Technical specifications:

Mesotriton alpestris qPCR detection kit

with eDNA qPCR hot start mix

#SYL166

Document date: 03 October 2022

For general laboratory and research use only

Index

1. Introduction

The *Mesotriton alpestris* 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 *Mesotriton alpestris*. The primers and probe are designed and validated to be used with eDNA samples.

The *Mesotriton alpestris* 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 full validation report can be found under “documents” on the *Mesotriton alpestris* detection Kit page (2)

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 *Mesotriton alpestris* detection kit.

1.1 Kit contents

- Positive control (cloned *Mesotriton alpestris* DNA)
- Primer/probe mix (10x) for detection of *Mesotriton alpestris* (FAM dye)
- eTaq DNA polymerase (hot start)
- eTaq qPCR 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 (SYL166), 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

In addition to the specific primer/probe mix, the kit contains all components of the eTaq qPCR hot start mix. The protocol associated with the eTaq qPCR hot start mix (SYL1003) is also valid for the *Mesotriton alpestris* detection kit. The complete protocol can be found under “Relevant documents” on the *Mesotriton alpestris* detection kit page (2

Thermal cycling conditions of The *Mesotriton alpestris* detection kit are as follows:

Step	**Temperature, °C**	**Time**	**Number of cycles**
Initial denaturation	95	10 min	1
Denaturation	95	15 s	50
Annealing	60	15 s	
Extension	72	30 s	

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

3 References

1. https://sylphium.com/webshop/product/syl009/
2. https://sylphium.com/webshop/product/syl166/