WHITE PAPER No. 29

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

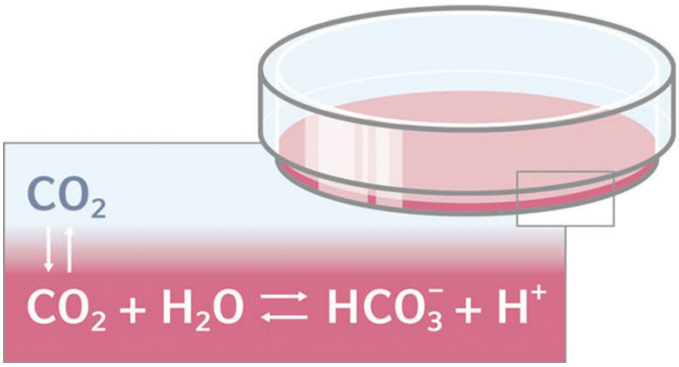
Ines Kristina Hartmann, Jessica Wagener
Eppendorf AG

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. 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 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 2). 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 for quick recovery of temperature after door opening, thus eliminating a traditional source of contamination and vibration.

Figure 2: 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 and Galaxy® 48 R series are 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. As an additional option, the incubators can be equipped with can be equipped with a 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.

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 stem cell growth and development, for instance. 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: some incubators display a very high consumption of the usually expensive N_2 gas for reducing the oxygen level inside the chamber.

The Eppendorf solution: The CellXpert C170i and the Galaxy 48R are available with oxygen control as an option to create hypoxic environments. Depending on the model, this option is offered either factory-installed or can be upgraded directly in your lab. The oxygen sensor provides precise measurement of O_2 level. With their low N_2 consumption and quick recovery after door opening, these Eppendorf CO_2 Incubators provide 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. As it has been described, relative humidity above 50 % adversely impacts the effectiveness of UV [1, 2]. UV light can only disinfect surfaces upon which it directly impinges. An incubator's interior is complex, so much so that UV light cannot reach and disinfect many of its surfaces. In addition, the UV lamp must be replaced periodically, adding a recurring cost with questionable 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 a minute.

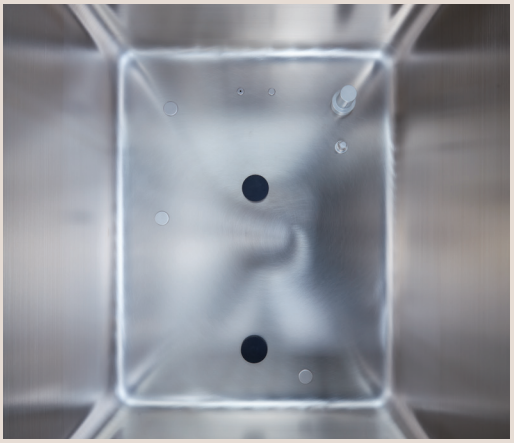


Figure 3: Eppendorf easy-to-clean incubator chamber. Deep-drawn chamber with rounded corners and smooth, seamless surface prevents contamination formation in hidden corners and allows quick and easy cleaning procedures.

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

Figure 4: Easily stack two CellXpert CO_2 incubators on a robust stand that can be quickly moved on its heavy-duty casters. Save space and adapt your configuration to your changing needs.

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. (Figure 5).

Figure 5: 4-segemented inner doors for CellXpert C170i incubators are equipped with the easy-to-operate magnetic latch design.

Lower capacity needs: A smaller incubator may be desirable for restricted lab space, low throughput, or personal workspaces. Also, 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 6). The Eppendorf Galaxy 48 R has many advanced features, 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 6: Eppendorf Galaxy 48 R, a small footprint CO_2 incubator for low capacity needs and limited lab space

Setting up a new incubator

When the decision for a certain incubator model has been made, the next step is installing the device in the cell culture lab. For gassed incubators, a risk assesment should be performed. A recommended measure are gas detectors that issue an alert when critical gas concentration is reached in the laboratory. In addition, a ventilation system ensures air exchange both during normal operation and in case a critical gas concentration is reached. When gas cylinders are used, they should be clearly labeled and securley anchored in suitable safety cabinets. The tubing connecting the incubator to the gas cylinder or the central gas supply should be appropiate for the pressure of the gas used to avoid any leakage of CO_2, which can cause suffocation if the concentration in the air is too high.

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 not only offers the possibilities of flexible movement and improved access to the backside 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. We recommend gas quality of 'high grade' (>99.5 %) for gas supplies. In some regulated fields, medical grade gas is required.

> Initially clean and disinfect the incubator interior and shelves, and other chamber equipment. 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'sinstructions. 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. Your incubator is now ready for use!

*Please note that following tips are general tips and do not replace reading the user manual [5] when installing a new unit in the lab.

