BAX® System Lysate Preparation - Uncapped Lysis

Introduction

Pathogen detection using the Polymerase Chain Reaction (PCR) requires sample lysis. A sample lysate is a fluid suspension containing the contents of lysed cells, including DNA. A small aliquot of an enriched sample is added to prepared BAX System lysis reagent (lysis buffer + protease), which is then heat-treated to break down the cell membrane and extract the DNA for subsequent amplification and detection by PCR.

During the lysis procedure, tubes are typically capped to minimize cross-contamination and maintain sample integrity. Some test kit manufacturers do not use caps which reduces the number of steps in the lysate preparation workflow. To add increased flexibility and minimize hands-on time of the BAX System lysis procedure, a study was designed to assess the performance of lysates prepared without caps compared to capped lysates.

Sample Preparation

Pure cultures of *Salmonella* Typhimurium DD13557 and *E. coli* O157:H7 DD916 were grown overnight in Brain Heart Infusion (BHI) broth at 35 °C. Each culture was then serially diluted 1:10 in additional BHI broth and plated in triplicate onto BHI agar for enumeration.

A total of 8 full racks of 96 cluster tubes were programed on the BAX Prep Xpress to prefill 200 µL of prepared lysis reagent (150 µL of protease to one 12 mL bottle of lysis buffer). Using 4 racks per organism, *Salmonella* (5 µL) or *E. coli* O157:H7 (20 µL) was added to every other cluster tube in a checker board pattern at a minimum concentration of 8 Log CFU/mL. For the remaining 48 cluster tubes per rack, Buffered Peptone Water (BPW) was added in 5 µL aliquots for *Salmonella*, and BAX System MP media was added in 20 µL aliquots for *E. coli* O157:H7. One set of lysates per organism-assay combination was capped while the second set of lysates were not capped (see Table 1). Lysis was performed for all samples on the automated thermal block by heating cluster tubes at 37 °C for 20 minutes and 95 °C for 10 minutes, and then cooling tubes at 4 °C.

Table 1. Organism-assay lysate combinations tested

Organism	Target concentration in Spiked Samples	Lysis Procedure	Assay Tested
Salmonella Typhimurium	5.2 x 10^8 CFU/mL	Capped	Real-Time *Salmonella* (KIT2006)
		Uncapped	Real-Time *Salmonella* (KIT2006)
		Capped	*Salmonella* 2 (KIT2011)
		Uncapped	*Salmonella* 2 (KIT2011)
E. coli O157:H7	1.4 x 10^8 CFU/mL	Capped	Real-Time *E. coli* O157:H7 Exact (KIT2039)
		Uncapped	Real-Time *E. coli* O157:H7 Exact (KIT2039)
		Capped	Real-Time *E. coli* O157:H7 (KIT2000)
		Uncapped	Real-Time *E. coli* O157:H7 (KIT2000)

Testing Method

Capped and uncapped lysates for each organism were hydrated on Real-Time or standard PCR tablets according to Table 1. All Real-Time PCR tablets were hydrated with 30 µL of the appropriate lysate, sealed with flat optical caps and held for 10 minutes on a chilled (4 °C) PCR cooling block if required. Standard PCR tablets were hydrated with 50 µL of the appropriate lysate and sealed with flat optical caps. All PCR tablets were then loaded into the Q7 instrument and a full process was run according to the instructions in the BAX System User Guide.

Results

Lysates prepared with and without caps returned the correct BAX System results for each organism. All spiked lysates returned positive results, and all media lysate controls returned negative results indicating no signs of cross contamination. Results are summarized below in Table 2 and Figures 1-4.

Table 2. Organism-Assay Lysate Results

Organism	Lysis Procedure-Assay Tested	Spike Level	Results (Positives/Total Samples)
Salmonella Typhimurium	Capped/Real-Time *Salmonella*	Media control	0/48
		Spiked	48/48
	Uncapped/Real-Time *Salmonella*	Media control	0/48
		Spiked	48/48
	Capped/*Salmonella* 2	Media control	0/48
		Spiked	48/48
	Uncapped/*Salmonella* 2	Media control	0/48
		Spiked	48/48
E. coli O157:H7	Capped/Real-Time *E. coli* O157:H7 Exact	Media control	0/48
		Spiked	48/48
	Uncapped/Real-Time *E. coli* O157:H7 Exact	Media control	0/48
		Spiked	48/48
	Capped/Real-Time *E. coli* O157:H7	Media control	0/48
		Spiked	48/48
	Uncapped/Real-Time *E. coli* O157:H7	Media control	0/48
		Spiked	48/48

Salmonella

Figure 1: BAX System Real-Time PCR assay for *Salmonella* (KIT2006) Capped (Left) and Uncapped (Right) Lysates

