infoPoint

Nucleic acid decontamination with DNA/RNA-ExitusPlus™

Application

The complete decontamination of equipment and surfaces from any DNA and RNA molecules is important for biological containment and safety, as well as preventing false results in PCR amplification experiments. DNA/RNA-ExitusPlus™ is a safe nucleic acid decontamination solution for molecular biology laboratory. Catalytic and cooperative effects of the components of the solutions cause a very rapid non-enzymatic, non-sequence-specific degradation of DNA and RNA molecules.

DNA removal - all or nothing at all

Comparing DNA/RNA-ExitusPlus™ to conventional products, we can demonstrate that it is fast and efficient in destroying nucleic acids without harmful or toxic effects on lab workers, equipment and the environment. Most decontamination reagents are based on several molecular principles for the destruction or inactivation of genetic material: Modification and denaturation can mask, but do not destroy the genetic information encoded in DNA strands and there is the risk that they may be chemically re-activated. Thus, safe and complete DNA decontamination depends on the degradation of DNA into very small fragments. Fig. 1 shows comparison of the fragmentation process produced by DNA/RNA-ExitusPlus™ with conventional reagents. Complete degradation was only obtained with DNA/RNA-ExitusPlus™, whereas only partially degraded DNA fragments (some of which contained complete genetic information) were found in the other decontamination products that rely on modification or denaturation methodology.

Keywords

- Nucleic acid decontamination
- DNA degradation test
- PCR, qPCR

Non specific degradation of DNA strands

Only our patented DNA/RNA-ExitusPlus™ rapid and efficient degradation of nucleic acids, because its unique method of action is based on chemical and not enzymatic activity. Therefore, its effects on fragmentation are totally independent of the sequence and size of DNA fragments.
Larger plasmids require a longer incubation time than smaller ones (e.g. primers). The nicks will be introduced statistically at any site, leaving not a single class of fragments.

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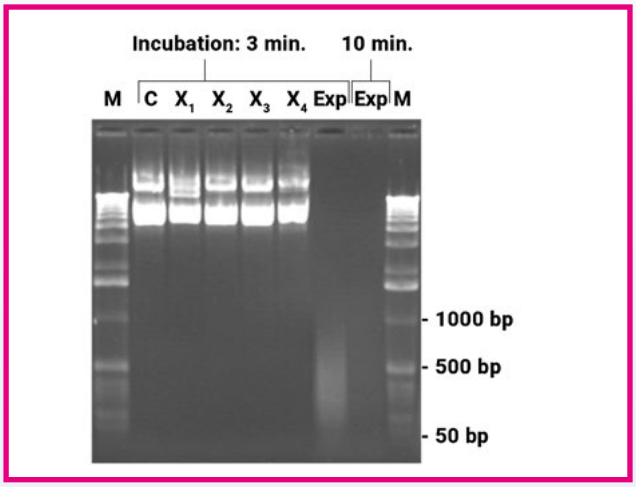

Figure 1. Comparison of DNA degradation by DNA/RNA-ExitusPlus™ and conventional DNA decontamination reagents.
200 ng of CCC plasmid DNA each, were treated with 5 µl of the indicated reagent for 3 or 10 min respectively. M = Molecular size marker, C = control (treated with water), X1, X2, X3, X4 = other products, Exp = DNA/RNA-ExitusPlus™.

