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WHITE PAPER No. 51

CO_2 Incubator Maintenance – Guide for Set-up and Care

Ines Kristina Hartmann, Jessica Wagener
Eppendorf AG

Executive Summary

CO_2 incubators are required to maintain an optimal environment for cell growth, by providing carbon dioxide control in a humidified atmosphere with constant temperature. In this CO_2 incubator maintenance guide we give you some best practices and tips, ranging from installation and daily operation to the maintenance required to keep a contamination-free environment for reliable cell growth.

Setting up a new CO_2 incubator

When the decision for a certain CO_2 incubator model has been made, the next step is installing the device in the cell culture lab. For gassed CO_2 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 CO_2 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. This CO_2 incubator maintenance guide will help you with general tips on installation, set-up, and care of your new CO_2 incubator.

Figure 1: CO_2 incubators CellXpert C170i (left) and C170 (right)

Tips on installation and initial set-up*

> Avoid placing your CO_2 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 CO_2 incubator directly on the floor. Use abase with casters, which 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 CO_2 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 CO_2 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 CO_2 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 CO_2 incubator is equipped with one.

> Fill the water tray with warm sterile distilled water, adjust the program set-points if required, and leave the CO_2 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 [8] when installing a new unit in the lab.

Proper handling and cleaning of CO_2 incubators

> To keep the risk of introducing contamination into the CO_2 incubator as low as possible, only touch the incubator with fresh or disinfected gloves.

> Keep your CO_2 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 (Fig. 2).

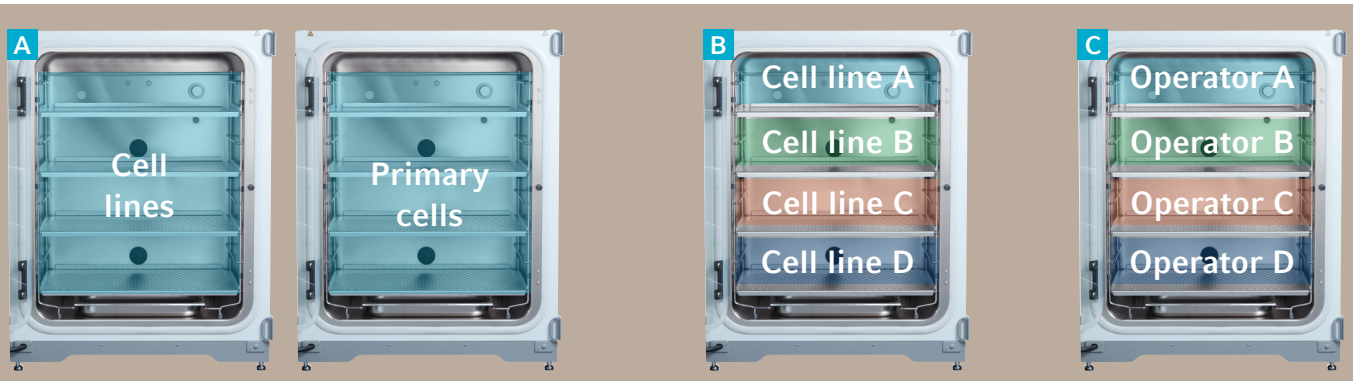

Figure 2: Organize your CO_2 incubator. Depending on the routine in the laboratory there are different ways to organize incubator contents to reduce the risk of contamination (**A**) Dedicated incubators for keeping cell lines and primary cells separately, (**B**) Dedicated shelves for different cell lines, (**C**) Dedicated shelves for different operators

> A regular cleaning schedule for your CO_2 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.

