MANUAL

Complete eDNA sampling set

SYL009

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Sylphium
molecular ecology

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Table of Contents

1. Introduction 4

1.1. Specifications 4

1.2. Kit contents 5

1.3. Notices and disclaimers 5

2. Sampling guidelines and precautions 6

3. Sampling protocol 7

3. Shipping of samples 11

Appendix A. Removing air bubbles from the eDNA Dual Filter capsule 12

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1. Introduction

This single-use DNA filter capsule is specially designed for on-site filtering of large quantities of (environmental) water with a negligible risk of cross-contamination. The enclosed filter membranes have an optimal pore size for environmental DNA (eDNA) filtration to collect all the eDNA without rapid clogging. The filter capsule is compact due to the design with two separate membranes that provide a huge membrane surface. The eDNA double filter capsule can be closed with “luer lock caps” after use, is suitable for in capsule lysing and preserving and can be stored at room temperature. During eDNA extraction from the capsule, all solid residues remain on the membrane and do not interfere with further downstream processing. The eDNA dual filter capsule meets all requirements as stated in CEN/TC 230 – Water analysis – N 1229.

1.1. Specifications

• Filter unit type:	Closed capsule
• Effective filter area	69 cm^2 (dual filter system)
• Filter membrane	Polyethylene sulfon (PES)
• Pore size	0.8 µm
• Maximum inlet pressure	3.1 Bar (45 psi)
• In and outlet fittings	Luer-lock female
• Size:	108 mm x 52 mm x 130 m (L x W x H)
• Cartridge material	Non-toxic Acrylonitrile butadiene styrene (ABS)
• Sterilization method	Production sterile

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1.2. Kit contents

- eDNA Dual filter capsule with valve connector
- 50 mL syringe
- 3 mL sample preservation solution in a 5 mL syringe (with a xenobiotic internal positive control)
- Luer-lock male caps (2x)
- Storage bag

Optional equipment and disposables:

- Sampling rod (SYL003)
- Waste bag

1.3. Notices and disclaimers

This product is developed, designed and sold for research purposes only. Sylphium Molecular Ecology B.V. does not take any responsibility and is not liable for any damage caused through use of this product, be it indirect, special, incidental or consequential damages (including but not limited to damages for loss of business, loss of profits, interruption or the like).

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2. Sampling guidelines and precautions

Successful eDNA detection depends not only on the sensitivity of the laboratory analysis, but also on the sampling strategy used in the field. Since eDNA is not evenly distributed in water, a well-designed sampling plan is essential to maximise the probability of detecting the target species.

Before starting fieldwork, establish a sampling plan and strategy. This plan should specify the number and distribution of sampling locations, the number of sub-samples per composite sample, the total volume of water to be filtered, and the habitats to be targeted. The sampling strategy should take into account the characteristics of the waterbody, the ecology of the target species, and the objectives of the survey.

For detailed recommendations on sampling strategies for rivers, streams, ponds and lakes, consult Sylphium's **eDNA Sampling strategy for aquatic environments (2026)**, available on our website: www.sylphium.com.

In addition to a well-designed sampling strategy, the precautions should be followed to minimise cross-contamination, ensure proper sample handling and maintain reliable sample records:

- **Avoid disturbing bottom sediments.** Historical eDNA can persist in sediments for years. Disturbing sediments may release this DNA into the water and affect the interpretation of results.
- **Minimise suspended particles and plant material.** Avoid highly turbid or recently disturbed water. Particles can clog filters and may contain substances, such as humic acids, that inhibit DNA analysis. When possible, collect water near the surface to minimise the intake of sediment and debris.
- **Clean reusable equipment between sampling locations.** Use a 1:10 dilution of regular household bleach to prevent DNA cross-contamination. **Important:** Avoid thickened or scented bleach, as additives may interfere with DNA analysis.
- **Ensure proper sample registration.** Record the sampling location, date and relevant details. Each sample analysed by Sylphium is assigned a unique identification number (E-number) for tracking and reporting.

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3. Sampling protocol

1. Connect the filter capsule

Connect the eDNA Dual Filter Capsule to the 60 mL syringe using the valve connector (**Figure 1A**).

2. Attach the sampling rod (optional)

Insert the 60 mL syringe with the attached filter capsule into the sampling rod (**Figure 1B**). Endure that the syringe plunger handle fits securely into the groove of the inner tube of the sampling rod.

