BINDER

CELL CULTIVATION: FOR AND AGAINST PARTICULAR TECHNICAL SOLUTIONS IN CO_2 INCUBATORS

THE PARAMETERS TO CONSIDER

Introduction

The aim of cell culture is to achieve and maintain good cell growth and, depending on the task, to attain appropriate cell differentiation. This involves taking numerous parameters and requirements into account and technically implementing them.

The CO2 incubator or CO2/O2 incubator is a key element in cell culture. Various technical solutions are used to achieve the right temperature, humidity, CO2 and/or O2 concentration, and interior design. This white paper compares various commercially available solutions for the four parameters and interior design.
Always make sure you are comparing like with like.

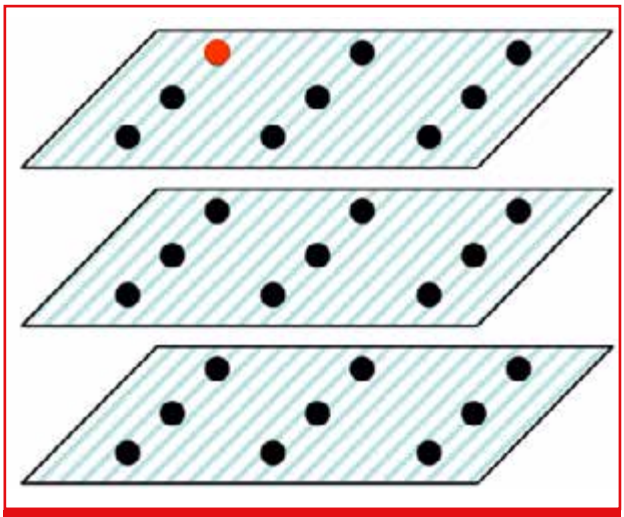
As cell and tissue cultures have no immune system, the problem of contamination is widespread. Our **white paper on "Cell cultivation without contamination"** presents various solutions, and explores the international standards and anti-contamination designs in detail.

Table of contents

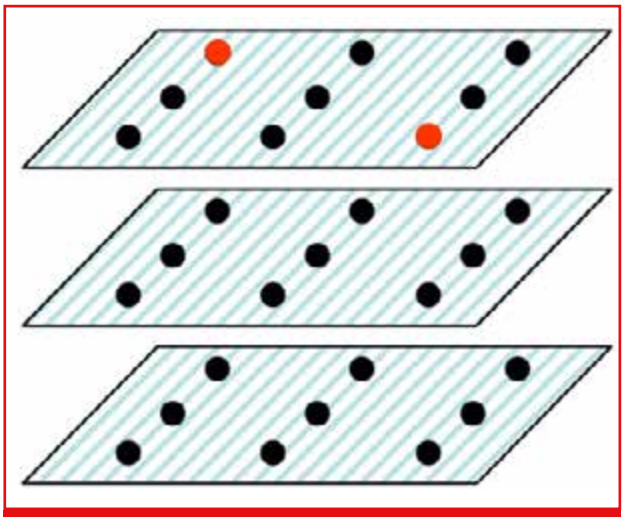
Temperature

The most important temperature specifications are the temporal and spatial temperature deviation, the recovery time, the cooling-down time in the event of a power failure, and the heating-up times on start-up. Some related definitions:

Temporal and spatial temperature deviation as per DIN 12880:2007-05
The name “27-point measurement” stems from the fact that there are nine measuring points on three measurement planes. Temperature deviations are specified in ±K.



The **temporal** temperature and humidity deviation is the difference between the measured values taken at a single point at different times, e.g., ±0.1 K at 40°C and 75% relative humidity.



The **spatial** temperature and humidity deviation is the difference between the measured values taken at two points at the same time, e.g., ±0.2 K at 40°C and 75% relative humidity.

The spatial deviation is always greater than the temporal deviation.

Recovery time as per DIN 12880:2007-05

An hour after the set value is reached inside the unladen CO2 incubator, the glass door is opened for 30 seconds and then quickly closed again. The recovery time is how long it takes to return to the initial value (typically 37°C in the case of a CO2 incubator). Differences are to be expected depending on the laboratory temperature, the size of the load, and the culture media volume.

Comparison between three different types of temperature control in CO2 incubators: water, air, silicone jacket

Type of temperature control	Water jacket	BINDER air jacket	BINDER silicone jacket
Description	A water-filled container wrapped around the interior	An air-filled container wrapped around the interior	A semifluid-filled container wrapped around the interior
Operating weight	Heaviest	Lightest	
Mobility during operation	Most difficult, emptying and filling may be required	Easiest	
Heating-up time from RT to 37°C	Longest, as a large quantity of water has to be heated	Shortest, as only air has to be heated	
Risk of contamination due to maintenance	Highest, as regular filling results in contaminated air escaping from the water jacket	Lowest, as no maintenance is required	Lowest, as no maintenance is required
Decontamination routine	Not possible	180°C possible	100°C possible
How a power failure lasting a couple of minutes affects temperature	Barely any temperature drop	Barely any temperature drop	Barely any temperature drop

The details applicable to individual units may differ from this general overview. We will be happy to assist you further in this regard.

