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CO_2 Incubators – Best Practices for Selection, Set-up and Care

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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, available in many sizes and configurations, 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, ranging from model selection, installation, and daily operation to the maintenance required to keep a contamination-free environment for reliable cell growth.

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. The specific arrangement of the heating elements creates a temperature differential between the top and the bottom of the inner chamber, which results in natural and gentle convection circulation of the chamber atmosphere (Figure 1). This helps avoid "cool spots" in the chamber 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, thus eliminating a traditional source of contamination and vibration.

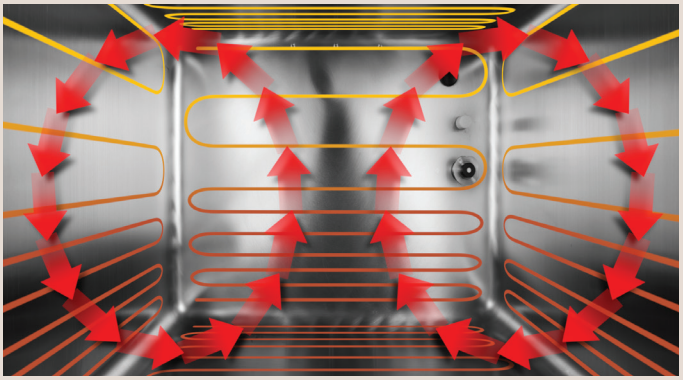

Figure 1: Eppendorf 6-sided direct-heating technology creates a gentle convection circulation of the chamber atmosphere. This maintains stable temperatures and CO_2 control throughout the chamber.

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 an advanced infrared (IR) sensor which offers the convenience of programmable automatic self-calibration with the auto-zero function. Once programmed, automated self-calibration of the sensor ensures long-term, drift-free, accurate measurement of CO_2. The sensor can withstand high heat, and can stay in place during the high-temperature disinfection cycle.

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 Eppendorf Galaxy® R-series can be equipped with an optional humidity monitoring system: a water level sensor which activates acoustic and display alarm when the water level gets too low, and a humidity sensor monitoring the relative humidity in the chamber.

Extra tip: A split inner door can help to keep chamber atmosphere undisturbed; it reduces the risk of germs getting into the incubator and can also help to reduce gas consumption. The most common options are 2-, 4- and 8-split doors depending on the model (Figure 2).

Figure 2: Eppendorf 4-split inner door for Galaxy 170 Liter incubators is equipped with the easy-to operate "click lock" design.

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 3). 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 3: 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, butrequires 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 disinfection cycle is started by pressing just one button. It heats the inner chamber up to 120 °C, and holds it for 4 hours. The whole process duration, of about 12 hours, can be conveniently completed overnight. Antimicrobial efficiency of the HTD cycle has been tested and validated using heat-resistant spore strips [4].

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 4). The lower base includes heavy duty castors, and can be ordered separately, for use with a single incubator.

Figure 4: Eppendorf Stacking Stand. For stacking two Eppendorf Galaxy CO_2 Incubators.

Lower capacity needs: A smaller incubator may be desirable for certain applications, such as cell cultivation under hypoxic conditions, or at varying temperatures. A quarantine incubator is recommended for untested or freshly isolated primary cells, both of which should be considered potentially contaminated until proven otherwise [3].

The Eppendorf solution: A small footprint 48 Liter model is available for users with smaller capacity needs (Figure 5). The Eppendorf Galaxy 48 R has the same advanced features as the Galaxy 170 R model, including an integrated 72-hour data-logging function. It can be optionally equipped with humidity monitoring and a water level sensor, and is available with hypoxic control. Two units may be stacked to save space.

Figure 5: Eppendorf Galaxy 48 R is equipped with a heated viewing window in the outer door that reduces full door openings

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 [5]. 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.

Note that oxygen control is always a factory-installed option. It is not possible to upgrade a standard model, as hypoxic incubators are much more complex, being equipped with an additional gas port, valve, regulator, and an oxygen sensor. If you use an incubator under hypoxic conditions we strongly recommend the use of split inner doors to maintain atmosphere stability, especially if you work with very low oxygen levels.

The Eppendorf solution: The Eppendorf Galaxy R-series is optionally available with oxygen control to create hypoxic environments. They have been shown to be effective for the cultivation of stem cells [6], and the generation of induced pluripotent stem cells (iPSCs) [7]. Available oxygen concentration spans are 0.1-19 % and 1-19 %.

Tips on installation and initial set-up*

> Avoid placing your incubator in direct sunlight, or close to vents, air-conditioning ducts or the exhaust of heat- or cold-generating equipment, as these can interfere with chamber conditions. Follow manufacturer's specifications on allowable room temperature to facilitate stable incubation at 37 °C.

> Do not place your incubator directly on the floor. Use a base with castors, which can be ordered separately or as one part of the stacking stand. It offers not only the possibilities of flexible movement and improved access to the back side for cleaning and service, but also keeps the unit away from dust and dirt on the floor that can enter when opening the door.

> Position the incubator to allow clearance for opening the door, access to the CO_2 sampling port (if an external gas analyzer is used to measure gas concentration), and access to any other port.

