

Ultrapure water free from biologically active impurities suitable for PCR

Abstract

PCR techniques are in widespread use through the life science sector for amplification of genetic material, from fundamental genomic research through to advanced biomedical and forensic applications. The need for reagents and solutions free from nucleases (DNase, RNase) which could cause breakdown of oligonucleotides is widely recognized, however, the influence of other waterborne contaminants is rarely considered. These could cause problems with the test results so it is vital that water should be used which is free from waterborne contaminants.



Introduction

The advent of polymerase chain reaction (PCR) techniques revolutionised genomics in the early 1980s, and PCR-based methods, such as reverse transcription PCR (RT-PCR) and quantitative PCR (qPCR), are now essential in a wide range of medical and biological research applications.

DNA amplification by PCR relies on the use of DNA polymerase enzymes to synthesise doublestranded DNA molecules from single-stranded 'templates', using short oligonucleotide primer sequences to target the gene of interest. Although this process generally uses thermostable DNA polymerases, both the target specificity and efficiency of the enzyme catalysed reaction is highly dependent on the physical conditions (pH, temperature, etc.) and composition of the reaction mixture¹. The presence of nucleases – enzymes which cleave the phosphodiester linkages between nucleic acid subunits – within the reaction vessel will lead to severe disruption of the PCR process, as genetic material will quickly become fragmented under reaction conditions. It is therefore vital to ensure that all reagents and solutions used in PCR applications are free from nucleases. The need for 'nuclease-free' water in PCR applications is widely recognized, however, there are a number of other contaminants commonly found in water that can impede DNA amplification² by PCR, including:

Bacteria

The presence of bacterial DNA can lead to erroneous results, particularly for qPCR techniques, as well as amplification of non-target sequences. In addition, many bacteria release extracellular nucleases and other small molecules and ions which interfere with DNA polymerization.

Ions

Thermostable DNA polymerases require a magnesium (Mg^{2+}) co-factor for effective substrate binding. The concentration of Mg^{2+} is therefore crucial for optimization of polymerase activity³. Polymerase enzymes are also highly sensitive to other common divalent cations (Cu^{2+} , Fe^{2+} , Ni^{2+} , etc.), which interfere with co-factor co-ordination and disrupt substrate binding within the active site. In addition, the presence of even trace amounts of heavy metal ions (such as cadmium) will inhibit enzyme activity.

Organic compounds

Because DNA molecules carry a negative charge, other negatively charged biomolecules within the reaction environment can interfere with substrate binding by entering the positively charged active site and causing steric interference, leading to reduced substrate turnover.

Purifying water for PCR

Optimization of PCR processes requires the use of water free from nucleases, micro-organisms, organic compounds and trace elements for constitution of all reagents and buffers. To ensure that these contaminants are not present, ultrapure grade (Type I) water – with a resistivity of 18.2 MΩcm, a low total organic carbon (TOC) value of less than 20 ppb, bacteria levels of below 1 CFU/ml and endotoxins below 0.01 EU/ml – is highly recommended for PCR applications⁴. ELGA's PURELAB Ultra Genetic uses a multi-step purification process to produce ultrapure grade water suitable for PCR, including:

Ultraviolet (UV) radiation

Passing water through a beam of ultraviolet light effectively breaks down organic compounds including bacteria and bio-active molecules such as nucleases and endotoxins. A wavelength of 185 nm oxidises carbon-containing macromolecules, yielding ionized fragments for subsequent removal by ion exchange, whereas longer wavelength UV radiation (254 nm) disrupts the activity of bacterial enzymes, preventing replication. To maximize breakdown of organic molecules, the PURELAB Ultra Genetic uses a full spectrum UV lamp with an ultra-high purity synthetic quartz sleeve.

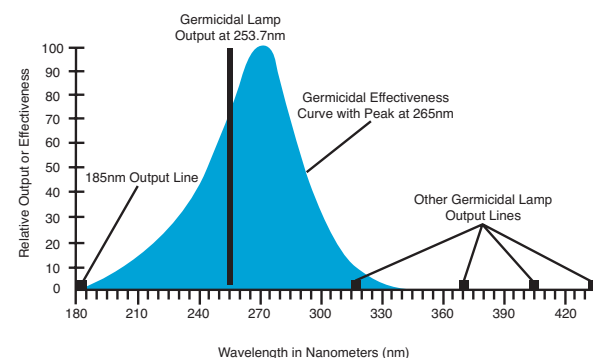


Figure 1: Germicidal efficiency of UV radiation

Ultrafiltration

Ultrafilters (UFs) are membrane filters with pores of typically 1 to 50 nm, often in the form of hollow fibres to give higher flow-rates per unit volume. They are usually made from asymmetric fibres with the active layer, with the finest pores, on one side. UFs are characterized by the efficiency with which they remove specified contaminants to acceptable levels. Ultrafiltration is a useful adjunct to UV to maximize removal of macromolecules and bacteria.

ELGA's PURELAB Ultra Genetic uses UV photo-oxidation, high capacity purification cartridges, combined with an ultrafilter, to reliably deliver endotoxin-free ultrapure water (see table). Real time resistivity and TOC monitoring ensure verifiable removal of inorganic and organic contaminants and the system offers validated traceability and guaranteed quality.

	Product water Endotoxin (EU/ml)
Pre-challenge	<0.001
During challenge after 1 litre	<0.001
During challenge after 2 litres	<0.001
During challenge after 3 litres	<0.001
During challenge after 4 litres	<0.001
During challenge after 5 litres	<0.001
Post challenge after 5 litres	<0.001
Post challenge after 15 litres	<0.001
Post challenge after 25 litres	<0.001

Table 1: Efficiency of endotoxin removal
PURELAB Ultra Genetic challenged with 5 litres of partially purified water spiked with 10,000 EU/ml endotoxins, achieving a residual endotoxin level of <0.001 EU/ml, even after 25 litres.

Conclusion

Ultrapure water with a high resistivity (>18.2 MΩcm) and free from nucleases, organic compounds and endotoxins should be used for all PCR applications, to ensure optimized amplification of target sequences.

To find out more about ELGA LabWater's water treatment technologies and solutions for life science applications, visit www.elgalabwater.com

References

1. Bogetto, P et al. (2000). Helpful tips for PCR. Focus, 22 (1), 12
2. Sambrook, J and Russel, DW (2001). Chapter 8: In vitro Amplification of DNA by the Polymerase Chain Reaction, Molecular Cloning: A Laboratory Manual (3rd ed)
3. Yang, L et al. (2004). Critical role of magnesium ions in DNA polymerase beta's closing and active site assembly, J Am Chem Soc, 126 (27), 8441-8453
4. ASTM "Standard Guide for Bio-applications Grade Water", D 5196-06.

About ELGA LabWater

ELGA specializes in pure and ultrapure water purification products for the laboratory, research, healthcare and clinical markets. ELGA has over 50 years experience in developing and manufacturing high quality products and an in-depth understanding of application and laboratory requirements. With a worldwide network ELGA supports its customers through the development, installation and servicing of purification systems as well as large projects involving consultation with architects, consultants and clients. ELGA is accredited to ISO9001 and ISO14001 standards.

ELGA is an integral part of Veolia Water Solutions and Technologies. Veolia Water Solutions & Technologies (VWS), subsidiary of Veolia Water, is a leading design & build company and a specialized provider of technological solutions in water treatment. With over 9,500 employees in 57 countries, Veolia Water Solutions & Technologies recorded revenue of 62.5 billion Euros in 2009.

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