Sylphium
molecular ecology

eDNA sampling set

Instructions and validation

#SYL009

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For general laboratory and research use only.

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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

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 (Trade name of Eelco Wallaart 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).

2. Sampling guidelines and precautions

All Sylphium eDNA detection kits are designed and validated to have a single DNA molecule sensitivity (see specific validation reports associated with each analysis). Maximization of the chance of detection is therefore completely dependent on the success of getting the target DNA molecules into the eDNA Dual Filter Capsule. The maximum detection probabilities of different species groups with eDNA are shown in **Table 1**.

Species group	Detection probability	Reference
Semi-aquatic mammals	≥95%	Sales et al. 2015
Fish	≥95%	Wood et al. 2010
Amphibians	≥95%	Caren et al. 2018
Mollusks	91.7%	Marshall et al. 2022

Table 1. Detection probability of different species groups with eDNA.

The maximum probability of detection can only be achieved with the correct sampling method. The following sampling guidelines (based on literature and experiments) should be taken into account to have the maximum detection probability, no eDNA cross-contamination between samples, and a proper registration of samples:

- **Minimum sample volume of 0.75 liters.** Take a new capsule if the eDNA Dual Filter Capsule becomes clogged with particles before the minimum sample volume is reached. The eDNA Dual Filter Capsules from the same location can be pooled afterwards and analyzed as one sample.
- **A new sample must be taken every 300 meters.**
- **A sample consists of sub-samples of 60 mL**. These are preferably taken every 10 meters along the shore to bridge the distance between two samples.
- **Ecological knowledge is important**. Samples should be taken at the preferred niche (location) and seasonal occurrence based on ecological knowledge of the target organism.
- **Historical DNA can be preserved in sediment layers for several years**. Avoid sampling or disturbing sediments to only gain knowledge of recent occurrences of the target organism.
- **Ensure proper registration of the samples**. Sample sets supplied for analysis in Sylphium's laboratory have an unique sample number. This sample number (E number) is used in Sylphium's analysis reports.
- **A sampling rod can be used for sampling from shore**. If the water is easily accessible, the sampling rod does not need to be used, but can still be useful.

- **The samples contain as few particles or plant material as possible**. Do not sample in water that has recently been stirred or otherwise disturbed and therefore contains a lot of soil particles. Surface water is also preferred because the upper part of the water column contains the fewest particles. These particles may contain, among other things, humic acids, which can disrupt the analyses.

- **Clean reusable material with 10 times diluted thin bleach** (available in the supermarkets). This destroys DNA and thereby prevents cross-contamination between sample locations. **Important**: do not use thick bleach, as it contains disruptive factors that interfere with the analysis process.

3. Protocol

1. Connect the valve connector to the syringe (**Figure 1**).

2. (Optional) Place the 60 mL syringe with the attached eDNA filter capsule into the opening of the sampling rod (**Figure 2**). Make sure that the handle of the plunger slides into the groove of the inner tube.

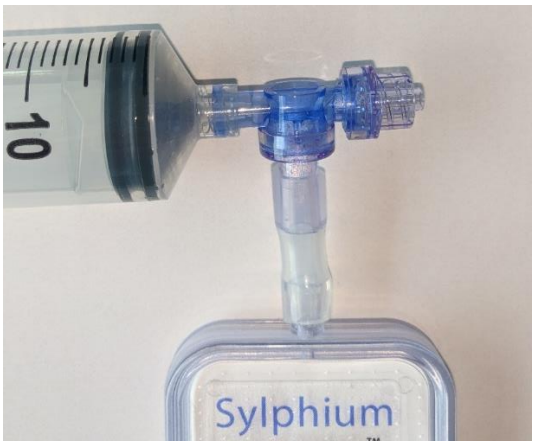

Figure 1. Syringe connected to an eDNA filter capsule.

Figure 2. Syringe placed into a sampling rod.

3. Place the inlet of the eDNA dual filter capsule into the water and ensure the syringe and sampling rod do **not** come into contact with the water. This prevents DNA contamination between sample locations. Draw water into the syringe by pulling back the plunger (**Figure 3A**) or the inner tube on the other end of the sampling rod (**Figure 3B**). **Important**: Avoid air bubbles in the filter, as they can cause it to malfunction. To prevent air from being drawn in, release the syringe plunger or inner tube from the sampling rod before lifting the dual filter capsule out of the water. If air bubbles do appear in the filter, they can easily be removed (see Appendix A for instructions).