Daily: Inspect CO_2 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 CO_2 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 a CO_2 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 CO_2 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 CO_2 incubator is equipped with an automatic disinfection program, first reinstall all parts that can withstand the disinfection program. Check if all sensors can stay inside. 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)

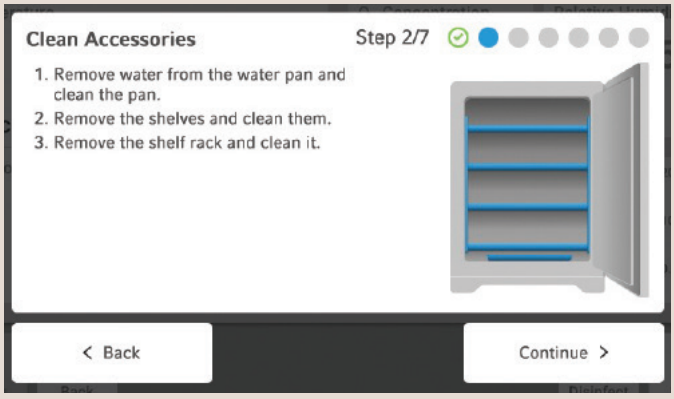

Figure 3: Modern CO_2 incubators have clear step-by-step instructions for standardized cleaning procedures to be carried out before sterilization at high temperatures. To further reduce the probability of user errors the process should be sensor supported.

See here for more information

CO_2 Incubator Maintenance - References

[1] Operating Manual Eppendorf CO_2 incubators.
www.eppendorf.com/co2-incubators

CO_2 Incubator Maintenance – Further Resources

Video: Your CO_2 incubator: How to minimize atmosphere disruptions - Cell Culture Do's and Don'ts

Video: How to correctly refill the water reservoir in your CO_2 Incubator - Cell Culture Do's and Don'ts

White paper: CO_2 Incubators - Making the Best Choice for Your Lab

White paper: CO_2 Incubator Temperature Control: What Is the Best Place For Your Cell Culture Vessels?

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

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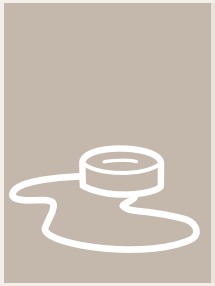
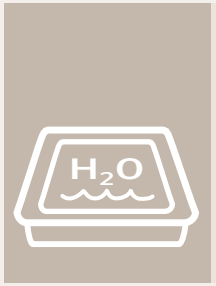
How to Clean Your Incubator

Best practices for CO_2 incubator cleaning

Immediate and weekly measures



Remove any spills immediately.



Check water tray weekly. Clean and refill with sterile distilled water.

General information

! Wear fresh gloves and avoid spray disinfection. Use lint-free cloth. For cleaning use a mild cleaning-detergent. Only use non-corrosive disinfection agents.

! If other than alcohol 70% (e.g. Ethanol, Isopropanol) is used, rinse with sterile distilled water followed by 70% alcohol afterwards.

For professional services please contact your local Eppendorf service at www.eppendorf.com/epServices

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Regular cleaning measures (recommended every 6-8 weeks)

1. 

Turn off the incubator. Clean the exterior surfaces you touch, like doors and handles.

2. 

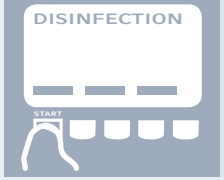
Remove shelves, rack, water tray and empty the incubator.

3. 

Clean and disinfect removed parts and chamber interior.*

4. 

Reinstall chamber parts.

5. 

Start disinfection cycle, if available.** When completed, refill water tray and return cultures.

* For chamber cleaning, place protective cover on gas sensors of Eppendorf Galaxy® CO_2 incubators and New Brunswick™ S41i CO_2 incubator shakers.

** Remove O_2 sensor from Galaxy CO_2 incubators before high temperature disinfection and reinstall afterwards. Reference Galaxy sensors from display before use.

Cleaning Schedule CO_2 Incubators

When?	What?	Disinfection reagent*	How?*			Who?	Comments
Date	Device (e.g. incubator 2)	What was used for disinfection?	Cleaning/disinfection of exterior, interior, and removable parts	Automatic disinfection routine	Water tray refilled	Name	Special occurences, observations, incidents etc. (e.g. water tray was contaminated)

Maintenance Interval:

Room:

* According to manufacturer´s instructions