Application Note: SSL2453

Validation of Thermo Scientific Barnstead Nanopure® UV/UF Water- Nucleases (Ribonuclease and Deoxyribonuclease)

The nucleases - ribonculease (RNase) and deoxyribonuclease (DNase) - are ubiquitous contaminants in the laboratory environment. Glassware and solutions must be rendered nuclease-free when working with RNA or DNA to ensure good recoveries. Therefore, it is critical that ultrapure water used for reagent preparation and glassware rinsing is free of nucleases. In this validation study, a Nanopure UV/UF ultrapure water system was spiked with feedwater containing high levels of RNase and DNase (in two separate experiments) and the product water was analyzed for their presence. The experiments were performed by Mo Bio Laboratories, Inc. in Solana Beach, CA. The results indicate that the Nanopure UV/UF effectively removed the nucleases from the water to levels below the sensitivity limit of the test.

Introduction

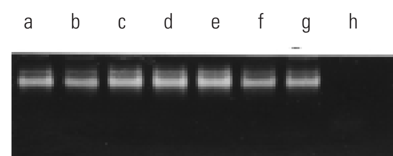
Nucleases are small enzymes present in all cell types and are responsible for the destruction of RNA and DNA. They are resistant to heating, are active over a wide pH range, renature readily, and can easily be transferred with an analyst's fingers. Consequently, nucleases are found on most laboratory glassware and in most solutions. Traditionally, a 0.1% solution of diethyl pyrocarbonate (DEPC) has been used to inactivate nucleases in buffers and water. However, since DEPC is a nonspecific inhibitor, it must be removed from treated solutions before being used with nucleic acids. This is typically done by autoclaving at 70° C for 1 hour. DEPC treatment is not only time consuming, it is also toxic. A point of use ultrapure water system that can produce nuclease free water without DEPC treatment can be a valuable piece of equipment in the laboratory for saving time and producing quality results.

The Ultrapure Water System

Our Nanopure UV/UF water system uses carbon adsorption, ion exchange, UV oxidation, and ultrafiltration (UF) to produce ultrapure water. This serial approach ensures the removal of a broad range of contaminants found in water. It is likely that each of these technologies is involved with the removal of nucleases. In this study, a Nanopure UV/UF was spiked with RNase and DNase in two separate experiments and the product water was evaluated for RNase and DNase activity using gel electrophoresis. The analysis was performed by Mo Bio Laboratories in Solana Beach, CA.

Our Nanopure UV/UF (D11931) was set up and installed with a Nanopure cartridge pack (D50281). An "air purge" of the cartridge pack was performed with the menu selection, and then 10 L of water was dispensed to drain to rinse and wet the 0.2 µm hollow-fiber final filter. Next, a system sanitization was performed. The sanitization cycle is software driven – the analyst simply needs to inject a liquid sanitant provided in a syringe (CMX25) and allow the cycle to run for 4 hours. The cycle sanitizes the ultrafilter and the system tubing up to the point of use. After the sanitizaton cycle the system was allowed to operate for two days in order to fully equilibrate the resins and ultrafilter. In this normal mode of operation the system continuously recirculates pure water.

FIGURE 1.



Gel electrophoresis results for RNase. The identity of the lanes is as follows: a) blank, b) 2.0 L sample, c) 2.5 L sample, d) 5.0 L sample, e) 10.0 L sample, f) 20.0 L pooled sample, g) negative control, h) low-level positive control, i) high-level positive control.

RNase Analysis

Spike solutions. RNase is measured in Kunitz units (Ku) which is the amount of RNase that completely hydrolizes 1 µg of RNA in 15 minutes at 37° C. A 500 ml spike solution with a concentration of 9.00x10-4 Ku/µL was prepared from US Biologicals RNase A. If this 500 mL spike were diluted to 20 L a 2.25x10-5 Ku/µL solution would result which is well above the 10-9 Ku/µL sensitivity of the analysis. The intent was to collect a 20 L pooled sample from the system after spiking, which would be above the sensitivity of the analysis if the spike broke through the system.

