Performance validation of the BAX® System Free DNA Cleanup Kit to eliminate PCR false-positives caused by external contaminant DNA

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INTRODUCTION:

The Polymerase Chain Reaction (PCR) allows for exponential amplification of DNA into many copies. However, it does not differentiate between DNA from living cells and, external contaminant DNA resulting from procedures such as bacteriophage interventions. Any contaminating target DNA can be amplified during the PCR reaction and consequently cause false-positive-results. To address the problem, a universal Free DNA Cleanup kit has been developed for use with the BAX® System PCR assays.

PURPOSE:

To validate the performance of the BAX® System Free DNA Cleanup Kit for removal of any free, contaminant DNA and eliminate potential PCR false-positive-results.

METHODS:

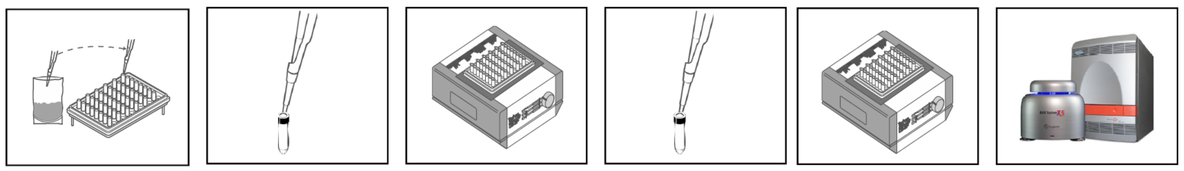
Demonstration of presence of bacterial DNA in bacteriophage solutions: Commercial bacteriophage solutions for *Listeria* and *Salmonella* were serial diluted and tested on BAX® System Real-Time Genus *Listeria* and *Salmonella* PCR assays respectively.

Elimination of DNA from phage solutions: The bacteriophage solutions for *Listeria* was diluted to 10^{10} PFU/ml and then treated using the DNA Cleanup procedure.

Determination of amount of DNA that can be eliminated: Efficiency of the procedure in removing external contaminating DNA was determined by testing the treatment on BHI broth samples containing up to 400 ng of *Salmonella* or *Listeria* gDNA.

Validation of performance on contaminated environmental samples: To further validate the performance, 16 environmental swabs from surfaces treated with a *Listeria* bacteriophage solution were obtained from a food processing facility. The swabs were enriched in either 40 ml or 90 ml of 24 LEB *Listeria* Enrichment Media at 35ºC for 22 h and treated using the DNA Cleanup procedure. Effectiveness of treatment in all studies was assayed using PCR.

BAX® System Free DNA Cleanup Kit Protocol:



Transfer 40µl of enriched sample to cluster tube

Add 5µl BAX® System Free DNA Cleanup agent & 5µl BAX® System Free DNA Cleanup agent buffer

Incubate at 37ºC for 15 min

Add 5µl inactivation agent

Incubate at 55ºC for 15 min

BAX® System Lysis Method

RESULTS:

Serial dilutions of commercial bacteriophage solutions tested on the BAX® System PCR assays resulted in positive PCR results (Figure 1).

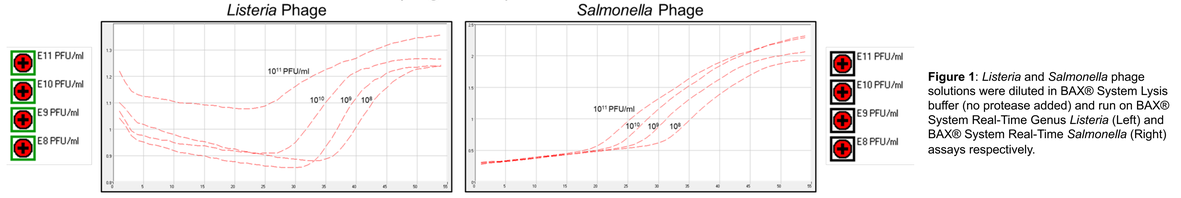


Figure 1: *Listeria* and *Salmonella* phage solutions were diluted in BAX® System Lysis buffer (no protease added) and run on BAX® System Real-Time Genus *Listeria* (Left) and BAX® System Real-Time *Salmonella* (Right) assays respectively.

