ThermoFisher SCIENTIFIC

CO_2 incubators

Mastering CO_2 incubator care

A comprehensive guide to optimal cell culture conditions

thermo scientific

You trust your work – your cells – to your incubator

There are many CO_2 incubator designs today in different labs worldwide. They all share some similarities. Having a well-designed, properly functioning incubator means that your cells will grow well, contamination problems are rare, and the incubator is easy to clean and maintain.

Everyone using a cell culture incubator needs to understand a few important points to keep any incubator working its absolute best. The information presented here can help you to avoid common mistakes that can result in project delays.

Please note that while these recommendations are applicable for any CO_2 incubator, we suggest that you consult your user manual to help ensure you are also following the manufacturer's best practices.

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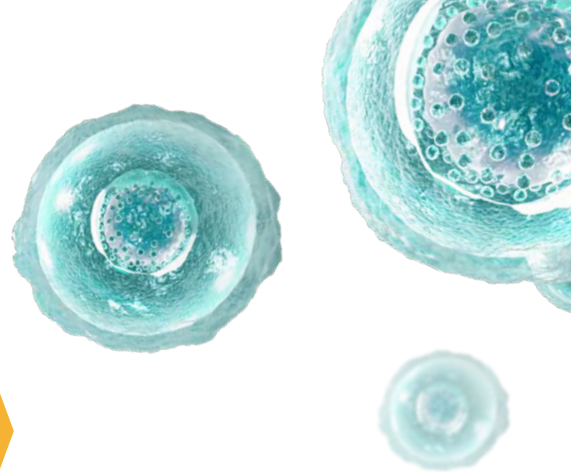
A comprehensive guide to optimal cell culture conditions

1 Positioning and installing your CO_2 Incubator



The placement of the incubator can significantly impact its performance. Factors such as ventilation, air flow, room temperature, and exposure to direct sunlight can influence its ability to maintain optimal temperature and humidity levels.

Lift

- Lift only by the sides of the bottom.
- Never lift front and back – and do not ever lift using the door.

Position

- Position the incubator on a firm, level surface away from any vibration source.
- Do not place any incubator directly on the floor. Instead, use a support stand to prevent dust and contaminants from entering when the door opens.
- Ensure that the incubator is level by adjusting the leveling feet or stand.

Stack

- Only stack similar brands. If you stack dissimilar units, you risk the stack tipping over or the top unit slipping off.
- Ensure adequate clearance on all sides of the incubator. This allows for ventilation and access to power cords and connectors including gas hookup.

Placement

- Place the incubator away from traffic areas, but avoid damp, humid corners that may harbor fungal growth.
- Shelter the unit from ventilation and other airstreams, because these air currents can direct contamination carrying dirt and dust into the incubator.

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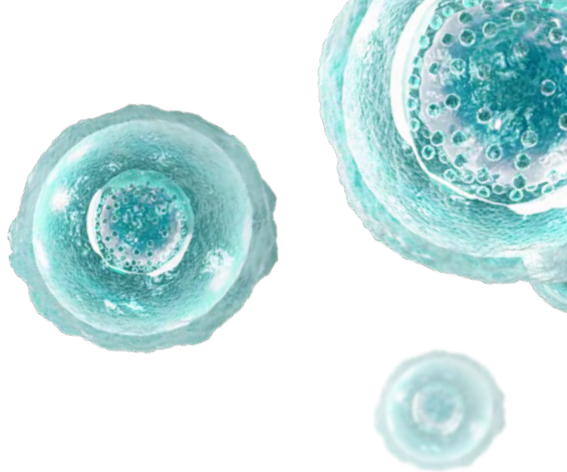
An in-chamber HEPA filtration system in any Thermo Scientific CO_2 incubator will filter the entire chamber air volume every 60 seconds, establishing ISO Class 5 cleanroom air quality in the chamber in 5 minutes after every door opening.[1]



Clean

- Clean and disinfect the incubator interior, the shelves and shelf supports, and air ducts.