Figure 3. Taking water samples using the eDNA dual filter capsule without (**A**) and with (**B**) sampling rod.

4. Empty the syringe by pushing the syringe plunger or the inner tube at the other end of the sampling rod (**Figure 4**). It is not necessary to disconnect the syringe from the sampling rod or to keep the inlet of the dual filter capsule submerged in water.

Figure 4. Emptying syringe to prepare for the next sub-sample.

Figure 5. Example of sub-sample locations.

5. Repeat this operation until the filter is clogged. One liter of water (approximately 16 full syringes) is sufficient in in most cases for a maximum detection probability. Take a new filter capsule when the filter becomes clogged. It is recommended to take multiple sub-samples at the same location. For example, take a sub-sample (1 full syringe of 60 mL) every 10 meters (**Figure 5**).

6. When finished, remove all water from the eDNA dual filter capsule by lifting the filter out of the water and using the syringe to draw air through the filter (**Figure 6**). Keep the eDNA dual filter capsule connected to the valve connector and the syringe during this step. **Important: failing to remove all water will result in degradation of eDNA.**

Figure 6. Drying eDNA dual filter capsule.

Figure 7. Sample preservation of filter membrane.

Figure 8. Ready for send-off.

7. Remove the double filter capsule from the silicone tubing connected to the connector. Place the blue cap of the 5 mL syringe on the outlet of the filter capsule. Connect the 5 mL syringe with the sample preservation solution to the inlet of the capsule (**Figure 7**). Hold down the filter capsule and push the preservation solution into the capsule.

8. Pull the plunger back to 3 mL to release pressure and remove the syringe from the dual filter capsule. It is not necessary to remove all the air from the dual filter capsule. Place the other blue cap on the inlet of the filter capsule.

9. Place the eDNA Dual filter capsule, both syringes and valve connector back into the sample bag. **These materials are required during eDNA isolation.** The sample bag is now ready to be send to the lab (**Figure 8**). If samples are analysed by Sylphium molecular ecology, please send the samples by regular mail (preferably by registered mail) to the following address:

Sylphium molecular ecology
PO Box 11107
9700 CC Groningen
The Netherlands

Please inform us that the samples have been sent by sending an email to info@sylphium.com

4. Validation and comparison of eDNA filter systems

4.1. Experimental set-up

The eDNA dual filter capsules were compared with commonly used filtering methods used in the eDNA research, namely the Sterivex ™ filter units and PES filter funnels (**Table 1**). Quantitative detection of the fish species *Rutilus rutilis* (SYL139) was used to compare the recovery of eDNA with these filter systems.

	eDNA Dual filter	Sterivex ™	Filter funnel
Manufacturer	Sylphium	Merck	Sarstedt
Filter area (cm²)	69	10	65
Material	PES	PES	PES
Pore seize	0.8	0.22	0.2
Closed capsule	Yes	Yes	No

Table 2. Filters used in this study.

4.2. Filtration

Water was collected with barrels from the river “De beek”, Drenthe, Netherlands. After collection, the vessels were taken to the laboratory and filtered. Each filter system was tested in triplicate. A vacuum pump with a vacuum of 0.85 bar was used during the filtration. Filtration was stopped after 10 minutes. The maximum volume of filtered water was recorded per filter (**Table 1**). The water was stirred with a magnetic stirrer during the filtration to avoid sedimentation. The filter capsules are filled with Sample preservation solution according to the Sylphium manuals (SYL001 and SYL009). The filter membrane of the filter funnel was cut with a scalpel from the funnel and transferred with all residues to a 15 mL tube containing 2 mL of Sample preservation solution.

4.3 DNA isolation

eDNA was isolated from the samples with the Environmental DNA isolation kit (Sylphium, SYL002) according to the protocol. One mL of each isolate was processed, the remaining isolate was kept as a backup.

4.4. Analysis

The efficiency of the filters was compared with a quantitative eDNA analysis (SYL139) targeting the common fish species *Rutilius rutilis*. The analysis was performed according to the protocol associated with this qPCR kit. Each filter was determined 8-fold compared to a simultaneously analyzed calibration line with known target DNA concentrations.

4.5. Results

4.5.1. Filtration volume

All filters were clogged within 10 minutes and the maximum filtration volume was reached for each type of filter. The mean maximum filtration volumes were; 2333 mL for the eDNA dual filter capsule, 95 mL for the Sterivex ™ and 277 mL for the filter funnel (**Figure 9**).

