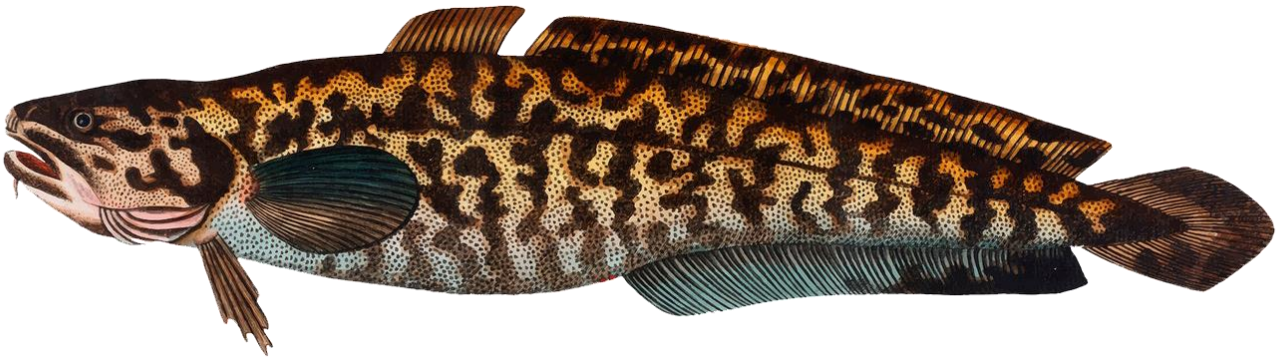
Technical specifications:

Lota lota qPCR detection kit

with eDNA qPCR master mix



#SYL115

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1. Introduction

The *Lota lota* detection kit with eDNA qPCR master mix provides excellent performance for sensitive, robust and accurate qPCR detection. eDNA qPCR master mix is highly resistant to inhibitory factors, such as humic acids present in environmental samples. In addition to all components of the eDNA qPCR master mix, the kit contains primers and a probe for detecting a highly specific sequence present on the mitochondrial DNA of the species *Lota lota* . The primers and probe are designed and validated to be used with eDNA samples.

The *Lota lota* detection kit has:

- **High resistance to inhibiting factors**. Environmental DNA (eDNA) extracts contain often multiple qPCR inhibiting factors. Normal qPCR master 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.

The full validation report can be found under “documents” on the *Lota lota* Detection Kit page at sylphium.com/webshop

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 *Lota lota* detection kit.

1.1 Kit contents

- Positive control (cloned *Lota lota* DNA)
- Primer/probe mix (10x) for detection of *Lota lota* (FAM dye)
- eTaq DNA polymerase
- eDNA qPCR master mix (2x)
- PCR water

The kit contains materials for 200 reactions of 25µl.

1.2 Equipment Required

- qPCR machine capable for detection of the FAM dye
- Microcentrifuge (13,000 x g)
- Pipettors
- -20 °Freezer
- 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 (SYL115), a wide variety of other eDNA qPCR detection assays, eDNA sampling materials/ equipment, qPCR master 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

In addition to the specific primer/probe mix, the kit contains all components of the eDNA qPCR master mix. The protocol associated with the eDNA qPCR master mix (SYL1003) is also valid for the *Lota lota* detection kit. The complete protocol can be found under “Relevant documents” on the *Lota lota* detection kit page at sylphium.com/webshop

Thermal cycling conditions of The *Lota lota* detection kit are as follows:

Step	Temperature, °C	Time	Number of cycles
Initial denaturation	95	10 min	1
Denaturation	95	15 s	50
Annealing	58	15 s	
Extension	72	30 s	

Table 1: Thermal cycling conditions of the *Lota lota* detection kit. Fluorogenic data should be collected during the extension step. *Lota lota* DNA detection via FAM channel.