Sylphium
molecular ecology

# *Lampetra fluviatilis & lampetra planeri* qPCR detection kit

**#SYL144**

Document date: 10 November 2025

For general laboratory and research use only.

# Table of Contents

# 1. Introduction

The *Lampetra fluviatilis & lampetra planeri* detection kit with eTaq qPCR hot start mix provides excellent performance for sensitive, robust and accurate qPCR detection. eTaq qPCR hot start mix is highly resistant to inhibitory factors, such as humic acids present in environmental samples. In addition to all components of the eTaq qPCR hot start mix, the kit contains primers and a probe for detecting a highly specific sequence present on the mitochondrial DNA of the species *Lampetra fluviatilis & lampetra planeri*. The primers and probe are designed and validated to be used with eDNA samples. The full validation report can be found under "*documents*" on the *Lampetra fluviatilis & lampetra planeri* detection Kit page (2).

The *Lampetra fluviatilis & lampetra planeri* detection kit has:

- **High resistance to inhibiting factors.** Environmental DNA (eDNA) extracts contain often multiple qPCR inhibiting factors. Normal qPCR hot start mixes are sensitive to these substances.
- **Strong fluorescence signal with low background noise.** Isolated environmental samples contain residues of naturally occurring auto fluoresce substances that will interfere with the measurements. In order not to experience background interference a strong fluorescence signal from the assays is required for this kind of samples.
- Highest possible sensitivity (1 DNA copy per reaction). Environmental water samples may contain very low amounts of target DNA.
- **100% specificity**. Isolated DNA from environmental samples contains billions of DNA fragments from bacteria, protozoa, plants, animals, etc. Not only animals from the same order (fish, amphibians, reptiles, mammals, etc.) must be taken in account during primer/probe design, but all known DNA sequences must be checked for nonspecific binding of the primers and probe. This is validated by experimental and bioinformatical studies.
- **≥ 95% detection probability.** The maximum probability of detection can only be achieved with the correct sampling method (1).
- **Hot-start properties.** The DNA polymerase enzyme has been genetically modified to have similar or better hot-start properties when compared to antibody-mediated hot-start enzymes. Working on ice is not necessary during the preparation of the PCR mixture.

The kit is developed and optimized to be used with eDNA isolates purified using the eDNA isolation kit (#SYL002) from Sylphium molecular ecology. Other eDNA isolation methods/kits can be used as well. Please send an e-mail to info@sylphium.com to get more information how to use the obtained isolates from other methods/kits to get reliable results with the *Lampetra fluviatilis & lampetra planeri* detection kit.

## 1.1. Kit contents

- Positive control (cloned *Lampetra fluviatilis & lampetra planeri* DNA)
- Primer/probe mix (10x) for detection of *Lampetra fluviatilis & lampetra planeri* (FAM dye)
- eTaq DNA polymerase (hot start)
- eTaq qPCR mix (2x)
- PCR water

The kit contains materials for 200 reactions of 25µl.

## 1.2. Required equipment and additional kits

**Equipment**

- Freezer -20°C
- Pipettors
- Personal protection equipment
- qPCR machine
- PCR cooling rack

## 1.3 Kit Storage

Store all reagents and kit components after arrival in dark in a freezer (-20°C).

## 1.4 Ordering information

This qPCR detection kit (SYL144), a wide variety of other eDNA qPCR detection assays, eDNA sampling materials/ equipment, qPCR hot start mixes and eDNA isolation kits can be found and ordered at sylphium.com/webshop.

## 1.5 Notices and disclaimers

This product is developed, designed and sold for research purposes only. Sylphium Molecular Ecology (Trade name of Eelco Wallaart bv) 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. Protocol

1. Completely thaw the eDNA PCR mix (2x), primer/probe mix, samples and positive controls to dissolve all precipitates.

2. Mix all tubes by shaking and briefly pulse-spin them in a centrifuge before opening. This ensures that the tube contents settle in the bottom and prevents spillage when opening the tubes. Prepare only as much mixture needed for the analysis, with a few additional reactions to account for pipetting errors.

| Component | Reaction volume 25 µl | Reaction volume 50 µl |
|---|---|---|
| eTaq qPCR mix (2x) | 12.5 µl | 25 µl |
| eTaq DNA polymerase | 1 µl | 2 µl |
| 10x Primers/probe mix* | 2.5 µl | 5 µl |
| Sample | 2 - 8 µl | 2 - 16 µl |
| ROX Reference Dye§ | X µl | X µl |
| PCR grade water | Fill to 25 µl | Fill to 50 µl |

**Table 1. Components per well for 25 µl and 50 µl reaction volume**. *If using custom primer/probe mixes, formulate to 10x initial concentration. **§** Not supplied in kit, see manual qPCR machine if needed.

3. Mix thoroughly but gently to minimize air bubbles in the mixture.

4. Pipette the prepared PCR mix into the wells of a PCR plate (total reaction volume minus the sample volume per well). To avoid pipetting errors, use a minimum sample volume of 2 µl for qualitative measurements and at least 5 µl for quantitative assays. Place a seal cover on top of the 96-well PCR plate to protect against airborne contaminations.

5. Add template DNA (sample or positive control) to the PCR plate according to the desired qPCR plate setup. **Note:** all hot-start enzymes exhibit some background activity at room temperature. To maximize sensitivity, keep the mixture at room temperature or lower for as little time as possible after adding the sample. Using a cooling block (4 °C) is recommended after adding the sample.

6. Briefly spin the plate if necessary to remove air bubbles, as these can interfere with fluorescence detection.

7. Program the thermal cycler according to the scheme in **Table 2**.

In addition to the specific primer/probe mix, this kit contains all components of the eTaq qPCR hot start mix. For a complete description of the eTaq qPCR hot start mix (SYL1003), which is also used in this kit, please

refer to eTaq qPCR hot start mix manual available under "*relevant documents*" on the *Lampetra fluviatilis & lampetra planeri* detection kit page (2).

| Step | Temperature | Time* | Number of cycles |
|---|---|---|---|
| **Initial denaturation** | 95 | 10 min | 1 |
| **Denaturation** | 95 | 15 s | 45 |
| **Annealing/Extension** | 62.5 | 30 s | |

**Table 2.** Thermal cycling conditions of the *Lampetra fluviatilis & lampetra planeri* detection kit. Fluorogenic data should be collected during the extension step *Lampetra fluviatilis & lampetra planeri* DNA detection via FAM channel.

## 3. References

1. https://sylphium.com/webshop/product/syl009/
2. https://sylphium.com/webshop/product/syl144/

# 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*