Axygen® PCR 8-Strip Tubes are Comparable with Competitors

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SnAPPShots
A brief technical report from the Corning Applications Group

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Introduction

Strip tubes for polymerase chain reaction (PCR) are an ideal choice for molecular biology applications with low throughput and individualized experiments. The thinness and consistency of PCR tube walls are important to allow for accurate and precise thermal transfer for optimum results. To that end, Corning offers Axygen 0.2 mL, thin wall PCR 8-strip tubes, which are ideal for smaller studies and compatible with widely used thermal cyclers. In this study, we evaluated Axygen 0.2 mL, thin wall PCR 8-strip tubes with comparable PCR 8-strip tubes from three other manufacturers (Competitors A, B, and C). All PCR 8-strip tubes were evaluated for accuracy and consistency in amplifying DNA using Real-Time PCR, as well as for reaction loss due to evaporation. These results demonstrate that Axygen PCR 8-strip tubes display consistent and accurate functionality for PCR applications.

Materials/Methods

Evaluation of PCR 8-strip Tubes

Axygen 0.2 mL, thin wall PCR 8-strip tubes (Corning Cat. No. PCR-0208-C) were compared to PCR 8-strip tubes from three other manufacturers (Competitors A, B, and C). For each brand, the corresponding optically clear, Real-Time PCR compatible caps were used. For Competitor B, the tubes had opaque attached caps that were cut off and replaced with the optically clear caps.

Real-Time PCR

To evaluate the performance of various PCR 8-strip tubes, Real-Time PCR reactions were performed in the Bio-Rad CFX-96 Touch™ Real-Time PCR Detection System (Bio-Rad Cat. 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. For each of the PCR 8-strip tube products, a 96-well format was created using 12 of the 8-strips for each replicate. 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 PCR tube types. Two β-actin DNA samples of unknown concentration (Unknown 1 and Unknown 2) were included for each PCR 8-strip tube setup in 8 replicates (unknowns supplied in TaqMan DNA Template Reagents kit). The starting concentration values for the unknowns were calculated using the standard curves. In addition, the quantification cycle (Cq) values obtained during amplification for the unknown samples were evaluated for consistency for each PCR 8-strip tube type. All experiments were performed three independent times.

Reaction Volume Loss Due to Evaporation

PCR 8-strip tubes were weighed before and after thermal cycling to evaluate reagent loss due to evaporation.

Results/Discussion

Function

The starting concentrations of two samples, Unknown 1 and Unknown 2, were determined by Real-Time PCR using all four brands of PCR 8-strip tubes. For samples Unknown 1 and Unknown 2, there was no significant difference in the concentrations determined between Axygen and Competitors A and C (Figure 1). These concentrations also match those previously calculated using 96-well PCR microplates (Corning literature codes: CLS-A-AN-247 and CLS-A-AN-248). However, the concentrations determined for Unknown 1 and Unknown 2 for Competitor B were more variable and significantly different from Axygen.

The quantification cycle (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 within each PCR 8-strip tube type for samples Unknown 1 (Figure 2A) and Unknown 2 (Figure 2B) were comparable and consistent, with the Cq values for Axygen 8-strip tubes displaying the lowest variability. For sample Unknown 1, Axygen 8-strip tubes displayed a standard deviation of 0.37 whereas Competitors A, B, and C demonstrated standard deviations of 0.54, 0.49, and 0.38, respectively. Similarly, for Unknown 2, Axygen 8-strip tubes demonstrated a standard deviation of 0.27 whereas Competitors A, B, and C had standard deviations of 0.77, 0.45, and 0.50, respectively. This low standard deviation resulted

in the significant differences observed in Cq values for Axygen compared with the three competitor PCR 8-strip tubes (Figure 2).

Representative standard curves, heat curves, and melt curves are shown in Figure 3. As can be seen, the R^2 values generated by the standard curves for all four brands were all above 0.92, indicating the Axygen® PCR 8-strip tubes are comparable with competitors. Further, the standard melt curves and heat curves generated from Real-Time PCR show that Axygen is comparable to competitors.

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 microcentrifuge tubes. In this study, sets of twelve PCR 8-strip tubes, used to mimic a microplate format, were weighed before and after thermal cycling to assess evaporation. As displayed in Figure 4, Axygen PCR 8-strip tubes, as well as Competitors A and C, displayed less than 5% evaporation of reaction volume. However, Competitor B exhibited a significantly greater volume loss after thermal cycling.

