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DNA de_ _ation: DNA-ExitusPlus in comparison with co_ _agents

Here we preser_ _contamination reagent DNA-ExitusPlus. In comparison with conventior_ _uarantees fast and efficient destruction of nucleic acids without corrosive_ _lus was developed by AppliChem GmbH, Darmstadt, in cooperation wi_ _nd.

The polymerase chain reac_ amplification technology[1], a_ gies has resulted in multiple ne_ and enzyme variants for PCR re_ single DNA molecules[1]. One impo_ sensitivity in DNA amplification is th_ nation from unwanted external DNA r_

The latest research in genetic techno_ the presence of free DNA molecules can_ Infections, biological transformations or rec_ can be generated by free DNA plasmids or fr_ in viral and bacterial infectivity are observed and_ phenomenon of resistance against multiple antibiot_ the detection of DNA contamination or prevention of a_ artifacts in PCR expreiments is essential for all applications_ biological containment and safety.

Investigations of the properties of conventional DNA decontamination reagents revealed two major problems. First, none of the reagents studied destroyed DNA molecules efficiently, and second, existing reagents typically contain components with corrosive or even toxic properties. As a consequence, we saw the necessity to develop new solutions for effective DNA decontamination.

DNA decontamination reagents use three different molecular principles for destruction or inactivation of genetic material: modification, denaturation and degradation. Safe DNA decontamination depends on the degradation of DNA into very small fragments. We developed a DNA degradation test to compare conventional decontamination reagents with the new DNA-ExitusPlus. This test allows sensitive quantification of the fragmentation process (**Fig. 1**).

Figure 1 | Comparison of DNA degradation for selected conventional DNA decontamination reagents and DNA-ExitusPlus. 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.

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However, even reagents that degrade DNA ca_ and partial destruction. Hence, very large DNA fragments contai_ ing the complete genetic information still survive treatment. Only DNA-ExitusPlus achieves rapid and efficient degradation.

Another severe disadvantage of conventional reagents is revealed in a test of their corrosive potential. For this purpose different metal plates were incubated with aliquots of the reagents (**Fig. 2**). This test demonstrates that all currently available products contain aggressive chemicals with corrosive, harmful or even toxic effects. Known ingredients of conventional reagents are azides, mineral acids like

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phosphoric acid or hydrochloric acid, aggressive peroxides or strong alkaline substances like sodium hydroxide. Therefore, after only 20 min of incubation, irreversible damage of metal surfaces is observed (**Fig. 2**). The newly developed reagent DNA-ExitusPlus exhibits its unique characteristics especially in this test. For all metal surfaces no corrosion is observed; DNA-ExitusPlus was also tested on many different plastic surfaces without any indication of damage (data not shown). DNA-ExitusPlus offers an efficient, gentle and environmentally safe alternative and proves its superiority to other available decontamination reagents. DNA-ExitusPlus not only degrades and removes all DNA molecules with high efficiency but is neither toxic nor corrosive.

In summary, one observes the following new and unique characteristics: (i) catalytic and cooperative effects guarantee rapid nonenzymatic degradation of nucleic acids, (ii) all components of DNA-ExitusPlus are biodegradable and not harmful or toxic, (iii) no aggressive mineral acids or alkaline substances are used. Equipment and materials are not damaged or corroded even after prolonged incubation

Currently, the most effective method for decontamination appears to be autoclaving. Under standard conditions for autoclaving, DNA molecules are degraded into fragments of 20 to 30 base pairs. However, recent investigations with highly sensitive PCR analysis demonstrate that even after autoclaving larger DNA fragments can persist[9]. Furthermore, autoclaving can only be used for decontamination of 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 (**Fig. 3**) proving that no amplifiable DNA templates are present. Today, only very different nonstandardized PCR tests are used as controls for successful DNA decontamination. In the case of large DNA control templates, low DNA concentrations and high dilutions in the washing steps, evidence for successful DNA decontamination is very limited. Therefore one has to be very cautious about using a single PCR test as evidence for complete decontamination because such a PCR test would also be negative in the case where 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.

The tests described here reveal the unique characteristics of DNA-ExitusPlus. These properties offer new opportunities for potential applications in the health sector, the life sciences, medical hygiene, food production and the household. We are convinced that this product defines a new standard for efficient, rapid and gentle DNA decontamination. According to the latest results on biological activities of free DNA molecules, such a product is critical for the new tasks concerning biological containment and safety.

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