Applications

12/02 eppendorf twin.tec PCR Plate No. 45

An Assessment of eppendorf twin.tec PCR Plate performance in PCR applications.

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Abstract

To evaluate the performances of eppendorf 96-well twin.tec PCR Plates we carried out PCR reactions with twin.tec plates and PCR microplates from another commercial microplate supplier and compared the reaction products by agarose gel analysis. Additionally, simultaneous temperature profiles were recorded in both plates using dual temperature probes during the course of a PCR reaction. As a result of these experiments, we found differences in efficiencies of the amplification of a 2 kb target. Further, there were also differences in temperature profiles between the two plates. In this article, we present evidence to substantiate that eppendorf twin.tec PCR Plates have excellent temperature transfer characteristics and outperform competitor plate.

Introduction

There are several suppliers of 96 well microplates for performing PCR reactions, and this format has become the most popular for high throughput research. One of the prime requirements for a good PCR microplate is the efficient transfer of block temperature to the reaction sample. This feature translates into a robust and reliable PCR reaction. Another desirable feature in a microplate is the mechanical sturdiness for unimpeded handling by robotic arm for automation. Eppendorf recently introduced the 96-well twin.tec PCR Plates to life science research community with the goals of addressing the aforementioned requirements. Twin.tec PCR Plates represent a new generation of 96-well plate for PCR applications, which combines the advantages of two materials – polycarbonate and polypropylene. Polycarbonate is a rigid and stiff material and is therefore used to impart the required mechanical stability for the frame and plate surface before, during and after PCR. The walls of the wells are made of polypropylene, an ideal material for achieving rapid heat transfer from the block to the sample thus leading to optimal results. Further, the manufacturing process and use of special “virgin” propylene provides thin walled wells and a “snug fit” in the thermocycler metal block resulting in uninterrupted heat transfer from the block to the sample. The well-walls of twin.tec PCR Plates are 20 % thinner than the conventional thin-walled tubes and plates and this translates into more efficient heat transfer to sample as evidenced in the results.

In this article, we have made a comparative assessment on the performance of eppendorf 96-well twin.tec PCR Plate with a microplate from a commercial supplier. We amplify a 2 kb fragment from the beta-globin region using both these plates. The gel analysis indicates that the amplified products are formed with better consistency in twin.tec PCR Plates. We also measure the temperature profile and present data that supports the superior heat transfer characteristics of eppendorf 96 well twin.tec PCR Plates.

Materials and Methods

Genomic DNA from blood was isolated using Eppendorf Perfect gDNA Blood Mini Kit. All PCR reactions were performed in four sets on the Eppendorf Mastercycler® gradient thermocycler using human genomic DNA as template. The primers were synthesized by Sigma Genosys.

The PCR target that was selected to compare the microplates was a 2.0 kb fragment from the human beta-globin gene. It was amplified using Taq DNA polymerase (eppendorf) and the same protocols were followed for both twin.tec PCR Plate and competitor plates. All reactions were performed in four sets and were run on a 1 % agarose gel for analysis.

For amplification the following primers, reaction components and cycling conditions were used:
Forward primer: 5’-GAA GAG CCA AGG ACA GGT AC-3’
Reverse primer: 5’-CCT CCA AAT CAA GCC TCT AC-3’
Reaction components:
1.68 x PCR buffer
(2.5 mM $MgCl_2$ final concentration)
0.2 µM of each primer
0.2 mM dNTP’s
1.25 units eppendorf Taq DNA polymerase
50 ng human gDNA
MBG Water to 25 µl total reaction volume
PCR program:

94 °C	2 minutes initial denaturation
35 cycles:	
94 °C	1 minute denaturation
55.5 °C	20 seconds annealing
72 °C	2 minutes extension
65 °C	5 minutes final extension
10 °C	hold

Measurement of temperature profile during PCR cycle:

The temperature profiles in the wells of microplates were measured during the course of PCR cycling with Testo 945 instrument (Test, Inc. NJ, USA). This unit was calibrated and certified to NIST standards, and is routinely used to calibrate cyclers in laboratories. The temperature measurements were carried out simultaneously in both the plates. Our goal was to record the temperature profiles on the same instrument with both the plates. To address this objective, the plates were cut in half and placed at the center of the thermal block. The high precision thermocouple probes were placed at the center of the wells and the temperature was recorded following the start of a PCR cycle. The real time recording of the temperature profile was viewed using Comsoft 3 software, also from Testo, Inc.

