

Technical specifications:

***Cottus perifretum/rhenanus* qPCR detection kit**

with eTaq qPCR master mix



#SYL143

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For general laboratory and research use only

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1. Introduction

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

The *Cottus perifretum/rhenanus* 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 *Cottus perifretum/rhenanus* 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 *Cottus perifretum/rhenanus* detection kit.

1.1 Kit contents

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

The kit contains materials for 200 reactions of 25µl.

1.2 Equipment Required

- qPCR machine multiplex 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 (SYL143), 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 eTaq qPCR master mix. The protocol associated with the eTaq qPCR master mix (SYL1003) is also valid for the *Cottus perifretum/rhenanus* detection kit. The complete protocol can be found under “Relevant documents” on the *Cottus perifretum/rhenanus* detection kit page at sylphium.com/webshop

Thermal cycling conditions of The *Cottus perifretum/rhenanus* detection kit are as follows:

Step	Temperature, °C	Time	Number of cycles
Initial denaturation	95	5 min	1
Denaturation	95	15 s	50
Annealing	58	15 s	
Extension	72	30 s	

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