WHITE PAPER No. 50

CO_2 Incubators – Making the Best Choice for Your Lab

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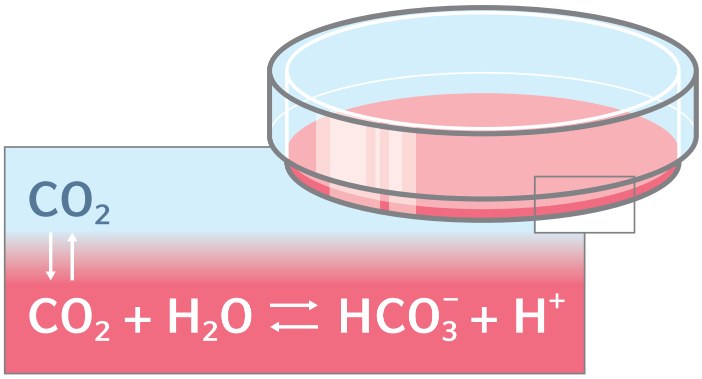
Executive Summary

The purpose of a CO_2 incubator is to maintain an optimal environment for cell growth, by providing carbon dioxide control in a humidified atmosphere with constant temperature. Modern CO_2 incubators offer specialized solutions for contamination prevention, limited lab space, and even specific needs, like support of hypoxic applications. In this guide we give you some best practices and tips to choose the best incubator for your needs.

Selecting the appropriate model

Selecting a CO_2 incubator used to be considered a routine administrative decision, often based on what was used in the past. Now, facing a wide range of specifications and specialized features, it is worthwhile to consider your needs and choose your incubator with careful analysis. This guide will help you with that process.

In-chamber atmosphere control
Controlling temperature and levels of CO_2 and humidity in the incubator is critical to the health and growth of cultured cells. For the majority of mammalian cell lines the optimal growth temperature is 37 °C. A humidified atmosphere of approximately 95 % avoids desiccation of the cultures. CO_2 is needed as part of the media buffer system to regulate the pH. The most commonly used CO_2 - bicarbonate buffering system depends on a chamber atmosphere of 5 - 10 % CO_2, providing a pH of 7.2 to 7.4.



Temperature: Although there are still water-jacketed incubators on the market, most modern systems work either with direct heat, an air-jacket, or a combination of both. In a directly heated incubator, the chamber is warmed by electrical heating elements placed directly on its outside surface. In an air-jacketed heating system, warm air is circulated in the air gap between the exterior of the chamber and an insulating layer. Both systems require less maintenance than water-jacketed incubators, as there are no water-jackets to fill and empty; they are lighter in weight, more compact, and take up less lab space. Furthermore, with no water present outside the chamber, the incubator can be self-sterilizing, using high temperature disinfection.

The Eppendorf solution: With the Eppendorf 6-sided direct-heating technology, the incubator chamber is heated from all six sides, including the door. Multiple fast-feedback temperature sensors and advanced microprocessor control regulate the 4 individual heating circuits to guarantee a homogenous temperature in the chamber. The specific arrangement of the heating elements creates natural and gentle convection circulation of the chamber atmosphere (Figure 1). This helps avoid "cool spots" in the incubator and results in excellent temperature stability (± 0.1 °C at 37 °C) and uniformity (± 0.3 °C). It also protects against wide temperature fluctuations that can stress the cells. No fan is needed for quick recovery of temperature after door opening, thus eliminating a traditional source of contamination and vibration.

Figure 1: Eppendorf 6-sided direct-heating technology creates a gentle convection circulation of the chamber atmosphere. This maintains stable temperatures and CO_2 control throughout the chamber.

CO_2 sensor: Measurement of CO_2 level with an infrared (IR) sensor is not impacted by fluctuations in temperature and humidity, in contrast to Thermal Conductivity (TC) sensors. Frequent door openings can cause fluctuations in temperature and relative humidity; they also affect the accuracy of a thermal conductivity sensor. Low levels of CO_2 may remain undetected. IR sensors are also less susceptible to drift over time. Some can even withstand high temperatures, and are able to remain in the incubator during the high-temperature disinfection cycle, if it is available.

The Eppendorf solution: For precise CO_2 control, Eppendorf CO_2 incubators are equipped with a dual-channel infrared (IR) sensor and advanced microprocessor control which ensure highly homogenous atmosphere and fast recovery after door opening. The advanced sensor technology ensures long-term, drift-free, accurate measurement of CO_2.