Install

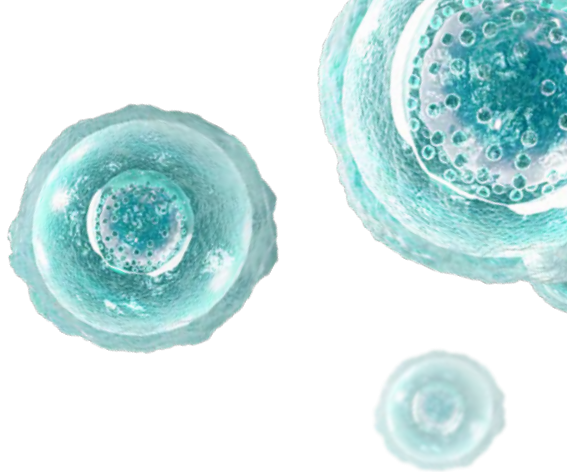
- Install internal components, following the manufacturer's directions.
- Install any additional optional parts such as an oxygen sensor or HEPA filter.

Setup

- If you have a water jacketed incubator, fill the water jacket at this time.
- Fill the humidity pan with sterile distilled water, to ½ inch (1.25 cm) from the top, or fill only to the "max" indicator. Keeping the water pan full is important for good, consistent humidity and best growth conditions.
- Following the manufacturer's directions, set the temperature, over-temperature, under-temperature, CO_2 and O_2 setpoints, and the appropriate alarms.

The Thermo Scientific™ iCAN™ interface, allows you to set the data logging parameters. Depending on your settings, the iCAN will collect data to be stored onboard for up to a week or exported to Microsoft™ Excel™ spreadsheet. This information can often be very useful, not only for GMP (Good Manufacturing Practice) monitoring, but also in helping to pinpoint the start of any problems.

2 CO_2 monitoring



There are two kinds of CO_2 gas sensors. The first is the T/C (thermal conductivity) sensor. Thermo Scientific incubators offer a T/C sensor and electronics that are very stable, accurate and economical. In fact, T/C sensors are our most popular method of CO_2 control. They are long-lasting, comparatively inexpensive and robust. The second kind of gas sensor is an IR sensor, which is preferred for some applications including GMP monitoring, or when the incubator door is opened repeatedly over a short period; for example, when performing time lapse expression analyses.

Some manufacturers have a design that places the sensors outside the incubation chamber to avoid the need to remove them during sterilization; this is not a good option because exterior sensors will not experience the same conditions as your cells do.[2] Thus, these incubators will not respond quickly to changes that affect cell growth.

We recommend using industrial grade gas that is at least 99.5% pure, because impurities in the gas can negatively affect your cells. The CO_2 gas tank should not contain siphon tubes. Use a 2-stage regulator on the tank, and set the input pressure to the incubator at 15 psi (1 barr). Connect the tubing to the air filter and then to the labeled serrated fitting on the back of the incubator. Be sure to use a hose clamp and to check for gas leaks, which could be dangerous to you and your labmates. When the incubator has been operating normally for 24 hours, check the calibration of the temperature using a National Institute of Standards and Technology (NIST) or other certified thermometer, and check the CO_2 using a fyrite or external IR tester.

For connecting CO_2 tanks to Thermo Scientific incubators, you must use a two-stage CO_2 pressure regulator on the outlet valve of the gas cylinder. This is because the input pressure on our incubators must be maintained at 15 +/- 5 psi (pounds per square inch) (1 barr) for the proper functioning of the CO_2 control system. A single stage regulator simply will not maintain this pressure, with resulting inaccurate CO_2 levels.

