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APPLICATION NOTE No. 482

Improved Sustainability, Identical Performance: Comparison of Eppendorf twin.tec® Trace PCR Plates BioBased and Standard

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Abstract

With increasing environmental requirements imposed on life science laboratories, reduction of lab plastics-related carbon footprint without compromising their performance represents a collective challenge. To comprehensively address that, Eppendorf offers a constantly growing range of lab consumables made of polypropylene based on renewable feedstocks and designed to achieve identical performance.
In this study, the performance of Eppendorf twin.tec® Trace PCR Plates BioBased made of ISCC PLUS certified biobased material was assessed.

In the comparative evaluation of the PCR-relevant parameters: sealing properties, leaching levels, residual volume, and qPCR assay performance, the biobased twin.tec® plates showed performance identical to their standard counterparts made of fossil-sourced polypropylene. This confirms that the biobased material regarding its physical and chemical properties may be regarded equal to fossil-sourced polypropylene. Eppendorf twin.tec Trace PCR Plates BioBased offer a major improvement of renewable properties of lab plastics, making them more sustainable without compromising product quality and performance.

Introduction

Reduction of consumable-related carbon footprint in the lab poses increasing challenge. One of the most effective, and in life science still rare approaches to improve sustainable properties of lab plastics, is to use renewable feedstocks in their production process.

Taking lead in the global sustainability initiative, Eppendorf offers a continuously growing portfolio of various lab consumables made of an ISCC PLUS (International Sustainability and Carbon Certification) certified polypropylene applying the mass balance approach [1, 2]. The recent addition to this portfolio are Eppendorf twin.tec® Trace PCR Plates BioBased – a sustainable alternative to the well-established and known since decades standard Eppendorf twin.tec® PCR Plates.
In this Application Note we investigated the performance of Eppendorf twin.tec® Trace PCR Plates BioBased made of ISCC PLUS certified biobased material in comparison to their standard counterparts made of fossil-sourced polypropylene.
We assessed key, PCR-relevant, parameters: sealing/evaporation properties, residual volume, leachables levels and finally also qPCR assay performance.

Material and Methods

All tests described below were performed in three replicates and as three independent experiments. Following PCR Plates were assessed: Standard Eppendorf twin.tec® 96 well PCR Plates (order nr. 0030 128 648) and Eppendorf twin.tec® Trace 96 well PCR Plates Biobased (order nr. 0030 129 857).

Sealing/evaporation test
The Plates were filled with 50 µL of 0.5 % Orange G solution and sealed with Eppendorf Heat Sealing Films. After brief centrifugation the plates were weighted and cycled in PCR thermocycler: 1 x 94°C/2 min, 30 x (94°C/15 sec, 50°C/15 sec, 72°C/30 sec), 1 x 72°C/2 min. After cycling, the plates were cooled, weighted again and the rate of evaporation was calculated.

Leaching
48 wells of each PCR plate were filled with 100 µL of ultra-pure water in a chessboard pattern. The plates were sealed with the Eppendorf Heat Sealing Film and placed in Eppendorf Mastercycler® X50s for 40 min at 96°C. After cooling down and short mixing/spin down aliquots of 90 µL were transferred from each well into an assay plate (Greiner UV-Star Microplate 96). The absorbance spectra (220 nm to 400 nm) were measured for each well using Bio-Rad Microplate Spectrophotometer xMark™.

Residual volume
A working solution of 5 mM ABTS (2.2'-azino-bis (3-ethylbenzothiazolin-6-sulfonic acid)) was used. All pipetting steps were performed in the Eppendorf epMotion® 5075vt, using pipetting mode described in Eppendorf Userguide 005. 50 µL of ABTS solution were pipetted into each well and the exact same volume was subsequently removed during the next step and 100 µL of ultra-pure water was added. Subsequently, 50 µL from each well was transferred into an assay plate (Greiner UV-Star® Microplate 96). The concentration of ABTS in each well was assessed using Bio-Rad Microplate Spectrophotometer xMark™. For all experiments, the residual liquid remaining in each well was quantified by comparison to a standard curve.

qPCR assay performance
The qPCR assays detecting gDNA bacteria A. baumannii were processed using CFX96 Touch™ (Bio-Rad®) in a 20 µL reaction per well and using respective Eurogentec qPCR reagent system.