Positive controls. An undiluted spike was used as a high-level positive control (9.00x10-4 Ku/µL) and a 20x dilution was used as a low-level positive control (2.25x10-5 Ku/µL).

Negative control. A negative control was prepared by treating deionized water with DEPC.

Spike procedure. To initiate the procedure, about 500 mL of water was sent to drain to rinse the final filter. It is a good practice to rinse the final filter before collecting water for any application. A 500 mL blank sample of

water was collected from the system in a nuclease free bottle before spiking and saved for subsequent analysis. To initiate the spike procedure, a 500 mL spike solution was fed to the system. After the spike was in the system, the water source was switched to DEPC-treated, nuclease-free, deionized water. A total volume of 20 L was allowed to run through the system and was collected in a nuclease free vessel. The vessel was treated by rinsing with first 1 N NaOH and then several times with DEPC treated water. Point samples (500 mL) were collected directly from the dispensor after 2, 3.5, 5 and 10 L had passed through the system. Since the void volume of the system is approximately 2 L, these samples should identify breakthrough of the nucleases, even assuming a wide range of dispersion and adsorption properties. For analysis, 100 µL was sampled from each 500 mL point sample. The remainder of each sample was pooled with the collected volume in the 20 L vessel. A sample was also taken from this pooled sample for analysis. The spike was prepared such that, if diluted to 20 L, it would still be above the sensitivity of the analysis.

RNase test method. A 10 µL aliquot from each sample of water was exposed to 1 µg of RNA (1 µg/µL) in a 15 µL vial. The vials were incubated for 1 hour at 37° C. The products of the reaction were examined by electrophoresis on a 1.2% agarose gel in 1/2 X TAE (80 volts for 20 minutes). A positive control sample and a negative control sample were treated and analyzed simultaneously.

Results. The nine lanes of the electrophoresis gel representing the samples, blank, and controls are displayed in Figure 1. Negative RNase activity is defined by samples appearing equal in intensity and band integrity as the negative control. Positive RNase activity is defined by smearing of the RNA band. From the figure, it is evident that the blank and all our Nanopure UV/UF samples exhibited no band smearing relative to the negative control and were therefore free of RNase.

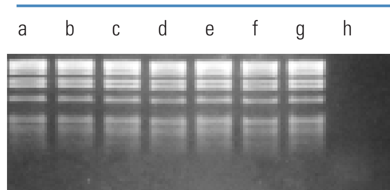
DNase Analysis

Spike solutions. DNase is also measured in Kunitz units (Ku) which is the amount of DNase that completely hydrolizes 1 µg of DNA in 15 minutes at 37° C. A 500 ml spike solution with a concentration of 2.00x10-2 Ku/µL was prepared from DNase I Sigma. If the 500 mL spike were diluted to 20 L, a 5.00x10-4 Ku/µL solution would result which is well above the 10-7 Ku/µL sensitivity of the analysis.

Positive controls. An undiluted spike was used as a highlevel positive control (2.00x10-2 Ku/µL) and a 20x dilution was used as a low-level positive control (5.00x10-4 Ku/µL).

Negative control. A negative control was prepared by treating deionized water with DEPC. Spiking and analysis. The spiking procedure and sample analysis were identical to that of RNase except that DNase solutions were substituted for RNase solutions and DNA was substituted for RNA. Results. The nine lanes of the electrophoresis gel are displayed in Figure 2. Negative DNase activity is defined by samples appearing equal in intensity and band integrity as the negative control. Positive DNase activity is defined by smearing of the DNA band. From the figure, it is evident that the blank and all our Nanopure UV/UF samples exhibited no band smearing relative to the negative control and were therefore free of DNase.

FIGURE 2.



Gel electrophoresis results for DNase. The identity of the lanes is as follows: a) blank, b) 2.0 L sample, c) 2.5 L sample, d) 5.0 L sample, e) 10.0 L sample, f) 20.0 L pooled sample, g) negative control, h) low-level positive control, i) high-level positive control.

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