3 Water – a crucial element

To provide the proper humidity required by your cultured cells, we recommend only sterile distilled water. Tap water with even small amounts of chlorine can corrode stainless steel or pure copper. Also, tap water can contain lots of bacteria and minerals. Since salts and minerals are precisely balanced in cell growth media, adding minerals via the water to the humidified atmosphere can cause poor cell growth.

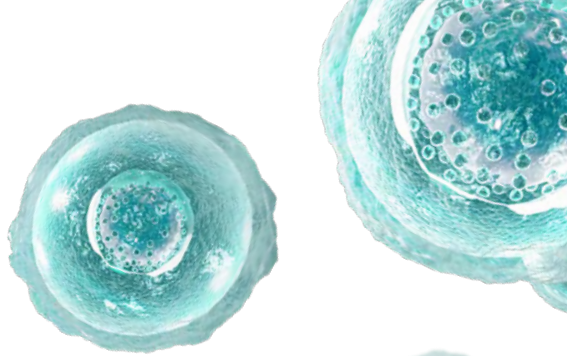
Deionized (DI) or ultra-pure Type 1 water is very aggressive. It corrodes stainless steel because the water, containing very few ions, actively pulls ions from the stainless steel, pure copper, glass door, and other incubator components. Reverse osmosis (RO) water can vary tremendously in terms of quality because the purification is a percent-removal process. Thus, if the starting water has 500 ppm (parts per million), the finished water might be 50 ppm, but if the starting water has 150 ppm, the finished water would only have 15 ppm.

Thermo Fisher Scientific scientists have done extensive water quality testing. Based on this testing, and for the sake of incubator longevity, we recommend sterile distilled water with a pH of 7-9 and a conductivity of 1-20 microsiemens /cm (resistivity of 50 K-1 megaohm-cm). Even sterile distilled water can have a pH that is too low, so be sure to test this.

If only DI or ultra-pure water is available in the lab, we recommend using water with a resistance range of 1-10 megaohm-cm. Ensure that the pH is 7-9, and then sterilize the water before using it in the incubator. Note that sometimes your building source of Type 1, DI, or ultra-pure water actually came from a still to start with, so as long as the parameters we have laid out are met, this water would be acceptable.

If you do not have access to distilled water, one option for using Type 1, DI, or ultrapure water in the incubator is to add a little sodium bicarbonate to the water to raise the pH and to add some ions. But it must be a sterile solution of the salt, and you must ensure the final pH of the water is in the 7-9 range.

4 Cleaning your incubator



Regular cleaning of the incubator is a necessity to help protect your cells from contamination and to keep the incubator functioning properly.

Prep

- Move all the cultures to a different incubator.
- Switch off the incubator, including turning off the gas supply.
- Remove all shelves, shelf supports, and any brackets or air ducts.
- Empty the water reservoir and wipe it dry with a clean, lint-free cloth.

Clean

- Clean all the internal surfaces, ducts, shelves, shelf supports, inner door, fan and door gaskets with mild, soapy water. A mild dish detergent works well for this.
- Be sure to reach all the corners and crevices where dirt, dust, and germs can hide.

Rinse

- Rinse these surfaces and parts using distilled water and wipe them dry again using a clean, lint-free cloth.

Disinfect

- Wipe the interior surfaces and parts with a diluted quaternary ammonium disinfectant (concentration 10% or less).
- Follow this by wiping with 70% alcohol to remove any remaining traces of the disinfectant.
- Be sure to reach all the corners and remember to treat the door gasket as well.

Replace

- Replace the internal parts.
- Turn the incubator heat back on and allow the incubator to dry completely.
- DO NOT leave the door open – that would reintroduce new dust and contaminants!

Sterilize

- If you have an automated decontamination/sterilization cycle, you can run it now.