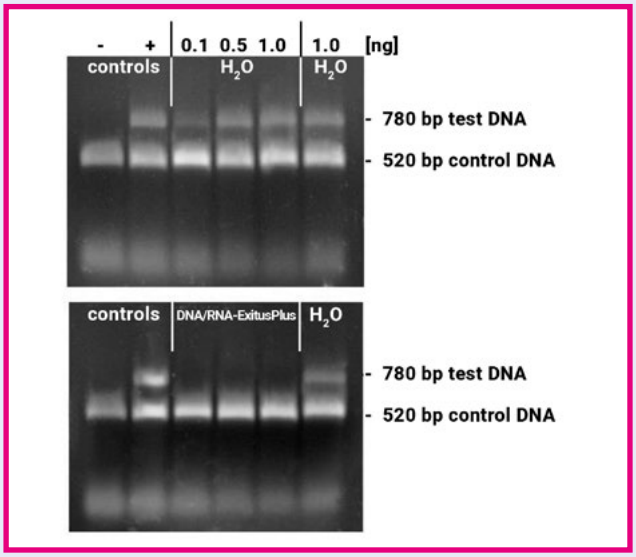

Figure 2. Complete removal of DNA contaminations by DNA/RNA-ExitusPlus™ determined by sensitive PCR assay.
Experimental procedure: Test DNA (0.1 to 1 ng) was lyophilized on the inner surface of PCR tubes, incubated for 20 secs with sterile water or DNA/RNA-ExitusPlus™, then washed twice with 100 µl of sterile water. For the PCR test we used 50 µl of each of the reaction mixtures , containing the appropriate primers for the amplification of the control and test DNA sequences. Control DNA (1 ng) in each sample proves that the PCR reaction is not inhibited.
Results: Amplification of a DNA band, corresponding to the test DNA, indicates that intact DNA molecules are present. Conversely, if no amplification DNA bands are present, it indicates complete degradation of the test DNA. The negative control with sterile water (H_2O) exhibits DNA bands for the test and control templates whilst after treatment with DNA/RNA-ExitusPlus™ only the fragment of the control DNA is amplified.

Sensitive PCR analysis (Fig. 2) shows that, after treatment with DNA/RNA-ExitusPlus™, no amplifiable DNA templates are present, proving there was a highly efficient degradation of DNA molecules. In this experiment, defined DNA samples were dried on the inner surface of reaction tubes, followed by treatment with DNA/RNA-ExitusPlus™. Only the positive controls, and the water treated controls showed amplification of the test DNA whereas the DNA/RNA-ExitusPlus™ treated samples did not show amplified sequences.

Spraying DNA/RNA-ExitusPlus™ on lab surfaces will ensure complete nucleic acid decontamination. The reaction time for DNA/RNA-ExitusPlus™ corresponds to the normal drying time (10 to 20 minutes).

Unwanted side effect of conventional reagents: Corrosion

Most conventional DNA decontamination products contain aggressive chemicals with corrosive, harmful or even toxic effects. Ingredients such as azides, mineral acids like phosphoric acid or hydrochloric acid, aggressive peroxides or strong alkaline substances like sodium hydroxide are in use. With such products irreversible damage of metal surfaces can be observed even after a short incubation time. In contrast, DNA/RNA-ExitusPlus™ shows no metal corrosion (Fig. 3).

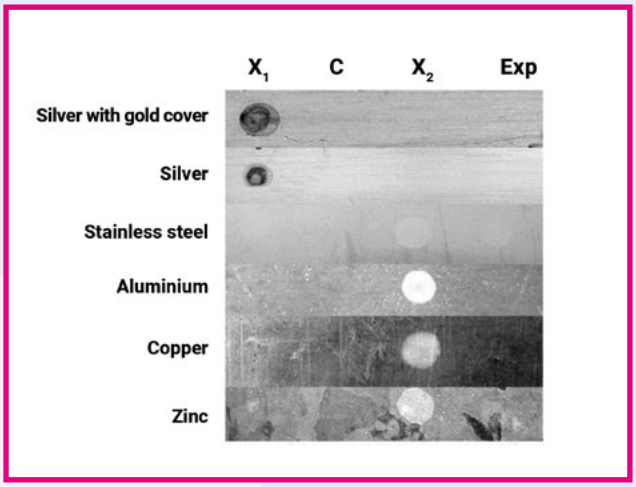

Figure 3. DNA/RNA-ExitusPlus™ has no corrosive potential compared to conventional DNA decontamination reagents.
Metal plates representing typical laboratory materials and equipment were treated with 10 µl of each indicated reagent for 20 minutes. No corrosive effects were observed when using DNA/RNA-Exitus™ (in some cases one observes a polishing effect by the removal of dirt or oxide layers).
C = water, Exp = DNA/RNA-ExitusPlus™, X1, X2 = other commercially available products.

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Autoclaving does not fully destroy nucleic acids

Finally, autoclaving is believed to be an effective method for DNA decontamination although limited for use with heat-resistant materials and equipment that fit into the autoclave. Under the standard autoclaving conditions, DNA molecules are believed to be degraded into very small fragments. But PCR analysis demonstrates that even after autoclaving, larger DNA fragments can be identified [1], especially when nucleic acids are protected by protein envelopes (e.g. viruses) or within microorganism cell walls (e.g. bacteria).
We therefore designed Autoclave-ExitusPlus™, an ExitusPlus™-based powder mixture, to be used as an additive for the decontamination of liquid waste. Due to its chemical composition, Autoclave-ExitusPlus™ (as it holds true for all other ExitusPlus™ reagents) is not heat-sensitive and does not contain volatile and harmful ingredients.
Figure 4 shows the effects of Autoclave-ExitusPlus™ on bacterial cultures and nucleic acids after autoclaving. Only with addition of Autoclave-ExitusPlus™ complete degradation of bacterial DNA is achieved, while under standard conditions, i.e. autoclaving in aqueous solution or culture medium, remains always undegraded and partially degraded DNA. Therefore, the use of autoclaving to eliminate DNA from microorganisms must be re-evaluated. The latest data shows that the nucleic acids from viruses and bacteria are not properly inactivated by simply autoclaving.

