VALIDATION

Internal Positive Control (IPC) qPCR quantification kit qPCR detection kit

SYL113

Document date: 13 November 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

© 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)	22	20	33
GC %	45	50	45
Stability	2.5	2.2	-
T_m (°C)	60	61	74
Target region	-	-	-
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)	117
Combined dimer formation	No
***In silico* PCR on Genbank**	Artemisia annua amorpha-411-diene synthase (ADS)
Date of in silico PCR	May 2023
DNA sequence	GACGCCTAAATGATCTCATGACCCACAAGGCCGAGCAAGAAA GAAAACATAGTTCATCGAGCCTTGAAAGTTATATGAAGGAATA TAATGTCAATGAGGAGTATGCCC

© 2026 Sylphium Molecular Ecology

2. Experimental validation

All validation experiments were conducted using the Sylphium qPCR mix and analyzed with the Applied biosystems 7300 qPCR instrument.

2.1. Analytical sensitivity, fluorescence output signal and efficiency

Standard solutions containing nominal target concentrations of 10^4, 10^3, 10^2, 10, 1 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 **1-10 target copies 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 **97.2%**, 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

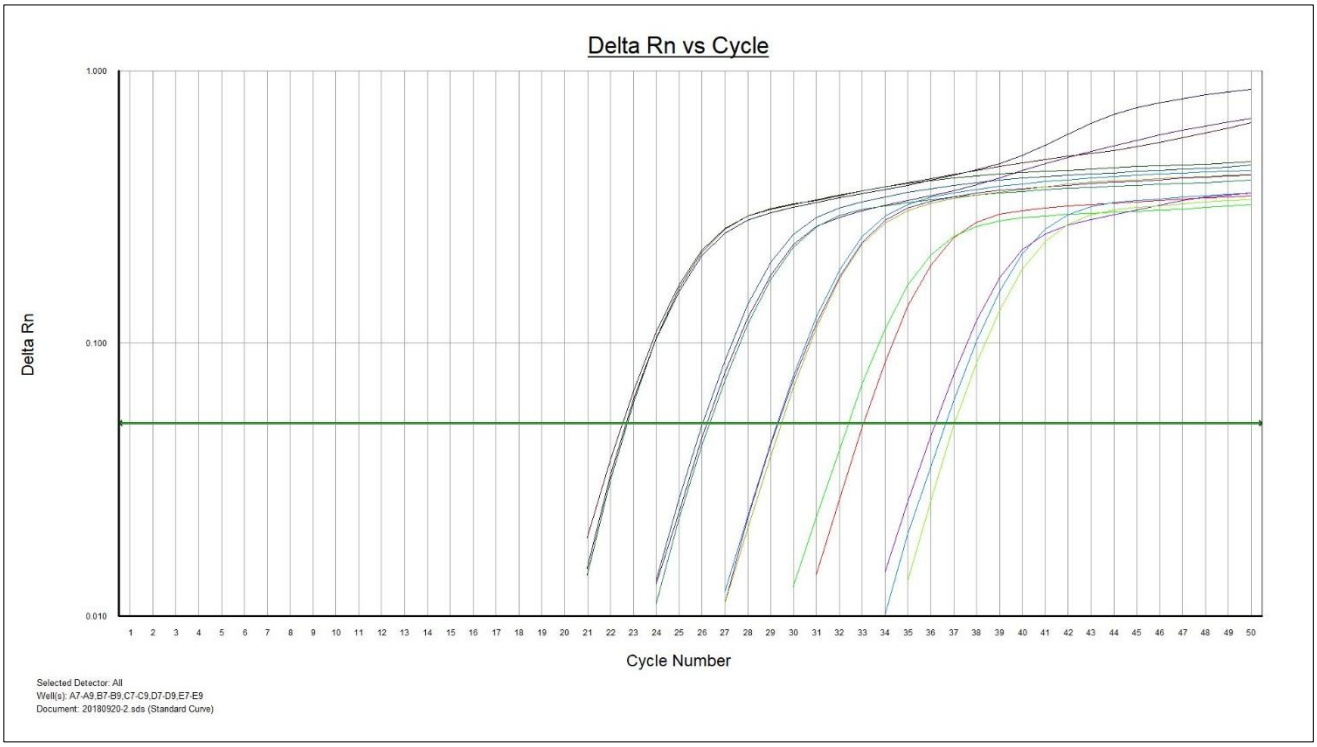


Figure 1. Amplification curves obtained at the optimal primer and probe concentrations and an annealing temperature of 55°C.

