Technical Application Bulletin

RNase FREE Water... Who Said It Couldn't Be Done?

RNase is an enzyme that causes the degradation of RNA molecules. It is a critical regulator of life processes in the cell. As critical as it is to this process, it is devastating to any RNA studies that rely on the presence of RNA.

Research involving RNA has become extremely important in the molecular biology field. RNA is an extremely unstable and difficult molecule to work with. The presence of RNase in nature and in the laboratory as well as RNA degrading on its own makes satisfactory yields difficult to obtain. With its increased importance in the research community, it has become necessary to ensure that a RNase-free environment exists and that all reagents utilized are prepared to ensure that RNase is not present. RNase, if present even in trace quantities, will severely limit the ability of researchers to conduct studies where RNA is essential to the results. Yields and product quality are drastically reduced in the presence of even a small amount of RNase. An example of this is in metabolism studies where they rely on the presence of mRNA whose levels are at or below detectable limits in cell extracts, any loss due to the presence of RNase will make achieving acceptable results impossible. RNA mapping, ribonuclease protection assays and northern blot hybridization are also affected by the presence of RNase.

Water is an essential reagent for most molecular biology applications. Ensuring that RNase is not present in the water has long plagued researchers. Up to this time, research has relied on DEPC to inactivate RNase. Using DEPC is expensive, time consuming and toxic. For these reasons, researchers would prefer not to depend on this chemical. DEPC also alters the chemistry of the water and could potentially affect results by the addition of both inorganic and organic impurities.

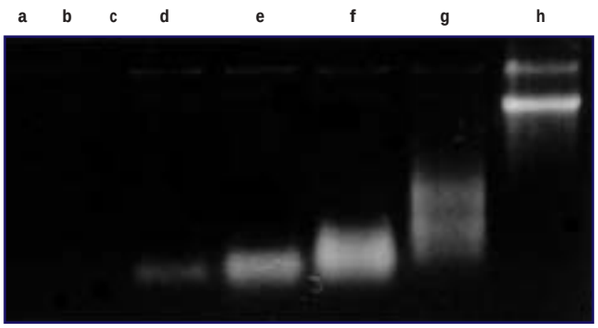
The Barnstead NANOpure® DIamond™ and EASYpure™ II UV/UF water purification system were designed to remove RNase from purified water without the use of DEPC. The technologies incorporated in the water purifier have demonstrated, through an independent detailed study, that RNase is completely removed by the system. Both systems incorporate adsorption, deionization, UV oxidation, and ultrafiltration within the same unit. We believe that ultrafiltration is the component that is primarily responsible for the majority of RNase removal. We also know that ultrafiltration is not absolute in its ability to remove impurities above the rated pore size of the filter. If no other technologies were present, a small amount of the challenged RNase would escape from the system and contaminate the product water. This was not the case as will be proven by the reported results. We believe that the carbon adsorption process, the strong base anion exchange and UV oxidation compliments the ultrafilter in the removal of RNase.

An independent two phase study was conducted at MO BIO Laboratories in Solana Beach, California. In the first phase, RNA spiked water was processed through the systems and the product water from the system was tested for detectable RNase activity. The detection of RNase activity is based on electrophoresis of RNA standards which were incubated with test effluent water samples from the UV/UF system. The RNA standard was a 7 kb poly (A) tailed mRNA. If RNase was present during the incubation, the RNA standard would be degraded by enzymatic activity.

In phase two, the systems were repeatedly challenged with RNase spiked feed water over a four month period. The system was challenged, once a week, for three weeks followed by twice a month for 3 additional months.

Sensitivity of the test method was determined by making ten-fold serial dilutions of RNase cocktail stock solutions (10 mg/ml RNase A, 2.6 mg/ml RNase T1). Serial dilutions were incubated separately with a 7 kb RNA standard in 100 mM NaCl, 10 mM MgCl2 at 37°C for 60 minutes. The reactions were then loaded in separate lanes from the highest to the lowest concentration of RNase added and run on a 1.2% Agarose gel in 0.5 x TAE containing 5 mg/ml of ethidium bromide. The gel banding of the RNA standard was dependent on the level of degradation caused by RNase. As the level of RNase decreased, the RNA banding was less smeared. The level of the sensitivity of the test method was represented by the lane where smearing still occurred. RNA incubated with DEPC treated water showed no smearing and was used for comparison. The sensitivity was confirmed to be 10-12 g (1 picogram) of RNase A/0.26 picograms RNase T1. The gel photograph shown below (Gel 1) shows the confirmation result of the reported sensitivity.

Gel 1



Lane (a) 1 mg/ml RNase A, 0.27 mg/ml RNase T1
Lane (b) 1 x 10-1 mg/ml RNase A, 3x10-2 mg/ml RNase T1
Lane (c) 1 x 10-2 mg/ml RNase A, 3x10-3 mg/ml RNase T1
Lane (d) 1 x 10-3 mg/ml RNase A, 3x10-4 mg/ml RNase T1
Lane (e) 1 x 10-4 mg/ml RNase A, 3x10-5 mg/ml RNase T1
Lane (f) 1 x 10-5 mg/ml RNase A, 3x10-6 mg/ml RNase T1
Lane (g) 1 x 10-6 mg/ml RNase A, 3x10-7 mg/ml RNase T1
Lane (h) Negative control, or zero RNase

The photograph shows levels of detection as low as 10-6 mg (1 picogram) /ml RNase A and 3x10-7 mg (0.3 picogram)/ml RNase T1.