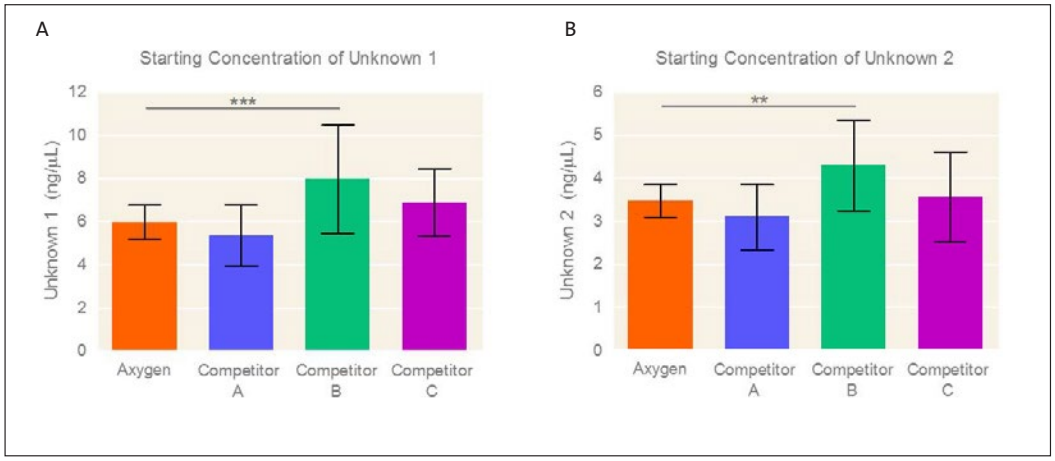


Figure 1. Determining the Concentration of Unknown Samples. The starting concentration of two unknown samples was determined using a standard dilution series. For (A) Unknown 1 and (B) Unknown 2, there were no significant differences in the concentrations determined between Axygen and Competitors A and C. However, the concentration determined for Competitor B was significantly greater than that of Axygen. Data shown with Standard Deviation (SD). One-way ANOVA with Newman-Keuls Post Test **p<0.01, ***p<0.001. n= 24.

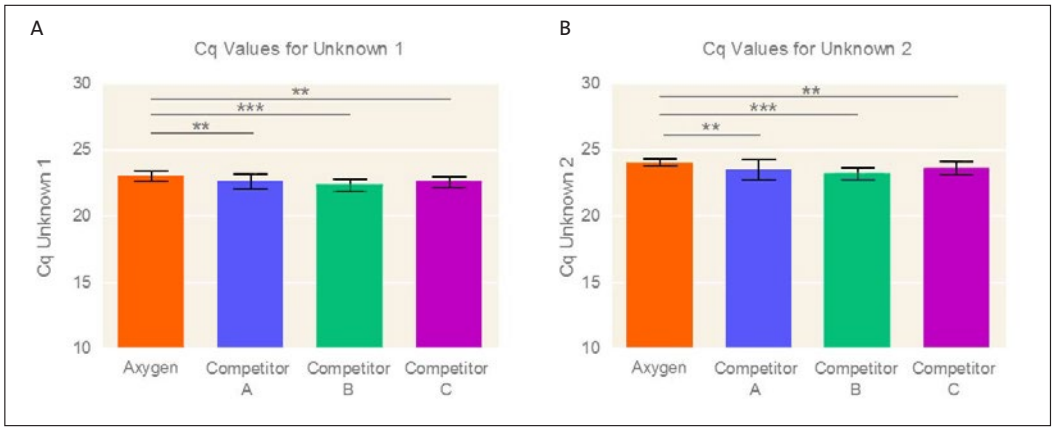


Figure 2. Evaluating the Consistency of Cq Values. The Cq values for samples (A) Unknown 1 and (B) Unknown 2 were consistent across each PCR 8-strip tube type. For (A) Unknown 1 and (B) Unknown 2, there were significant differences in the Cq values determined between Axygen and the Competitor PCR 8-strip tubes. Data shown with SD. One-way ANOVA with Newman-Keuls Post Test **p<0.01, ***p<0.001. n= 24.

