucleoMag B-Beads and MWA2 mix

NucleoMag B-Beads and MWA2 mix sediment quickly, vortex between each samples to ensure homogeneity

- Vigorously vortex **NucleoMag B-Beads** tube
- For each tube containing MWA2 buffer (previously prepared), add the appropriate volume of **NucleoMag B-Beads** (*see Table 3 above*)
- Vortex

▪ NucleoMag B-Beads - MWA2 mix and lysate distribution

- Remove the film from the plate
- Add the appropriate solution into each well of the plate

1st column / 7th column:

500 µL of **NucleoMag B-Beads and MWA2 mix**

450 µL of **Lysate** (supernatant from the 2 mL tubes)

	1	2	3	4	5	6	7	8	9	10	11	12
Solution	NucleoMag B-Beads and MWA2 mix	Plate preparation 1/2	Plate preparation 1/2	Plate preparation 1/2	x	Plate preparation 1/2	NucleoMag B-Beads and MWA2 mix	Plate preparation 1/2	Plate preparation 1/2	Plate preparation 1/2	x	Plate preparation 1/2
Volume (µL)	500				x		500				x	
Solution	Lysate				x		Lysate				x	
Volume (µL)	450				x		450				x	
Column name	L				x		L				x	
	Samples between 1 to 8						Samples between 9 to 12					

Table 4: Plate preparation 2/2

Extraction step performed in the MagnetaPure 32 System

6 ▪ Place the plate into the MagnetaPure 32 System and insert the magnetic rod coverfor – *see instrumen* 40m
manual

- **Select the appropriate program to the chosen elution volume and elution temperature**

Step	Well	Name	Mix time (min)	Magnet (sec)	Wait time (min)	Volume (µL)	Mix speed	Temp (°C)
1	1	Binding	8	10	0	950	8	OFF
2	2	MWA3	2,3	5	0	850	8	OFF
3	3	MWA3	2,3	5	0	850	8	OFF
4	4	MWA4	2,3	5	15	850	8	OFF
5	6	Elution	5	20	0	50 or 65	8	56°C or OFF
6	4	Release	0.5	0	0	850	10	OFF

Table 5: MagnetaPure 32 System program for NucleoMag DNA/RNA Water Kit DNA extraction

Note: Heating to 56°C during elution gives a higher yield of DNA, but there is a risk of evaporation which reduces the volume of eluted DNA recovered

- Start the run *(The run lasts approximately* 00:40:00 *)*

Transfer of DNA extracts

30m

7 ▪ At the end of the run, remove the plate and place it into the DNA-workstation
- Remove the magnetic rod cover and start UV for decontamination – *see instrument manual*
- In the DNA-workstation, transfer each **DNA extract** into a 1.5 mL tube previously annotated

Note: DNA concentration and quality can be measured at this step (e.g. Nanodrop)

▪ Store DNA extracts at 4 °C for immediate use, or at -20 °C or -80 °C for long-term preservation