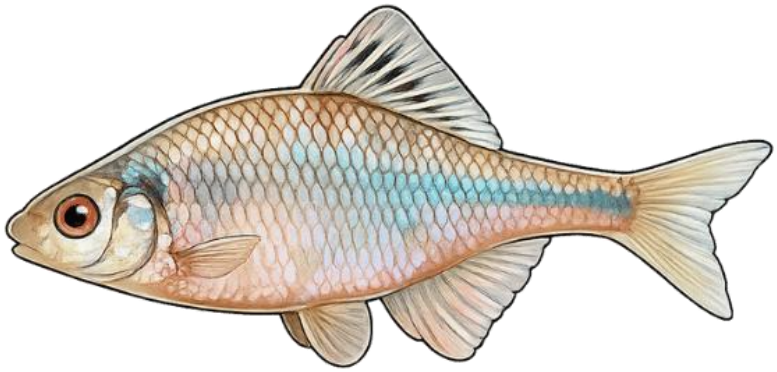
VALIDATION

Rhodeus amarus qPCR detection kit

SYL111

Document date: 28 August 2026



Sylphium
molecular ecology

For general laboratory and research use only.

© 2026 Sylphium Molecular Ecology

Table of Contents

1. *In silico* validation 4

2. Experimental validation 5

2.1. Analytical sensitivity, fluorescence output signal and efficiency 5

2.2. Detection confirmation of *Rhodeus amarus* in environmental samples 8

© 2026 Sylphium Molecular Ecology

1. *In silico* validation

Table 1. *In silico* validation of primers and probe

Parameter	Forward primer	Reverse primer	Probe
Length (bp)	23	23	26
GC %	43	52	50
Stability	1.3	1.8	-
T_m (°C)	62	62	73
Target region	CytB(mtDNA)	CytB (mtDNA)	CytB (mtDNA)
Dimer formation	No	No	No
Nucleotide runs	No	No	No

*Probe labelled with FAM.

Table 2. *In silico* validation primers and probe combination

PCR product size (bp)	113
Combined dimer formation	No
***In silico* PCR on Genbank**	*R. amarus, R. colchicus, R. meridionalis, R. sericeus*
Date of in silico PCR	December 2020

Conclusion:

The *in silico* analysis exclusively identified the target organism as well as *R. amarus, R. colchicus, R. meridionalis,* and *R. sericeus*.

© 2026 Sylphium Molecular Ecology

2. Experimental validation

All validation experiments were conducted using the Sylphium qPCR mix and analyzed with the Bio-Rad CFX96 Touch Real-Time PCR Detection System.

2.1. Analytical sensitivity, fluorescence output signal and efficiency

Standard solutions containing nominal target concentrations of 10^5, 10^4, 10^3, 10^2, 10, and 0 target copies per reaction were analyzed at the optimal primer and probe concentrations. Fluorescence output was compared with the background signal, with a minimum 100-fold increase in fluorescence considered acceptable. Reaction conditions were optimized when fluorescence signals were insufficiently distinguishable from background noise.

The amplification efficiency of the primer and probe set was calculated from the slope of the standard curve and was considered acceptable when between 90% and 110%.

Results

Positive amplification was observed down to the lowest tested nominal concentration of **1 target copy per reaction**, demonstrating single-copy detection capability under the tested conditions (**Figure 1; Table 3**). The fluorescence output of positive reactions exceeded the predefined criterion of a 100-fold increase relative to the background signal.

The amplification efficiency of the assay was **90.3%**, indicating that the primer and probe set performed within the predefined acceptable efficiency range of 90-110% (**Figure 2; Table 4**).

