Instructions for use

RNase AWAY™

A998

For efficient removal of RNases and DNA-contamination from surfaces

I. Introduction

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 280 °C for several hours is required. Diethyl pyrocarbonate (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.

While these techniques are successful at removing RNase contamination, they require extensive time and careful execution.
A viable substitute for these procedures employs the direct application of *RNase AWAY*™ to surfaces and laboratory equipment that are subjected to risk of RNase contamination. We present the test results from the applications of *RNase AWAY*™ on intentionally contaminated beaker bases which had been subsequently treated with an RNA standard comprised of 7.5 kb poly (A)-tailed RNA and incubated one hour.

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Furthermore, the *RNase AWAY*™ performance assay also details the success of *RNase AWAY*™ in the degradation of "unwanted" DNA through the insertion of one microliter aliquots of a linear 1 kb DNA ladder into micro centrifuge tubes and allowed to evaporate overnight. Finally, our evaluation of *RNase AWAY*™ residues on DNA has shown that *RNase AWAY*™ yields no effect on "wanted" DNA.

II. Use

II.I. Cleaning of surfaces by wiping
Apply undiluted *RNase AWAY*™ to the surface and incubate for approx. 1 minute. Wipe the surface with a clean paper towel and rub down.
Alternatively, clean surface thoroughly with *RNase AWAY*™ wipes and rub down.
II.II. Incubation
Incubate all items over night in undiluted *RNase AWAY*™. Rinse with distilled, RNase-free water and let dry.

III. Test Performed

Test for *RNase AWAY*™'s ability to remove RNase contamination from glass surfaces and its effectiveness at eliminating DNA.

IV. Findings

When directions are followed, *RNase AWAY*™ effectively eliminates RNase contamination from the surface of glassware. *RNase AWAY*™ effectively eliminates DNA. There appears to be no effect of "wanted" DNA being degraded by residual *RNase AWAY*™ after a surface has been washed with the product. *RNase AWAY*™ should not be diluted.

V. Testing Procedure

To test for *RNase AWAY*™'s ability to remove RNase from glass surfaces, the bases of several class 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 possible 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.
To test for it'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*™ at 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*™.

VI. Methods

VI.I. Complete Removal of RNase Contamination with RNase AWAY™:
All apparatus was treated with Diethyl pyrocarbonate (DEPC) to inhibit possible RNase contamination from sources outside the experiment. The bases of 5 small beakers were contaminated with RNase by extensive handling with ungloved hands. Two of these beakers had *RNase AWAY*™ placed on them and were left to soak overnight. The following day, the solution of the beakers was just poured away, leaving a film of *RNase AWAY*™ (a), while the other one was rinsed with distilled, RNase-free water (b). Two of the remaining beakers were treated with *RNase AWAY*™ and wiped clean with "Kimwipes" (c,d). One of these two was additionally rinsed with distilled, RNase-free water (d). One microgram of an RNA standard comprised of 7.5 kb poly (A)-tailed RNA in a buffer solution with both sodium and magnesium ions were placed on all beaker bases and left for a period of 1 minute and then were placed back into a test tube. An RNA standard unexposed to RNase was included to represent a negative control (e). An RNA standard exposed to the remaining beaker, which was contaminated but not washed with *RNase AWAY*™, was included to represent a positive control (f). All samples were incubated one hour at 37 °C and then run on a 1.2 % agarose gel containing ethidium bromide in 0.5x TAE for twenty minutes at 80 volts. The gel was then photographed and the samples were evaluated for degradation.

VI.II. Complete Degradation of „unwanted“ DNA Contaminants with RNase AWAY™:
One microliter aliquots each of a linear 1 kb DNA ladder (1 mg/ml) were placed in three microcentrifuge tubes and left to evaporate overnight. The following day one hundred microliters of *RNase AWAY*™ were placed into each of the first two tubes and left to stand for five minutes. The solution was then poured off and the tubes were spun in a centrifuge for one minute. The remaining solution was extracted with a pipet tip (a,b). One of the tubes was rinsed with distilled water and again poured off, spun down, and the final volume of the rinse was extracted with a pipet tip (b). Ten microliters of distilled water were added to both tubes to reconstitute any DNA which may have remained. Ten

microliters of *RNase AWAY*™ were added to the third tube and mixed by pipeting up and down (c). One microliter of the same 1 kb DNA ladder was placed into each of two additional tubes (d,e). Nine microliters of *RNase AWAY*™ were added to one tube (d) and nine microliters of distilled water (as a control) were added to the other tube (e). All samples were then run on a 1.2 % agarose gel containing ethidium bromide in 0.5xTAE, for twenty minutes at 80 volts, photographed and evaluated.

