APPLICATION NOTE

Purified water for mammalian and bacterial cell culture

Abstract

To achieve success in mammalian cell culture it is necessary to use extremely pure media and buffers. The use of Type I ultrapure water to effectively eliminate the presence of biologically active species and other contaminants which could interfere with the culturing process is essential. General laboratory grade purified water (Type II) with low levels of bacterial and organic contamination is usually sufficiently pure for bacterial cell culture.

Introduction

Cell culture is the process of growing mammalian or bacterial cells under controlled conditions. Primary cells isolated from tissue have a limited lifespan, while immortalized cell lines can, in principle, proliferate indefinitely. Bacterial cell culture entails culturing microbial organisms in predetermined culture media under controlled laboratory conditions.

Mammalian cell culture has many applications. Cells may be used for research purposes, for example in the pharmaceutical industry, where they can provide models for the study of disease, or for target identification and validation. They are also used in cell-based assays for high throughput screening, in permeability assays and in the production of therapeutic proteins. Biotechnology applications include the production of antibodies, hormones, proteins, enzymes and vaccines. Bacterial cell cultures are used as a diagnostic test in microbiology to determine the type of organism responsible for an infectious disease, and as a research tool in molecular biology.

Purified water is vital for successful cell culturing. Water is the main component of buffers and media and may be used for dissolution of additives and drugs. Contaminating micro-organisms, biologically active cell debris and by-products are particularly damaging, but organic and inorganic compounds can also adversely affect the culture.

Bacteria

Bacteria are detrimental to cell cultures. They thrive in typical cell culturing conditions and can quickly outgrow the cells of interest, causing nutrient levels to fall and toxic by-products to increase. Bacterial contamination may also result in sudden changes in media pH and the contamination of previously pure cultures.

Endotoxins

Endotoxins from bacteria have been shown to seriously affect cell growth and function, production of recombinant proteins and the efficiency of cloning[1].

Organic compounds

Small organic compounds present in water can affect cell development. Dissolved organic compounds are an uncontrolled source of nutrients for bacterial growth and should be removed from water used for preparation of materials for cell culturing. They can also cause problems such as poor detection limits and decreased reproducibility with trace HPLC and GC analyses.

Ions

Levels of ionic contaminants, particularly multivalent ions and heavy metals, must be kept low. Heavy metals – for example, mercury and lead – are known to be cytotoxic[2].

Purifying water for cell culture

Ultrapure water should be used for the preparation of media and buffers for cell culture to ensure that cells are free from bacterial, yeast and viral contaminants[3,4]. Water for mammalian cell culture typically requires apyrogenic, Type I ultrapure water[5]. Recommended acceptable levels of contaminants in water for mammalian cell culture are shown in Table 1. Pharmacopoeial Water for Injection (WFI), or water of equivalent purity, is used for larger scale production units. ELGA's PURELAB® Classic UVF, with a resistivity of 18.2 MΩ.cm, a very low total organic carbon (TOC) value of less than 3 ppb, bacteria levels below 0.1 CFU/ml and endotoxins below 0.001 EU/ml, is highly recommended. ELGA's PURELAB flex is suitable for the less sensitive bacterial cell culture, which requires Type II water with a resistivity of > 10 MΩ.cm, a TOC below 50 ppb and a bacterial count of less than 1 CFU/ml.

Parameter	Mains water	Water for cell culture	Reduction (%)
Conductivity (µS/cm)	50 – 900	< 0.055	> 99.99
Calcium (mg/l)	20 – 150	< 0.001	> 99.999
Sodium (mg/l)	20 – 150	< 0.001	> 99.999
Iron (mg/l)	0.01 – 0.1	< 0.001	> 98
Bicarbonate (mg/l)	30 – 300	< 0.001	> 99.999
Chloride (mg/l)	10 – 150	< 0.001	> 99.999
Sulfate (mg/l)	1 – 100	< 0.001	> 99.998
TOC (mg/l)	0.2 – 5	< 0.01	> 99.6
Free chlorine (mg/l)	0.1 – 0.5	< 0.01	> 97
Bacteria (CFU/100 ml)	10 – 1000	< 10	> 98
Endotoxin (units/ml)	1 – 10	< 0.001	> 99.98
Turbidity	0.1 – 2	< 0.01	> 99

Table 1: Typical mains water impurities and recommended values for mammalian cell culture[6].

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Ultraviolet (UV) radiation

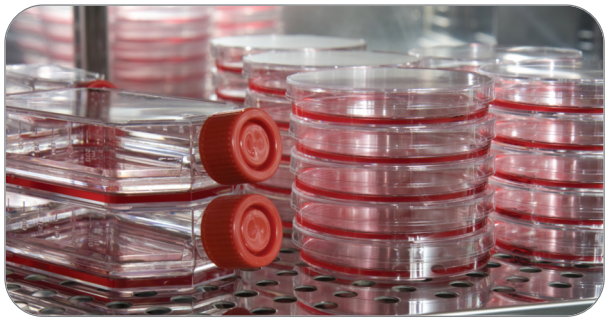
Passing water through ultraviolet light at a wavelength of 185 nm effectively breaks down and oxidizes carbon-containing molecules, yielding ionized fragments for subsequent removal by ion exchange. Longer wavelength UV radiation (254 nm) disrupts the activity of bacterial enzymes, preventing replication. To maximize breakdown of organic molecules, the PURELAB Classic UVF and PURELAB flex use a full spectrum UV lamp.

Ultrafiltration

Ultrafiltration of water removes the bacteria, endotoxins and other macromolecules which can affect cell culture. ELGA's PURELAB Classic UVF contains an ultrafilter to effectively remove these sources of contamination according to their size. The PURELAB flex uses charged point-of-use filters to filter out and remove particles, macromolecules and bacteria.

Media

The media cartridges in the PURELAB Classic UVF and PURELAB flex contain synthetic, activated carbon beads which adsorb a wide variety of organic compounds, as well as high purity ion exchange resins to minimize the release of impurities.



Conclusion

Water for bacterial cell culture should be at least Type II quality, while the more sensitive mammalian cell culture requires Type I ultrapure water with high resistivity (> 18 MΩ.cm) and free from bacteria, endotoxins and other contaminants to avoid adversely affecting the culture.

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

References

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About ELGA LabWater

ELGA LabWater manufactures supplies and services laboratory, healthcare and clinical water purification systems. ELGA offices and distributors are located in more than 60 countries worldwide. ELGA is the global laboratory water brand name of Veolia Water Solutions & Technologies.

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