The plates were sealed with the Masterclear® real-time PCR film self-adhesive and after brief spin placed in thermocycler with the following qPCR program: 1 x 50°C/2 min, 1 x 95°C/3 min, 40 x (95°C/10 sec, 60°C/60 sec).

The selected fluorophore was SYBR (Ex 450-490 / Detection 515-530). The Cq-values and amplification data were analyzed using the CFX Maestro™ software from (Bio-Rad). Cq values were determined with PCR Base Line Subtracted Curve Fit and automatic threshold analysis method of the software.

Result and Discussion

The aim of this study was to comprehensively evaluate PCR-relevant features of the Eppendorf twin.tec® Trace PCR Plates made of biobased material in comparison to the well-established, standard Eppendorf twin.tec® PCR Plates produced from fossil-sourced polypropylene.

Sealing performance

Tight sealing of the PCR plates is a critical prerequisite to prevent excessive sample loss due to evaporation and is necessary for optimal performance of the PCR reaction and sample integrity. Frequent and fast temperature shifts during the PCR reaction pose considerable challenge to PCR sealing.

The results of sealing tightness test using a standard PCR cycling program are presented in figure 1 and indicate virtually no difference between fossil-sourced and biobased Eppendorf twin.tec® PCR Plates with evaporation values of 1.30% and 1.07% of sample loss respectively. Noticeably, the values observed for evaporation loss were well below the test acceptance level (3.00%), which assures a very good level of sample safety and PCR reaction reliability.

Leaching

Scientific evidence indicates that various compounds used during production process of consumables may be washed out of the plastic material (leach) and adversely affect various assay systems [3, 4]. In this Application Note we comparatively evaluated leachables levels of fossil-sourced and biobased Eppendorf twin.tec® PCR Plates. Figure 2 shows absorbance values obtained at various wavelengths for water samples incubated at 95 °C for 40 min. The absorbance values obtained at these wavelengths may yield false elevated results during photometric analyses of molecules such as nucleic acids and proteins, which are primarily conducted at 260 nm – 280 nm range. As depicted in figure 2, the absorbance values obtained for both fossil-sourced and biobased Eppendorf twin.tec® PCR Plates were similar and well below levels considered being critical [5]. This indicates that biobased material provides same low-leaching profile and minimization of negative influence on PCR assays as the well-established standard material.

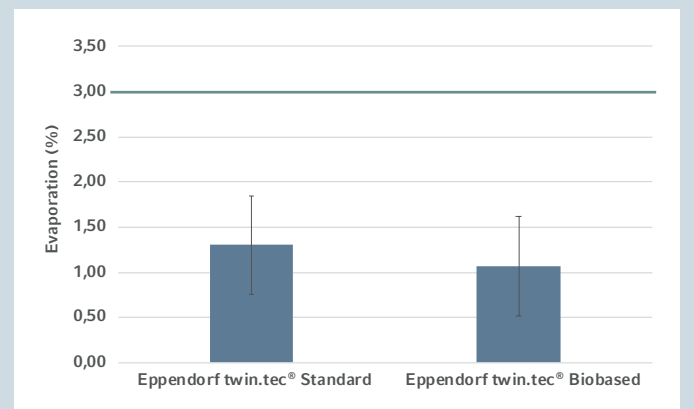

Figure 1: Average evaporation rates [%] of water samples thermocycled in fossil-sourced and biobased Eppendorf twin.tec® PCR Plates. Green line (3%) depicts an internal acceptance level (n=288).

