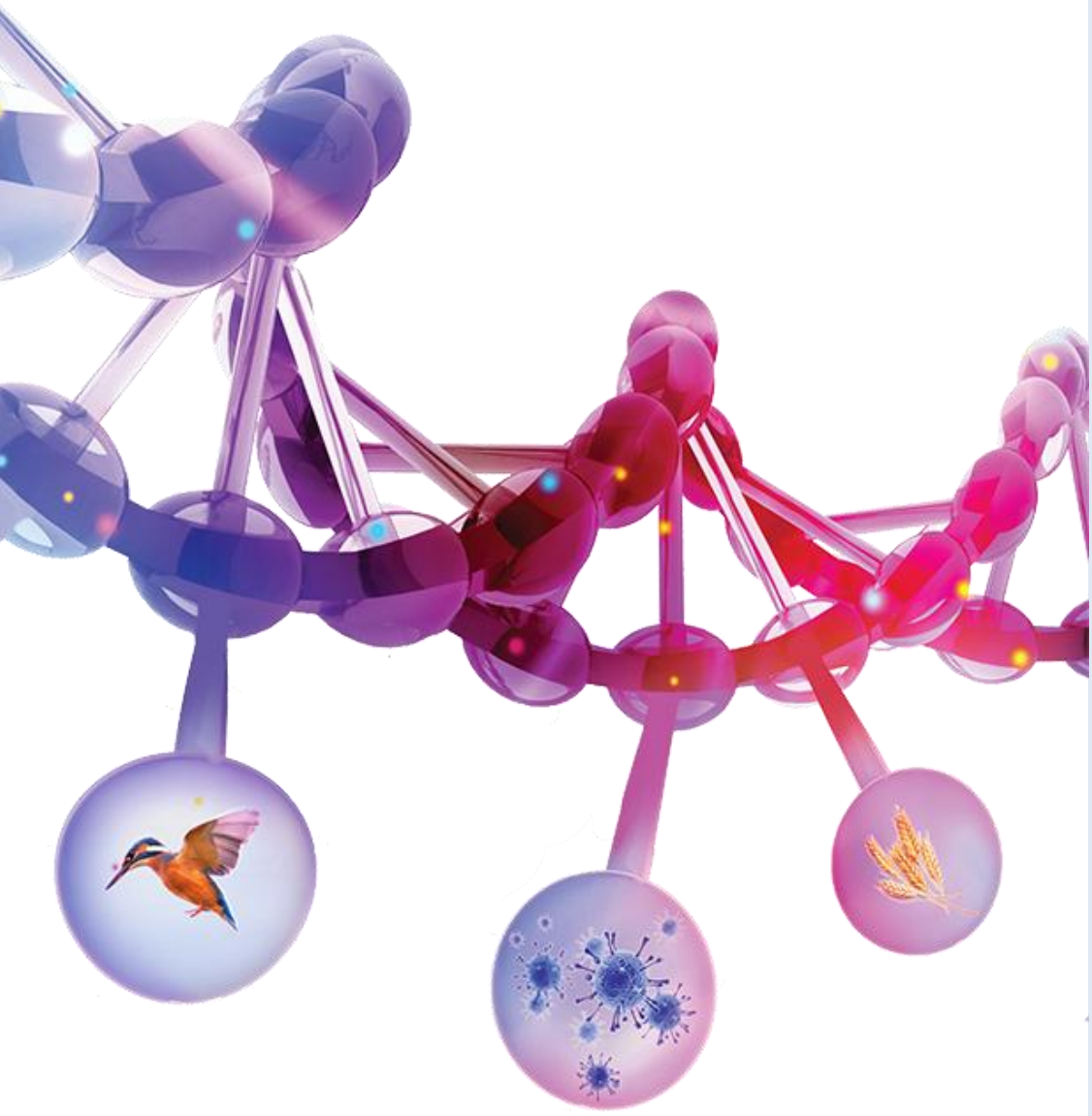
VALIDATION

eTaq flex kit

SYL1003

Document date: 27 augustus 2026



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1. Materials and setup

The validation of the eTaq flex kit was performed with the **SYL113 IPC quantification kit**[1].

Table 1. Specific components covered in this validation

Component	Manufacturer	Reference number
eTaq flex buffer	Sylphium	SYL1003/1
eTaq DNA polymerase	Sylphium	SYL1003/2
PCR grade water	Sylphium	SYL1003/3

Table 2. General components, consumables and equipment used in this validation.

