DNA Decontamination: Novel DNA-ExitusPlus™ in comparison with conventional reagents

Dr. Karl-Heinz Esser[1], Dr. Wolfram H. Marx[2] and Prof. Dr. Thomas Lisowsky[1]
[1]multiBIND biotec GmbH, Otto-Hahn-Str. 15, D-44227, Dortmund, Germany
[2]AppliChem GmbH, Ottoweg 4, D-64291 Darmstadt, Germany

Advanced experiments in gene technology demonstrate that even small amounts of free DNA molecules are sufficient to cause infections, recombinations or biological transformations and new methods with extremely low detection limits for the presence of DNA molecules have been developed. Therefore, detection of DNA contaminations or prevention of amplification artifacts in PCR experiments are essential in all applications. Accordingly, the complete decontamination of equipment and laboratory surfaces from any DNA molecules is important for biological containment and safety. These new developments motivated us to investigate the molecular mechanism of action of our product DNA-Exitus™ in comparison to other commercially available DNA decontamination reagents. Two major problems were apparent: First, none of the conventional reagents destroyed DNA molecules efficiently and second, all reagents contained components with corrosive or even toxic properties. Consequently, we saw the necessity to develop new solutions for effective DNA decontamination. For the first time we present the completely new DNA decontamination reagent DNA-ExitusPlus™. In comparison with conventional products, DNA-ExitusPlus™ guarantees fast and efficient destruction of nucleic acids without corrosive or toxic properties.

Polymerase Chain Reaction (PCR) has set a new milestone for the technology of DNA amplification [1]. Rapid extension of PCR technologies resulted in multiple new applications [2, 3]. Latest protocols and enzyme variants for PCR reactions allow the detection of even single DNA molecules [1]. One important consequence resulting from the improved sensitivity in DNA amplification is the necessity to avoid any contaminations from unwanted external DNA molecules.

In addition advanced experiments in gene technology demonstrate that often even free DNA molecules cause biological effects [4, 5]. Thereby infections, biological transformations or recombination of genomes are generated by free DNA plasmids or fragments [4 - 7]. Changes in viral and bacterial infectivity are observed and are related to the phenomenon of multiple resistances against antibiotics [8].

With respect to these findings, the decontamination of even the smallest quantities of DNA molecules from surfaces and equipment is critical. DNA decontamination reagents use three different molecular principles for destruction or inactivation of genetic material: modification, denaturation and degradation (see Figure 1). Safe DNA decontaminations depend on the degradation of DNA into very small fragments. We developed a DNA degradation test to compare conventional decontamination reagents with the novel DNA-ExitusPlus™. This test allows sensitive quantification of the fragmentation process (see Figure 2).

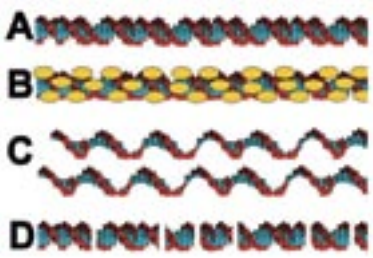

Fig. 1A - D. Scheme for the different principles of action for DNA decontamination. Intact double-strand molecules (a) are chemically modified (b) to block amplification reactions. Denaturation (c) separates double-strands into single-strand molecules. In both cases (b and c) the genetic information is not destroyed. Only DNA degradation (d) destroys the encoded genetic information. The efficiency of any DNA decontamination depends on the rapid degradation of the molecules.

One surprising result was the finding that some of the conventional reagents only use the principle of modification or denaturation for the inactivation of DNA molecules. This was determined in the test by the complete absence of any degraded DNA molecule. The genetic information encoded in these DNA strands is only masked but not destroyed. By chemical demasking reactions the genetic information of these molecules would be available again and could also be amplified by enzymatic reactions. Based on our knowledge about gene technology and the principles of recombination, we concluded that these reagents are no longer appropriate.

But even reagents that show degradation of DNA cause only limited and partial destructions. Hence, very large DNA fragments, even containing the complete genetic information, still survive treatment. Only our novel DNA-ExitusPlus™ achieves rapid and efficient degradation.