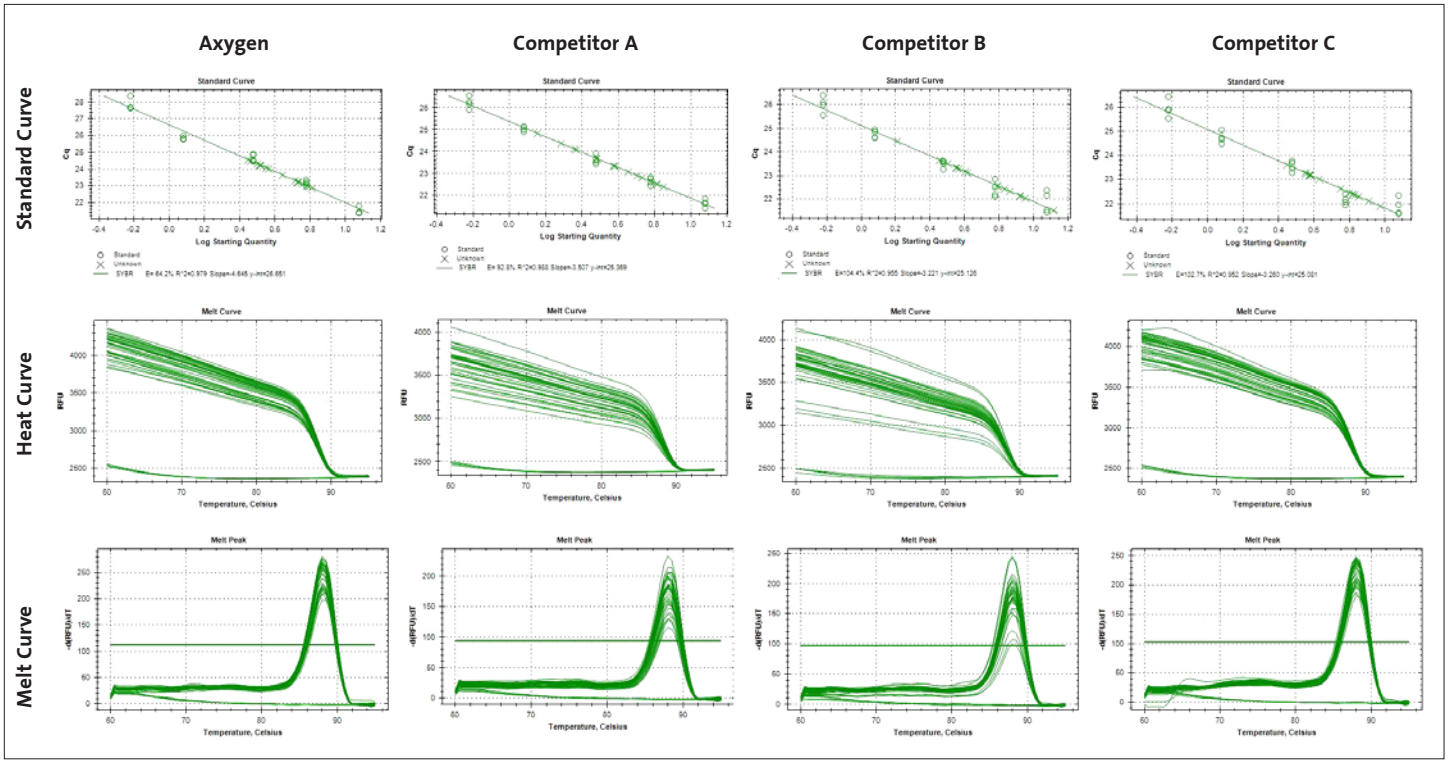


Figure 3. Standard Curves, Melt Curves, and Heat Curves Generated During Real-Time PCR. Standard curves generated by each PCR 8-strip tube type display R^2 values above 0.92. The melt curves and heat curves were consistent between each PCR 8-strip tube type.

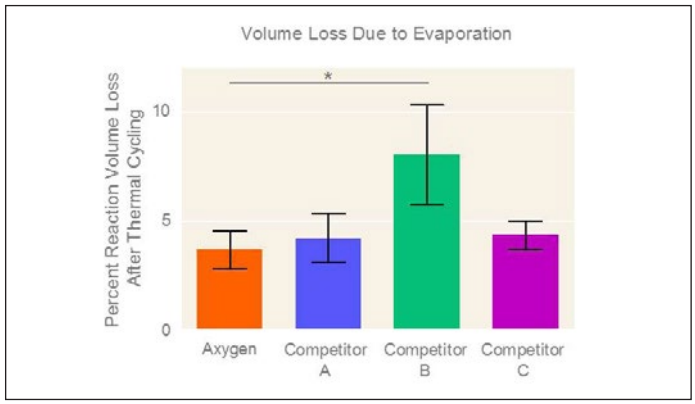
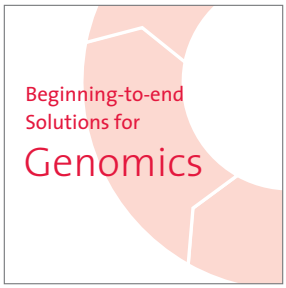


Figure 4. Reaction Volume Loss Due to Evaporation. Reaction volume loss was determined by weighing the PCR 8-strip tubes before and after thermal cycling. The Axygen PCR 8-strip tubes and Competitors A and C exhibited less than 5% volume loss after thermal cycling. Competitor B exhibited significantly more volume loss. Data shown with SD. One-way ANOVA with Newman-Keuls Post Test *p<0.05. n= 24.

Conclusions

- Axygen® PCR 8-strip tubes consistently display accurate functionality for PCR.
- Axygen PCR 8-strip tubes display comparable function to Competitors A and C and higher precision than Competitor B.
- Axygen PCR 8-strip tubes display comparable evaporation loss to Competitors A and C and significantly lower evaporation loss than Competitor B.



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