Component	Manufacturer	Reference number
Primers & probe mix (10x)	Sylphium	SYL113/1
Positive control 10^7/2 µL	Sylphium	SYL113/2
Real-Time PCR System	Applied Biosystems	7300
Rox Reference dye	Gene-on	F153
PCR plate without skirt	Sarstedt	721.978.202
SafeSeal micro tube 2 mL	Sarstedt	72.695.500
Biosphere filter tips	Sarstedt	70.760.213
Biosphere filter tips	Sarstedt	70.760.211
Biosphere filter tips	Sarstedt	703.050.255
Sealing tape	Sarstedt	951.994

[1] https://sylphium.com/article-page/?apn=61

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2. Analytical sensitivity, fluorescence output signal and efficiency

Standard solutions containing nominal target concentrations of 10^6, 10^5, 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 target copy per reaction**, demonstrating single-copy detection capability under the tested conditions (**Figure 1**). 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 eTaq flex kit in combination with the **SYL113** primer and probe set was 98.4%, which was within the predefined acceptable efficiency range of 90-110% (**Figure 2; Table 2**).

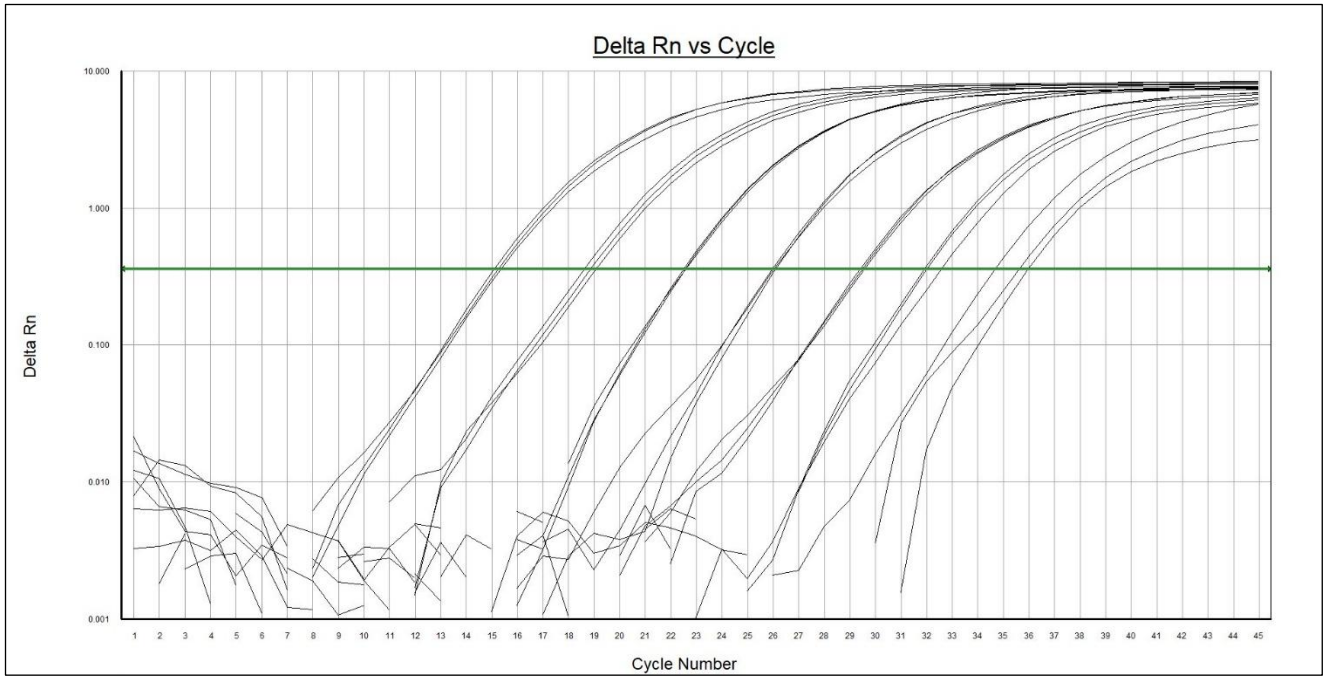


Figure 1. Amplification curves obtained with SYL113 using the eTaq flex kit.