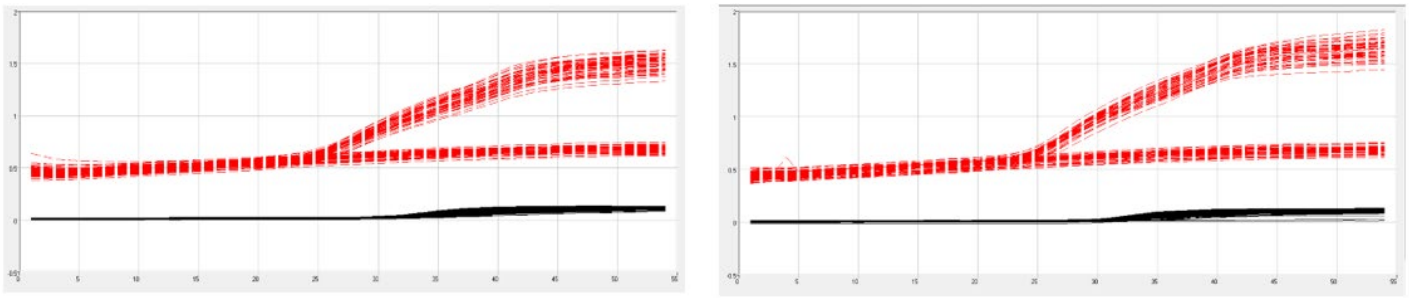
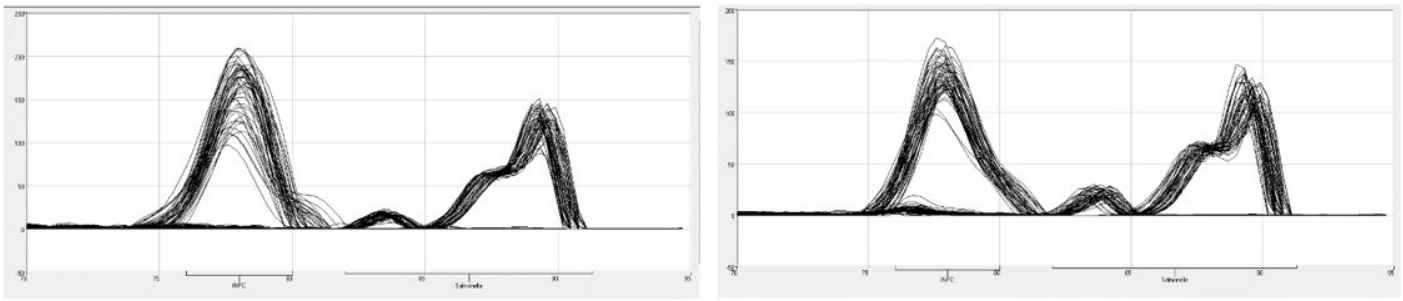


Figure 2: BAX System PCR Assay for Salmonella (KIT2011) Capped (Left) and Uncapped (Right) Lysates



E. coli O157:H7

Figure 3: BAX System Real-Time PCR Assay for *E. coli* O157:H7 Exact (KIT2039) Capped (Left) and Uncapped (Right) Lysates

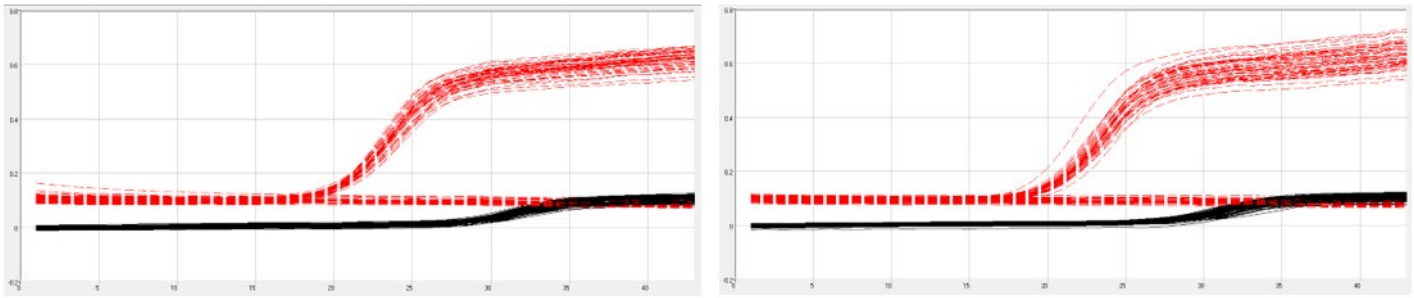
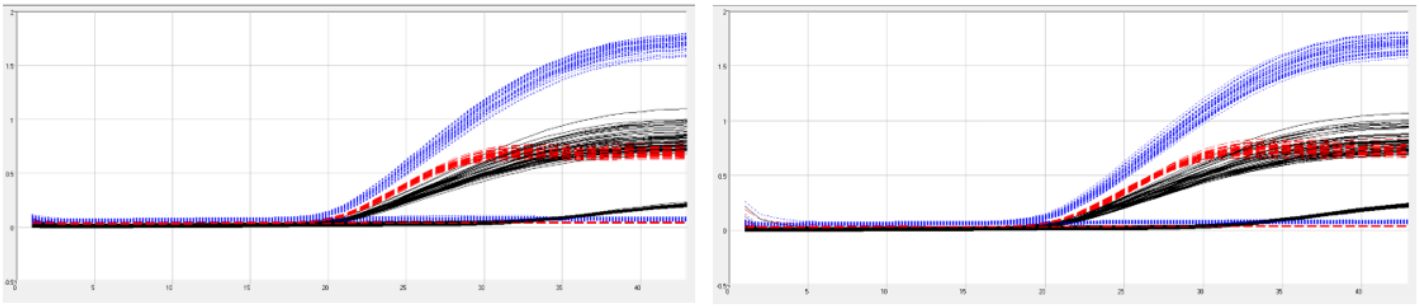


Figure 4: BAX System Real-Time PCR assay for *E. coli* O157:H7 (KIT2000) Capped (Left) and Uncapped (Right) Lysates



Conclusions

The results of this study demonstrate equivalent performance between lysates prepared with or without caps during the lysis procedure. Overall, using the BAX System lysis procedure with no caps simplifies the work flow, reduces repetitive motions and shortens the hands-on time for users during the preparation of lysates for BAX System PCR assays.

Note: If lysates are kept at 4 °C after tablet hydration, it is recommended to cap lysates to prevent spills.