© 2026 Sylphium Molecular Ecology

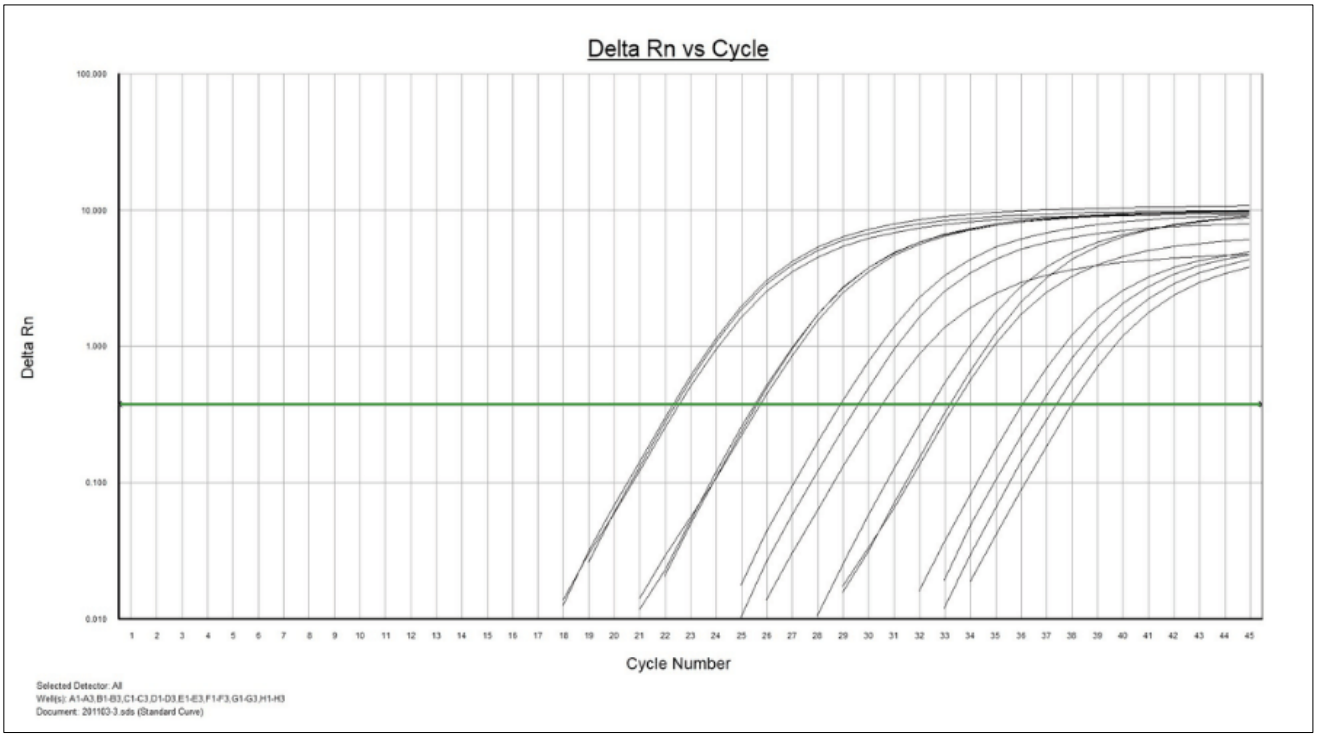


Figure 1. Amplification curves obtained at the optimal primer and probe concentrations and an annealing temperature of 58°C.

Table 3. Detection results and Cq values obtained at the optimal primer and probe concentrations and an annealing temperature of 58°C

Nominal target copies per reaction	Target detected	Mean Cq ± SD
0	No	n.a.
1	Yes	n.d.
10	Yes	36.7 ± 0.6
10^2	Yes	33.0 ± 0.5
10^3	Yes	29.6 ± 0.8
10^4	Yes	25.6 ± 0.1
10^5	Yes	22.4 ± 0.1

n.d.: not determined, very low amounts of DNA could not be quantitively detected in a reliable manner with the used qPCR instrument.