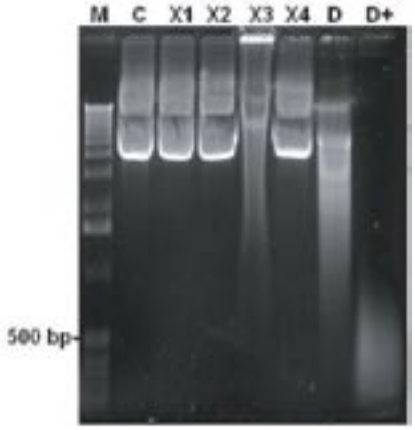

Fig. 2. Comparison of DNA degradation for selected conventional DNA decontamination reagents and DNAExitusPlus™. For each sample, aliquots of 1 µg covalent, closed, circular (CCC) plasmid DNA dissolved in 5 µl sterile Tris buffer (1 mM; pH 8.0) were treated with 5 µl of the listed reagents, respectively, for 2 min at 20°C. The control (C) contains intact CCC plasmid DNA after treatment with sterile water. The products X1, X2 and X4 cause no detectable degradation of the test DNA. For the product X3 and D (conventional DNA-Exitus) only partial degradation is observed. Only DNA-ExitusPlus™ (D+) causes very rapid and nearly complete DNA degradation.

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 reagents (see Figure 3). This test demonstrates that all conventional products contain aggressive chemicals with corrosive, harmful or even toxic effects. Known ingredients of conventional reagents are azides, mineralic acids like phosphoric acid or hydrochloric acid, aggressive peroxides or strong alkaline substances like sodium hydroxide. Therefore, even after only 20 minutes of incubation irreversible damages of metal surfaces are observed (see Figure 3). The newly developed DNA-ExitusPlus™ exhibits its unique characteristics especially in this test. For all metal surfaces no corrosions are observed. DNA-ExitusPlus™ was also tested on many different plastic surfaces without any indication of damages (data not shown). DNA-ExitusPlus™ offers an efficient, gentle and

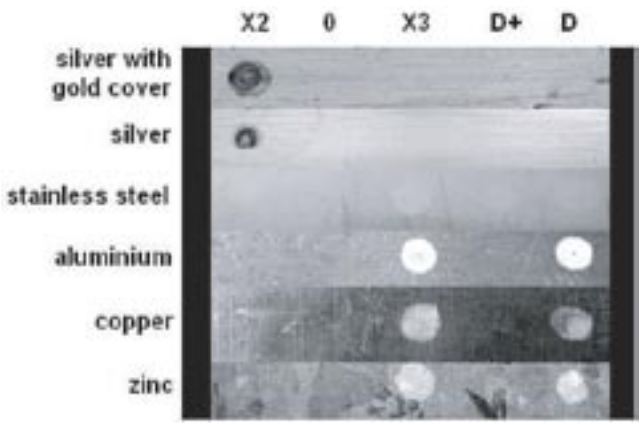

Fig. 3. Test of the corrosive potential of selected conventional DNA decontamination reagents in comparison with DNA-ExitusPlus™. For this test metal plates were chosen that are typical for laboratory materials and equipment. Aliqouts of 10 µl from each listed reagent were applied on the different metal surfaces. Sterile water was used as a control (C). After an incubation of 20 minutes the reagents were wiped off and the metals were briefly washed with sterile water. The reagents X2, X3 and D (see figure 2) for DNA decontamination cause irreversible corrosion and damages to many of the metal surfaces. For DNA-ExitusPlus™ (D+) no damage of any of the surfaces is observed. In some cases one observes a polishing effect by the removal of dirt or oxide layers.