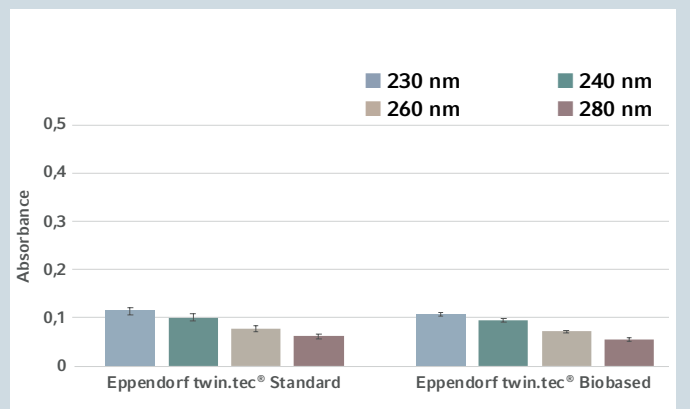

Figure 2: Leachable test. Absorbance values measured at various wavelengths of water samples incubated 40 minutes at 95 °C in standard (fossil-sourced) and biobased Eppendorf twin.tec® PCR Plates (n=144).

Residual Volume

PCR plates are often used in automatic workflows, where samples are taken from the PCR plates for downstream steps. Here, the remaining, or dead sample volume is of essential importance since it contains valuable samples or reagents and complete retrieval in automatic setup is often not trivial.

Data obtained here for both biobased and standard twin.tec® PCR Plates demonstrate that using automated pipetting system (Eppendorf epMotion® 5075vt) the residual volumes remaining inside the PCR plates are equivalent (figure 3). On average, approximately 0.25 µL remained in each well of an Eppendorf twin.tec® plate, either standard or biobased. Noteworthy, the remaining volume was highly homogenous showing almost no variation between wells of a given plate (figure 4). This very low rest volume and high uniformity is a hallmark of Eppendorf twin.tec® PCR Plates and is result of the high-quality material and the state-of-the-art production process of the twin.tec PCR plates.

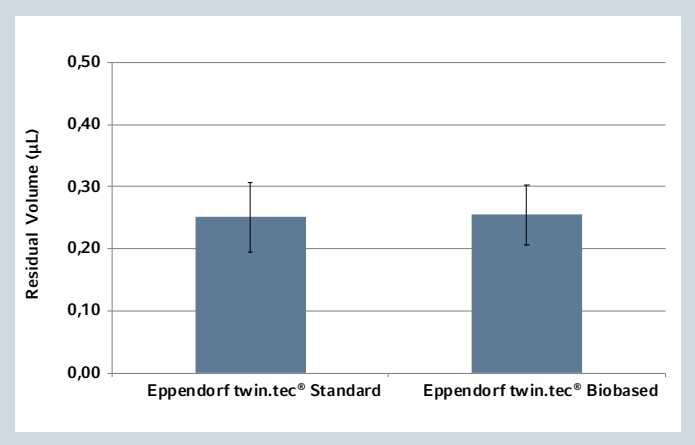

Figure 3: Average of residual volumes in Eppendorf biobased and standard twin.tec® PCR Plates after retrieving samples using automated pipetting system Eppendorf epMotion® 5075vt (n= 288).

