Autoclave-ExitusPlus™

Additive for the destruction of nucleic acids in culture media and buffers at elevated temperatures (e.g. autoclaving)
Product code A7600

Instructions for use

General notes

The product is suited for use at elevated temperatures (50°C to 133°C, inclusive autoclaving). Completely dissolve the powder before autoclaving. Assure good mixing of media, buffers and cell suspensions before starting autoclaving. During autoclaving, Autoclave-ExitusPlus™ permeates into microorganisms and viruses and destroys DNA, RNA and recombinant constructs. Larger cell pellets have to be resuspended completely to assure full access of the reagent to the cells.

The standard autoclaving

The standard procedure for autoclaving culture and media residues (waste) and for the sterilization of solutions has the following parameters: Autoclave for at least 15 minutes at a temperature of 121°C and a pressure of approx. 2 bar (or 200 kPa). This is a so-called liquid cycle, at which air is replaced by steam. The effective sterilization temperature within the waste is reached later than within the rest of the autoclave chamber. Therefore, the period of autoclaving and the quantity of the waste have to be adjusted.

Note: Do not add Autoclave-ExitusPlus™ directly to the deionized water of the autoclave and to the heating system - Residues of salts and staining by the color indicator would be observed.

For which applications can Autoclave-ExitusPlus™ be used?

Autoclave-ExitusPlus™ is used for the removal of nucleic acids from normal buffers and from resuspended cells or cell residues in standard growth media. Large, non-resuspended cell pellets have to be diluted and resuspended in water. Otherwise, the access of the reagent to the single cells is not given.

Solutions with an alkaline pH value (e.g. alkaline lysis of bacteria) have to be neutralized. Highly concentrated buffers and solutions of salts, acids or bases may reduce the effectivity or even inhibit the reagent. These solutions have to be diluted and thereafter the pH value has to be 4 to 8 - if necessary, adjust with HCl.

Solutions containing high concentrations of chaotropic salts have to be diluted to a final concentration of 50 to 100 mM. Make sure that the pH is in the range of 4 to 8 or adjust it.

Removal of residual Autoclave-ExitusPlus™

Residues of Autoclave-ExitusPlus™ on surfaces: To detect unwanted residues of Autoclave-ExitusPlus™ on surfaces, the reagent was supplemented with a color indicator. After complete drying, residues become purple to blue. Residues may be removed with either sterile water or TE buffer and a paper towel.

Disposal of solutions containing Autoclave-ExitusPlus™: Since Autoclave-ExitusPlus™ only contains environmentally friendly ingredients, there are no special measures for disposal required.

Application

Autoclave-ExitusPlus™ is supplied as a *ready-to-use* powder mixture. The pack sizes are designed to dissolve the complete content and not to take out aliquots. We do not recommend using aliquots. During storage the powder mixture may turn to a slight brownish color. The effectivity is neither influenced nor reduced!

Recommended use: Add the complete content of a pack directly to the corresponding volume of the liquid to be autoclaved. Smaller volumes may be chosen too. An increased concentration has positive effects. We recommend dissolving the powder by stirring.

Liquid waste of several cultures may be collected in one large bucket or glass beaker and autoclaved. Dirty vessels (e.g. Erlenmeyer flasks, test tubes, autoclavable centrifuge tubes), may be decontaminated when collected in one stainless-steel bucket or a large beaker, covered with water and Autoclave-ExitusPlus™.

Does autoclaving destroy nucleic acids completely?

The accepted opinion that autoclaving completely destroys nucleic acids, shall be a thing of the past. For most of us, it may be even surprising to learn that autoclaves are a rich source of nucleic acid contaminations in laboratories! As good as the autoclave is for inactivating live microorganisms, it is poorly suited to destroy nucleic acids (Espy et al. 2002, Elhafi et al. 2004, Simmon et al. 2004). During autoclaving, DNA molecules in recombinant microorganisms become fragmented only, and are released in large quantities with the steam while opening the autoclave. The statistical size distribution of such fragments is 1 to 2 kb, ideal for amplification in PCR reactions or transformations. High purity DNA analysis laboratories therefore removed autoclaves from the DNA work areas.

A fundamental problem is that no standardized detection assay for the effective destruction of nucleic acids exists. Just a DNA strand-break assay could show that most of the commercially available DNA decontamination reagents do not degrade DNA. DNA simply becomes non-amplifiable! Principally, two mechanisms exist to make DNA non-amplifiable:

1. degradation of DNA (e.g. by the addition of DNases or chemical destruction) or
2. by modification of the bases - i.e. the DNA strand is still intact but reading is blocked!

DNA/RNA-ExitusPlus™ (A7089) effectively destroys DNA and RNA on different surfaces. DNA/RNA-ExitusPlus™ not only introduces strand breakes into DNA and RNA, but splits DNA / RNA into its components. An amplification by PCR is impossible. Based on this patented product, Autoclave-ExitusPlus™ was developed. Addition of Autoclave-ExitusPlus™ to culture media residues or buffer solutions effectively degrades nucleic acids during autoclaving.

1. Cooperative and catalytic effects of the reagent components lead to a fast, non-enzymatic, non-sequence specific degradation of DNA and RNA molecules.
2. All components of Autoclave-ExitusPlus™ are biodegradable, non-harmful and non-toxic for humans.
3. No aggressive mineral acids or bases are included, so that equipment and materials won't be destroyed even after long incubation.
4. No organic solvents: No volatile components or toxic fumes.
5. Temperatures above approx. 50°C increase the reaction rate and efficiency!