Humidity: In most systems humidity pans – filled with sterile distilled water - produce humidity through passive evaporation. They maintain relative humidity levels of about 95 %.

The Eppendorf solution: The CellXpert® C170i incubator is equipped with a single piece water tray that can be easily removed for emptying, cleaning, and refilling. There are no additional drain valves that need to be cleaned.

Oxygen control: Atmospheric air contains approximately 21 % oxygen. Physiological oxygen concentrations of cells can typically range from 1 % to 13 %. It has been found that oxygen concentration is a critical environmental component that influences e.g. stem cell growth and development [4]. That is why scientists in a variety of emerging fields, like stem cell research, are coming to understand the value of controlling oxygen in addition to CO_2 and temperature. Today, most incubators offer additional oxygen control. The oxygen level in the incubator is controlled by supplying Nitrogen to the chamber. Depending on the incubator this can add a substantial cost factor, because some incubators show a very high consumption of the usually quite expensive N_2 gas for reducing the oxygen level inside the chamber.

The Eppendorf solution: The CellXpert C170i is available with oxygen control as an option to create hypoxic environments. This option is off ered either factory-installed or can be upgraded directly in your lab. The oxygen sensor provides precise measurement of O_2 level and can stay inside the chamber during high-temperature disinfection cycles. With its low N_2 consumption and quick recovery after door opening the CellXpert C170i provides an optimal environment for hypoxic applications, e.g. cultivation of stem cells or tumor cells.

Contamination control

Besides reliable chamber atmosphere control and built-in automatic self-disinfection, the design of an incubator can help beat one of the biggest challenges of a cell culture researcher – contamination.

One such measure is to install a HEPA (High Efficiency Particulate Air) filter, as used in a biosafety cabinet. Doing so requires the addition of many complex components to the chamber, including fans and ducts to aspirate air through the filter and redistribute it in the chamber. The air is filtered, but there are several disadvantages. Given the more complex interior, there are more places, including seams and corners, for contaminants to hide. Splashes may stay undetected, providing a breeding ground for germs, and more time has to be spent dismantling the unit for cleaning and disinfection. The forced air flow may also disturb cultures and lead to desiccation of culture media. Apart from all that, it is essential to schedule regular maintenance and invest in new filters. Otherwise, the filter can become a source of contamination, doing more harm than good.

Another measure is adding a UV light to the chamber, which is claimed to eliminate both airborne and waterborne organisms that may have entered the chamber. The UV lamp is usually isolated from the cell culture chamber by a plenum cover over the humidity pan. The lamp automatically switches on for a specified period after each door opening and is directed at the circulated, humidified air and the water in the humidity pan. Directional airflow needs an additional fan and a duct at the back of the incubator. Although UV treatment of the air and the water in incubators has been found effective [1], relative humidity above 70 % was found to adversely impact the effectiveness of UV [2]. UV light can only disinfect surfaces upon which it directly impinges. An incubator's interior is complex, so that UV light cannot reach and disinfect many of its surfaces. In addition, the UV lamp must be replaced periodically to maintain its effectiveness.

Unlike the forced airflow in a fan-assisted incubator, the fan-less incubator circulates air gently, by convection. The potential risks of turbulent airflow – drying of samples, vibrations and further spread of contaminants – are fully eliminated. And the chamber design has no complex interior structures where germs can hide. By its plain design, with no seams and hidden corners, contaminants rarely have the chance to grow without being detected.

If any spillages occur they can be disinfected immediately, as all surfaces of the incubator chamber are easily accessible. A recently published guideline for good cell culture practice recommends non-fan-assisted incubators to reduce the airborne spread of contamination within the incubator [3].

The Eppendorf solution: Less is more in our chamber design. The deep-drawn fan-less chambers of Eppendorf CO_2 incubators are made from single sheets of stainless steel, with no seams or sharp corners (Figure 2). Eppendorf direct-heating technology avoids fans and complex interior parts. This elegant and minimalistic design strategy eliminates the chance for the growth of microorganisms in hidden corners or behind ducts, and makes cleaning and disinfection exceptionally easy. Spills can be detected and eliminated on the spot, and all surfaces areas are easily accessible for wiping and disinfection. The racking system and the shelves are designed to be removed in less than 2 minutes.

