

# BAX<sup>®</sup> System Free DNA Cleanup Kit Performance Validation

## The Challenge

Bacteriophage treatments of food products and work environments in the food industry has gained popularity for effective control of pathogens such as *Salmonella*, *E. coli* and *Listeria*. Industrial phage products also contain remnants of lysed host cells including large amounts of their DNA. Consequently, customers using phage treatments have encountered several unconfirmable positives in PCR assays when testing for residual viable pathogens in both product and environmental samples.

To address the problem, a universal Free DNA cleanup kit has been developed for use with the BAX<sup>®</sup> System method.

## Protocol

A simple 2-step enzymatic procedure has been developed to remove extraneous DNA from samples.

### Required Equipment

- BAX<sup>®</sup> System Free DNA Cleanup Kit
- Thermal block or incubator maintaining 37°C and 55°C
- Cluster tubes
- 5-50 µl micropipette and sterile filter-tips
- BAX<sup>®</sup> System PCR assay

### Step 1

- In a cluster tube, combine 40µl enriched sample, 5µl BAX<sup>®</sup> System Free DNA Cleanup Agent, and 5µl BAX<sup>®</sup> System Free DNA Cleanup Agent Buffer.
- Incubate at 37°C for 15 min

### Step 2

- Add 5µl BAX<sup>®</sup> System Free DNA Cleanup Inactivation Agent
- Incubate at 55C for 15 min

## Performance

The BAX® System Free DNA Cleanup Kit 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 (Tables 1 and 2).

**Table 1.** PCR Signal from serial dilution of *Salmonella* genomic DNA with and without pre-treatment using the BAX® System Free DNA Cleanup Kit. Samples were tested using the BAX® System Real-Time PCR assay for *Salmonella*.

Amount of DNA in sample kit	PCR Signal without BAX® Free DNA Cleanup, Ct values	PCR Signal with BAX® Free DNA Cleanup, Ct values
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 ✓

**Table 2.** PCR Signal from serial dilution of *Listeria* genomic DNA with and without pre-treatment using the BAX® System Free DNA Cleanup Kit. Samples were tested using the BAX® System Real-Time PCR assay for *Listeria monocytogenes*.

Amount of DNA in sample kit	PCR Signal without BAX® Free DNA Cleanup, Ct values	PCR Signal with BAX® System Free DNA Cleanup, Ct values
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 ✓

## Conclusion

The BAX® System Free DNA Cleanup Kit can be used to remove DNA not protected by living cells from food and environmental matrices. This easy to use kit removes all extraneous DNA, including DNA from dead cells and DNA residuals from bacteriophage interventions, and is designed for easy integration with the BAX® System method.

The BAX® System Free DNA Cleanup procedure has been validated internally for poultry, poultry rinses and environmental surface testing. Data available upon request.