# On-demand Nuclease- and Endotoxin-Free Lab Water Using a Thermo Scientific Barnstead GenPure UV/UF Water Purification System

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Application Note

## Key Words
Water purification, lab water, ultrapure water, nuclease, endotoxin, pyrogen, RNase, DNase, Thermo Scientific Barnstead, GenPure Pro

## Abstract
A Thermo Scientific™ Barnstead™ GenPure™ Pro UV/UF water purification system was challenged with RNase, DNase and endotoxins to evaluate the effectiveness of its ability to reduce these impurities below detectable limits and produce nuclease- and endotoxin-free ultrapure water.

## Introduction
Nuclease is the general name which includes both ribonuclease (RNase) and deoxyribonuclease (DNase), the enzymes responsible for degrading RNA and DNA respectively. In controlled experiments, these enzymes can be very beneficial as they are used in many life science experiments to cleave specific links on RNA and DNA strands. In contrast, nucleases can also be detrimental to experiments if they are present in applications that require the RNA or DNA to be whole. Controlling nuclease contamination can be a challenge, but it is necessary for accurate and reproducible results in these types of experiments. Very durable, nucleases are resistant to heating, are active over a wide pH range, re-nature readily and are easily transferable.[1] They can be plentiful on counter tops, centrifuges, laboratory glassware, buffer and reagent solutions. They can even be found on gloved hands that have touched hair or skin.

It is important to reduce possible contamination of nucleases, and there are multiple ways to achieve this. Using nuclease-free water for buffers and reagents is a good first step. Traditional practices include inactivating RNase in water with the use of the inhibitor Diethyl Pyrocarbonate (DEPC) followed by autoclaving the water to destroy the inhibitor. DEPC hydrolyzes when exposed to trace levels of moisture, so proper storage requires a layer of inert gas in the bottle after each use. DEPC can only be used with glass pipettes as it will dissolve some plastics and is not recommended to be used with common buffers such as Tris. Lastly, if exposed to ammonia, DEPC can decompose to a possible carcinogen, urethane.[2] Alternatively, bottled nuclease-free water is also available, but this adds more consumables to manage, with the additional risk of contaminating the bottle during each use, taking time and resources away from valuable research.

Just as nucleases can be detrimental to many life science experiments, so can endotoxins. Endotoxins are lipopolysaccharides in gram negative bacteria, which are left behind during the course of the bacteria's life cycle. Endotoxins (also referred to as pyrogens), can induce a high fever when injected into mammals. When present in vitro, endotoxins can interfere with the growth of tissue cultures. To utilize water that is endotoxin-free, many labs utilize endotoxin-free bottled water. While this can be convenient, the bottle can also become contaminated and is another consumable that needs to be ordered, shipped, and stored.

Point of use ultrapure water purification systems with ultrafiltration (UF) are designed to effectively reduce nuclease and endotoxin macromolecules to below detection limits. Ultrafilters used in Thermo Scientific water purification systems use polysulfone hollow fibers to provide a powerful and consistent barrier to trap these particles. In the Barnstead GenPure Pro UV/UF system this filter is strategically placed in-line, at the end of the water system's flow path, to help ensure the complete elimination of all nucleases and endotoxins without possible outside contamination. Proper maintenance of the water system, such as regular system cleaning and prompt filter replacement as specified in the operational manual, helps to ensure the ultrapure water remains contaminate free.

Systems also incorporating an ultraviolet (UV) light with an ultrafilter create a powerful component to further purify the water. A dual wavelength UV light uses its 185 nm wavelength to reduce total organic carbon (TOC) levels to 1 - 5 ppb, and its 254 nm wavelength to maintain an

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aseptic environment as the water is circulated throughout the system.[3] A GenPure water system with UV and UF is designed to effectively deliver high quality, ultrapure water on demand with ultralow TOCs, and free of bacteria, nuclease and endotoxin contaminants. This allows for efficient work flow, and productive use of resources and space.