VI.III. The Effects of RNase AWAY™ Residues on DNA:
A series of *RNase AWAY*™ dilutions of 100 %, 50 %, 25 %, 10 % and 1 % were prepared. Five microliters of each dilution were added to five microliters of water containing one microgram of linear 1 kb DNA ladder. This yielded final dilutions of 50 %, 25 %, 12.5 %, 5 % and 0.5 % (a-e). Additionally, two tubes received five hundred microliters of *RNase AWAY*™. The tubes were left to stand for five minutes at room temperature. The solution was then poured off, the tubes were centrifuged for one minute and the remaining solution was extracted with a pipet tip. One of the tubes was rinsed once with de®stilled water (f), the other tubes was rinsed twice (g). One microgram of linear 1 kb DNA ladder in ten microliters of distilled water was placed into each of the tubes. An identical aliquot of the DNA was placed in an untreated tube as a control (h). These samples were run on a 1.2 % agarose gel in 0.5xTAE for twenty minutes at 80 volts, photographed and evaluated.

VII. Results

VII.I. Removal of RNase with *RNase AWAY*™

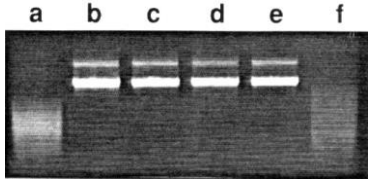

Fig.1: 1.2 % agarose gel showing samples tested for RNase activity.
a-d: 7.5 kb poly (A)-tailed RNA was exposed to:
(a) contaminated glass surface soaked overnight in *RNase AWAY*™ and not rinsed
(b) contaminated glass surface soaked overnight in *RNase AWAY*™ and rinsed with distilled water
(c) contaminated glass surface wiped with *RNase AWAY*™ and a paper towel
(d) contaminated glass surface wiped with *RNase AWAY*™ and a paper towel and then rinsed with distilled water
e,f: Standards
(e) unexposed RNA standard as a negative control
(f) RNA standard exposed to a contaminated glass surface (untreated) as a positive control.
Lanes (a) which represent the overnight soak without a rinse shows severe degradation of the RNA by the chemical compound. 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 (c) 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.

VII.II. Eliminating DNA Contamination with *RNase AWAY*™

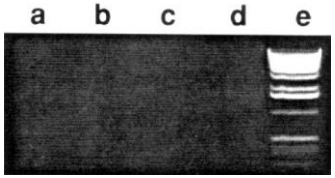

Fig.2: 1.2 % agarose gel showing samples tested for DNA degradation.
(a) tube containing residual DNA to which *RNase AWAY*™ was added, DNA eluted with water
b) tube containing residual DNA to which *RNase AWAY*™ was added, rinsed with distilled water, DNA was eluted with water
(c) tube containing residual DNA to which ten microliters of *RNase*
(d) 1 µg DNA in solution + 9 µl *RNase AWAY*™
(e) 1 µg DNA in solution + 9 µl distilled water

Lanes (a) through (d) show no DNA at all, while lane (e) which represents the negative control is intact.

VII.III. Effects of Residual *RNase AWAY*™

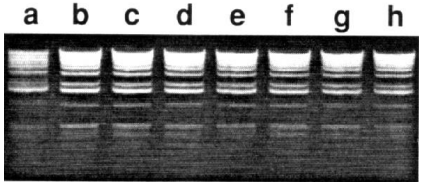

Fig.3: 1.2 % agarose gel showing samples tested for DNA degradation.
(a) 50 % dilution of *RNase AWAY*™ added to one µg of DNA
(b) 25 % dilution of *RNase AWAY*™ added to one µg of DNA
(c) 12.5 % dilution of *RNase AWAY*™ added to one µg of DNA
(d) 5 % dilution of *RNase AWAY*™ added to one µg of DNA
(e) 0.5 % dilution of *RNase AWAY*™ added to one µg of DNA
(f) one µg of DNA in ten microliters of water placed in a tube which was washed with *RNase AWAY*™ and rinsed once
(g) one µg of DNA in ten microliters of water placed in a tube which was washed with RNase A WAY and rinsed twice
(h) one µl of DNA in ten µl of water as a control.
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 %-0.5 % 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.

VIII. Conclusions

- An overnight soak with *RNase AWAY*™ with a subsequent rinse is effective at removing all RNase contamination from a glass surface.
- Wiping with *RNase AWAY*™ and a paper towel is effective at removing RNase contamination from glass surfaces with or without a rinse with RNase-free water.
- *RNase AWAY*™ is effective at eliminating DNA provided it is used at full strength.
- *RNase AWAY*™ does not appear to have any residual effect on "wanted" DNA which comes in contact with an apparatus which has been washed according to label directions.

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IX. Reference

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

⚠ **Warning** H315-H319
P280-P302+P352-P305+P351+P338

RNase AWAY™	250 ml	**A998.1**
	1 l	**A998.2**
	4 l	**A998.3**
	Spray (475 ml)	**A998.4**