Comparison of efficiency of amplification

eppendorf twin.tec PCR Plate and a commercially available PCR plate were evaluated for the amplification of a 2 kb human beta-globin fragment. The main criterion for this evaluation is efficiency of the plates for target amplification. Upon comparison of the yields of the product in gel electrophoresis, (Fig. 1) it was obvious that eppendorf twin.tec PCR Plates performed well in terms of yield of PCR products. Note that the samples shown in this analysis are from the same wells in both plates, D5 – D8 and were chosen as good representatives of the entire plate.

Figure 1: Amplification of 2.0 kb human beta globin target using eppendorf 96 well twin.tec PCR Plate compared to a commercially available polypropylene microplate.
Lanes 1 and 6: MWM (1 KB Ladder, Invitrogen)
Lanes 2–5: Competitor polypropylene microplate
Lanes 7–10: twin.tec PCR Plates

The results demonstrate that eppendorf twin.tec PCR Plate facilitates a highly efficient amplification of the 2 kb fragment with a consistent product yield in all the four reactions (Fig. 1). These results also show that the yield in twin.tec PCR Plate is higher as evidenced by the higher intensity of the bands in all the four wells. This observation prompted us to further examine any differences in temperature profiles in both the plates.

Comparison of temperature profiles

The simultaneous sample temperature measurements during the course of PCR cycle in both plates are shown in Figure 2. The green line represents the temperature trace of eppendorf twin.tec PCR Plate while red represents the trace of competitor plate. It is very clear from this figure that there are differences in the temperature attained by individual wells in both the microplates. An annealing temperature of 55.5 °C was set for this experiment. Note that the red line representing the competitor does not reach this temperature, while the green trace (twin.tec) does. Following annealing is the elongation step, for which a temperature of 72 °C was selected. Again, the red trace does not reach this temperature, suggesting that a sample in that well would not be exposed to the set temperature. The same is also true for the denaturation step. We see a very clear pattern in the temperature transfer abilities between the twin.tec PCR Plate and the competitor's plate (Figure 2). It is obvious that the twin.tec PCR Plates attained the desired temperature exactly in every step and every cycle of the PCR reaction, while the competitor plate always lagged behind. We can attribute this result to the fact that the walls of the wells of twin.tec PCR Plates are even thinner than the conventional thin wall tubes and this feature enables rapid heat transfer. This feature ensures more robust conditions for the PCR reaction and thus more reliable results.

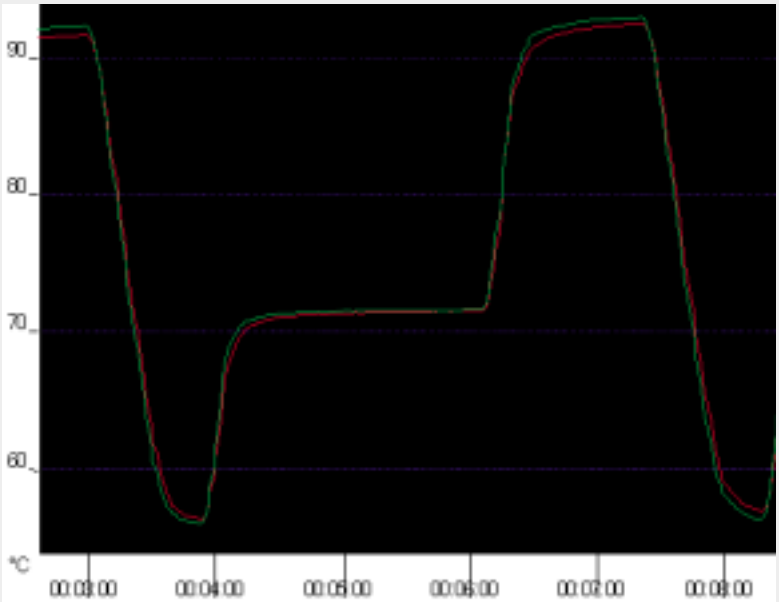

Figure 2: Temperature profile of twin.tec PCR Plate vs. a competitor's PCR plate.
Green trace = twin.tec
Red trace = competitor plate

When we take a closer look at a specific part of this graph, the difference in temperature transmission is even more obvious. An enlarged area of the annealing step is shown in Figure 3. A temperature difference of ~1 °C can be clearly seen in the annealing step of the PCR reaction between the two plates. The samples in the competitor's plate cannot attain the set temperature (55.5 °C) because of this inefficient heat transfer. This difference in temperature transfer between the plates helps to explain the results in previous experiment about the robust product formation with twin.tec PCR Plate.