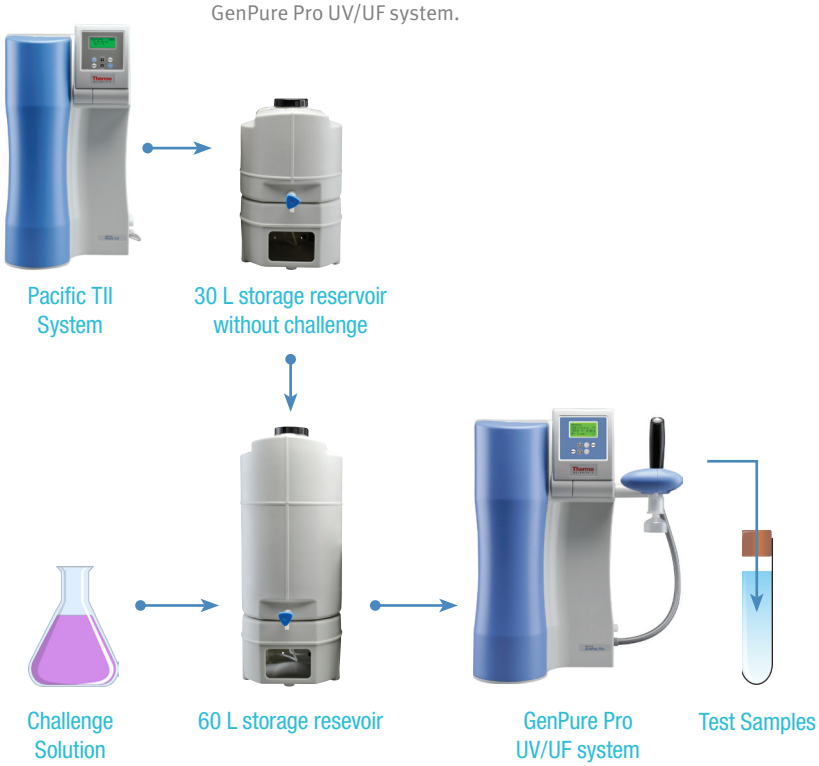
A Barnstead GenPure Pro UV/UF water system was challenged with RNase A and DNase I, nucleases commonly used to qualify ultrapure water systems for nuclease reduction. The GenPure Pro UV/UF system was also challenged with *E. coli* O55:B5 endotoxin. This system was chosen from the family of Barnstead GenPure systems, which also includes the GenPure UV/UF and GenPure xCAD Plus UV/UF models. All of these systems have the same feed water requirements, basic water flow pattern, ultrafilter filtration, UV lamp and dispense water through a 0.2 µM final filter. To challenge the system, the GenPure Pro UV/UF system was connected to a storage tank with a high concentration of solution containing RNase, DNase, and endotoxins to determine if the increased bio-load would impact the system's ability to reduce these impurities.

## Methods

### Nuclease and endotoxin performance testing in GenPure Pro UV/UF system

The GenPure Pro UV/UF system is an ultrapure water system, which requires ASTM Type II pre-treated feed water. A Thermo Scientific™ Barnstead™ Pacific™ TII 20 UV system with a 30 L Thermo Scientific reservoir was used to pre-treat tap water and was set up according to the operational manual.[4] A 60 L Thermo Scientific storage reservoir was set up to directly feed water into the GenPure Pro UV/UF system to introduce known challenge solutions and the GenPure Pro UV/UF system was set up per its operation manual.[4] The systems were set up as demonstrated in Figure 1.

Figure 1. Diagram of a Pacific TII system with a 30 L reservoir without challenge, 60 L reservoir for challenge solutions and GenPure Pro UV/UF system.




Clean techniques were used throughout to reduce the chance of nuclease or endotoxin contamination. Three samples were routinely collected so that one sample was sent for nuclease analysis, one for endotoxin analysis, and one was archived to protect against shipping errors. The samples were stored at -20°C until they were analyzed.

### Negative Controls:

A 60 L reservoir was filled with 15 L of Pacific TII system product water and 10 L was rinsed through the GenPure Pro UV/UF system without the 0.2 µM final filter, followed by 1 L rinse with the 0.2 µM final filter. After a 0.2 L rinse from the 60 L reservoir spigot, three 10 ml samples were taken directly from the reservoir to determine the nuclease and endotoxin levels of the water feeding the GenPure Pro UV/UF system (see "Feed Water" in Table 1). After 0.2 L of water was dispensed from the GenPure Pro system, three 10 ml samples were collected to establish a non-challenged baseline ("Pre-Challenge Water" samples listed in Table 1).

### Nuclease Challenge Protocol

A challenge solution with 1 µg/mL RNase and 100 U/L DNase was prepared by adding 500 µL of 10 mg/mL RNase A stock solution and 110 µL of 4.5 U/µL DNase to 5 L of UltraPure™ DNase/RNase-Free distilled bottled water from Life Technologies. The 60 L storage reservoir was drained of remaining water, and the challenge solution was introduced to the reservoir that was connected to the GenPure Pro UV/UF system. Water was dispensed continuously from the GenPure Pro UV/UF system dispenser and three 10 mL samples were taken at specific volume intervals: 2.5 L, 5 L, 10 L, 20 L, 30 L, 40 L, and 50 L. The Pacific TII system was used to replenish water in the 60 L challenge reservoir to complete the sampling. After collecting all samples, the GenPure Pro UV/UF system was sanitized and consumables changed per the system's operational manual[5] and the entire procedure was repeated to create the run 2 data set. Samples were shipped to Thermo Fisher Scientific Baltics UAB, Lithuania for analysis.