> Gas connections: Gas connection set-up depends on the manufacturer, so follow instructions in the operating manual. In Eppendorf CO_2 incubators, separate in-line pressure regulators control secondary gas pressure. We recommend gas quality of 'high grade' (> 99.5 %) for CO_2, O_2, and N_2 supplies. In some regulated fields, medical grade gas is required.

> Initially clean and disinfect the incubator interior and shelves, and other chamber equipment. After cleaning, remove the sensor protective cover (if present) and store it for future use. Install all internal components and make sure your incubator is level, with the help of a small water level placed on the second shelf of the incubator. Level the incubator by adjusting the feet or the base of stacking stand, according to the manufacturer's instructions. Don't forget to lock the leveling feet in place by tightening the locking nuts on each foot!

> Run the automatic self-sterilization program, if your incubator is equipped with one.

> Fill the water tray with warm sterile distilled water, adjust the program set-points if required, and leave the incubator running for at least two hours (preferably overnight) to allow conditions to stabilize. Prior to cultivating cells, auto-zero the CO_2 sensor once manually, and program the frequency and time for continuous CO_2 sensor self-calibration. Your incubator is now ready for use!

*Please note that following tips are general tips and do not replace reading the user manual [8] when installing a new unit in the lab.

Proper handling and cleaning and maintenance schedules

> Only touch the incubator with fresh or disinfected gloves.
> Minimize the frequency and length of door openings. Keep your incubator contents organized to easily relocate cells and to avoid long and frequent door openings.
> Implement a regular cleaning schedule for your incubator to ensure a contamination-free environment for your cells. We offer suggestions below, but please decide on frequencies according to your own risk management policies, as they depend on multiple factors, including the number of users, their aseptic skills, and the probability that the cells are contaminated.

Daily: Inspect incubator contents! Remove and disinfect any spills immediately with 70 % Ethanol or Isopropanol. Prefer wipe to spray disinfection. This prevents the formation of aerosols which may be harmful to you and your cells, and enables complete wetting of the surface for proper disinfection.

Weekly: Replace water in water tray, and clean and wipe disinfect the tray using alcohol 70 %. Most suppliers recommend sterile distilled water. If you have ongoing contamination problems, it may be helpful to add copper sulfate (~1.0 g per liter) to the water, for example.

Monthly: Once a month, up to every 6–8 weeks, empty the incubator fully. Place a protective cover over the CO_2 sensor, if one is present, prior to cleaning the incubator. Using a lint free cloth, clean the chamber interior with soapy water and rinse with water, followed by wiping the surfaces with alcohol 70 % or an equivalent non-corrosive disinfectant. If you have an incubator with many hidden corners, fissures, ducts, or seams, you should pay special attention to these areas, as germs can hide there.

Clean and disinfect the removed shelves and racking similarly. Clean, as well, the exterior of the incubator, especially the surfaces you touch, like the doors. Take care to keep the solutions from coming into contact with any mains electrical outlets or assemblies. If your incubator is equipped with an automatic disinfection program, first reinstall all parts that can withstand the disinfection program. Check if the sensor can stay inside, remove the sensor protection cover, and keep the HEPA filter out, if the unit is equipped with one. Then run the disinfection program overnight, following the manufacturer's instructions.

Every 6 months: Replace the HEPA filter if your unit is equipped with one.

Annually: Service should be done at least once a year by an authorized service engineer. Suppliers offer flexible service performance plans and contracts according to your needs, from basic checks of sensors and functional parts up to replacement of worn parts.
(For Eppendorf Service packages please visit www.eppendorf.com/incubator-service)

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[8] Operating Manual Eppendorf CO_2 incubators. www.eppendorf.com

Ordering information

Description	Order no. international	Order no. North America
Galaxy® 48 R CO_2 incubator (48 Liter, with high temperature disinfection)	CO48310001	CO48210005
Galaxy® 170 R CO_2 incubator (170 Liter, with high temperature disinfection)	CO17311001	CO17211005
Galaxy® 170 R CO_2 incubator (170 Liter, with high temperature disinfection, 1-19 % O_2 control, 8-split inner door)	CO17338001	CO17238005
Galaxy® 170 S CO_2 incubator (170 Liter, with high temperature disinfection)	CO17111001	CO17011005

CO_2 Incubator Accessories		
Lower stacking frame, with castors (for Galaxy® 170)	6710 070.219	6710070219
Upper stacking frame (for Galaxy® 170)	6710 070.200	6710070200
4 split inner doors, retrofit (for Galaxy® 170)	6710 866.005	6710866005
8 split inner door, retrofit (for Galaxy® 170)	6710 868.008	6710868008
Lower stacking frame, with castors (for Galaxy® 48 R)	6705 070.103	6705070103
Upper stacking frame (for Galaxy® 48 R)	6705 070.111	6705070111

All variants and accessories (with 4-split door, humidity monitoring kit, copper chamber etc.) are available on our website: www.eppendorf.com

Learn more about our incubators using the interactive product model here: www.eppendorf.com/co2

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 about 2,900 employees worldwide. The company has subsidiaries in 25 countries and is represented in all other markets by distributors.

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