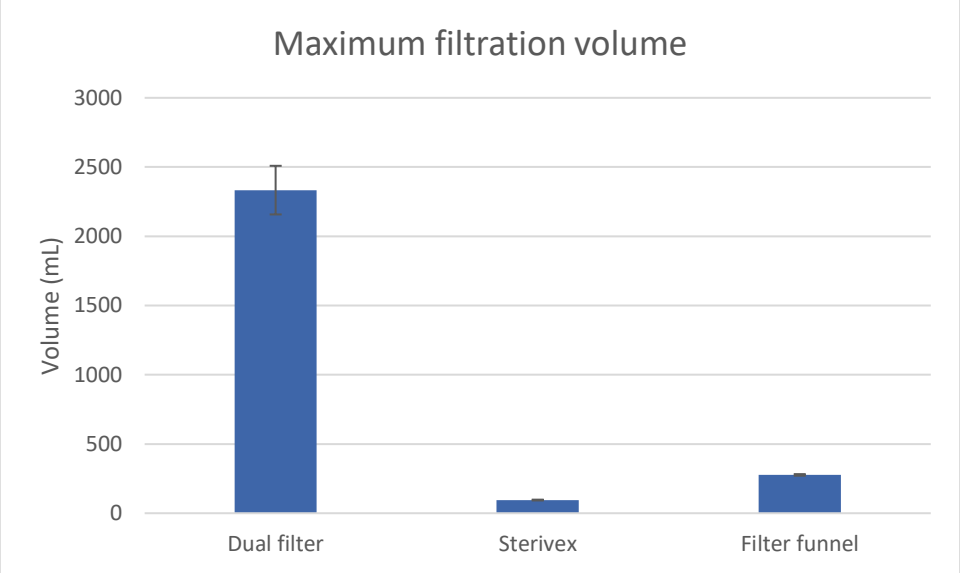

Figure 9. Maximum filtration volume of the eDNA Dual filter capsule, Sterivex ™ and filter funnel determined in triplicate. The error bars represent the standard deviation.

4.5.2. Quantitative eDNA analysis

The absolute amount of eDNA collected and isolated from the filter systems was determined with a quantitative eDNA analysis (SYL139) targeting the common fish species *Rutilius rutilis* for each filter type. This resulted in the following values; 1.2 x 10^5 fragments for the eDNA Dual filter capsule, 5.2 x 10^3 fragments for the Sterivex ™ and 1.3 x 10^4 fragments for the filter funnel (**Figure 10**).

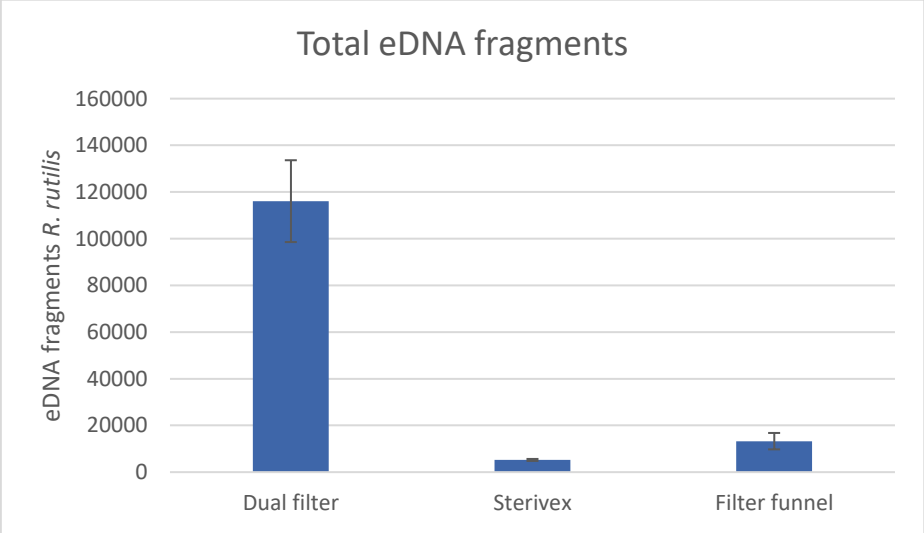

Figure 10. Total eDNA fragments per filter type. The error bars represent the standard deviation.