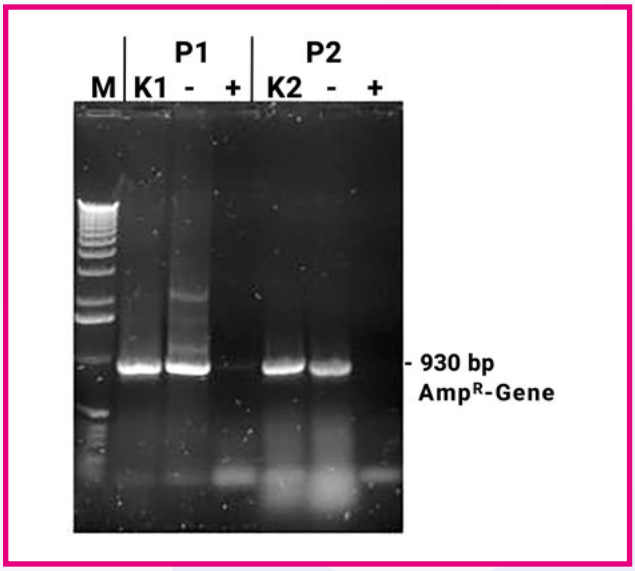

Figure 4. Analysis of two *E. coli* cultures by PCR, after autoclaving with or without Autoclave-ExitusPlus™.
Two recombinant *E. coli* cultures (P1 and P2) containing a plasmid with the Ampicillin resistance gene (AmpR-Gene) were autoclaved. Aliquots (2 µl) of the autoclaved cultures were analyzed by PCR for the presence of the complete AmpR gene.
(M) molecular size marker, (-) = *E. coli* plus water, (+) *E. coli* plus Autoclave-ExitusPlus™, (K1 and K2) = PCR amplification controls: *E. coli* plus Autoclave-ExitusPlus™ plus 2 ng template for the AmpR gene.

Summary

Only PCR analysis in combination with a DNA degradation test will show the true decontamination potential of a reagent, as it will avoid false results from masking or modifying of the nucleic acid material.

DNA/RNA-ExitusPlus™ solutions employ a mild and non-corrosive chemistry for a rapid non-enzymatic degradation of nucleic acids. Already short incubation times with DNA/RNA-ExitusPlus™ completely remove unwanted DNA and RNA from work surfaces and tools.

References

[1] Elhafi, G. et al. (2004) Microwave or autoclave treatments destroy the infectivity of infectious bronchitis virus and avian pneumovirus but allow detection by reverse transcriptasepolymerase chain reaction. Avian Pathology **33, 3003-306.**

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Outstanding and unique characteristics of DNA/RNA-ExitusPlus™

- Catalytic and cooperative effects cause a very rapid non-enzymatic, non-sequence- specific degradation of DNA and RNA molecules.
- All components of DNA/RNA-ExitusPlus™ are readily biodegradable and not harmful or toxic for humans.
- The solutions do not include aggressive mineral acids or alkaline substances. Equipment and materials are not damaged or corroded even after prolonged incubation times.

Products available

Product code	Product name	Pack sizes
A7600,1000	Autoclave-ExitusPlus™	Powder to treat 6 x 1 L of medium or cell culture
A7089,0100	DNA/RNA-ExitusPlus™	100 mL
A7089,0500		500 mL
A7089,1000RF		1 L
A7089,2500RF		2.5 L
A7409,0100	DNA/RNA-ExitusPlus™ IF	100 mL
A7409,0500		500 mL
A7409,1000RF		1 L
A7409,2500RF		2.5 L
A7409,5000		5 L

Note: DNA/RNA-ExitusPlus™ contains a light yellowish color indicator.
Typically, DNA/RNA-ExitusPlus™ comes in a spray bottle.
IF = "Indicator-Free" version
RF = Refill bottle, without spray pistol.

IP-020EN

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