Influence of PCR consumables on the accuracy of real-time PCR experiments

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Introduction

Classical PCR and qPCR plates are one-component plates made out of polypropylene (PP). PP is the best plastic material for PCR tubes as it is chemically inert and allows for the production of ultra-thin tube walls which is important for fast temperature transfer. While PP has become the standard material for PCR consumables, some of its properties question its suitability for applications like qPCR and NGS. The material characteristics of PP exhibit a Vicat Softening Temperature (VST) of 90°C and a coefficient of thermal expansion of 180x10-6 K-1 which are potential weaknesses for its usage at typical (q)PCR temperatures. Thermal expansion of plate seals does not match the expansion of the plates and therefore is likely to lead to weakening of the seal and evaporation of the well contents, particularly in the corner and outer wells[1,2]. Two-component PCR plates offer improved properties for PCR with a rigid frame made of polycarbonate (PC) and tubes made from PP.

Evaporation has a significant effect on the reaction conditions resulting in noticeable effects, especially for qPCR. Identical samples can exhibit significant differences in their Ct values, depending on their position on the plate. This often remains unnoticed as triplicates are typically placed in neighbouring wells which are affected by similar levels of evaporation.

PP plate properties during PCR

Plastic polymers have differing VSTs leading to varying stabilities at higher temperatures. PP has a VST which falls in the critical range of a PCR reaction, whereas the VST of PC is greater than this.

Vicat Softening Temperature

Softening point for materials with no definite melting point

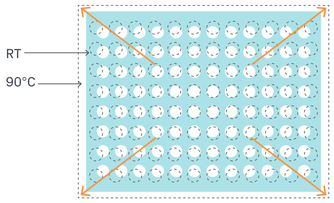
Polypropylene >90°C

Polycarbonate >145°C

Typical distortion of 96-well plates during PCR was measured by filling each well of either 1- or 2-component plates with 5µl water and running them in a Thermo Px2 Cycler for 1min 94°C, 10x cycles of (30sec 94°C, 30sec 55°C, 30sec 72°C), 5min 72°C, holding at 4°C. Average measurements of 10 plates are shown. X- and Y- measurements were taken at the middle section of the plate skirt and distortion was calculated as the maximum deviation from a flat plane.

Plate type	X-axis	Y-axis	distortion
FrameStar 96, skirted	0.02mm	0.02mm	0.03mm
1-component 96 well PP plate	1.18mm	1.69mm	1.80mm
Framestar 384	0.02mm	0.02mm	0.03mm
1-component 384 well PP plate	1.22mm	1.82mm	2.20mm

Table 1. 2-component Framestar plates show minimal thermal expansion after PCR compared to 1-component PP plates.



Expansion and contraction will weaken the contact between the seal and plate, particularly in the corner positions and outer rows. This increases the risk of evaporation in these areas. This effect is more pronounced when adhesive seals are used (opposed to heat seals).

Figure 1. Standard PP plates expand by up to 2mm during thermal cycling leading to movement of the wells away from the centre.

60% of evaporation issues reported to 4titude involve the outer wells of the plates, with only 10% involving the inner wells.

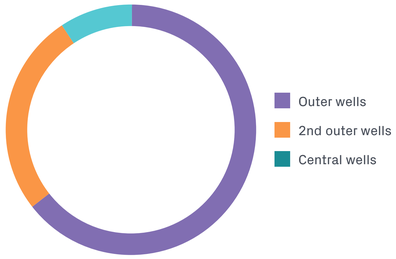
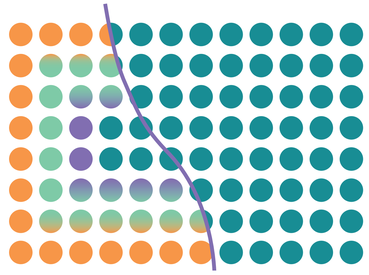


Figure 2. Frequency of evaporation issues in different areas of 96 well PCR plates based on technical enquiries.



Polypropylene / Polycarbonate

Figure 3. Risk of evaporation in wells during a (q)PCR run in a 1-component PP plate (left) and a 2-component PP (wells) and PC (frame) plate (right). Red denotes high risk, yellow is medium risk and green is low risk of evaporation.

Evaporation during PCR

Evaporation from 1- and 2-component plates were tested by determining the weight loss during a mock PCR run. The outer 64 wells (2 rows) of each 96 well, 1- or 2- component plate were filled with 10µl H2O and sealed with an adhesive qPCR seal (4titude, cat no 4ti-0560). The weight of the plates were measured before and after a PCR run of 30x cycles of (15secs 95°C, 15secs 55°C) and the change converted into water volume loss. This experiment was then repeated with only the inner 32 wells filled with 10µl water.

Plate type		Starting weight	Weight post PCR	Weight loss	Volume loss total / well	Percentage loss
1-component PP plate	Outer 64 wells	17.299	17.118	0.181	181µl / 2.8µl	28%
	Inner 32 wells	17.132	17.078	0.054	54µl / 1.69µl	16.9%
FrameStar 2-component	Outer 64 wells	26.230	26.193	0.037	37µl / 0.57µl	5.7%
	Inner 32 wells	25.841	25.824	0.017	17µl / 0.53µl	5.3%

Table 2. Weight and volume loss from 1- and 2- component 96 well PCR plates. Results shown are averages from 5 plates of each type.

Percentage volume loss in 1-component plates compared to 2-component plates

- Almost 5 times greater in the outer wells
- 3 times greater for the inner wells
- Consistently low across FrameStar plates
- Greater in the outer rows for standard plates compared to the inner wells

Significant loss of water in PCR reactions can alter the pH value of the reaction and consequently the enzyme efficiency. Differential evaporation across the plate has been linked with greater variation in Ct values which is particularly prevalent in the corner and outer wells.

Sealing

Choosing the best sealing method for your PCR plates is equally important as selecting the correct plate. The use of heat seals leads to a greater number of wells from a 384 well plate with detectable liquid in them following a PCR run with very low starting volumes. The best combination for optimal PCR performance are 2-component plates with heat seals.

	Standard PCR Plate		Two-component PCR Plate	
	Reaction volume (nl)	% wells with detectable liquid	Reaction volume (nl)	% wells with detectable liquid
Adhesive sealing	500	81.97	500	96.83
	1000	80.74	1000	100.00
	2000	82.34	2000	100.00
Heat sealing	500	90.63	500	97.27
	1000	99.61	1000	100.00
	2000	98.44	2000	100.00

Table 3. Sealing efficiencies with standard and FrameStar 384 well PCR Plates using adhesive or heat sealing

Conclusion

A solution to the problem of evaporation related qPCR inaccuracies is the usage of two-component plates. These plates consist of tubes made out of PP but a frame made out of PC. PC does not show significant temperature-dependant expansion and contraction as the VST of this material is greater than those used in PCR cycles. The use of a more rigid frame which does not alter size or shape during PCR reduces the distortion and also weakening of the seal attachment to produce more reliable data for quantification. Reduced risk of evaporation with 2-component plates facilitates the miniaturisation of reaction volumes and therefore provides opportunities for savings within labs.

References

[1]Reiter, M and Pfaffl, M.W. (2008) Biotechnology & Biotechnological Equipment 22:3, 824-828

[2]Eppendorf AG (2009) Biotechniques Protocol Guide 2009, 49

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