Table 3. Detection results and Cq values obtained at the optimal primer and probe concentrations and an annealing temperature of 55°C

Nominal target copies per reaction	Target detected	Mean Cq	SD
0	No	-	-
1	Yes	36.6	0.4
10	Yes	32.8	0.3
10^2	Yes	29.3	0.1
10^3	Yes	26.2	0.1
10^4	Yes	22.6	0.1

© 2026 Sylphium Molecular Ecology

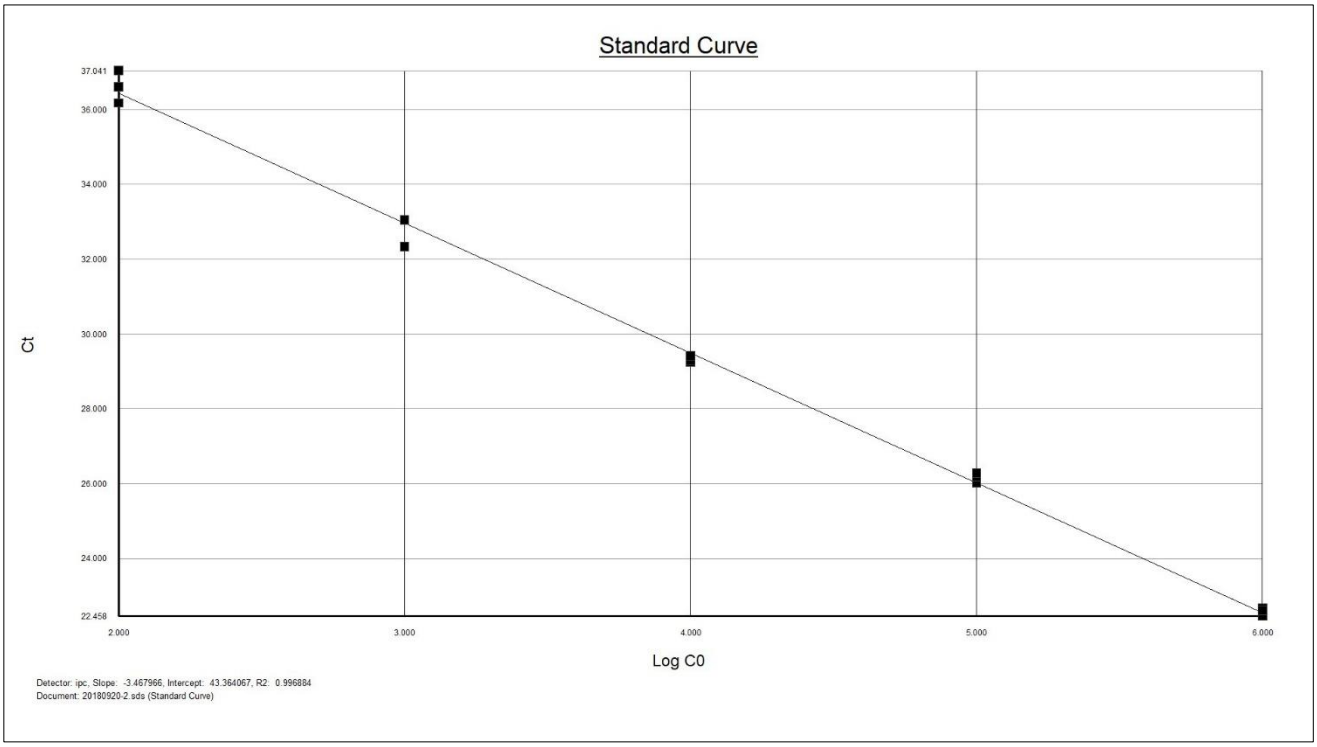


Figure 2. Standard curve generated from nominal target concentrations of 1, 10, 10^2, 10^3, and 10^4 copies per reaction.

Table 4. Standard curve parameters obtained with the Internal Positive Control (IPC) qPCR quantification kit

Parameter	Result
Slope	-3.39
Amplification efficiency (%)	97.2
R^2	0.995

© 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