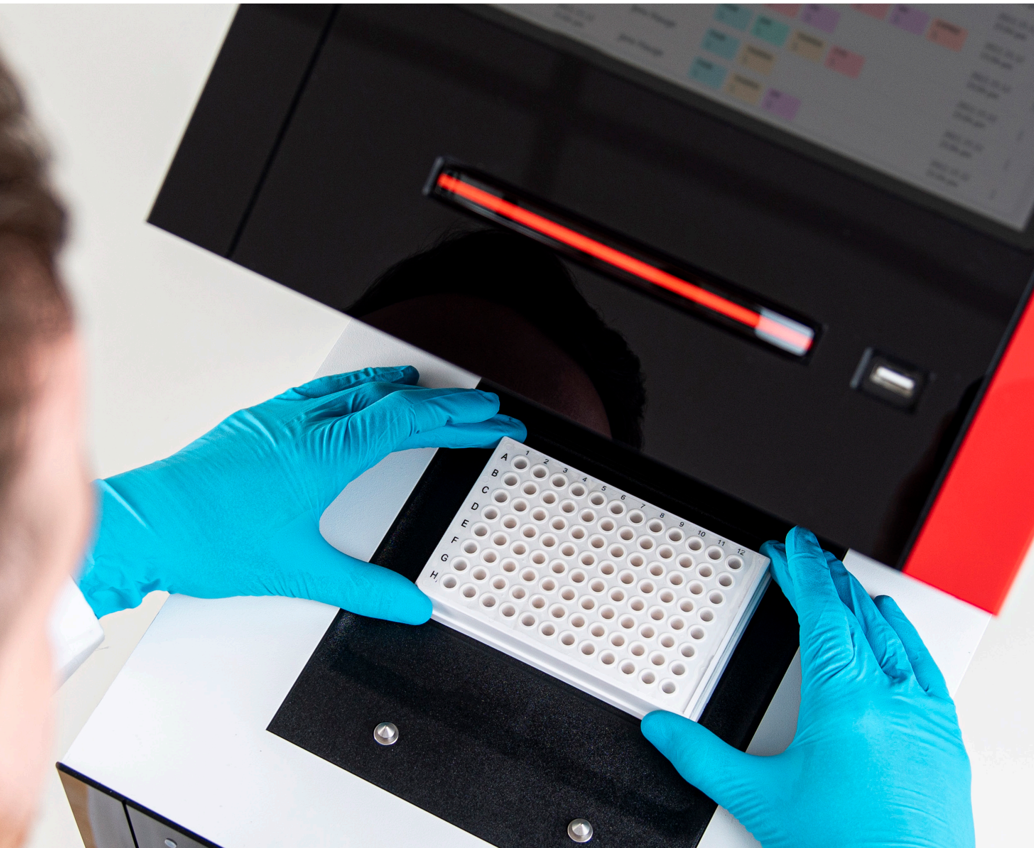


Challenge
Simple and easy improvement of real-time PCR results.

Solution
Using optimized plastic ware, up to four times stronger real-time PCR signals can be generated.

Intended audience
qPCR users in general

Plastic makes the difference: Ideal real-time PCR signals with optimized plastic products

Introduction

For quantitative PCR in 96 well format a variety of microplates provided by different suppliers are available. The most important difference in those plates is their color, as they are available in standard clear, opaque black and white. Although clear plates are easier to use due to the better visibility of liquids through the well walls and black plates give the lowest background signals, white microplates stand out in performance. Clear plates struggle with light-scattering through the plastic which results in loss of intensity. In contrast, white plates reinforce signal intensities due to reflection of light in the wells which leads to enhanced signal intensity and Ct values for the real-time assays.
When using white microplates, significant differences in signal intensity as well as in shifts of the Ct values can be shown. Hence, white microplates optimize qPCR experiments enormously without effecting costs and handling.

