

BAX® System DNA Cleanup Kit

performance validations from industry,
government, and Hygiena scientists

Bacteriophage treatments of food products and environmental surfaces in the food industry has gained popularity for its ability to effectively control a variety of bacterial pathogens including *Salmonella*, *E. coli* and *Listeria*. These industrial phage products contain remnants of lysed host cells including significant amounts of their DNA. Other processes, such as using disinfectants or treatments such as heat or UV sterilization, and under certain conditions adding spices to food products, also can result in an extraneous amount of non-cellular DNA.

Due to these host DNA remnants, customers using phage treatments and other processes have encountered several unconfirmable positives in PCR assays when testing for residual viable pathogens in both product and environmental samples.

The new BAX® DNA Cleanup Kit addresses this issue by removing all “free” extraneous DNA (not within a cell) from samples, allowing for more confidence in test results. Because this treatment does not affect cells with intact cell membranes, the detection of pathogens is not impacted.



Customer impressions

- A U.S. poultry plant that tested 120 samples (Table 1) and found 69 “false” positives to be negative with the cleanup kit termed the cleanup kit treatment as a “magic solution.” With treatment performed using a competitor’s DNA cleanup product, the customer reported experiencing 10 – 15 “false” unconfirmable positives post-treatment.
- A Canadian reference laboratory gave high scores for ease of use (86/100) and overall performance (87/100) of the cleanup kit.
- A Southeast Asian government laboratory reported that the BAX® Free DNA Cleanup Kit could eliminate “free” *Vibrio* DNA up to 10^5 CFU/ml exponentially higher than the 10^4 CFU/mL typically seen by seafood processors (Table 4).

Customer research

Two Hygiena customers—one of the largest poultry processors in the United States, and the second a prominent southeast Asian government agency—tested the performance of the BAX® Free DNA Cleanup kit. Hygiena scientists also conducted their own tests.

Poultry processing plant

The US poultry processor tested 120 swab samples of areas that were treated with *Listeria* bacteriophage solution, widely used in poultry plants to disinfect environmental surfaces. Of those, 88 were positive for *Listeria*. Of the 88, 19 were “true” positives, confirmed by cell culture. All 69 unconfirmable “false” positives were determined to be negative by the BAX® Free DNA Cleanup Kit (Table 1).

Table 1. Non-culturally confirmed “positive” *Listeria* results found to be negative by BAX® Free DNA Cleanup Kit.

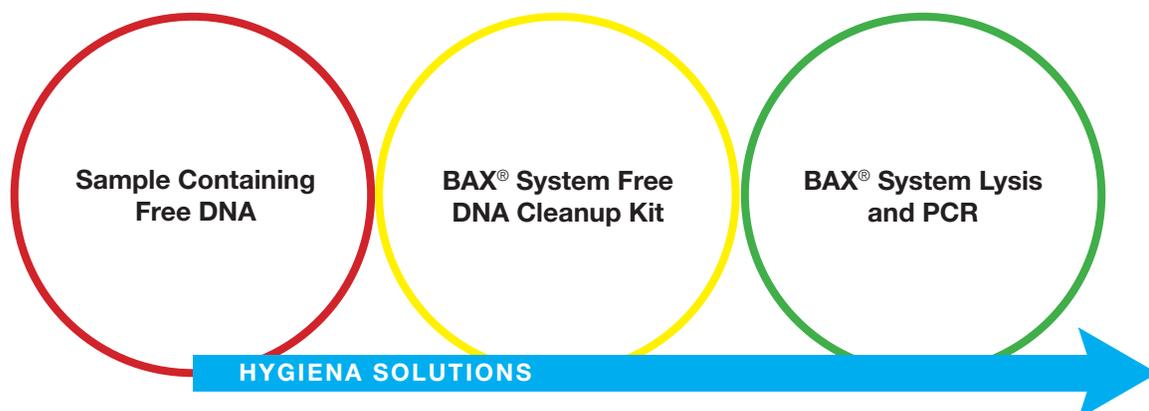
Total samples (swabs)	Initial Positive samples	“True” positives	Remaining “False” positives	“False” Positives Confirmed Negatives with DNA Cleanup prior PCR
120	88	Culturally Confirmed	Culturally Confirmed	
		19	69	69

In another study, swabs from a poultry processor were tested for the ability of the kit to clear “free” DNA from a *Listeria* bacteriophage solution (Table 2). Cycle threshold (Ct) values indicate the presence of bacterial DNA (in this case from remaining bacteriophage DNA). The use of the DNA Cleanup kit provided a solution to resolve unconfirmable positives caused by the extraneous DNA in the bacteriophage solution.

Table 2. Detection of *Listeria* bacteriophage by BAX *Listeria* assay, before and after treatment with BAX® Free DNA Cleanup kit

Enrichment	Number of samples	Ct value average, no DNA cleanup, no lysis	Ct value average, no DNA Cleanup, <i>Listeria</i> lysis	Ct, DNA cleanup, lysis	Culture confirmation (MOX)
40 mL 24 LEB	8	44.59	45.07	0	Negative
90 mL 24 LEB	8	44.65	46.20	1* + result	1* + result

*The sole positive result after DNA cleanup was confirmed to be *Listeria*.



Asian government laboratory

This southeast Asian customer regularly encountered dead *Vibrio* cells of up to 10⁴ CFU/mL. They wanted to eliminate unconfirmable positives caused by DNA from dead cells after testing cooked shrimp. The lab tested the BAX[®] DNA Cleanup Kit for its ability to remove “free” DNA from dead *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* from frozen shell-on and de-shelled shrimp. The BAX[®] DNA Cleanup Kit effectively removed dead cell DNA up to 10⁵ CFU/mL. The table compares shelled and unshelled shrimp without and with treatment by the BAX[®] DNA Cleanup Kit.

Table 2. Detection of *Listeria* bacteriophage by BAX *Listeria* assay, before and after treatment with BAX[®] Free DNA Cleanup kit

Product Type	No Treatment Positives	With DNA Cleanup Kit Positives ¹
Shelled Cooked Shrimp	8	4*
Un-shelled Cooked Shrimp	16	3**

¹Culturally confirmed *Vibrio* samples

*≥10⁵ CFU/mL Free DNA removed

**10⁶ CFU/mL Free DNA removed

Hygiena laboratory testing

Hygiena’s researchers performed experiments testing genomic *Salmonella* DNA at different concentrations. This testing was completed with and without the BAX[®] Free DNA Cleanup Kit and then detection with the BAX[®] Real-Time PCR assay for *Salmonella*. The results in Table 4 demonstrate that although the genomic “free” *Salmonella* DNA was detected without the cleanup kit, once the BAX[®] DNA Cleanup step was introduced, no “free” DNA was detected.

Table 4. BAX[®] Real-Time *Salmonella* detection of Genomic *Salmonella* DNA with and without BAX[®] Free DNA Cleanup Kit

<i>Salmonella</i> DNA inoculation	PCR Ct value without cleanup	PCR Ct value with cleanup
400 nanogram*	25.5	0.0
40 ng	31.1	0.0
4 ng	34.6	0.0
400 picogram	38.8	0.0
40 pg	41.2	0.0
4 pg**	43.5	0.0

*≥10⁵ CFU/mL Free DNA removed

**10⁶ CFU/mL Free DNA removed