Proper handling and cleaning and maintenance schedules

> To keep the risk of introducing contamination into the incubator as low as possible, only touch the incubator with fresh or disinfected gloves.

> Keep your incubator contents organized to easily relocate cells and to avoid long and frequent door openings. This reduces the risk of air-borne microorganisms to enter the incubator chamber. Depending on the routine in your lab there are different ways to organize the incubator contents (Figure 7).

> A regular cleaning schedule for your incubator prevents contaminations within the 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.

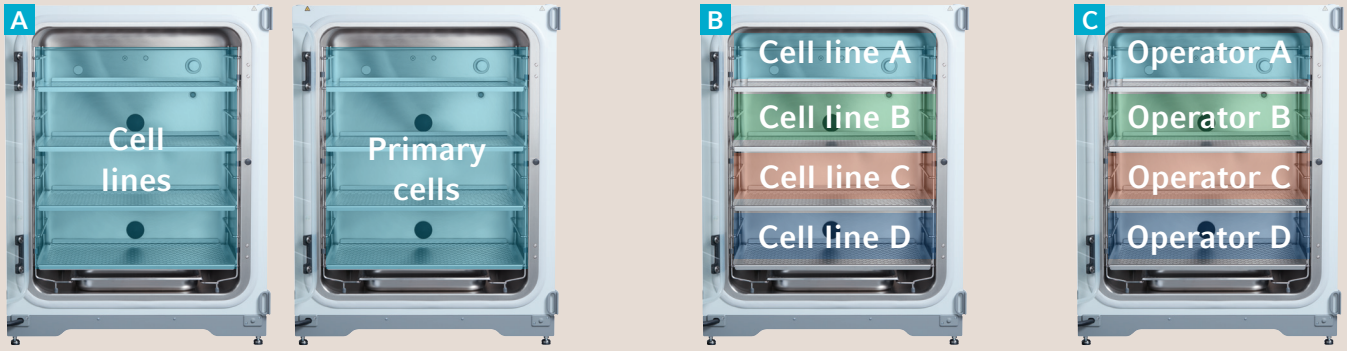


Figure 7: Organize your incubator. Depending on the routine in the laboratory there are different ways to organize incubator contents to reduce the risk of contamination (**A**) Dedicated incubators for keeping cell lines and primary cells separately, (**B**) Dedicated shelves for different cell lines, (**C**) Dedicated shelves for different operators.

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.

Monthly: Once a month, up to every 6–8 weeks, empty the incubator fully. 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)

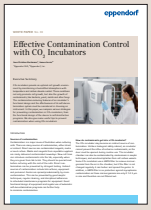
References

[1] Peccia J., Werth H., Miller S. , Hernandez M. Effects of Relative Humidity on the Ultraviolet Induced Inactivation of Airborne Bacteria. Aerosol Science and Technology (2001) 35 : 72 8–740
[2] Burgener J., Eli Lilly and Company, Indianapolis, Position Paper on the Use of Ultraviolet Lights in Biological Safety Cabinets 228 Applied Biosafety (2006), 11 (4), 228-230
[3] Geraghty RJ, Capes-Davis A, Davis JM, Downward J, Freshney RI, Knezevic I, Lovell-Badge R, Masters JRW, Meredith J, Stacey GN, Thraves P, Vias M. Guidelines for the use of cell lines in biomedical research, British Journal of Cancer (2014), 1–26 | doi: 10.1038/bjc.2014.166
[4] Mohyeldin A., Garzón-Muvdi T., Quinones-Hinojosa A. Oxygen in Stem Cell Biology: A Critical Component of the Stem Cell Niche, Cell Stem Cell (2010) 7(2), 150-161
[5] Operating Manual Eppendorf CO_2 incubators. www.eppendorf.com/co2-incubators

Want to learn more about the new family of Eppendorf CO_2 Incubators and compare with other manufacturers?

Video: Easy cleaning and reliable contamination prevention with the new CellXpert CO_2 incubators

Video: Cell Culture Reproducibility – Support with the CellXpert CO2 incubator from Eppendorf