Setup

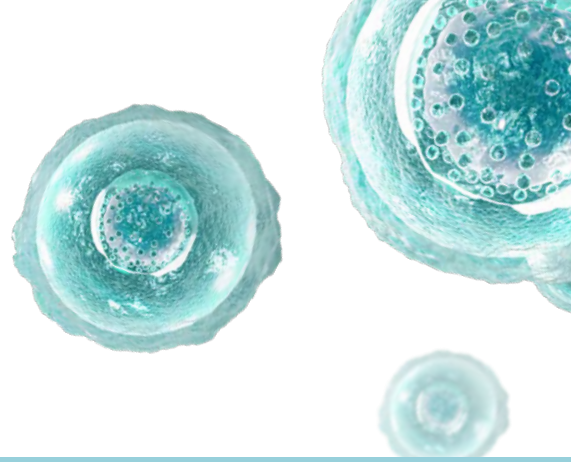
- Fill the water reservoir with sterile distilled water.
- Turn on the gas supply.

Exterior

- Clean the incubator exterior to eliminate dirt and microorganisms that could find their way inside.
- Use a lint-free cloth dampened in mild soapy water.
- Wipe clean using a clean cloth slightly dampened in clear water.
- Dry the outside with a clean, dry cloth. Pay special attention to the door handles where everyone touches.
- Use a dry microfiber cloth to clean a touch sensitive surface or display.

You can use the same procedure to clean copper that you would with stainless steel, including mild soapy water followed by a 70% ethanol wipe. It is not necessary to use a quaternary ammonium disinfectant, due to copper's inherent properties. How to clean a 100% pure copper incubator.

5 Choosing the correct incubator disinfectants



Many researchers ask: which disinfectants are okay to use in the CO_2 incubator? The truth is that there are many disinfectant options available, but not all are safe for your cells or for incubator components. Disinfection practices must include proper use of chemical cleaners and disinfectants, since overuse or improper application can damage the facility and equipment. Some strong disinfectants may give off fumes that enter the incubator and then affect cell growth. These fumes contain volatile organic compounds (VOCs) that can induce expression of heat shock and other stress proteins. Common laboratory chemicals such as phenol, isoamyl alcohol, and betamercaptoethanol are VOCs, but also laboratory cleaning products and disinfectants, and even floor cleaners and waxes produce harmful vapors. In short, if it smells strong or bad to you, it can also be bad for your cultured cells. We evaluated a number of different chemical disinfectants for use in a CO_2 incubator. A disinfectant that is effective against a range of microorganisms; and harmless (non-corrosive) to incubator components is a quaternary ammonium disinfectant.

This basic type is available in all regions from different manufacturers. Some examples include Lysol™ No Rinse Sanitizer, Decon™ Conflikt™ Detergent Disinfectant (North America) and Fermicidal-D2™ (Europe). It is important to check the material safety data sheet. Ensure that the concentration of quaternary ammonium is 10% or less. Higher concentrations can harm cells and incubator components over time. Use a 2:98 dilution of the same quaternary ammonium disinfectant that you used to disinfect the incubator interior, as a disinfection additive in the water pan.[3] Water bath disinfectant additives often contain very high concentrations of quaternary ammonium or other chemicals and should not be used in the CO_2 incubator water. These concentrations are too high for the warm, humid and slightly acidic atmosphere (due to the CO_2 gas + humidity making weak carbonic acid) in a CO_2 incubator. Such additives are very likely to cause incubator corrosion over time. Similarly, copper sulfate should not be used in the water long term.

We must stress that you should never use bleach-containing chemicals. Chlorine bleach and its derivatives with oxidizing activity corrode stainless steel and copper. In addition, these chemicals are very toxic to your cells!

VOCs are an increasing concern for cell culture scientists. Recognizing this, some manufacturers offer flexible incubator designs that fit specialized HEPA filters that capture VOCs, as well as standard HEPA filters.

6 Reducing contamination

To reduce chances of contamination in your cultures, the cleanliness of your lab is important. Dust and dirt can fly around in the lab, carried by air currents created when people move about in the room or open and close doors. Normal indoor room air contains 100-1,000 microorganisms per cubic meter,[4] all circulating at any given moment. Most of these come from the trillions of normal flora that live in and on our skin. This means that every time you open any incubator door, contaminants can enter.

