Axygen® 96 Well Full-Skirt Microplates for PCR are Comparable with Competitors

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A brief technical report from the Corning Applications Group

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Introduction

Microplates for polymerase chain reaction (PCR) are an ideal choice for molecular biology applications requiring automation compatibility and throughput. The thinness and consistency of PCR microplate walls are important to allow for accurate and precise thermal transfer for optimum results. To that end, Corning offers Axygen 96 well full-skirt PCR microplates, which are ideal for smaller studies, compatible with several thermal cyclers, and are automation friendly. In this study, we evaluated the Axygen 96 well full-skirt PCR microplates with comparable PCR microplates from two other manufacturers (Competitor A and Competitor B). The results demonstrate that Axygen 96 well full-skirt PCR microplates display consistent and accurate functionality for PCR applications and are comparable with competitor PCR microplates.

Materials and Methods

Microplates Evaluated

Axygen 96 well full-skirt PCR microplate (Cat. No. PCR-96-FS-C) with comparable 96 well PCR microplates from two manufacturers (Competitor A and Competitor B). Corning Universal Optical microplate sealing tape (Cat. No. 6575) was used for all microplates.

Real-Time PCR

To evaluate the performance of various PCR microplates, Real-Time PCR reactions were performed in the Bio-Rad CFX-96 Touch™ Real-Time PCR Detection System (Bio-Rad Part No. 185-5195) and prepared in accordance with the instructions contained in the SYBR® Advantage® GC qPCR Premix kit (Clontech Cat. No. 639676) for 25 µL reaction volume. The TaqMan® DNA (β-actin) template reagents kit (Life Technologies® Cat. No. 401970) was used to create standard curves and the resulting R^2 values were evaluated for consistency between microplate types. Two unknown samples were included for each microplate in 8 replicates and their starting concentration values were calculated using the standard curves. Two β-actin DNA samples of an unknown concentration were evaluated to determine whether similar results could be obtained using various PCR microplates (unknowns supplied in TaqMan DNA Template Reagents kit). In addition, the quantification cycle (Cq) values were evaluated for consistency for Unknown samples 1 and 2 for each plate type. All experiments were performed three independent times.

Reaction Volume Loss Due to Evaporation

PCR microplates were weighed before and after thermal cycling to evaluate reagent loss due to evaporation.

Results and Discussion

Function

As demonstrated in Figure 1, comparable concentrations for samples of Unknown 1 (Figure 1A) and Unknown 2 (Figure 1B) were determined amongst all three PCR microplates (Axygen, Competitor A, and Competitor B). For each plate, regardless of manufacturer, the starting concentrations of the unknown samples determined by Real-Time PCR were consistent (Figure 1). As demonstrated by representative standard curves in Figure 3, the R^2 values generated by the standard curves were all above 0.95.

The Cq value represents the number of cycles needed to reach a set threshold fluorescence signal level and is a commonly used metric to analyze Real-Time PCR results. The Cq values across each microplate for samples Unknown 1 (Figure 2A) and Unknown 2 (Figure 2B) were comparable and consistent, displaying low variability. This is also displayed by representative heat curves and melt curves for each microplate, as shown in Figure 3.

Reaction Volume Loss Due to Evaporation

Loss of PCR reaction volume due to evaporation can affect the accuracy of DNA quantification and thus must be considered when selecting PCR microplates. In this study, PCR microplates were weighed before and after thermal cycling to assess evaporation across each microplate. As shown in Figure 4, Axygen® microplates, as well as the two other brands, displayed less than 5% evaporation of reaction volume. There was no significant difference in the volume loss due to evaporation between the microplate types.