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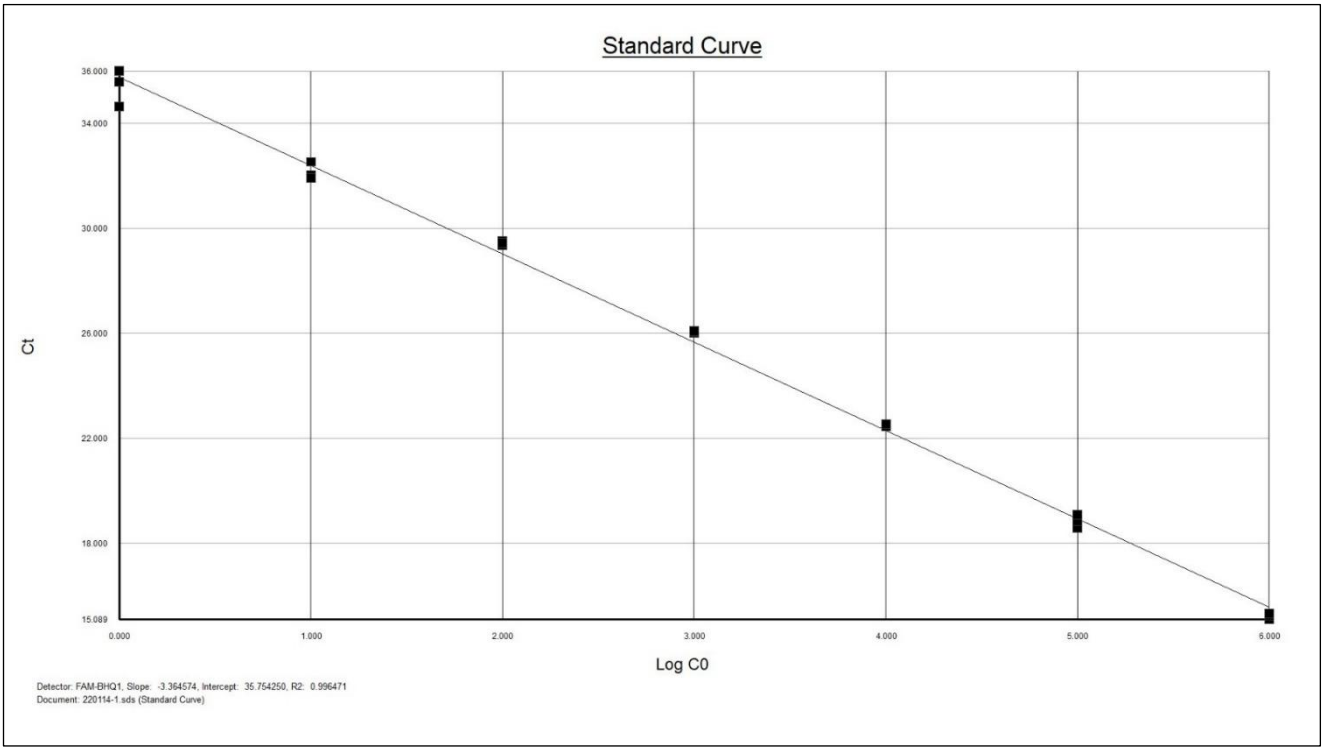


Figure 2. Standard curve obtained with SYL113 using the eTaq flex kit, based on nominal target concentrations of 10^6, 10^5, 10^4, 10^3, 100, 10, and 1 copies per 25 µL reaction.

Table 2. Standard curve parameters obtained with SYL113 using the eTaq flex kit.

Parameter	Result
Slope	-3.36
Amplification efficiency (%)	98.4
R^2	0.996

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3. Resistance to humic acids

The performance of the eTaq flex kit was evaluated in the presence of increasing concentrations of humic acids (Sigma-Aldrich). Samples were prepared according to Uchii et al.[2] and contained 0, 250, 500, or 1,000 ng/µL humic acids, together with a fixed concentration of 200 target DNA copies/µL.

qPCR reactions were performed in duplicate using a total reaction volume of 50 µL and a sample volume of 5 µL. The eTaq flex kit was compared with the best-performing PCR hot start mix (EMM), as reported by Uchii et al.[2]. Both mixes were used according to their respective manufacturer's protocols[3].

Results:

The eTaq flex kit successfully detected the target DNA in all samples containing 0–1,000 ng/µL humic acids. In contrast, EMM was unable to detect the target DNA at humic acid concentrations above 500 ng/µL.

At all tested humic acid concentrations above 0 ng/µL, the eTaq flex kit showed greater resistance to inhibition than EMM, as indicated by lower Cq values and successful amplification at 1,000 ng/µL humic acids (**Figures 3-5**).

