

RNase and DNA Contamination Effectively Removed with Application of RNase AWAY[®]

ABSTRACT

Subjectivity of genetic experiments to RNase and DNA contamination leads to concern for the possibility of false positive and negative signals. Techniques to minimize chances of RNase introduction from exogenous sources such as autoclaving tubes and aliquoting reagents are recommended for preventing ribonuclease contaminations. Unfortunately, such treatments do not fully inactivate all RNases.

To further ensure contamination-free surfaces, extensive baking of glassware at 300° F for several hours is required. Diethylpyrocarbonate (DEPC) treatments are also effective in the removal of RNase from likely contaminated equipment and areas, but DEPC is suspected to be carcinogenic and must be handled with gloves and under approved fume hoods at all times.

INTRODUCTION

While the techniques listed above are successful in removing RNase contamination, they are also time-consuming and require careful execution. RNase AWAY provides a fast, easy and safe alternative to these methods while completely eliminating RNase and DNA contamination from laboratory surfaces and apparatus. To demonstrate this, tests were conducted to determine RNase AWAY's efficacy in eliminating unwanted RNase and DNA contamination from laboratory surfaces. Furthermore, tests were conducted to demonstrate RNase AWAY's ability to rinse away completely, leaving no damaging residues.

OBJECTIVE

Experiments were designed and conducted to test RNase AWAY's ability to remove RNase contamination from glass surfaces and its effectiveness at eliminating DNA.

MATERIALS AND METHODS

Experiment #1: Complete Removal of RNase Contamination with RNase AWAY

To test for RNase AWAY's ability to remove RNase from glass surfaces, the bases of several glass beakers were contaminated with RNase and then washed with RNase AWAY. RNA standards were placed on the beakers and left for a period of time. The RNA was then placed back in a test tube and incubated in the presence of mono and divalent cations for one hour, allowing any nuclease present to degrade the nucleic acid in the tube. After incubation, the RNA was evaluated by electrophoresis on an agarose gel. Appropriate controls were run to check for false positives and negatives.

Data

All apparatus were treated with diethylpyrocarbonate (DEPC) to inhibit possible RNase contamination from sources outside the experiment. The bases of five small beakers were contaminated with RNase through extensive handling with ungloved hands. Two of these beakers had RNase AWAY placed on them and were left to soak overnight. The following day, two of the remaining beakers were treated with RNase AWAY and wiped clean with Kimwipes[®]. One remaining beaker was used as a positive control for "hand" RNase activity.

After a variety of rinsing conditions, 1 µg of an RNA standard, comprised of 7.5 kb poly (A)-tailed RNA in a buffer solution with both sodium and magnesium ions, was placed on all beaker bases and left for a period of one minute and then placed back into a test tube. An RNA standard unexposed to RNase was included to represent a negative control. An RNA standard exposed to the remaining beaker, which was contaminated but not washed with RNase AWAY, was included to represent a positive control. All samples were incubated for one hour at 37° C then run on a 1.2% agarose gel containing ethidium bromide in 1/2X TAE for 20 minutes at 80 volts. The gel was then photographed and the samples were evaluated for degradation.

Experiment #2: Complete Degradation of "Unwanted" DNA Contaminants with RNase AWAY and Residual Effect on "Wanted" DNA

To test for RNase AWAY's ability to eliminate DNA contamination, small aliquots of DNA were placed into test tubes and allowed to evaporate overnight leaving behind residual DNA. The next day, the tubes were filled with RNase AWAY. The product was then extracted and water was added to reconstitute any DNA which may have remained within the test tube.

A series of RNase AWAY dilutions was also prepared to test the effectiveness of RNase AWAY in eliminating DNA when added to DNA in water. The product was then tested for any residual effect it may have on "wanted" DNA which would come in contact with a surface that had been treated with RNase AWAY.

Data

One microliter aliquots of a linear 1 kb DNA ladder (1 mg/ml) were placed in three microcentrifuge tubes and left to evaporate overnight. The next day, 100 µl of RNase AWAY were placed into each of the first two tubes and left to stand for five minutes. The solution was poured off and the tubes were spun in a centrifuge for one minute. The remaining solution was extracted with a pipet tip.

One of the tubes was rinsed with distilled water, poured off and spun down again. The final volume of the rinse was again extracted with a pipet tip. Ten microliters of RNase AWAY were added to the third tube and mixed by pipetting up and down. One microliter of the same 1 kb DNA ladder was placed into each of the two additional tubes. Nine microliters of RNase AWAY were added to one tube and 9µl of distilled water (as a control) were added to the other tube. All samples ran on a 1.2% agarose gel containing ethidium bromide in 1/2X TAE for 20 minutes at 80 volts. The samples were then photographed and evaluated.

A series of RNase AWAY dilutions of 100%, 50%, 10% and 1% were prepared. Five microliters of each dilution were added to 5 μ l of water containing 1 μ g of linear 1 kb DNA ladder. This yielded final dilutions of 50%, 25%, 12.5%, 5% and 0.05%. Additionally, 500 μ l of RNase AWAY were added to two tubes.

