AppliCations
No.1

Nucleic Acid Decontamination with The ExitusPlus™ Technology

Advanced experiments in gene technology demonstrate that even small amounts of free DNA molecules are sufficient to cause infections, recombination or biological transformation [1,2]. The complete decontamination of equipment and surfaces from any DNA molecules is important for biological containment and safety, as well as preventing artifacts in PCR amplification experiments. Using new methods that detect extremely low levels of DNA molecules, we investigated the molecular mechanism of action of various commercially available DNA decontamination reagents. We found that when using high concentrations of DNA and short incubation times, none of the conventional reagents destroyed DNA molecules efficiently, despite their corrosive or even toxic properties.

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Keywords

Nucleic acid decontamination

DNA degradation test

Autoclaving DNA

PCR test

All or Nothing at All

Destruction and elimination of nucleic acids depends to this day, on bleach, a corrosive and toxic substance. To address this issue, AppliChem partnered up with multiBIND Cologne, to develop a new and unique nucleic acid decontamination technology: the ExitusPlus™ family of products, comprising DNA-ExitusPlus™, and Autoclave-ExitusPlus™. Comparing DNA-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. Figures 1 and 2 show the results of comparing the fragmentation process produced by the novel DNA-ExitusPlus™ with conventional reagents. Using highly sensitive detection assays, it is seen that an efficient and total fragmentation was only obtained with DNA-ExitusPlus™, whereas only partially degraded DNA pieces, some of which contained complete genetic information, were found in the decontamination products that relied on modification or denaturation methodology.

Sequence-Independent Degradation of DNA

Only Applichem's DNA-ExitusPlus™ is capable of achieving 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 size and sequence of the DNA fragments. Larger plasmids require a longer incubation time than smaller ones (e.g. primers). Assuming a theoretical nicking activity of 100,000 nicks per minute, all DNA fragments will be destroyed, after several minutes, regardless of their size. Smaller fragments will disappear before the larger ones. Applying this theory to a test molecule (ccc form, 6 kb plasmid) only a small fraction of fragments with 200 to 500 bp in size will remain after 5 minutes. The nicks will be introduced statistically at any site, leaving not a single class of fragments. Remaining fragments are fully destroyed after 10 minutes.

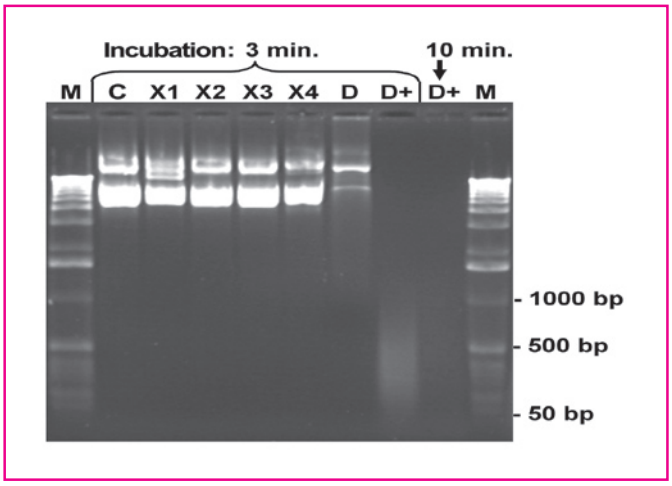

Figure 1. Comparison of DNA degradation by DNA-ExitusPlus™ and conventional DNA decontamination reagents. 200 ng of CCC plasmid DNA each, were treated with 5 µl of the indicated reagent for 3 or 10 min respectively.
C = control (treated with water);
M = Molecular weight marker;
X1, X2, X3, X4 = competitor's products;
D = conventional DNA-Exitus™ (discontinued);
D+ = DNA-ExitusPlus™.
For a complete protocol of the experiment please see our brochure: "Contaminations by Nucleic Acids: Problems & Practical Solutions".