Figure 2: Eppendorf easy-to-clean incubator chamber. Deep-drawn chamber with rounded corners and smooth, seamless surface prevents contamination formation in hidden corners and allows quick and easy cleaning procedures.

Built-in automated self-disinfection
All the described measures do not replace regular thorough cleaning and disinfection of the incubator, which includes cleaning and wipe disinfection of all parts of the unit. Incubators with integrated self-disinfection programs offer an additional safety measure. Today, incubators are available with various built-in automatic self-disinfection systems, from moist or dry heat to hydrogen peroxide (H_2O_2) nebulization.

H_2O_2 nebulization is quicker than heat decontamination, but requires handling of a toxic reagent, and periodic repurchase of the reagent specified by the manufacturer.

Moist heat disinfection requires a long and tedious procedure, including draining of the water, disinfecting surfaces, and refilling the reservoir. In addition, it leaves condensed water in the chamber at the end of the cycle, which increases the risk of recontamination. Condensed water has to be removed by a final wipe disinfection of the chamber.
Dry heat disinfection can be run overnight and has the shortest preparation time. It also has the lowest chance of recontamination, as the chamber can be used directly afterward. Note that HEPA filters cannot stay in the incubator during high temperature disinfection.

The Eppendorf solution: The optional automatic self-decontamination program with high heat offers additional safety against contamination. The high-temperature disin-fection cycle is started by pressing just one button. It heats the inner chamber up to 120–180 °C (depending on the model) and the whole process can be conveniently completed overnight. Antimicrobial efficiency of the HTD cycle has been tested and validated using heat- resistant spore strips.

Other selection criteria
Limited lab space: Today most incubator models can be stacked one above the other, to save valuable lab space. Stacking the incubators on a frame with casters is preferable, as it allows them to be moved for cleaning and servicing. This also prevents germs from entering the incubator as it is lifted.

The Eppendorf solution: Eppendorf offers a robust stacking stand (Figure 3). The lower base includes heavy duty casters, and can be ordered separately, for use with a single incubator.

Figure 3: Easily stack two CellXpert Incubators on with a robust stand that can be moved on heavy-duty casters. Save space and adapt your configuration to your changing needs.

References

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CellXpert® C170i Ordering Information

Device Options			Order no.				
Door Handle	O_2 Control	Copper	230 V, 50/60 Hz European	230 V, 50/60 Hz UK/HKG	230 V, 50/60 Hz Australia	230 V, 50/60 Hz China	100–120 V, 50/60 Hz USA/Japan
Right			6731 000.011*	6731 000.012*	6731 000.013*	6731 000.014*	6731 010.015*
Right		Yes	6731 000.511	6731 000.512	6731 000.513	6731 000.514	6731 010.515
Right	Yes		6731 001.011*	6731 001.012*	6731 001.013*	6731 001.014*	6731 011.015*
Left			6731 000.021*	6731 000.022*	6731 000.023*	6731 000.024*	6731 010.025*
Left		Yes	6731 000.521	6731 000.522	6731 000.523	6731 000.524	6731 010.525
Left	Yes		6731 001.021*	6731 001.022*	6731 001.023*	6731 001.024*	6731 011.025*

*Stock article; all others are built-to-order

CellXpert® C170 Ordering Information

Device Options	Order no.				
Door Handle	230 V, 50/60 Hz European	230 V, 50/60 Hz UK/HKG	230 V, 50/60 Hz Australia	230 V, 50/60 Hz China	100–120 V, 50/60 Hz USA/Japan
Right	6734 000.011	6734 000.012	6734 000.013	6734 000.014	6734 010.015

About Eppendorf

Eppendorf is a leading life science company that develops and sells instruments, consumables, and services for liquid-, sample-, and cell handling in laboratories worldwide. Its product range includes pipettes and automated pipetting systems, dispensers, centrifuges, mixers, spectrometers, and DNA amplification equipment as well as ultra-low temperature freezers, fermentors, bioreactors, CO_2 incubators, shakers, and cell manipulation systems. Associated consumables like pipette tips, test tubes, microtiter plates, and disposable bioreactors complement the instruments for highest quality workflow solutions.

Eppendorf was founded in Hamburg, Germany in 1945 and has more than 3,000 employees worldwide. The company has subsidiaries in 25 countries and is represented in all other markets by distributors.

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