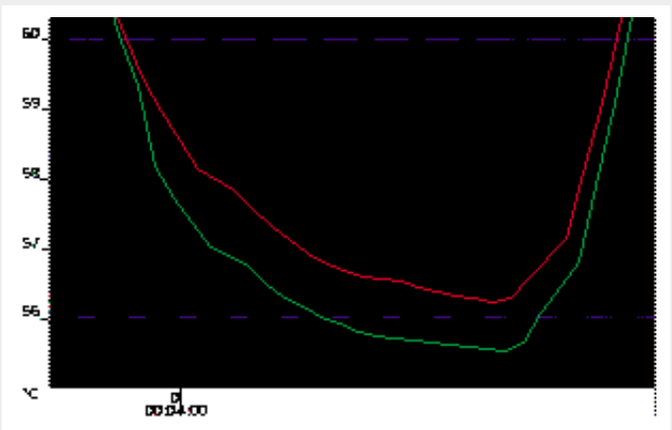

Figure 3: Closer view of the temperature recordings.
Green trace = twin.tec
Red trace = competitor plate

From the foregoing discussion, we can conclude that the ideal micro-plates for PCR application are those that can effectively transfer heat to the reaction mixture. All of the results from above experiments point towards this characteristic of twin.tec PCR Plates, which make it a perfect choice for all temperature sensitive applications.

Conclusions

Eppendorf twin.tec PCR Plates offer many advantages over other PCR plates in the market. Primarily, twin tec PCR Plates increase specific yields of some PCR products to a greater extent than the competing plates. This increase in yield is probably due to the excellent heat transfer properties of twin.tec PCR Plates, which are evidenced by temperature recordings. The superior performances of twin.tec PCR Plates, combined with its structural rigidity, make it an excellent choice for all 96 well PCR applications.

Ordering information

Article	International Order no.	North American Order no.
twin.tec PCR Plate 96, skirted (wells colorless)		
Colorless, 25 pcs	0030 128.684	951 02 040-1
Yellow, 25 pcs	0030 128.656	951 02 042-7
Green, 25 pcs	0030 128.664	951 02 044-3
Blue, 25 pcs	0030 128.672	951 02 046-0
Red, 25 pcs	0030 128.680	951 02 048-6
twin.tec PCR Plate 96, skirted (wells black)		
Yellow, 25 pcs	0030 128.800	951 02 039-7
twin.tec PCR Plates 96, semi-skirted (wells colorless)		
Colorless, 25 pcs	0030 128.575	951 02 030-3
Yellow, 25 pcs	0030 128.583	951 02 032-0
Green, 25 pcs	0030 128.591	951 02 034-6
Blue, 25 pcs	0030 128.605	951 02 036-2
Red, 25 pcs	0030 128.613	951 02 038-9
twin.tec PCR Plate 384 (well colorless)		
Colorless, 25 pcs	0030 128.508	951 02 070-2
Yellow skirt, 25 pcs	0030 128.516	951 02 071-1
Green skirt, 25 pcs	0030 128.524	951 02 072-9
Blue skirt, 25 pcs	0030 128.532	951 02 073-7
Red skirt, 25 pcs	0030 128.540	951 02 074-5
Mastercycler® gradient	5331 000.010	950 00 002-3 (220 V) 950 00 001-5 (115 V)
Eppendorf Taq DNA Polymerase (250 U)	0032 002.307	954 14 001-6
dNTP Mix 200 µl (10 mM)	0032 003.001	954 14 300-7
Water, Molecular Biology Grade, 1 litre	0032 006.159	955 15 500-9
Perfect gDNA Blood Mini, 50 samples	0032 007.864	955 15 030-9

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