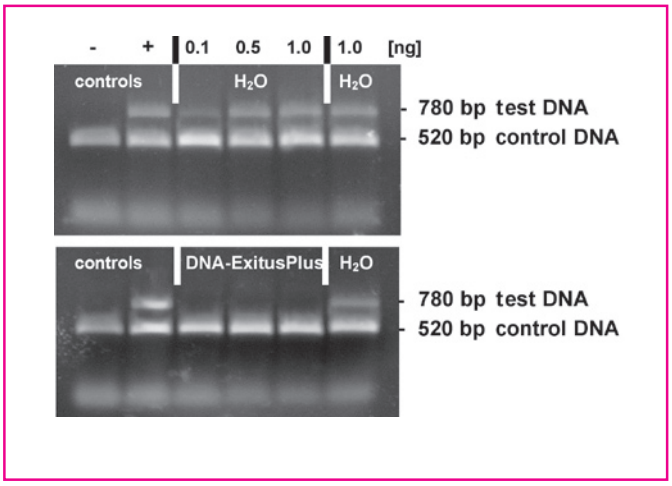

Figure 3. Complete removal of DNA contaminations by DNA ExitusPlus™ determined by PCR assay. Test DNA (0.1 to 1 ng) was lyophilized on the inner surface of PCR tubes, incubated for 20 secs with sterile water or DNA-ExitusPlus™, then washed twice with 100 µl of sterile water. For the PCR test we used 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. 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-ExitusPlus™ only the fragment of the control DNA is amplified.

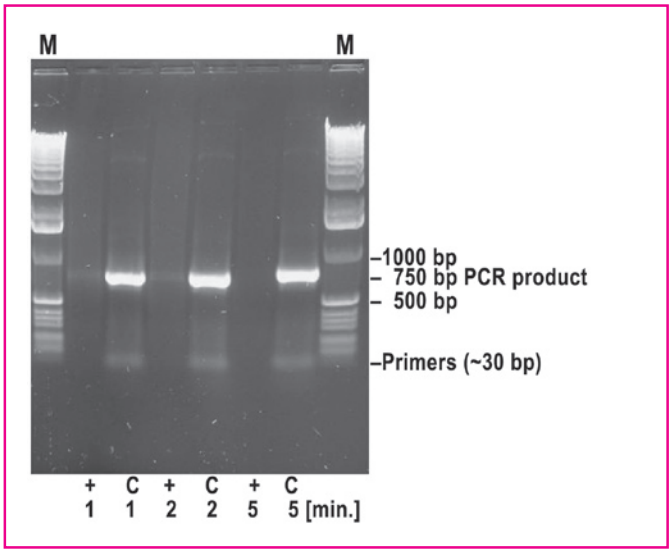

Figure 2. Degradation of small DNA fragments by DNA-ExitusPlus™.
500 ng of a 750 bp PCR product and the corresponding primers were treated for 1, 2, or 5 minutes with DNA-ExitusPlus™.
(+) 5 µl DNA with 5 µl DNA-ExitusPlus™;
(C) Control 5 µl DNA with 5 µl water;
(M) molecular weight marker (1 kb ladder).
After the treatment, the DNA was denatured for 2 minutes at 95°C.

In Figure 3, sensitive PCR analysis shows that, after treatment with DNA-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-ExitusPlus™. Only the positive controls, and the water treated controls showed amplification of the test DNA whereas the DNA-ExitusPlus™ treated samples did not show amplified sequences. These results are in stark contrast with other PCR tests that attempt to demonstrate successful DNA decontamination, by using large DNA control templates, low DNA concentrations and highly diluted samples. Only by using PCR analysis in combination with a sensitive DNA degradation test, can one be sure that the DNA is NOT merely modified or masked.

It is now clear that spraying DNA-ExitusPlus™ on lab surfaces will ensure complete decontamination. Moreover, the reaction time for DNA-ExitusPlus™ corresponds to the drying time after spraying on a surface (within 10 to 20 minutes).

Unwanted Side Effect of Conventional Reagents: Corrosion

Another severe disadvantage of conventional reagents is revealed in a test for their corrosive potential. For this purpose different metal plates were incubated with aliquots of the different products. Figure 4 shows that all conventional products contain aggressive chemicals with corrosive, harmful or even toxic effects including ingredients such as azides, mineral acids like phosphoric acid or hydrochloric acid, aggressive peroxides or strong alkaline substances like sodium hydroxide. Even after only 20 minutes of incubation, irreversible damage of metal surfaces can be observed, but DNA-ExitusPlus™ shows no metal corrosion. Other tests on different plastic surfaces show no damage either (data not shown). DNA-ExitusPlus™ therefore offers a gentle and environmentally safe alternative that degrades and removes all DNA molecules with high efficiency but is also neither toxic nor corrosive.