The *Listeria* phage solution was diluted to 10^{10} PFU/ml and then treated using the Free DNA Cleanup procedure. Untreated and treated samples were run on the Real-Time Genus *Listeria* assay. Treated samples were negative while target DNA was detected in untreated samples (Table 1).

Phage Dilution	No DNA Cleanup Treatment (Ct)		DNA Cleanup Treated (Ct)	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
10^{10} PFU/ml	39.9	40.0	No Target Detected ✓	No Target Detected ✓

Table 1: PCR results obtained on the BAX® System Real-time Genus *Listeria* assay with and without Free DNA Cleanup treatment

The Free DNA Cleanup procedure successfully removes up to 400 ng of free DNA completely from the sample kit. No amplification was detected post treatment using BAX® System PCR assay (Table 2 and 3).

Amount of DNA	No DNA Cleanup Treatment (Ct)	DNA Cleanup Treated (Ct)
400 ng	23.8	No Target Detected ✓
40 ng	29.0	No Target Detected ✓
4 ng	32.4	No Target Detected ✓
400 pg	34.9	No Target Detected ✓
40 pg	38.1	No Target Detected ✓
4 pg	40.9	No Target Detected ✓

Table 2 (Left): PCR Signal from samples containing *Listeria* gDNA with and without Free DNA Cleanup treatment.

Table 3 (Right): PCR Signal from samples containing *Salmonella* gDNA with and without Free DNA Cleanup treatment.

Amount of DNA	No DNA Cleanup Treatment (Ct)	DNA Cleanup Treated (Ct)
400 ng	25.5	No Target Detected ✓
40 ng	31.1	No Target Detected ✓
4 ng	34.6	No Target Detected ✓
400 pg	38.8	No Target Detected ✓
40 pg	41.2	No Target Detected ✓
4 pg	43.5	No Target Detected ✓

Environmental swabs from surfaces treated with a *Listeria* bacteriophage solution were obtained from a food processing facility and enriched in 24 LEB media. Enrichments were treated using the Free DNA Cleanup procedure. The Free DNA Cleanup treatment was able to successfully eliminate contaminant DNA from enrichments and consequently eliminated all "unconfirmable positives" experienced due the phage treatment of surfaces (Table 4 and 5). Additionally, we have also shown that the Free DNA Cleanup procedure does not affect the detection of the presence of any true-positive resulting due to intact cells in the matrix (Sample 15).

Sample	Enrichment Method	Ct values on BAX Real-time Genus *Listeria*		Culture Confirmation using MOX plates
		No Free DNA Cleanup Treatment	Free DNA Cleanup Treated	
		Avg Ct of replicates	Avg Ct of replicates	
1	40 ml 24 LEB Incubated @ 35C for 22 h	45.8	NEG	NEG
2		45.9	NEG	NEG
3		45.9	NEG	NEG
4		44.5	NEG	NEG
5		NEG	NEG	NEG
6		43.2	NEG	NEG
7		45.7	NEG	NEG
8		44.5	NEG	NEG

Table 4 (Left): Samples enriched in 40 ml of 24 LEB media.

Table 5 (Right): Samples enriched in 90 ml of 24 LEB media.

Ct values observed with/without Free DNA Cleanup treatment are shown. Modified Oxford (MOX) Agar was used for confirmation.

Sample	Enrichment Method	Ct values on BAX Real-time Genus *Listeria*		Culture Confirmation using MOX plates
		No Free DNA Cleanup Treatment	Free DNA Cleanup Treated	
		Avg Ct of replicates	Avg Ct of replicates	
9	90 ml 24 LEB Incubated @ 35C for 22 h	46.1	NEG	NEG
10		44.9	NEG	NEG
11		46.5	NEG	NEG
12		45.0	NEG	NEG
13		45.8	NEG	NEG
14		47.1	NEG	NEG
15		47.7	47.1	POS
16		NEG	NEG	NEG

SIGNIFICANCE:

The Free DNA Cleanup procedure has demonstrated robustness in elimination of contaminant DNA with the ability to remove 400 ng of DNA. Additionally, it has been verified that the treatment will eliminate only free contaminant DNA and will not interfere with DNA within living cells and their subsequent detection by PCR-pathogen-detection assays.

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