White paper: Effective Contamination Control with CO_2 Incubators

White paper: CellXpert CO_2 Incubators – Cut Costs & Deliver Results

Visit **www.eppendorf.com/co2-incubators**

CellXpert® C170i Ordering Information

Device Options					Order no.				
Door Segments	Door Handle	O_2 Control	Humidity Monitor/ Water Level Sensor	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
1	Right				6731 000.011*	6731 000.012*	6731 000.013*	6731 000.014*	6731 010.015*
1	Right			Yes	6731 000.511	6731 000.512	6731 000.513	6731 000.514	6731 010.515
1	Right		Humidity monitor		6731 000.111*	6731 000.112*	6731 000.113*	6731 000.114*	6731 010.115*
1	Right		Water level sensor		6731 000.211*	6731 000.212*	6731 000.213*	6731 000.214*	6731 010.215*
1	Right		Both		6731 000.311*	6731 000.312*	6731 000.313*	6731 000.314*	6731 010.315*
1	Right	Yes			6731 001.011*	6731 001.012*	6731 001.013*	6731 001.014*	6731 011.015*
1	Right	Yes		Yes	6731 001.511	6731 001.512	6731 001.513	6731 001.514	6731 001.515
1	Right	Yes	Both	Yes	6731 001.811	6731 001.812	6731 001.813	6731 001.814	6731 011.815
1	Left				6731 000.021*	6731 000.022*	6731 000.023*	6731 000.024*	6731 010.025*
1	Left			Yes	6731 000.521	6731 000.522	6731 000.523	6731 000.524	6731 010.525
1	Left		Humidity monitor		6731 000.121	6731 000.122	6731 000.123	6731 000.124	6731 010.125
1	Left		Water level sensor		6731 000.221	6731 000.222	6731 000.223	6731 000.224	6731 010.225
1	Left		Both		6731 000.321	6731 000.322	6731 000.323	6731 000.324	6731 010.325
1	Left	Yes			6731 001.021*	6731 001.022*	6731 001.023*	6731 001.024*	6731 011.025*
1	Left	Yes		Yes	6731 001.521	6731 001.522	6731 001.523	6731 001.524	6731 001.525
1	Left	Yes	Both	Yes	6731 001.821	6731 001.822	6731 001.823	6731 001.824	6731 011.825
4	Right				6731 000.041*	6731 000.042*	6731 000.043*	6731 000.044*	6731 010.045*
4	Right		Both		6731 000.341	6731 000.342	6731 000.343	6731 000.344	6731 010.345
4	Right		Both	Yes	6731 000.841	6731 000.842	6731 000.843	6731 000.844	6731 010.845
4	Right	Yes			6731 001.041*	6731 001.042*	6731 001.043*	6731 001.044*	6731 011.045*
4	Right	Yes	Both		6731 001.341	6731 001.342	6731 001.343	6731 001.344	6731 011.345
4	Left				6731 000.051	6731 000.052	6731 000.053	6731 000.054	6731 010.055
4	Left		Both		6731 000.351	6731 000.352	6731 000.353	6731 000.354	6731 010.355
4	Left		Both	Yes	6731 000.851	6731 000.852	6731 000.853	6731 000.854	6731 010.855
4	Left	Yes	Both		6731 001.351	6731 001.352	6731 001.353	6731 001.354	6731 011.355
4	Left	Yes			6731 001.051	6731 001.052	6731 001.053	6731 001.054	6731 011.055
8	Right	Yes			6731 001.081*	6731 001.082*	6731 001.083*	6731 001.084*	6731 011.085*
8	Left	Yes			6731 001.091	6731 001.092	6731 001.093	6731 001.094	6731 011.095

* 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

Galaxy® 48R Ordering Information

Device Options					Order no.				
Door Segments	HTD	O_2 Control	Humidity package	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
–	–	–	–	–	CO48300001*	CO48300002*	CO48300003*	CO48300004*	CO48200005*
–	–	1–19 %	–	–	CO48320001*	CO48320002*	CO48320003*	CO48320004*	CO48220005*
–	Yes	–	–	–	CO48310001*	CO48310002*	CO48310003*	CO48310004*	CO48210005*
2	–	–	–	–	CO48312001	CO48312002	CO48312003	CO48312004	CO48212005
–	Yes	0.1–19 %	–	–	CO48310041	CO48310042	CO48310043	CO48310044	CO48210045
–	Yes	0.1–19 %	Yes	–	CO48310061	CO48310062	CO48310063	CO48310064	CO48210065
2	Yes	0.1–19 %	–	–	CO48312041	CO48312042	CO48312043	CO48312044	CO48212045
2	Yes	0.1–19 %	Yes	–	CO48312061	CO48312062	CO48312063	CO48312064	CO48212065
–	Yes	1–19 %	–	–	CO48330001*	CO48330002*	CO48330003*	CO48330004*	CO48230005*
2	Yes	1–19 %	–	–	CO48332001	CO48332002	CO48332003	CO48332004	CO48232005
2	Yes	1–19 %	Yes	–	CO48332011	CO48332012	CO48332013	CO48332014	CO48232015

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. Consumables such as pipette tips, test tubes, microtiter plates, and single-use bioreactora vessels complement the range of highest-quality premium products.

Eppendorf was founded in Hamburg, Germany in 1945 and has more than 3,300 employees worldwide. The company has subsidiaries in 26 countries and is represented in all other markets by distributors.

Your local distributor: www.eppendorf.com/contact
Eppendorf AG · Barkhausenweg 1 · 22339 Hamburg · Germany
eppendorf@eppendorf.com · www.eppendorf.com

www.eppendorf.com