4.5.3. eDNA recovery

To determine differences in eDNA uptake and recovery of the filter systems used, the concentration of eDNA fragments was calculated per mL of filtered water and compared between each filter system (**Figure 11**). There were no statistically significant differences, in eDNA uptake and recovery, between filter systems, as determined by a one-way ANOVA (F (2,6) = 0.564, p = 0.596; **Figure 11**).

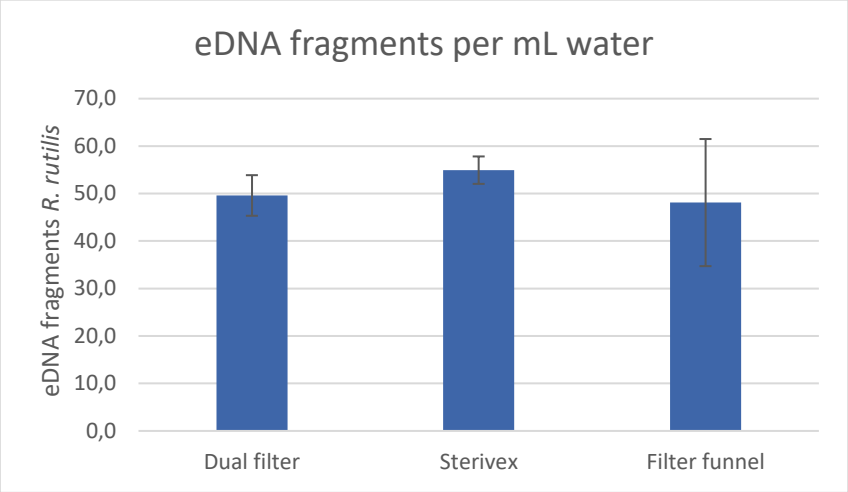

Figure 11. eDNA fragments per mL per filter type. The error bars represent the standard deviation.

4.6. Conclusions

The eDNA dual filter capsule is capable of filtering much more water than competing systems. The maximum filter volume is 10 times as high as a funnel filter and even 20 times as high as a Sterivex ™ capsule. The eDNA dual filter capsule also collects much more eDNA than the competing systems. The eDNA dual filter capsule collects 20 times more eDNA compared to a Sterivex ™ and 10 times more than a filter funnel. There is no difference between the filter systems in the amount of eDNA obtained per unit volume of the sample water. All eDNA is recovered with the used filter systems.

The eDNA double filter capsule and the Sterivex ™ meet all requirements as stated in CEN / TC 230 - Water Analysis - N 1229. Both systems are closed capsules, which prevents cross contamination during sampling. The filter funnel is an open system and requires additional measures to meet all criteria as stated in CEN / TC 230 - Water analysis - N 1229.

References

Caren S. Goldberg, Katherine M. Strickler, Alexander K. Fremier, Degradation and dispersion limit environmental DNA detection of rare amphibians in wetlands: Increasing efficacy of sampling designs. Science of The Total Environment. 2018; 633: 695-703

Marshall, N. T., Symonds, D. E., Dean, C. A., Schumer, G., & Fleece, W. C. (2022). Evaluating environmental DNA metabarcoding as a survey tool for unionid mussel assessments. Freshwater Biology, 67, 1483– 1507.

Sales, NG, McKenzie, MB, Drake, J, et al. Fishing for mammals: Landscape-level monitoring of terrestrial and semi-aquatic communities using eDNA from riverine systems. J Appl Ecol. 2020; 57: 707– 716.

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Appendix A:

Removing air bubbles from eDNA dual filter capsule

Air bubbles block the filter membrane and have a negative effect on the filter capacity. These air bubbles are easy to remove from the capsule.

Remove the 60 mL syringe from the sample rod and remove the dual filter capsule from the silicone tubing connected to the connector. Fill the syringe with sample water and connect the syringe to the outlet of the eDNA dual filter capsule. Hold the capsule upwards and push the syringe plunger until all air bubbles have been expelled from the capsule. Reconnect the capsule and syringe to the valve connector and place them back into the sample rod. Proceed with sampling.

Removing air bubbles from the eDNA dual filter capsule

Sylphium

The eDNA company

Sylphium molecular ecology

P.O. box 11107
9700 CC Groningen
Netherlands

Tel.: +31 6 82 42 64 44
E-mail: info@sylphium.com

www.sylphium.com