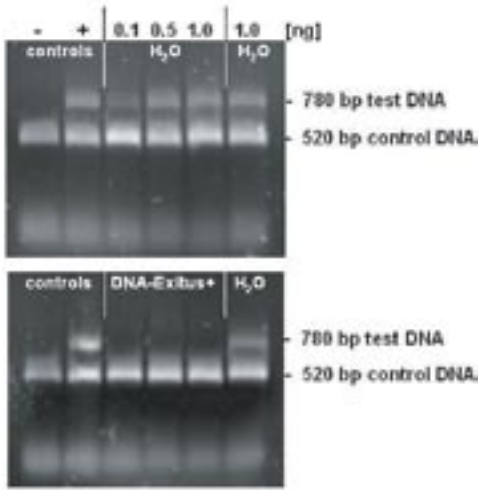

Fig. 4. PCR test for the complete removal of DNA contaminations by DNA-ExitusPlus™. Selected amounts (0.1 to 1 ng) of a test DNA were lyophilised on the inner surface of PCR tubes. PCR tubes with lyophilised DNA samples were incubated for 20 seconds with either sterile water or DNA-ExitusPlus™. Thereafter the tubes were washed twice with 100 µl of sterile water. For the PCR test reaction mixtures of 50 µl were added to each tube. This reaction mixture contains primers for the amplification of the control DNA (C) and the test DNA (T). The control DNA (1 ng) is added into each sample and proves that the PCR reaction is not inhibited. Amplification of a DNA band corresponding to the test DNA indicates that still intact DNA molecules of this template are present. Upon complete degradation and removal of the test DNA the PCR reaction should not amplify any DNA fragment for this template. After electrophoresis through a 1% agarose gel the gel was stained with ethidium bromide and documented. The negative control with sterile water (H2O) exhibits DNA bands for the test and control templates. The PCR reaction after tretament with DNA-ExitusPlus™ amplifies only the fragment of the control DNA. This demonstrates that the treatment with DNA-ExitusPlus™ destroyes and removes all traces of the test DNA template.

environmentally safe alternative and proves its superiority towards all other commercially available decontamination reagents. DNA-ExitusPlus™ not only degrades and removes all DNA molecules with high efficiency but in addition is neither toxic nor corrosive.

In summary one observes the following new and unique characteristics:

I. catalytic and cooperative effects of the components cause a very rapid non-enzymatic degradation of DNA and RNA molecules

II. all components of DNA-ExitusPlus™ are readily biologically degradable and not harmful or toxic for humans

III. no aggressive mineralic acids or alkaline substances are used. Equipment and materials are not damaged or corroded even after prolonged incubation times

Currently, the most effective method for DNA decontamination appears to be autoclaving. Under the standard conditions for autoclaving, DNA molecules are degraded into fragments of 20 to 30 base pairs. PCR analysis, however, demonstrate that even after autoclaving, larger DNA fragments can be identified [9]. Furthermore, autoclaving can only be used with heat-resistant materials and equipment that fit into the autoclave. Decontamination of laboratory benches or larger equipment is impossible.

Efficient degradation of DNA molecules by DNA-ExitusPlus™ was monitored by PCR analysis (see Figure 4) proving that no amplifiable DNA templates are present. Today, only very different non-standarized PCR tests are used as controls for successful DNA decontamination. In case of large DNA control templates, low DNA concentrations, and high dilutions in the washing steps, evidence for a successful DNA decontamination is very limited. Therefore one has to be very cautious about using a single PCR test as evidence for complete decontamination since such a PCR test would also be negative in case the DNA is only modified or masked. For complete evaluation of the potential of a DNA decontamination reagent one has to use PCR analysis in combination with a sensitive DNA degradation test.

All the current tests reveal the unique characteristics of DNA-ExitusPlus™. These characteristics provide new opportunities for potential applications in the health sector, life sciences, medical hygiene, food production and household. The development of DNA-ExitusPlus™ was achieved through a technological cooperation between multiBIND biotec GmbH, Dortmund and AppliChem GmbH, Darmstadt. We are convinced that this novel product defines a new standard for the efficient, rapid and gentle DNA decontamination. According to the latest results for the biological actions of free DNA molecules such a product is decisive for the new tasks concerning biological containment and safety in genetechnology and biomedical hygiene.

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