[2] Uchii, K., Doi, H., Okahashi, T., Katano, I., Yamanaka, H., Sakata, M. K., et al. (2019). Comparison of inhibition resistance among PCR reagents for detection and quantification of environmental DNA. *Environ. DNA* 1, 359–367.
[3] https://www.thermofisher.com/order/catalog/product/4396838

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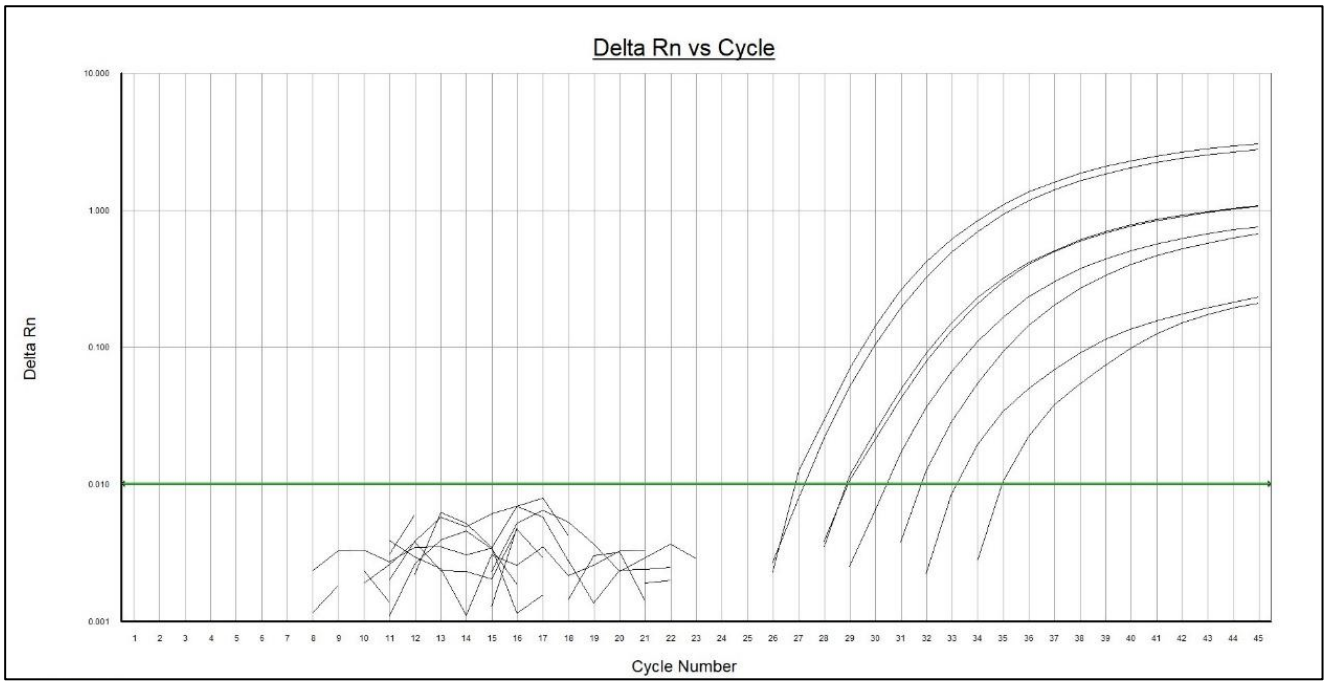


Figure 3. Logarithmic fluorescence amplification curves obtained with the eTaq flex kit at humic acid concentrations of 0, 250, 500, and 1,000 ng/µL sample.

