

Technology Note 40

Undetectable levels of DNase and RNase in purified water

PURELAB® range achieves nuclease specifications without point-of-use filters

Performance of ELGA's PURELAB® range of water purification systems specifications

- RNase <1 pg/ml
- DNase <5 pg/ml

The presence of DNase and RNase in laboratory water can be a major problem, especially with techniques such as PCR (polymerase chain reaction) where primers are used to amplify small segments of DNA. Their presence can have a significant impact on genetic analyses, rendering characterization difficult or even impossible. The introduction of point-of-use (POU) filters is often used to allay fears of potential analytical issues resulting from biologically active impurities in water. However, these filters may also have a negative impact on water quality, and can come at a cost, financially, environmentally, and in terms of maintenance requirements. Now, based on years of experience backed by laboratory tests, ELGA has been able to demonstrate that this is no longer a mandatory requirement for its water purification systems.

Clinical and Laboratory Standards Institute (CLSI) guidance for water purification system design specifies that water quality monitoring should be performed after the final purification step to ensure that specifications are met at the point of use. ELGA has demonstrated that well-designed water purification systems - such as those in the PURELAB range - can generate ultrapure water directly from tap water or a pretreated water supply, without the need for the traditional POU filter. The water produced is ideal for biochemical applications, with DNase and RNase concentrations of <5 and <1 pg/ml respectively. As the water treatment system does not require an additional filter, maintenance requirements, running costs and the environmental impact are also reduced

DNase and RNase

DNase and RNase are large, protein-based organic molecules in the region of 30,000 and 15,000 Da respectively, that are used by live cells to catalyze the cleavage of nucleic acid polymers in a variety of cellular functions. The ubiquitous presence of these enzymes is a particular concern in areas such as genetic analysis where a small amount of a 'template' nucleic acid is amplified to generate a much larger quantity for characterization. Enzymatic activity may damage the template material. This damage will then be replicated during the amplification process, which can make characterization difficult, or even impossible, and the experimental results meaningless.

Overcoming the DNase/RNase challenge

It is essential to avoid introducing nuclease contamination during experimental procedures, and this requires the use of laboratory water that meets DNase and RNase specifications for biochemical applications. While this has traditionally been achieved using POU filters, ELGA has successfully demonstrated that this is unnecessary in well designed water purification systems. These incorporate reverse osmosis (optional), ultraviolet light (UV) and ion exchange purification technologies. A variety of laboratory tests has been carried out to demonstrate water purification products achieving specifications (<1 and <5 pg/ml for RNase and DNase respectively), however, additional, artificial challenge work was also conducted to highlight the capabilities of these purification technologies. These tests used nuclease concentrations orders of magnitude higher than can be expected in reality.

Deactivation with UV light

RNase solutions in deionized water (100 and 1,000 pg/ml) were passed through a UV oxidation cell fitted with a dual wavelength (185/254 nm) UV lamp at a rate of 1 liter/min. The effluent water contained <2 pg/ml RNase.

Removal of DNase and RNase by UV light combined with ion exchange

DNase (1 mg/ml) was introduced into the distribution loop of an ELGA water purification system using a dosing pump. Following treatment by UV light and ion exchange media, samples were collected via the dispense tap and tested for DNase. A PURELAB Chorus 1 Complete and Analytical Research systems were tested, and all results showed a DNase concentration below the 5 pg/ml detection limit. RNase (1mg/liter) was added to the 25-liter reservoir of a PURELAB Type I system. After treatment with UV light and ion exchange media over a 45-minute period, the contents of the reservoir were drained via the dispense tap, taking samples regularly for RNase testing. The RNase concentration was below the 2 pg/ml detection limit in all samples.

Summary

As part of Veolia, ELGA Labwater a leading technology development company which specialises in the engineering, design, service and field support of laboratory water purification systems has been able to successfully demonstrate through the various test results that the PURELAB Type I range water purification systems can generate ultrapure water with DNase and RNase levels in-line with specifications without the need for additional POU filters. As total cost of ownership (TCO) is so important to many customers, ELGA has decided to remove the need for POU filters in order to achieve these DNase and RNase levels, however acknowledges that some customers require POU filters on a Type I system for other reasons (for microbiological control, for example) and so would continue to recommend their use for these purposes.

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