It takes more than 75 l of water to fill the water jacket on a water jacket CO2 incubator with an interior volume of 160 l. Moving models with a water jacket is a time-consuming process because it involves emptying and refilling the jacket, e.g., for service work. A model with an air jacket of similar size is 75 kg lighter and can be conveniently moved without any loss of time.

The process of regularly topping up the water jacket with 3-4 l of water creates an increased risk of contamination. While the filling nozzle is open, air escapes from the jacket water, which is not only foul-smelling but also a potential source of contamination. Topping up is a lengthy and tricky job (that no one enjoys doing), particularly in cases where water jacket CO2 incubators have been stacked up.

The larger the fill quantity of the water or silicone jacket, the longer the heating-up time, e.g., to get from RT to a stable 37°C. On some models with a water jacket, it can take up to 24 hours.

Water jacket CO2 incubators do not feature a thermal decontamination routine. Water vapor is generated at temperatures of 100°C or above. This is accompanied by an increase in pressure. In the case of a kettle with a pop-off valve, you would be able to hear it whistling. But a water jacket does not have a whistle and so would end up bursting.

In the event of a power failure lasting a few minutes, the temperature behavior of all three temperature control methods is comparatively good. Water and silicone jackets only have an advantage over an air jacket in the event of longer power failures.

Due to differences in the way recovery times are specified, the only way to conduct a proper comparison would be to consider each individual unit. This goes beyond the scope of this white paper. DIN 12880:2007-05 stipulates how the recovery time is to be measured.

Split glass doors that significantly reduce the access cross section can have a positive impact on the temperature recovery time. **The new CB 170** is the first ever product to feature the innovative “quick sample access” facility.

Do you have any questions relating to recovery time?
We will be happy to assist you further in this regard.

Humidity and dew point

Neither hypotonic nor hypertonic conditions are conducive to good cell growth. The set osmotic concentration (osmolarity) must be constantly maintained to ensure good cell growth. High humidity values minimize the rate of evaporation from the culture vessels, thereby preventing an overconcentration (an increase in the osmotic pressure occurs) of salts, amino acids, and other additives in the culture media. Values ≥ 95% RH are recommended.

Split glass doors that significantly reduce the access cross section can have a positive impact on the humidity recovery time. **What's more, the new CB 170** is the first ever product to feature the innovative "quick sample access" facility.

Temperature and humidity are connected via what is known as the dew point or dew point temperature. When the temperature at a certain location within the CO2 incubator reaches or falls below a specific value while at constant pressure (the dew point temperature), unwanted condensation forms at this point. Condensation increases the risk of contamination.

The type of air ducting in a CO2 incubator does not just affect the evaporation rate but also the risk of contamination. For more information, see point 7.0.

Comparison between three different types of humidification in CO2 incubators: evaporation, vaporisation, atomization

Type of humidification	Base pan	Water pan		Active		
	Evaporation			Vapor		Ultrasound
Description	Base reservoir without water heating	Removable	Permanently installed	Removable external water tank	Permanently installed external water tank	Externally generated atomized spray with permanently installed water tank
Cleaning capability Contamination risk	Easiest to access. Can be disinfected by wiping, not autoclavable, thermal decontamination possible. Reservoir is visible. Manageable risk of contamination	Easiest to access. Can be disinfected by wiping, autoclavable, thermal decontamination possible. Reservoir is visible. Manageable risk of contamination	Can be disinfected by wiping (laborious) or via thermal decontamination. Increased risk of contamination, as tank is not visible	Easier to access, replaceable tank with hoses. Reduced risk of contamination	Cleaning difficult to impossible. Some parts are barely accessible, including tank, valves, and hoses. Increased risk of contamination	Cleaning difficult to impossible. Some parts are barely accessible, including tank, valves, and hoses. Increased risk of contamination
Filling and emptying	Easy to fill. Can be laboriously emptied with a pump	Easiest	Easy to fill. Can be laboriously emptied via a pipeline	Easy to fill and empty	Filling process is laborious, emptying process is problematic	Filling process is laborious, emptying process is problematic
Heating-up time from RT to 37°C	Longest, as a large quantity of water has to be heated		Shortest, as only air has to be heated			
Risk of contamination due to maintenance	Highest, as regular filling results in contaminated air escaping from the water jacket		Lowest, as no maintenance is required			
Decontamination routine	Not possible		180°C possible			
Ease of servicing and maintenance	Problem-free	Problem-free	Laborious	Laborious	Extremely laborious	Extremely laborious
How a power failure lasting a couple of minutes affects temperature	Barely any temperature drop		Barely any temperature drop			

The details applicable to individual units may differ from this general overview. We will be happy to assist you further in this regard.