Investigations have shown that high temperatures, especially those during autoclaving, multiply the reaction rate. The DNA degradation and the "additional" sterilization effect are achieved even if the autoclaving temperature is not reached, due to wrong settings or a defect of the autoclave, or a larger quantity of the liquid simply was not heated to 120°C.

DNA degradation during autoclaving

Dried DNA residues are difficult to remove from surfaces. Even autoclaving does not result in a sufficient degradation. After autoclaving of virus particles, complete viral genomes were detected (Elhafi et al. 2004). The effectivity of Autoclave-ExitusPlus™ as additive to solutions during autoclaving was tested. Elevated temperatures increase the activity!

As shown in fig. 1, DNA of recombinat *E. coli* - cultures autoclaved under the standard autoclaving conditions did not sufficiently destroy the nucleic acids. The addition of Autoclave-ExitusPlus™ led to an almost complete DNA degradation. These small fragments aren't dangerous. The average size of the fragments was smaller than 20 base pairs. A PCR test for the detection of the ampicillin resistance gene was performed. Without the addition of Autoclave-ExitusPlus™, the gene was detectable (fig. 2)!

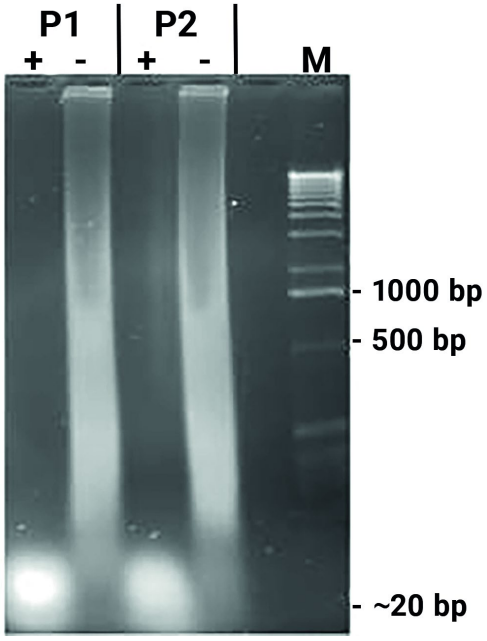

Fig. 1. Autoclaving of recombinant bacteria leads to a partial DNA degradation only.

50 ml cultures of recombinant *E. coli* cultures were autoclaved at 120°C, 1.2 bar for 20 minutes after addition of either water (-) or Autoclave-ExitusPlus™ (+). 10 µl aliquots of the cultures were analyzed on agarose gels. After addition of sterile water (-), large quantities of high molecular weight DNA fragments are detectable after autoclaving. An identical culture supplemented with Autoclave-ExitusPlus™ (+) reveals fragments smaller than 20 base pairs of degraded DNA. Two samples of the same reaction were loaded.

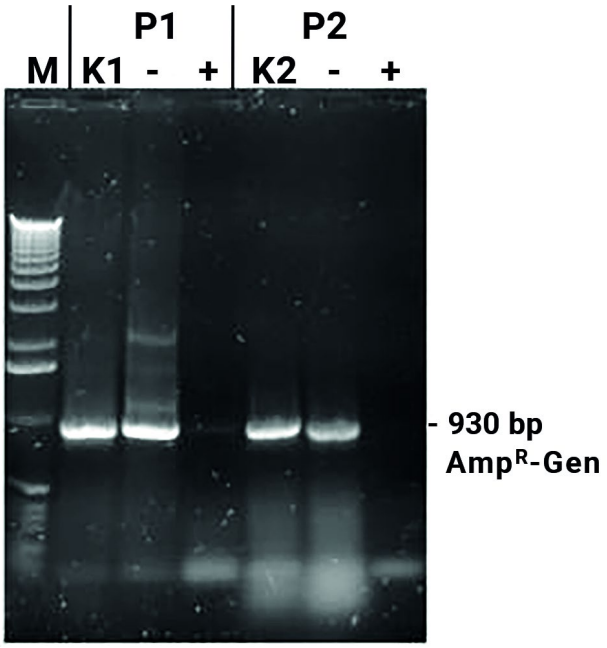

Fig. 2. PCR analysis of the autoclaved *E. coli* cultures from fig. 1.

Recombinant *E. coli* contain a plasmid carrying the ampicillin resistance gene (Amp^R-Gen). Therefore, 2 µl aliquots of the autoclaved cultures were tested in PCR reactions with primers covering the complete Amp^R gene. The sample of the reaction with addition of sterile water (-) showed a strong band for the complete Amp^R gene. The sample containing Autoclave-ExitusPlus™ (+) are negative for the Amp^R-PCR product. As a positive control (K) 2 µl aliquots of the Autoclave-ExitusPlus™ containing culture were supplemented with 2 ng template DNA for the Amp^R gene. The amplification of the corresponding DNA fragment in this reaction shows that the PCR reaction works under the experimental conditions. Two samples of the same reaction were loaded.

Literature:

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, 303-306.
Espy, M.J. et al. (2002) Detection of Vaccinia Virus, Herpes Simplex Virus, Varicella-Zoster Virus, and Bacillus anthracis DNA by LightCycler Polymerase Chain Reaction After Autoclaving: Implications for Biosafety of Bioterrorism Agents. Mayo Clin. Proc. 77, 624-628.
Simmon, K.E. et al. (2004) Autoclave method for rapid preparation of bacterial PCR-template DNA. J. Microbiol. Methods 56, 143-149.