Materials and Methods

Chemicals and consumables

- A&A Biotechnology RT PCR Mix SYBR®: 2008-100
- *E.coli* K12 plasmid (1x 10^6 copies)
- *E.coli* K12 specific primers
- Water for molecular biology
- 96 Well PCR Plate (0.2 mL; LP), full-skirted, white: 844-70038-S
- 96 Well PCR Plate (0.2 mL; LP), full-skirted, transparent: 844-70035-0

Sample preparation

Table 1: Preparation of qPCR master mix for 1 sample

	Stock conc.	Final conc.	Volume
A&A Biotechnology RT PCR Mix SYBR®	2x	1x	10 µL
E.coli K12 plasmid	1x10^6 copies 1:10 dilutions	-	0.2 µL
E.coli K12 specific primer FWD	50 µM	0.5 µM	0.2 µL
E.coli K12 specific primer REV	50 µM	0.5 µM	0.2 µL
Water for molecular biology	-	-	9.4 µL

Table 2: Preparation of dilution series

Dilution	Volume of plasmid	Volume of water	Final concentration
Dilution 1	1 µL of plasmid	9 µL	1x 10^6 copies
Dilution 2	1 µL of Dilution 1	9 µL	1x 10^5 copies
Dilution 3	1 µL of Dilution 2	9 µL	1x 10^4 copies
Dilution 4	1 µL of Dilution 3	9 µL	1x 10^3 copies
Dilution 5	1 µL of Dilution 4	9 µL	1x 10^2 copies
Dilution 6	1 µL of Dilution 5	9 µL	1x 10^1 copies

Instrumentation

The qTOWERiris including the Color Module 1 (455 nm / 515 nm) was used for the measurements.

Table 3: Temperature and time protocol

Step	Cycle	Profile	Temperature	Holding time	Ramp rate
1	1	Initial denaturation	95 °C	2 min	max.
2	50	Denaturation	95 °C	5 sec	max.
		Annealing	58 °C	5 sec	max.
		Elongation*	72 °C	20 sec	max.
3	1	Melting curve	60 °C - 95 °C		

* Data acquisition: qTOWERiris Color Module 1 with Gain 5

	1	2	3	4	5	6	7	8	9	10	11	12
A				White plate				Clear plate				
B			D1	D1	D1		D1	D1	D1			
C			D2	D2	D2		D2	D2	D2			
D			D3	D3	D3		D3	D3	D3			
E			D4	D4	D4		D4	D4	D4			
F			D5	D5	D5		D5	D5	D5			
G			D6	D6	D6		D6	D6	D6			
H												

Figure 1: Plate layout for the real-time PCR run

Results and Discussion

Figure 2 shows the amplification plots of dilution series (tenfold) from 10^6 to 10^1 copies using *E.coli* K12 plasmid and K12-gene specific primer. Using white microplates in quantitative real-time PCR experiments results in total fluorescence intensities that are more than four times higher compared to signals obtained with transparent plastic ware.