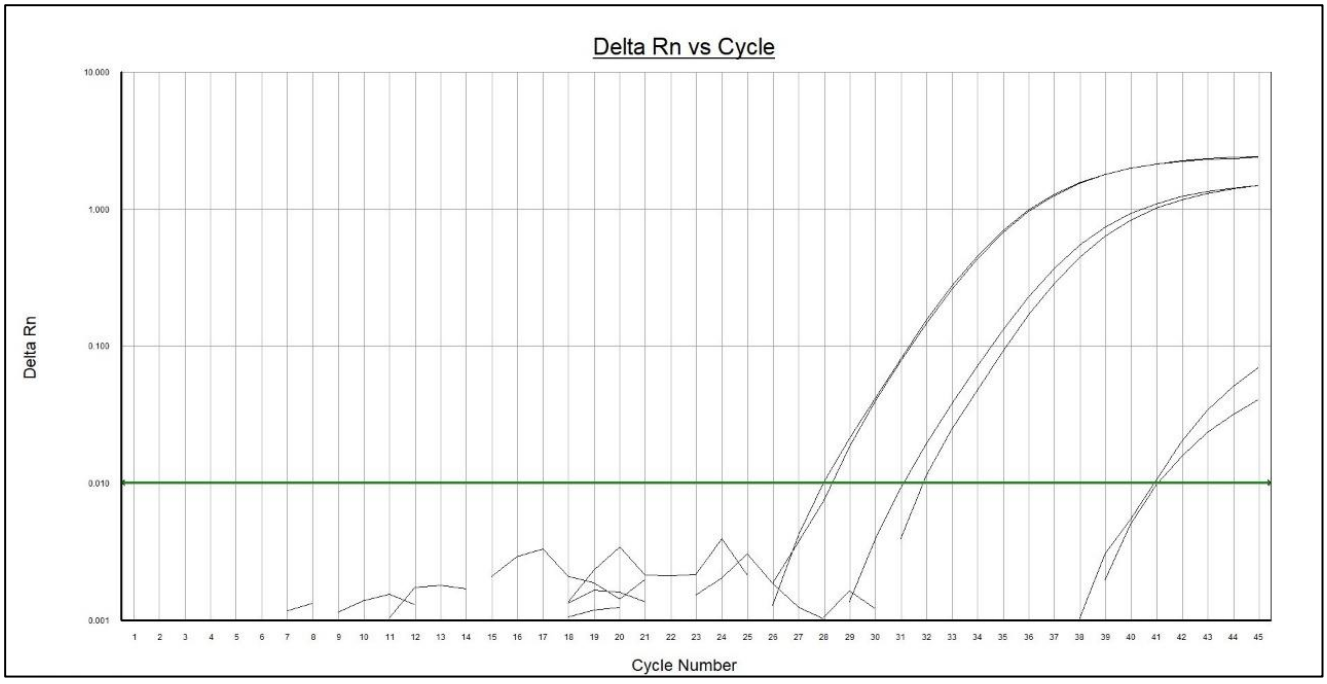


Figure 4. Logarithmic fluorescence amplification curves obtained with EMM at humic acid concentrations of 0, 250, 500, and 1,000 ng/µL sample.

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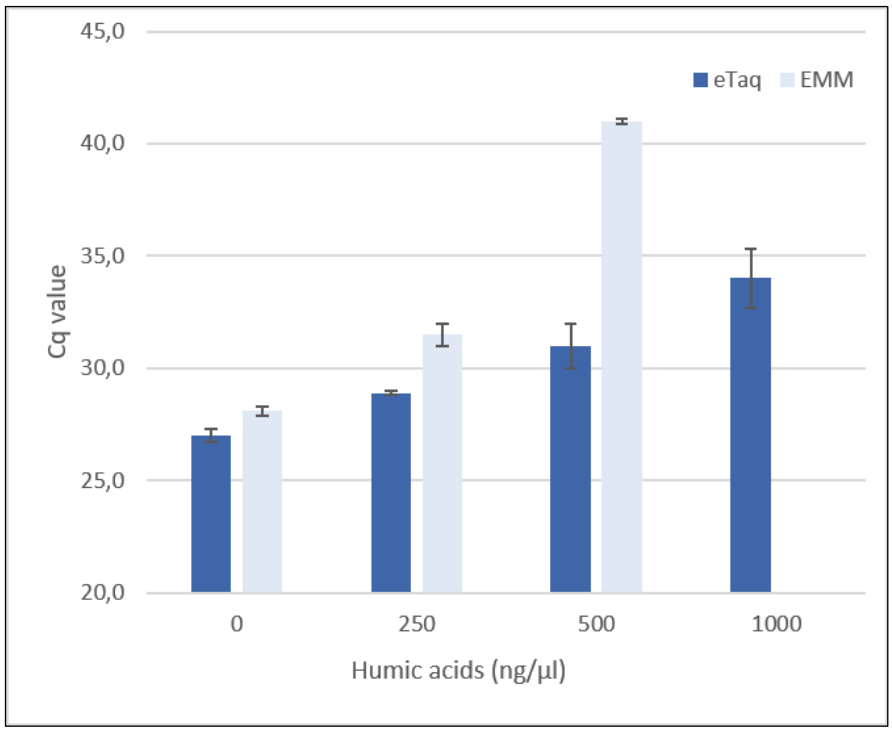


Figure 5. Cq values obtained with the eTaq flex kit and EMM at different humic acid concentrations and a fixed target DNA concentration. Lower Cq values indicate less inhibition. No fluorescent signal was detected with EMM at 1,000 ng/µL humic acids.

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