Challenge

Simple and easy improvement of real-time PCR results.

Solution

Using optimized plastic ware can generate up to four times stronger real-time PCR signals.

Comparison of Real-time Signals using White or Clear Microplates

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 readings, 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 cost and handling.

Materials and Methods

Chemicals and consumables

- 2x innuMIX qPCR MasterMIX SyGreen
- Tobacco gDNA (1x 106 copies), tobacco specific primers (actin gene) and water for molecular biology
- 0.2 mL 96 well microplate, white, frame

Table 1 : Preparation of qPCR master mix for 1 sample

	Stock conc.	Final conc.	Volume
innuMIX qPCR MasterMix SyGreen	2x	1x	10 µl
Water for molecular biology	-	-	9.4 µl
Tobacco gDNA	See below	See below	0.2 µl
Primer fwd (actin gene)	50 µM	0.5 µM	0.2 µl
Primer rev (actin gene)	50 µM	0.5 µM	0.2 µl
Final Volume			20 µl

Table 2 : Preparation dilution series

	Start conc.	Final conc.	Tobacco gDNA	Water mol. bio.
Dilution 1	1x 10^6 copies	1x 10^6 copies	10 µL	-
Dilution 2	1x 10^6 copies	1x 10^5 copies	1 µL	9 µL
Dilution 3	1x 10^5 copies	1x 10^4 copies	1 µL	9 µL
Dilution 4	1x 10^4 copies	1x 10^3 copies	1 µL	9 µL
Dilution 5	1x 10^3 copies	1x 10^2 copies	1 µL	9 µL
Dilution 6	1x 10^2 copies	1x 10^1 copies	1 µL	9 µL

Instrumentation

The qTOWER³ including the Color Module 1 (470 nm / 520 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	5 °C/s
2	45	Denaturation	95 °C	15 sec	5 °C/s
		Annealing	58 °C	15 sec	5 °C/s
		Elongation*	72 °C	45 sec	5 °C/s

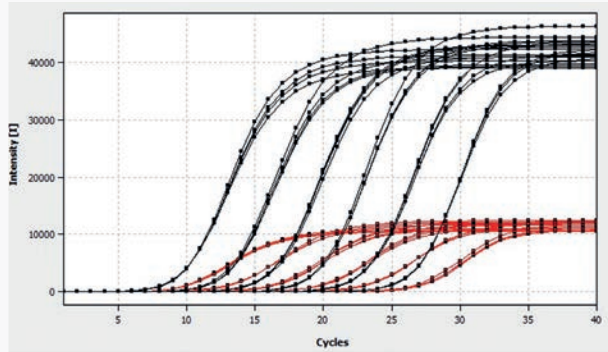
* Data acquisition: Color Module 1 (470 – 520 nm) and Gain 5

	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												

Fig. 1 : Plate layout for qPCR run

Results and Discussion

Figure 2 shows the amplification plots of dilution series (tenfold) from 10^6 to 10^1 copies using tobacco gDNA and actin-gene specific primer. Using white microplates in quantitative real-time PCR experiments leads to total fluorescence intensities, which are more than four times higher in comparison to the signals achieved by transparent plastic ware.



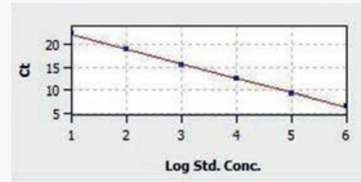


Fig. 2: Data analysis by using absolute quantification : Amplfiation plots of both plastic types and standard line of white plastic ware are shown

black plots: amplification by using white microplate, R^2 = 0.99943, efficiency = 0.99, signal intensity > 40.000 IU
red plots: amplification by using clear microplate, R^2 = 0.99931, efficiency = 0.98, signal intensity > 10.000 IU

Table 2 : Comparison of Ct values

	No. of copies	Mean Ct		ΔCt
		Clear	White	
D1	10^6	10.74	8.77	1.97
D2	10^5	14.67	12.06	2.61
D3	10^4	17.59	15.30	2.29
D4	10^3	20.73	18.50	2.23
D5	10^2	24.13	21.95	2.18
D6	10^1	28.07	25.61	2.46

Additionally all Ct values detected in clear microplates are shifted with approximately two cycles to higher values compared to the white plastic material.

Conclusion

The use of opaque white consumables (Analytik Jena) enhances the qPCR signal by optimizing the amount of light returned to the detector. In practice, as demonstrated in the tests described above, this has the effect of improving the sensitivity and intensity of qPCR assays which may be an important factor for low copy number detection.
In addition, the innuMIX qPCR MasterMIX SyGreen (Analytik Jena) is ideally adapted to real-time PCR using qTOWER³.
It provides a ready-to-use master mix including a highly specific Taq DNA polymerase with no additional activation time at 95 degrees Celsius, 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.

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