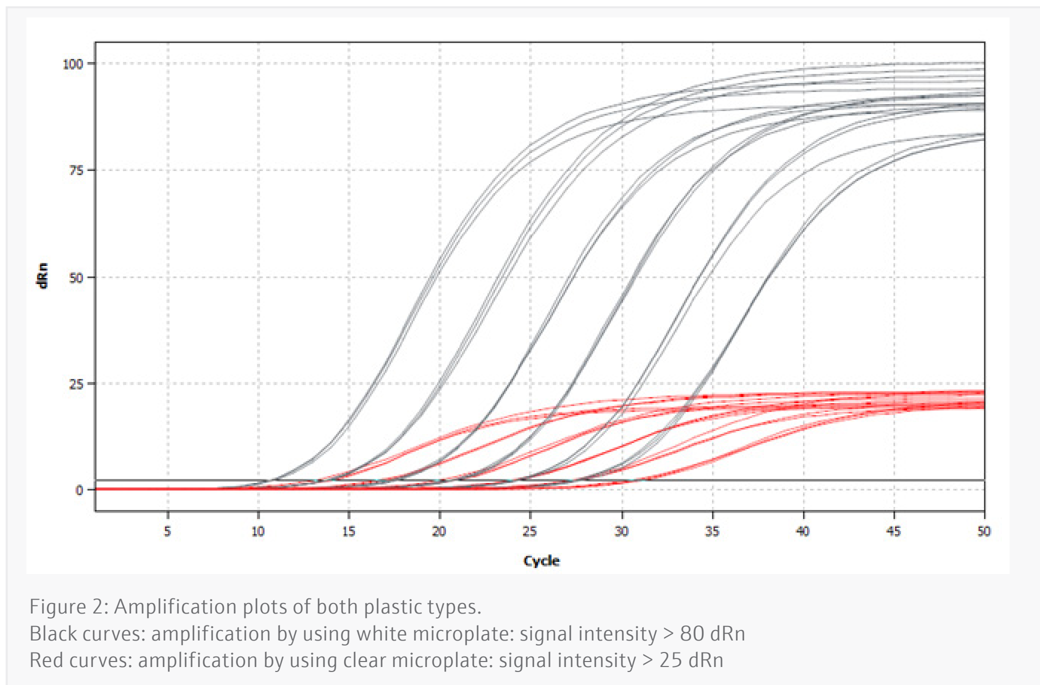
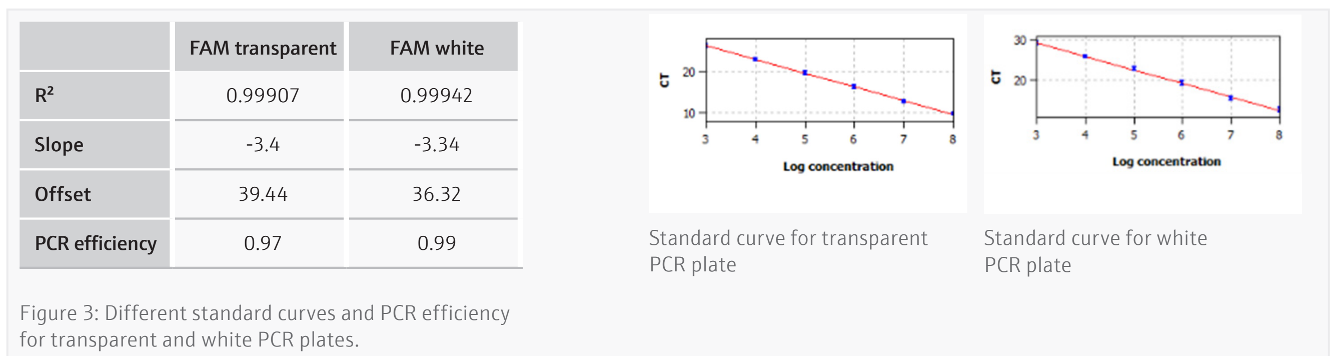


Figure 2: Amplification plots of both plastic types.
Black curves: amplification by using white microplate: signal intensity > 80 dRn
Red curves: amplification by using clear microplate: signal intensity > 25 dRn

Figure 3 shows the different standard curves for transparent and white PCR plates using *E.coli* K12 plasmid and K12-gene specific primer.



	FAM transparent	FAM white
R^2	0.99907	0.99942
Slope	-3.4	-3.34
Offset	39.44	36.32
PCR efficiency	0.97	0.99

Figure 3: Different standard curves and PCR efficiency for transparent and white PCR plates.

Standard curve for transparent PCR plate

Standard curve for white PCR plate

Additionally, all Ct values detected in clear microplates are shifted upwards with approximately two to three cycles compared to the white plastic material. These results are shown in table 3.

Table 3: Comparison of Ct values

	No. of copies	Mean Ct		ΔCt
		Clear	White	
D1	10^6	13.81	10.82	2.99
D2	10^5	16.88	13.89	2.99
D3	10^4	20.64	17.55	3.10
D4	10^3	24.28	20.88	3.40
D5	10^2	27.54	24.24	3.30
D6	10^1	30.98	27.71	3.27

Conclusion

The use of opaque white consumables (Analytik Jena) enhances the real-time PCR signal by optimizing the amount of light returning to the detector. In practical terms, as demonstrated in the aforementioned tests, this leads to an enhancement in the sensitivity and intensity of qPCR assays, which can be a crucial factor for detecting low copy numbers.

In addition, the A&A Biotechnology RT PCR Mix SYBR® (A&A Biotechnology) is ideally adapted to real-time PCR using qTOWERiris. It provides a ready-to-use master mix including a highly specific Taq DNA polymerase with no additional activation time at 95 °C, high-quality dNTPs, optimal $MgCl_2$ concentration and an intercalating fluorescent dye that doesn't inhibit qPCR.
The mix has been validated using the most common real-time PCR instruments and can be used to detect any DNA template including genomic, cDNA and viral sequences. Especially extremely low copy number targets can be amplified with high efficiency. The proprietary technology prevents formation of primer dimers and non-specific products leading to improved reaction sensitivity and specificity.

Figure 4: qTOWERiris Series

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