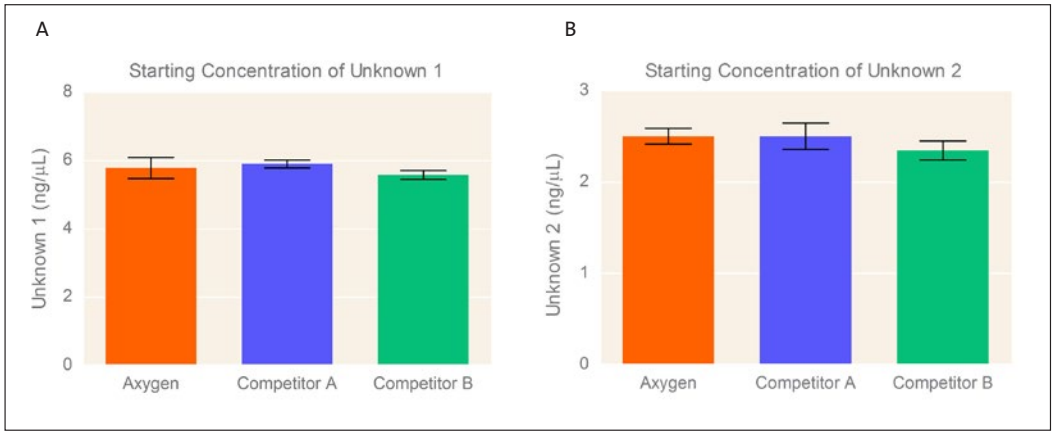


Figure 1. Determining the concentration of Unknown 1 and Unknown 2 samples. The starting concentration of two unknown samples was determined using a standard dilution series. There was no significant difference in the concentration determined for (A) Unknown 1 and for (B) Unknown 2 between each of the microplate types. Data shown with Standard Error of the Mean (SEM).

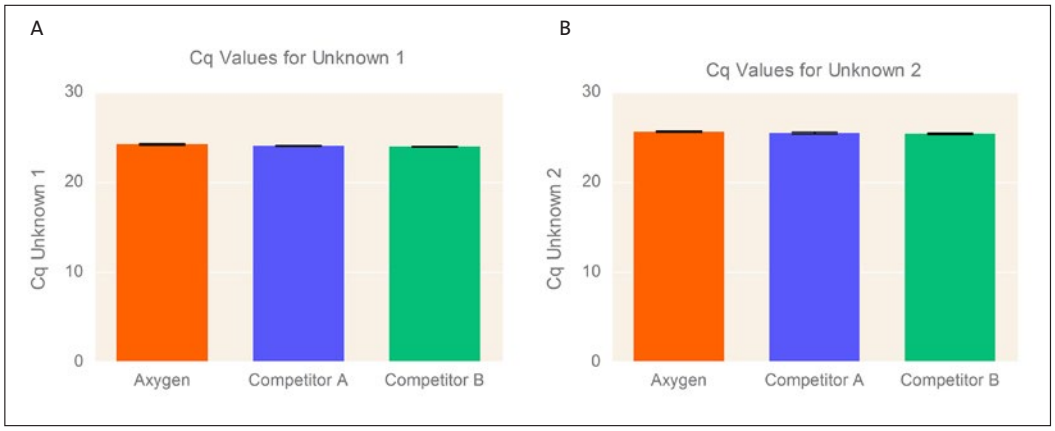


Figure 2. Evaluating the consistency of Cq values. The Cq values for samples (A) Unknown 1 and (B) Unknown 2 were consistent across each microplate type. There was no significant difference in Cq values between the microplate types. Data shown with SEM.