The tubes were left to stand for five minutes at room temperature. Then the solution was poured off and the tubes centrifuged for one minute. The remaining solution was extracted with a pipet tip. One microgram of linear 1 kb DNA ladder in 10 μ l of distilled water was placed into each tube. An identical aliquot of the DNA was placed into an unwashed tube as a control. These samples ran on a 1.2% agarose gel for 20 minutes at 80 volts. The samples were then photographed and evaluated.

RESULTS

RNase AWAY effectively eliminated RNase and DNA contamination from laboratory surfaces when directions for use were followed.

In Figure 1 below, lane (a), which represents the overnight soak without a rinse, shows severe degradation of the RNA. Lanes (b) through (d), which represent the various wash and rinse protocols as stated on the product label, show no signs of RNA degradation. Lane (e), which represents the unexposed sample as a negative control, shows no degradation of the RNA standard. Lane (f), which represents the contaminated glass surface as a positive control, shows severe degradation of the RNA standard.

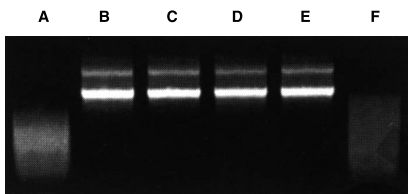


Figure 1. Removal of RNase with RNase AWAY

Figure 1 is a 1.2% agarose gel showing samples tested for RNase activity. The following lanes were exposed to 7.5 kb poly (A)-tailed: (a) contaminated glass surfaces soaked overnight in RNase AWAY and not rinsed, (b) contaminated glass surface soaked overnight in RNase AWAY and rinsed with distilled water, (c) contaminated glass surface "wiped" with RNase AWAY and a Kimwipe, (d) contaminated glass surface "wiped" with RNase AWAY and a Kimwipe then rinsed with distilled water, (e) unexposed RNA standard as a negative control, and (f) RNA standard exposed to a contaminated glass surface (unwashed) as a positive control.

In Figure 2 below, lanes (a) through (d) show no signs of DNA. Lane (e), which represents the negative control, is intact.

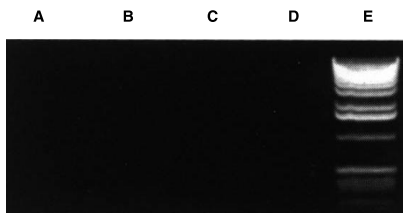


Figure 2. Eliminating DNA Contamination with RNase AWAY.

Figure 2 is a 1.2% agarose gel showing samples tested for DNA degradation. The following lanes show results from the: (a) tube containing residual DNA to which RNase AWAY was added then extracted, (b) tube containing residual DNA to which RNase AWAY was added, extracted and then rinsed with distilled water, (c) tube containing residual DNA to which 10 μ l of RNase AWAY were added, (d) tube containing 1 μ g of DNA in solution to which 9 μ l of RNase AWAY were added, (e) tube containing 1 μ g of DNA in solution to which 9 μ l of water were added as a control.

In Figure 3 below, lane (a), which represents the 50% dilution of the RNase AWAY, shows a slight degradation of the DNA. Lanes (b) through (e), which represent the 25%-0.05% dilution of the RNase AWAY, show no degradation of the DNA. Lanes (f) and (g), which represent the DNA exposed to the treated surface, show no degradation of the DNA, as does lane (h), which represents the negative control.

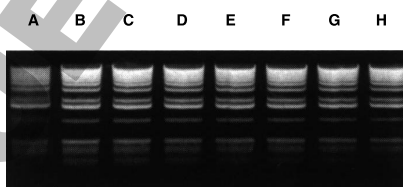


Figure 3. Effects of Residual RNase AWAY

Figure 3 is a 1.2% agarose gel showing samples tested for DNA degradation. The following lanes show results from: (a) 50% dilution of RNase AWAY added to 1 μ g of DNA, (b) 25% dilution of RNase AWAY added to 1 μ g of DNA, (c) 12.5% dilution of RNase AWAY added to 1 μ g of DNA, (d) 5% dilution of RNase AWAY added to 1 μ g of DNA, (e) 0.05% dilution of RNase AWAY added to 1 μ g of DNA, (f) one microgram of DNA in 10 μ l of water placed in a tube, which was washed with RNase AWAY and rinsed once, (g) one microgram of DNA in 10 μ l of water placed in a tube, which was washed with RNase AWAY and rinsed twice, (h) one microgram of DNA in 10 μ l of water as control.

CONCLUSIONS

RNase AWAY proved to be effective in eliminating RNase and DNA contamination from laboratory surfaces.

Unlike other products, RNase AWAY does not leave a harmful residue after a surface is washed with the product. Labware is decontaminated by soaking, wiping or spraying with RNase AWAY right from the bottle, then rinsing or wiping the surface dry. This enables researchers to safely work with "wanted" samples. No mixing or preparation is required since RNase AWAY is premixed. The process is fast and simple.

This study shows that RNase AWAY is an effective replacement for DEPC, a known carcinogen. RNase AWAY was proven to be ideal for cleaning pipettor barrels, gel boxes, benches and labware that cannot be autoclaved.

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References:

Fritsch E.F., T. Maniatis, J. Sambrook; *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989).

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