Validation of NANOpure® DIamond™ UV Water Trace Organic Compounds

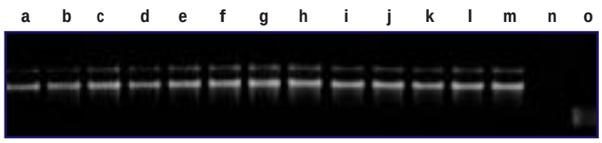
The testing protocol utilized for the 10 assays completed during the four month study is listed below. All the assays were performed according to this schedule.

1. Switch the EASYpure UV/UF from STANDBY to ON.
2. Draw off 200 ml. of water from the EASYpure UV/UF.
3. Prepare RNase cocktail: 100ml RNase stock: 50 mg/ml Lot: A2214A39 100ml RNase T1 Stock: 1000u/ml Lot: AM112, 500 ml DI water. Save 50 ml for positive control.
4. Spike system with the 500 ml of RNase cocktail as feed water, followed by deionized water.
5. Zero point is just before adding the spike. Take 1 ml samples of water after drawing off 1000 ml, 1500 ml, 1600 ml, 1700 ml, 1800 ml, 1900 ml, 2000 ml, 2100 ml, 2200 ml, 3 L, 5 L, and 10 L.
6. Prepare RNA/Salt Pool: 16 ml RNA Lot: EM4702, 16 ml 1M NaCl Lot: MB1, 16ml 100 mM MgCl2 Lot: MB1.
7. Into 0.5 ml tubes place 7 ml of each sample and add 3 ml of the RNA/Salt to each.
8. Negative control: 7 ml of DEPC treated water and 3 ml of RNA/Salt.
9. Positive Spike control: 7 ml of the RNase cocktail and 3 ml of the RNA/Salt.
10. Positive sensitivity control: 6 ml of DEPC treated water, 1 ml RNase A/T1 10-6 dilution and 3 ml of RNA/Salt. (Final RNase A/T1 conc. 1 picogram/ml/0.3 picogram/ml)
11. Incubate all tubes for 1 hr. at 37°C.
12. Prepare 1.2% agarose gel: 50 ml of _ X TAE Lot: MB1, 0.6 g agarose, 5 ml of ethidium bromide (5 mg/ml) Lot: MB1.
13. After 1 hour incubation, heat all tubes to 65°C for 5 minutes.
14. Spin all tubes to send condensation back into tube.
15. Add 2 ml of gel dye to each tube and load on gel in this order; Gel: Samples 1-12 in lanes 1-12 respectively, followed by, (-) control (lane 13), (+) Spike control (lane 14) and (+) sensitivity control (lane 15).
16. Run for 20 minutes at 80 volts.

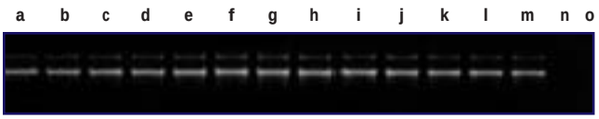
Results

The three gels shown below represent testing assays completed initially (Gel 1), after 45 days, (Gel 2), and after 4 months, (Gel 3). Identical results were reported on the 10 assays completed during the 4 month evaluation.

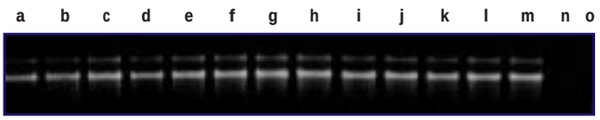
Gel 1



Gel 2



Gel 2



Lane

a. 1000 ml
b. 1500 ml
c. 1600 ml
d. 1700 ml
e. 1800 ml
f. 1900 ml
g. 2000 ml
h. 2100 ml
I. 2200 ml
j. 3 liters
k. 5 liters
l. 10 liters
m. Negative control
n. Positive control- Sample of RNase cocktail
o. Positive control- 1 picogram RNase A/0.3 picograms RNase T1

Conclusion

The NANO DIamond UV/UF and the EASYpure II UV/UF both demonstrated through empirical independent lab evaluation that it consistently produces RNase free water, even when challenged over an extended period of time with large quantities of RNase. The sensitivity of the procedure proved that if as little as 1 picogram of RNase was present in a 10 microliter water sample it could be detected after only an hour of incubation. The water was also tested under more sensitive assay conditions by incubating at 37°C for 24 hours. This showed that RNase activity was less than 0.5 picograms/microliter. RNA incubated with water from the EASYpure UV/UF showed no visible degradation even after the 24 hour incubation. The positive control which contained 0.5 picograms of RNase A showed very measurable degradation.

If you require a copy of the entire study for both the EASYpure UV/UF and NANOpure DIamond UV/UF it can be supplied to you by simply contacting Barnstead International at 1-800-446-6060, or by fax at 563-589-0516.