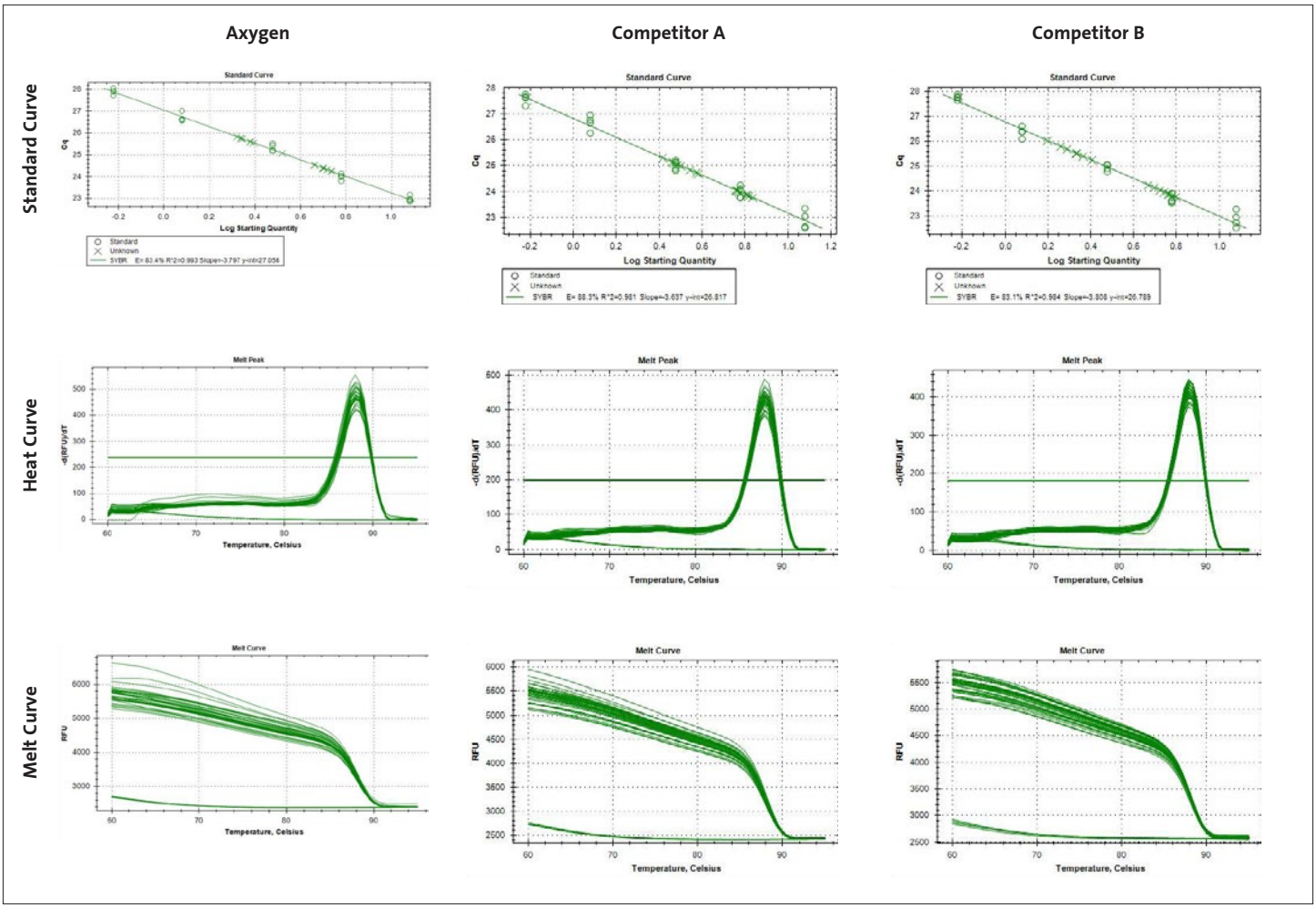


Figure 3. Standard curves, heat curves, and melt curves generated during Real-Time PCR. Standard curves generated by each microplate had R^2 values above 0.95. The heat curves and melt curves of standard dilution series and unknown samples 1 and 2 were consistent between each microplate type.

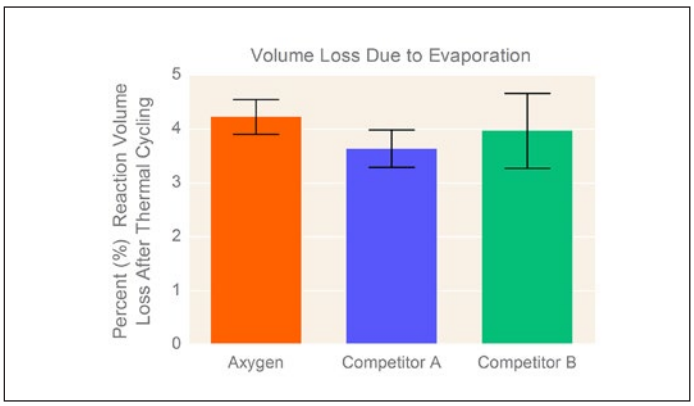
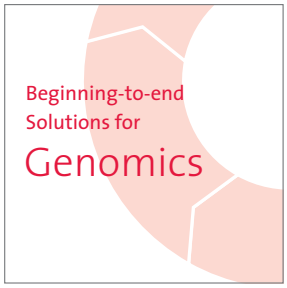


Figure 4. Reaction volume loss due to evaporation. Reaction volume loss was determined by weighing the PCR microplates before and after thermal cycling. The Axygen 96 well full-skirt PCR microplate and the two competitor microplates exhibited less than 5% volume loss after thermal cycling. Data shown with SEM.

Conclusions

- Axygen® 96 well full-skirt PCR microplates consistently display accurate functionality for PCR.
- No significant difference was observed in function between Axygen 96 well full-skirt PCR microplates and commercially available competitor PCR microplates.
- No significant difference was observed in evaporation loss between Axygen 96 well full-skirt PCR microplates and commercially available competitor PCR microplates.



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