When the water surfaces are not enclosed, e.g., in the case of the base reservoir and water pan, the humidification performance is primarily determined by the size of the water surface and the heating output.

BINDER water pans feature the unique Permadry™ dew point principle. The CO2 incubator has been designed so that the condensation is routed in the ideal manner and only passes into the water pan without reaching difficult-to-access locations such as the rear wall.

Maintaining and servicing CO2 incubators with permanently installed external water tanks is a laborious process. Contaminated water can only be detected via the small viewing window of the water level indicator – but by then it is usually too late. By contrast, water pans remain highly visible at all times.

The new CB 170 is the first product in the world to feature an easily accessible and replaceable external water tank. This allows potential contamination to be removed easily and effectively, unlike with inaccessible tanks that are permanently installed.

CO2 concentration

Fluctuations in the concentration of CO2 in the atmosphere result in undesirable changes to the pH value within the cell culture. For this reason, a carbon dioxide concentration of between 5 and 7 vol. % is constantly maintained inside CO2 incubators as appropriate for the specific cell culture concerned.

The CO2 concentration inside incubators is measured using either IR sensors (infrared sensors) or TCDs (thermal conductivity detectors).

Measuring principles

The measuring principle of a TCD relies on measuring the electrical conductivity as a function of the difference in thermal conductivity between the concentration of CO2 inside the incubator and that of a reference gas (usually the concentration of CO2 in the laboratory air).

The measuring principle of an IR sensor relies on the fact that carbon dioxide absorbs a specific electromagnetic wavelength of 4.3 µm. IR radiation is guided through the gas and hits an IR detector. High concentrations of CO2 absorb (swallow) more IR radiation than low CO2 concentrations. Therefore, the quantities that reach the IR detector are indicative of the concentration.

Comparison between two different types of CO2 sensor in CO2 incubators: light-based, conductivity-based

Type of CO_2 sensor	TCD	IR
Description	Measures thermal conductivity between gases	Measures the quantity of IR radiation absorbed by a gas
Reaction if door is opened frequently	Sluggish. Gradually settles at the initial value again	Very fast. PID controller returns to a stable initial value more quickly
Influenceability	Dependent on changes in the humidity and carbon dioxide concentration of the laboratory air (waste gas emissions)	Independent of changes
Precision	Less precise	More precise
Readjustment	Required more frequently	Required less frequently
Heat sterilizability	180°C	180°C

The details applicable to individual units may differ from this general overview. We will be happy to assist you further in this regard.

Good cell growth by ensuring an optimum supply of CO2

In order to restrict CO2 consumption, it is helpful to open and close the glass door quickly but cautiously. Split glass doors that significantly reduce the access cross section can have a positive impact on CO2 consumption.
What's more, the new CB 170 is the first ever product to feature the innovative "quick sample access" facility.

To ensure that CO2 continues to be supplied for a prolonged period, e.g., over a long weekend, we recommend connecting two gas tanks to one gas tank changer. When one of the CO2 tanks runs out, the changer automatically switches over to the full one, thereby ensuring a continuous supply of CO2.

When handling gases, it is essential to observe the manufacturer's safety information.

Depending on the type of cells, tissue or application, e.g., IVF, normoxic (21 vol. %), hypoxic (‹ 21 vol. %) or hyperoxic (› 21 vol. %) oxygen concentrations may be required. CO2/O2 incubators offer two ranges, e.g., 0.2 to 20 vol. % O2 or 10 to 95 vol. % O2.

To achieve hypoxic conditions, a nitrogen gas supply is absolutely essential. The inert nitrogen gas displaces the excess oxygen. That is why CO2/O2 incubators are also sometimes referred to as “tri-gas incubators”. The oxygen concentration is measured with a zirconium oxide sensor. This should likewise be hot-air sterilizable.

CO2/O2 incubators are more technically complex than CO2 incubators, and they cost more to purchase and operate.

In order to restrict O2/N2 consumption, it is helpful to open and close the glass door quickly but cautiously. Split glass doors that significantly reduce the access cross section can have a positive impact on O2/N2 consumption.

When handling gases, it is essential to observe the manufacturer’s safety information.