The RNase analysis was performed by incubation of 80 ng of 2 kb RNA transcript for 4 hours at 37°C with 8.2 µL of the water sample in RNase assay buffer with $Mg^{2+}$, in a 20 µL total reaction mixture. After incubation, the integrity of RNA was analyzed on a 1% agarose gel and stained with ethidium bromide. RNase contamination is not detectable with a detection limit of 1 x $10^{-7}$ Unit per reaction (0.003 ng/mL). The data for the RNase challenge is in Table 1.

DNase testing was conducted by incubation of 1.2 µg of supercoiled pUC19 DNA/SmaI with 15.6 µL of the water sample in DNase assay buffer with $Mg^{2+}$ for 17 hours at 37°C, a 24 µL total reaction mixture. After incubation the DNA was analyzed on 1% agarose gel and stained with ethidium bromide. DNase is not detectable with detection limit of 1 x $10^{-6}$ Unit per reaction (0.002 pg/µL). The data for the DNase challenge is in Table 1.

### Endotoxin Challenge of the GenPure Pro UV/UF system

A challenge solution was prepared adding 5 vials of 1,250,000 EU/vial *E.coli* O55:B5 Endotoxin to 5 L of

**Table 1. RNase, DNase, and endotoxin detection in ultrapure water produced by a Barnstead GenPure Pro UV/UF water purification system. Data for both runs were identical unless noted otherwise.**

| | RNase Concentration (ng/mL) | DNase Concentration (pg/µL) | Endotoxin Concentration (EU/mL) | |
|---|---|---|---|---|
| **Feed Water** | <0.003 | <0.002 | Run 1: 0.112 | Run 2: 0.0271 |
| **Pre-challenge water** | <0.003 | <0.002 | <0.001 | |
| **2.5 L post challenge** | <0.003 | <0.002 | Run 1: <0.002* | Run 2: <0.001 |
| **5 L post challenge** | <0.003 | <0.002 | <0.001 | |
| **10 L post challenge** | <0.003 | <0.002 | <0.001 | |
| **20 L post challenge** | <0.003 | <0.002 | <0.001 | |
| **30 L post challenge** | <0.003 | <0.002 | <0.001 | |
| **40 L post challenge** | <0.003 | <0.002 | <0.001 | |
| **50 L post challenge** | <0.003 | <0.002 | <0.001 | |

* Unknown interference in sample was detected. Sample was diluted and retested with different detection limit.

UltraPure™ DNase/RNase-Free distilled bottled water. The challenge solution was introduced to the 60 L reservoir that was connected to the GenPure Pro UV/UF system. Water was dispensed and sampled as above in the RNase/DNase challenge. The endotoxin analysis was conducted by Nelson Laboratory, Salt Lake City, UT. Samples were analyzed using the Bacterial Endotoxins Test: Kinetic Chromogenic Method or Limulus Amebocyte Lysate (LAL) test to detect and quantify bacterial endotoxin. Endotoxin was not detectable with detection limit of 0.001 EU/mL. The data for the endotoxin challenge is in Table 1.

## Results

Table 1 lists results from runs 1 and 2 for the RNase, DNase and endotoxin analysis. For results that were identical in both runs, only one result is reported. RNase and DNase levels were below detection limits in the Pacific TII 60 L tank, which is listed in the table as feed water. The pre-challenge water samples in the table refer to the samples collected from water dispensed from the GenPure Pro UV/UF system before the challenge solution was introduced. Here again, the levels were below the detection limit. After the challenge solution was introduced into the feed water for the GenPure Pro UV/UF system, samples were taken at timed intervals. All post-challenge samples were determined to be below the RNase/DNase detection limits.

The endotoxin analysis, on the other hand, indicated endotoxins were already present in the feed water to the GenPure system even before the system was challenged. The pre-challenge water sample was below the level of detection, so any endotoxins naturally present in the feed water was reduced by the system. All endotoxin levels in samples taken at specific volume intervals were found to be below the level of detection, and one sample at 2.5 L contained an unknown interference in the analysis, so the sample had to be diluted and retested with a result reporting as a different detection limit than other data points.

## Conclusion

In-line ultrafiltration combined with UV oxidation provided an easy and efficient method of reducing nucleases and endotoxins from water below detectable limits, even when the Barnstead GenPure Pro UV/UF water purification system was challenged with 5mg RNase A, 500 U DNase I, and 6,250,000 EU *E.coli* O55:B5 endotoxin.

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