

Axygen® PCR Tubes are Comparable with Competitors

Application Note



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Introduction

Single tubes for polymerase chain reaction (PCR) are an ideal choice for low throughput molecular biology applications and individualized experiments. The thinness and consistency of PCR tube walls are important to allow for accurate and precise thermal transfer and optimal amplification. Corning offers Axygen 0.2 mL thin-wall PCR tubes, which are compatible with widely used thermal cyclers. In this study, we evaluated Axygen 0.2 mL thin-wall PCR tubes with comparable PCR tubes from three different manufacturers (Competitor A, Competitor B, and Competitor C). All PCR tubes were evaluated for accuracy and consistency in amplifying DNA using PCR, as well as for reaction loss due to evaporation. These results demonstrate that the Axygen 0.2 mL thin-wall PCR tubes are consistent and accurate for PCR applications and are comparable with competitor PCR tubes.

Materials and Methods

Evaluation of PCR Tubes: Axygen 0.2 mL thin-wall PCR tubes with attached flat caps (Corning Cat. No. PCR-02-C) were compared to similar PCR tubes from three other manufacturers (Competitor A, Competitor B, and Competitor C).

Plasmid DNA Preparation

GC10 competent cells (Sigma-Aldrich Cat. No. G2544) containing gWIZ™ GFP plasmid DNA (Genewiz®) were cultured in Corning Miller's LB-Broth (Corning Cat. No. 46-050-CM) at 37°C for 16 hr at 250 rpm. Plasmid DNA was purified using a plasmid miniprep kit and quantified with the EnVision® multimode plate reader (PerkinElmer).

PCR

To evaluate the performance of various PCR tubes, PCR reactions were performed in the MultiGene™ OptiMax Thermal Cycler (Labnet International) and prepared using a TAQ Polymerase kit with 100 µL reaction volume. PCR reactions contained 200 ng of gWIZ GFP DNA, TAQ Polymerase buffer with 2 mM Mg²⁺, 20 µM dNTPs, TAQ Polymerase, and 250 nM of each primer. Primers were generated by Integrated DNA Technologies, Inc., to anneal to GFP

(~700 base pairs [bp]). Samples were added to PCR tubes and the following program was performed in the MultiGene™ OptiMax Thermal Cycler: 1 cycle of 2 minutes at 95°C for TAQ Polymerase activation, followed by 25 cycles for amplification (30 seconds at 95°C for denaturation, 1 minute at 59.5°C for primer annealing, 3 minutes at 72°C for extension), a final 10-minute extension at 72°C, and a hold at 4°C at the end. For each of the PCR tube brands, three tubes were evaluated concurrently. All experiments were performed three independent times.

DNA Quantification

All PCR product samples were loaded onto a 1% agarose gel (Corning Cat. No. AGR-LE-100) in 1X TBE buffer (Corning Cat. No. 46-011-CM) containing ethidium bromide (Fisher Cat. No. BP1302-10) in an electrophoresis unit. The unit was operated under constant voltage at 90 volts for 60 minutes. Following electrophoresis, the gel was imaged using the ENDURO™ GDS Touch Gel Documentation System (Labnet International) and quantified with ENDURO™ TotalLab 1D software (Labnet International).

Reaction Volume Loss Due to Evaporation

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

Results/Discussion

Function

The concentration of DNA after PCR was determined for all four brands of PCR tubes by performing gel electrophoresis and quantifying the pixel intensities of the PCR products. As displayed by the representative gel in Figure 1, DNA amplification using each brand of PCR tubes resulted in a single band, which represents a pure GFP DNA sample. The concentration of GFP DNA for each brand of tubes was calculated by analyzing the pixel intensity from the images of the gels (Figure 2). As displayed in Figure 2, there was no significant difference in the determined concentrations of GFP DNA between Axygen and Competitors A, B, and C.

Reaction Volume Loss Due to Evaporation

Loss of PCR reaction volume due to evaporation during amplification can affect the accuracy of DNA quantification and must be considered when selecting PCR tubes. In this study, for each replicate, three PCR tubes of each brand were weighed before and after thermal cycling to assess volume loss due to evaporation. For all brands of tubes no weight difference ≥ 0.01 g was observed. Therefore, loss of PCR reaction volume due to evaporation was insignificant and could not be determined.

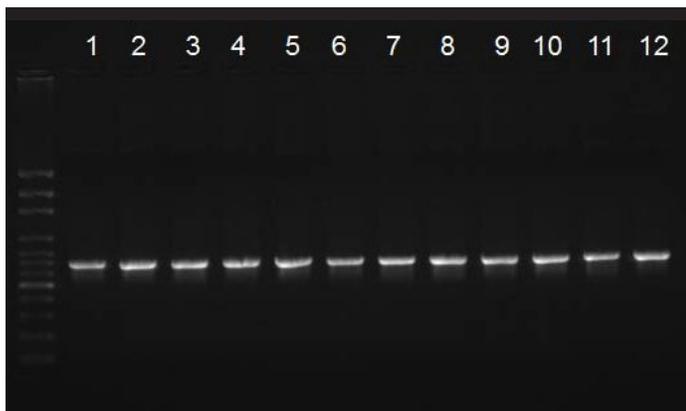


Figure 1. Representative Gel of PCR product. The post-PCR GFP DNA from each replicate was loaded onto a 1% agarose gel in an electrophoresis unit. Images of the resulting gels were used for the quantification of the PCR product concentration. One band representing the amplified GFP DNA was seen after using Axygen single PCR tubes (lanes 10-12), Competitor A (lanes 7-9), Competitor B (lanes 4-6), and Competitor C (lanes 1-3).

Conclusions

- ▶ Axygen PCR tubes consistently display accurate functionality for PCR.
- ▶ Axygen PCR tubes display comparable function to Competitors A, B, and C.
- ▶ Axygen PCR tubes do not display significant evaporation loss, which is comparable to Competitors A, B, and C.

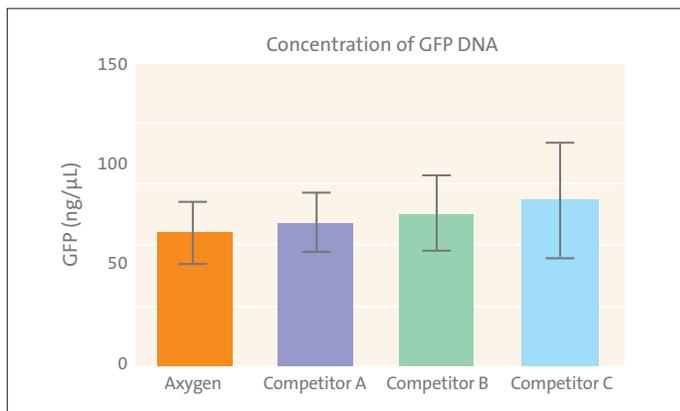


Figure 2. Concentration of PCR product. The GFP DNA concentration post-PCR was determined for each PCR tube brand. There were no significant differences in the concentration determined between Axygen single PCR tubes and Competitors A, B, and C single PCR tubes. $n = 9$ from three independent experiments.

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