Interior

Design features of the interior	Version A	Version B1	Version B2	Version B3
		Solid copper	Cu-coated stainless steel	Cu-stainless steel alloy
Interior material	Monochromatic stainless steel. Early detection of contamination sources. Very good cleaning capability. (That is why bioreactors and fermenter tanks are made from stainless steel.)	Scratch-proof bactericidal effect. Severe discoloration and scaling. Spills and sources of contamination go undetected. Difficult to clean	Coating scratches off. Bactericidal effect is lost. Cleaning plays a role in destroying the coating/ bactericidal effect	Bactericidal effect is inadequate. Good cleaning capability
Design of interior	Deep-drawn from one piece. No welding seams. Large, round corners. Very easy to clean	Welded together from multiple parts. The welding seam cavities pose a risk of contamination. Small, round corners. Poor cleaning	Welded together from multiple parts. The welding seam cavities pose a risk of contamination. Small, round corners. Poor cleaning	Welded together from multiple parts. The welding seam cavities pose a risk of contamination. Small, round corners. Poor cleaning
Shelving system	Deep-drawn shelves and nothing else (“less is more” principle)	Self-supporting shelving system with numerous fastening elements. Time-consuming disassembly, assembly, and cleaning	Self-supporting shelving system with numerous fastening elements. Time-consuming disassembly, assembly, and cleaning	Self-supporting shelving system with numerous fastening elements. Time-consuming disassembly, assembly, and cleaning
Air ducting	Uniform temperature control across all sides, and nothing else (“less is more” principle) Natural temperature distribution. Very easy to clean	Fans plus a system of air baffle plates ensure air circulation. The forced flow of air can increase the evaporation rates in the culture medium and the risk of contamination. Time-consuming disassembly, assembly, and cleaning	Fans plus a system of air baffle plates ensure air circulation. The forced flow of air can increase the evaporation rates in the culture medium and the risk of contamination. Time-consuming disassembly, assembly, and cleaning	Fans plus a system of air baffle plates ensure air circulation. The forced flow of air can increase the evaporation rates in the culture medium and the risk of contamination. Time-consuming disassembly, assembly, and cleaning

The details applicable to individual units may differ from this general overview. We will be happy to assist you further in this regard.

Sterilization:
The necessary sterile environment for every laboratory

As mentioned initially, the aim of cell culture is to achieve and maintain good cell growth and, depending on the task, to attain appropriate cell differentiation. In addition to the parameters already referred to above, another key factor is the constantly recurring topic of contamination and decontamination in cell and tissue culture laboratories. To address this, various solutions have been developed for CO2 incubators.

Explanation of terms

Decontamination is a general term that describes the removal of hazardous contaminants. This includes biological, chemical, and radioactive forms of contamination but no precise quantifiable conclusions can be drawn about its effectiveness.

Disinfection refers to a process that achieves a log 5 reduction in the number of microbes (a 100,000 fold reduction) in the course of a defined test procedure.

Sterilization (SIP, sterilization in place) refers to the complete elimination of viable microorganisms.

Our white paper on “Cell cultivation without contamination” explores this topic in detail from the perspective of CO2 incubators.

Decontamination as and when required	Continuous protection against contamination
Dry heat at 160–180°C	Minimized, seamless surfaces
Dry heat at 120–140°C	Humidity limitation
Moist heat at 90–95°C	Bactericidal surface material
Hydrogen peroxide gassing	Air filtration with HEPA filters
UV-C radiation	UV-C radiation

Table 1: Measures and methods for minimizing the risk of contamination

BINDER's 180°C hot-air sterilization method has become the gold standard. It is extremely effective and straightforward to use, and it always delivers reproducible results. Of the methods referred to above, it is the only one that meets the equipment sterilization guidelines for medical and pharmaceutical applications.

Examples of relevant international standards include the US, European, and Japanese pharmacopoeias.

Unless quality assurance measures are implemented as well, technical solutions within CO2 incubators quickly lose their effect. Examples of such measures are: protocols, SOPs (standard operating procedures), **GLP checklists**, and qualification documents (IQ, OQ, PQ). Our **white paper on "Validation and qualification"** provides further information in this regard.

Qualification and validation:
Support from incubator manufacturers

Both GLP and GMP laboratories must ensure that their equipment undergoes qualification. This encompasses DQ (design qualification), IQ (installation qualification), OQ (operational qualification), and PQ (performance qualification). It takes a long time to carry out the entire validation process and test all the functions, and every detail must be accurately recorded in the form of extensive documentation.

That is why service-focused incubator manufacturers are happy to provide their customers with a comprehensive set of validation documents and checklists.

The white paper describes the general approach to validation and qualification.

Everything from quality assurance measures, organizational aspects, and precautions through to back-up systems

WANT TO FIND THE RIGHT CO_2 INCUBATOR FOR YOUR CELL CULTURES?

FIND OUT ABOUT OUR DIFFERENT MODELS NOW!

BINDER