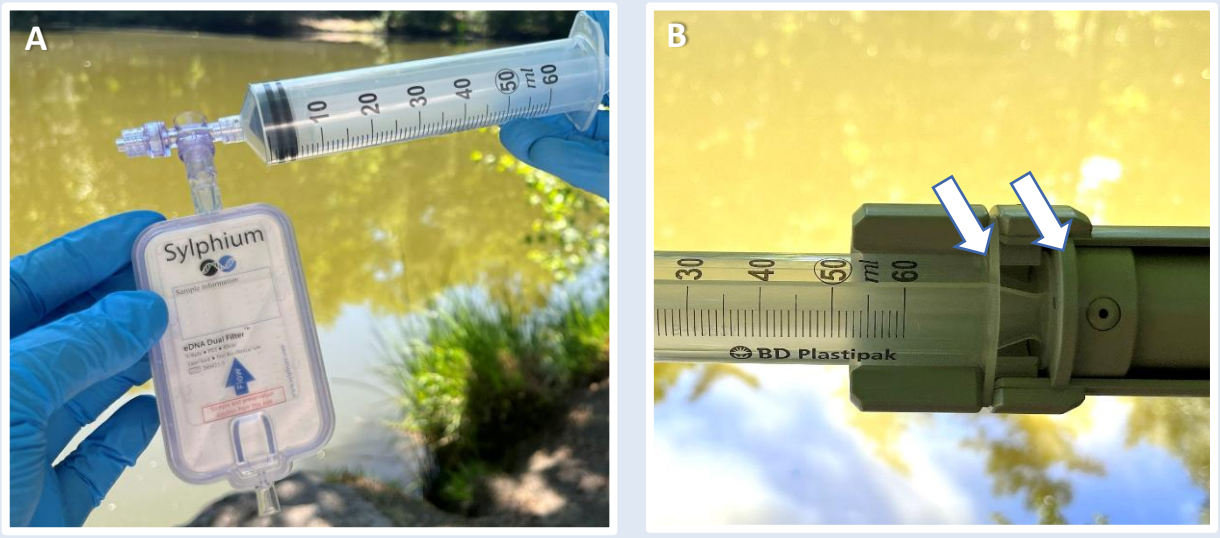


Figure 1. **(A)** The 60 mL syringe connected to the eDNA Dual Filter using the valve connector. **(B)** The 60 mL syringe and filter capsule inserted into the sampling rod.

3. Collect a water sub-sample

Submerge the inlet of the eDNA Dual filter capsule into the water. The filter capsule may almost be completely submerged if this makes sampling easier. However, always **keep the syringe and sampling rod out of the water** to prevent DNA cross-contamination between sampling locations.

Draw water through the filter capsule into the syringe by pulling back either the syringe plunger (**Figure 2A**) or the inner tube at the opposite end of the sampling rod (**Figure 2B**).

Important: avoid drawing air into the filter capsule, as trapped air bubbles can reduce filtration efficiency or cause the filter to malfunction. To prevent this, release the syringe plunger or the inner tube of the sampling rod before lifting the filter capsule out of the water. If air bubbles become trapped inside the capsule, follow the instructions in Appendix A to remove them.

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Figure 2. Collecting water sub-samples using the eDNA Dual Filter capsule **(A)** without and **(B)** with the sampling rod.

4. Empty the syringe

Push the syringe plunger or the inner tube of the sampling rod forward to expel the collected water (**Figure 3**).



Figure 3. Emptying the syringe before in preparation for the next sub-sample.

There is no need to remove the syringe from the sampling rod or to keep the filter capsule submerged in water while emptying the syringe (as depicted in the picture). Instead, you can lift the filter capsule from the water during this step.

5. Repeat sub-sampling

Repeat steps 3 and 4 until the desired volume of water has been filtered or the filter capsule becomes clogged. If the filter becomes clogged before the required volume has been collected, continue sampling with a new filter capsule.

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6. Remove residual water

After collecting the final sub-sample, lift the filter capsule out of the water and use the syringe to draw air through the filter until all residual water has been removed from the filter capsule (**Figure 4A**).

Keep the filter capsule connected to the valve connector and syringe during this step.

Important: remove all residual water before adding the preservation solution. Excess water compromises DNA stability and will result in degradation of the eDNA.

7. Add the preservation solution

Disconnect the filter capsule from the silicone tubing attached to the valve connector.

Seal the outlet of the filter capsule with one of the blue caps supplied with the 5 mL syringe containing the preservation buffer.

Connect the 5 mL syringe containing the sample preservation buffer to the inlet of the filter capsule.

Hold the filter capsule firmly in place and slowly push the syringe plunger to inject the preservation solution into the capsule (**Figure 4B**).



Figure 4. **(A)** Removing residual water from the eDNA Dual Filter capsule by pulling back the 60 mL syringe plunger. **(B)** Adding preservation solution to the eDNA Dual Filter capsule.

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8. Seal the filter capsule

Carefully pull back the plunger of the 5 mL syringe to the 3 mL mark to release any pressure inside the filter capsule.

Disconnect the syringe. It is not necessary to remove all air from the filter capsule.

Seal the inlet with the second blue cap. Ensure that both caps are securely fitted to prevent leakage.

9. Pack the sample for shipment

Place the sealed eDNA Dual Filter capsule, both syringes and valve connector back into the sample bag.

Keep all components together, as they are required for the subsequent eDNA isolation procedure in the laboratory.

The sample is now preserved and ready for shipment to the laboratory.

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3. Shipping of samples

If samples are analysed by Sylphium, please send the samples by regular mail (preferably with track and trace option) to the following address:

Sylphium Molecular Ecology

Diamantlaan 6b

9743BG Groningen

The Netherlands

Please inform us that the samples have been sent by sending an email to lab@sylphium.com or register the samples via the website.

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Appendix A. Removing air bubbles from the eDNA Dual Filter capsule

Air bubbles trapped inside the filter capsule can block the filter membrane and reduce filtration capacity. They can be easily removed following these steps:

1. Remove the 60 mL syringe from the sampling rod and disconnect the filter capsule from the silicone tubing of the valve connector.
2. Fill the syringe with sample water and connect it directly to the outlet of the filter capsule (**Figure 5**).
3. Hold the filter capsule upright with the inlet facing upwards. Slowly push the syringe plunger to push water backwards though the filter capsule, displacing and expelling any trapped air bubbles. Stop before water escapes through the inlet, as this may result in the loss of captured DNA.
4. Reconnect the filter capsule to the valve connector and optionally place the syringe back into the sampling rod.
5. Continue sampling as usual.



Figure 5. Removing trapped air bubbles by pushing sample water backwards through the filter capsule before collecting the next sub-sample, or alternatively, before sealing the filter capsule with the supplied caps.

Caution: do not push water beyond the dotted line, as this will result in the loss of captured eDNA.

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