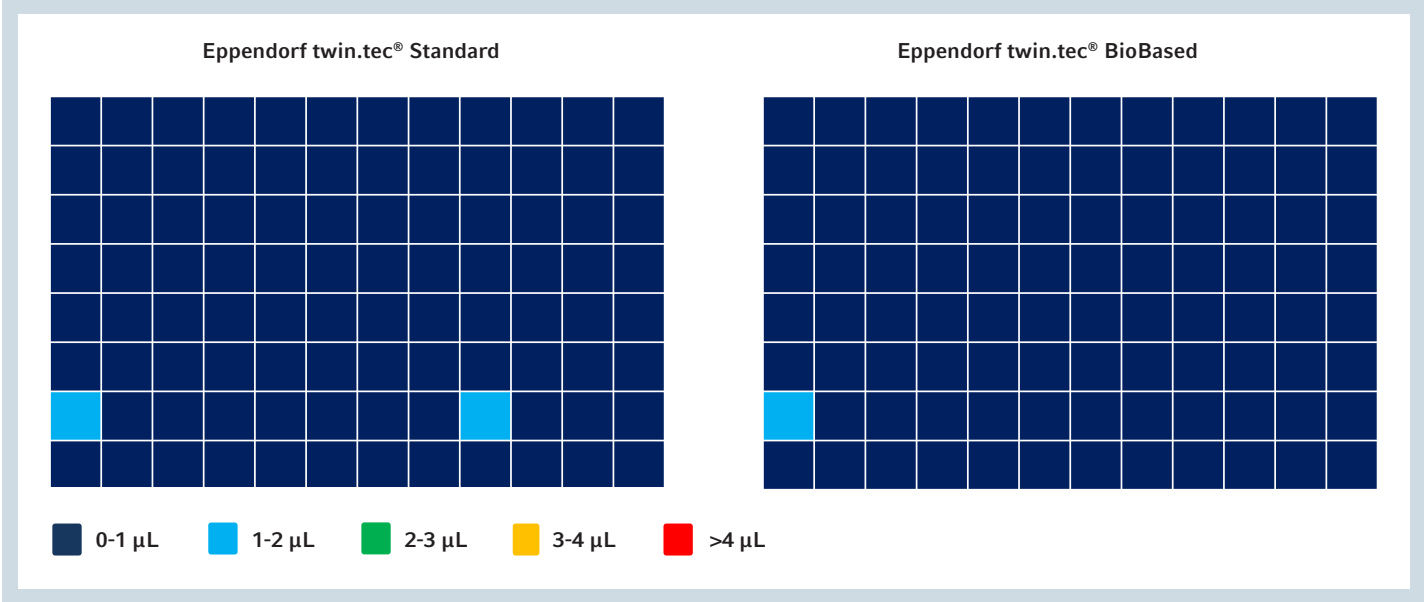

Figure 4: Depiction of average residual volumes per well in Eppendorf biobased and standard twin.tec® PCR Plates (n= 288).

qPCR assay performance
Ultimately, to investigate any possible effect of the biobased material on the overall assay reproducibility and performance, a standard qPCR test detecting bacterial gDNA was performed.

The intra- and inter-plate Cq mean values (figure 5) obtained for standard and biobased plates are virtually the same and equally reproducible (standard deviation ± 0.3 Cq). This confirms that Eppendorf twin.tec® Trace PCR Plates BioBased provide optimal and highly reliable assay performance.

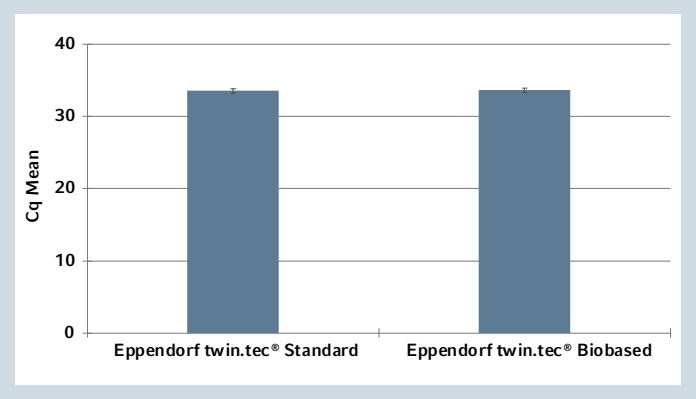

Figure 5: Mean Cq values in qPCR assay with A. baumannii gDNA using Eppendorf biobased and standard twin.tec® PCR Plates (n= 288).

Conclusion

In summary, the Eppendorf twin.tec® Trace PCR Plates BioBased have been evaluated and found to be virtually identical to fossil-sourced Eppendorf twin.tec® plates in terms of safety and PCR-parameter performance. This confirms that the biobased material has physical and chemical properties equal to fossil-sourced polypropylene. By using biobased consumables, you can make your lab plastics more sustainable without compromising product quality and performance.

Literature

[1] For more information on ISCC system please visit: www.iscc-system.org
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[5] Lewis LK, Robson M, Vecherkina Y, Ji C, Beall G. Interference with spectrophotometric analysis of nucleic acids and proteins by leaching of chemicals from plastic tubes. BioTechniques 2010; 48(4) 297–302

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