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

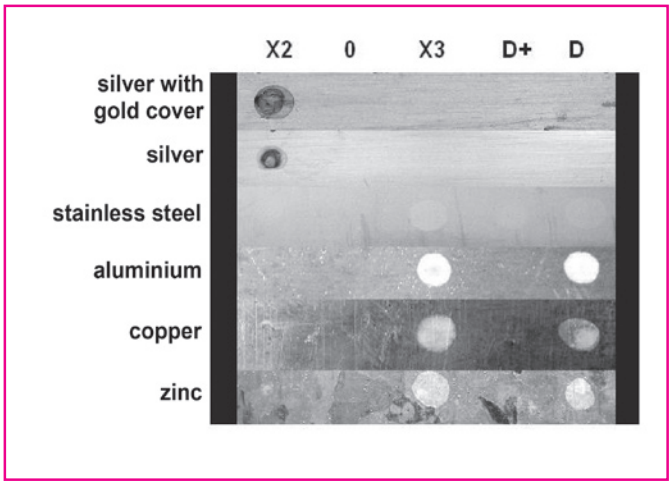

Figure 4. DNA-ExitusPlus™ has no corrosive potential compared to conventional DNA decontamination reagents. Metal plates representing typical laboratory materials and equipment were treated with 10 µl of each indicated reagent for 20 minutes. No corrosive effects were observed when using DNA-Exitus™ (in some cases one observes a polishing effect by the removal of dirt or oxide layers).
0 = sterile water;
X2, X3 = competitor's products;
D = conventional DNA-Exitus™ (discontinued);
D+ = DNA-ExitusPlus™.

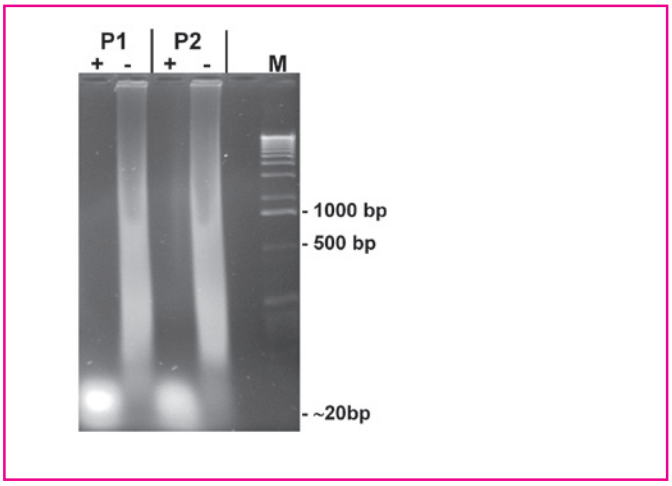

Figure 5. Autoclave-ExitusPlus™ leads to highly efficient bacterial DNA degradation. Cultures of recombinant *E. coli* (two comparable samples, 50 ml each) were subjected to autoclave treatment at 120°C over a period of 20 minutes, and 1.2 bar after adding either water (-) or Autoclave-ExitusPlus™ (+). 10 µl aliquots of these cultures were then examined in an analytical agarose gel. DNA degradation by Autoclave-ExitusPlus™ (+) results in fragments smaller than 20 base pairs, while treatment with water reveals high molecular weight DNA fragments.

the autoclave. Under the standard autoclaving conditions, DNA molecules are believed to be degraded into fragments of 20 to 30 base pairs. 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 created 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.
Figures 5 and 6 show the effects of Autoclave-ExitusPlus™ on bacterial cultures and nucleic acids at elevated temperatures, in autoclaves. Only the addition of Autoclave-ExitusPlus™ leads to an efficient degradation of bacterial DNA, while under standard autoclave conditions (aqueous solutions, culture medium) there is always undegraded / partially degraded DNA.