© 2026 Sylphium Molecular Ecology

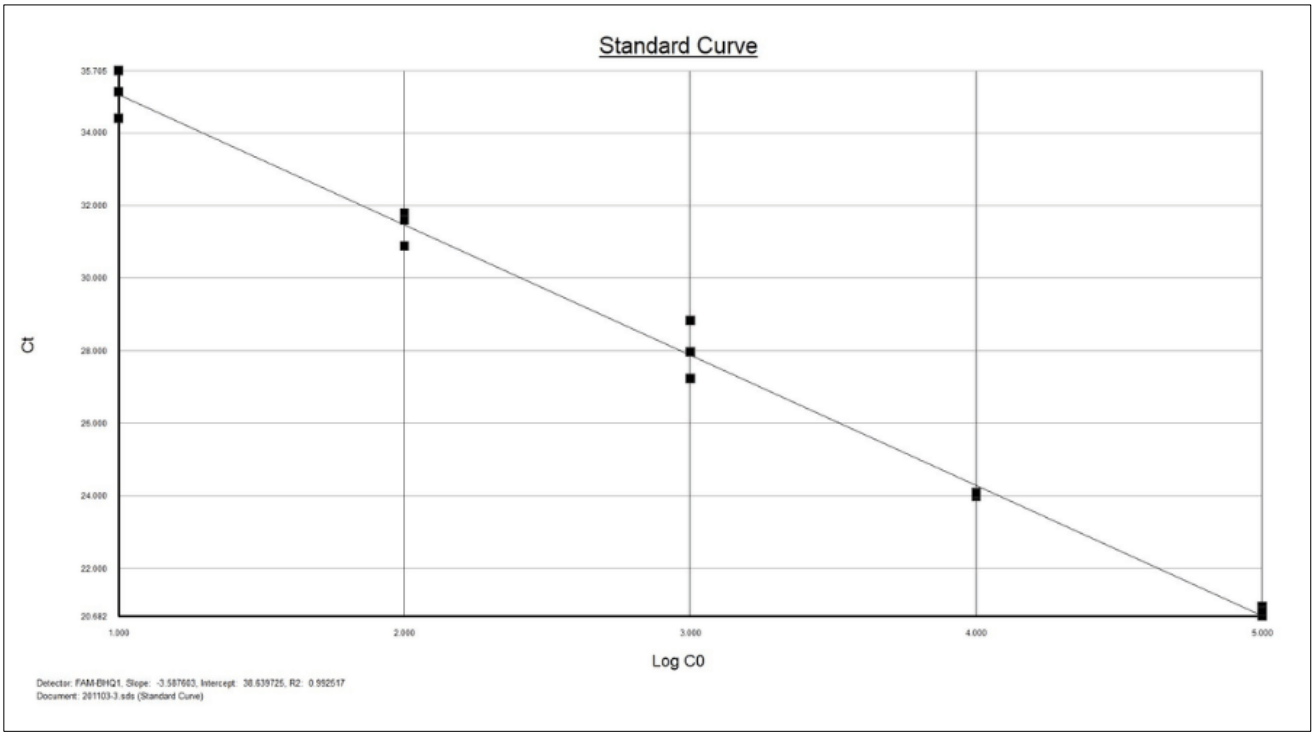


Figure 2. Standard curve generated from nominal target concentrations of 10, 10^2, 10^3, 10^4, and 10^5 copies per reaction.

Table 4. Standard curve parameters obtained with the *Rhodeus amarus* qPCR detection kit

Parameter	Result
Slope	-3.59
Amplification efficiency (%)	90.3
R^2	0.992

© 2026 Sylphium Molecular Ecology

2.2. Detection confirmation of *Rhodeus amarus* in environmental samples

The amount of eDNA released by a target organism into the environment depends on the target species, the environmental conditions, and season (CEN / TC230-water analysis, proposal documented in N 1229). Samples were collected with the **SYL009 Complete eDNA sampling set** and eDNA was isolated using the **SYL002 Environmental DNA isolation kit**.

Results:

The kit SYL111 - *Rhodeus amarus* detection kit successfully detected *Rhodeus amarus* DNA in environmental samples from different locations in the Netherlands when 100-200 mL water was filtered.

PCR products obtained from positive environmental samples were analyzed by Sanger sequencing. The resulting sequences were identical, and BLAST analysis confirmed that the amplified products originated from *Rhodeus amarus*. No amplification was observed in the tested target-organism-free environmental samples.

© 2026 Sylphium Molecular Ecology

Sylphium Molecular Ecology

The eDNA company

Sylphium Molecular Ecology

Diamantlaan 6b
9743 BG Groningen
Netherlands

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

www.sylphium.com

© 2026 Sylphium Molecular Ecology