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.
In addition the use of autoclaving to eliminate DNA from microorganisms must be re-evaluated, since the latest data shows that the nucleic acids from viruses and bacteria are not properly inactivated by simply autoclaving.

DNA-ExitusPlus™ has outstanding and unique characteristics:

I. Its catalytic and cooperative effects cause a very rapid non-enzymatic, non-sequence-specific degradation of DNA and RNA molecules.
II. All components of DNA-ExitusPlus™ are readily bio-degradable and not harmful or toxic for humans.
III. No aggressive mineral acids or alkaline substances are used. Equipment and materials are not damaged or corroded even after prolonged incubation times.
IV. No harmful aerosols are formed when spraying on surfaces.

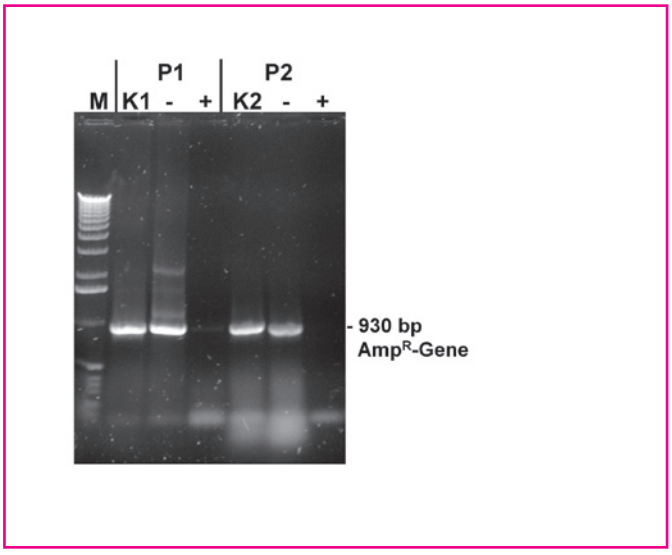

Figure 6. Analysis of E. coli cultures by PCR, after autoclaving with Autoclave-ExitusPlus™. Recombinant *E. coli* cultures containing a plasmid with the ampicillin resistance gene (Amp^R-Gene) were used. Aliquots (2 µl) of the cultures were analyzed by PCR for the presence of the complete Amp^R gene.
(-) = *E. coli* plus water;
(+) *E. coli* plus Autoclave-ExitusPlus™;
(K) = *E. coli* plus Autoclave-ExitusPlus™ plus 2 ng template for the Amp^R gene (positive control to prove that PCR is working); (M) molecular weight marker.

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 transcriptase-polymerase chain reaction. *Avian Pathology* **33**, 3003-306.

[2] Burns, P.A. *et al.* (1991) Transformation of mouse skin endothelial cells in vivo by direct application of plasmid DNA encoding the human T24 H-ras oncogene. *Oncogene* **6**(11), 1973-1978.

Product Name	Order No.	Quantity
Autoclave-ExitusPlus™	A7600,0100	1.2 L
	A7600,0250	3 L
	A7600,0500	6 L
	A7600,1000	6 L
DNA-ExitusPlus™	A7089,0100	100 ml
	A7089,0250	250 ml
	A7089,0500	500 ml
	A7089,1000	1 L
	A7089,1000RF*	1 L
	A7089,2500RF*	2.5 L
DNA-ExitusPlus™ IF**	A7409,0100	100 ml
	A7409,0250	250 ml
	A7409,0500	500 ml
	A7409,1000	1 L
RNase-ExitusPlus™	A7153,0100	100 ml
	A7153,0250	250 ml
	A7153,0500	500 ml
	A7153,1000	1 L
	A7153,1000RF*	1 L
	A7153,2500RF*	2.5 L

* Refill ** indicator free

For more information about nucleic acids decontamination please see our comprehensive brochure.

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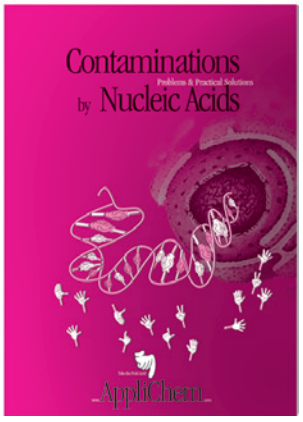

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