It's important to minimize contaminants and dirt in the lab by cleaning the lab often; at least one to two times per month.

- Clean and disinfect the biological safety cabinet (BSC), the water bath, the centrifuge, and microscope.
- Eliminate cardboard storage in or around refrigerators and freezers because the cardboard can get wet and then breed fungi.
- Do not store items on top of the incubator because dust and dirt among these items can be swept inside the chamber with air currents created during a door opening.
- Remember to also clean the corners of the lab and on top of and under equipment where dust can collect.

Every scientist in every lab deals with contamination. It can be a tricky factor of any experiment that impacts the consistency and repeatability of a protocol. Watch the webinar "Exploring and Preventing Culture Contamination" to better understand the types of contamination that scientists commonly face, the extent of the problem, and recommendations to reduce contamination – from prevention to elimination.

7 CO_2 calibration

You can calibrate your own CO_2, but there are a couple points you should know. If you have a T/C sensor, be sure to do any calibration first thing in the morning, because this provides at least 12 hours of uninterrupted equilibration to ensure stable temperature and humidity. Since the T/C measurement works in conjunction with temperature and humidity, it is important to keep the water pan full. If the water runs out, that will likely affect the T/C sensor calibration.

In case you have a tri-gas incubator with variable oxygen control, you must calibrate the O_2 first and let it stabilize before calibrating the CO_2. This is because changing the oxygen will change the CO_2 calibration.

How often should you calibrate?
You can calibrate the CO_2 once per month, using fyrite or an external infrared (IR) tester. However, if you properly maintain the incubator and keep the water pan full, you should only need to calibrate a few times per year, for example, once per quarter. And remember, if you have any concerns, you can contact Thermo Fisher Scientific's factory trained service specialists.

If you have a Thermo Scientific CO_2 incubator with a TC180 sensor, this will compensate for changes in humidity.

If you have a Thermo Scientific™ Heracell™ incubator, the AutoStart cycle will automatically zero the sensors.

Learn more about CO_2 sensors here.

8 Adding extra equipment

Some researchers want to add electrical equipment such as shakers, rotators or stirrers to the CO_2 incubator. The difficulty with this is that too much heat from added equipment raises the internal temperature in the incubator, and that extra heat makes it challenging for the incubator electronics to compensate. Some shakers create vibration as well as heat, so it's important to not set the rotation faster than about 200 RPM, to minimize motion that can affect adherent cell attachment and growth. Test your shaker at different speeds with your required volume of liquid and flasks to ensure no heat or vibration is produced before experimenting with cells. If you know you will be using electrical equipment inside, consider purchasing a water jacketed incubator configured with a cooling coil and chiller that will compensate for the extra heat generated.

As an alternative to a shaker, the Thermo Scientific™ Heracell™ 240i CO_2 incubator offers a roller bottle assembly, which does not produce heat. Each roller bottle assembly substitutes for a shelf, so you can add up to 4 of these units inside, giving you added culturing flexibility.

9 General maintenance

Ongoing maintenance of your incubator, other than an occasional CO_2 check and calibration, is very minor. If your incubator is equipped with HEPA filtration, you should replace the HEPA filter every 6 months to 1 year. The life of a filter will depend on the number of people using the incubator, the general cleanliness of it and the laboratory, as well as the manufacturer's design. Handle a HEPA filter only by the external housing; do not touch the filter medium. Before replacing a HEPA filter, visually inspect the filter medium to ensure there are no breaks or tears. If any breaks or tears are present, discard that filter and use another.

You should also replace the gas inlet filters (where the CO_2 gas enters the incubator) every 6 months to 1 year, and simply follow the manufacturer's recommendations for that procedure, or schedule a qualified service specialist to do it for you.

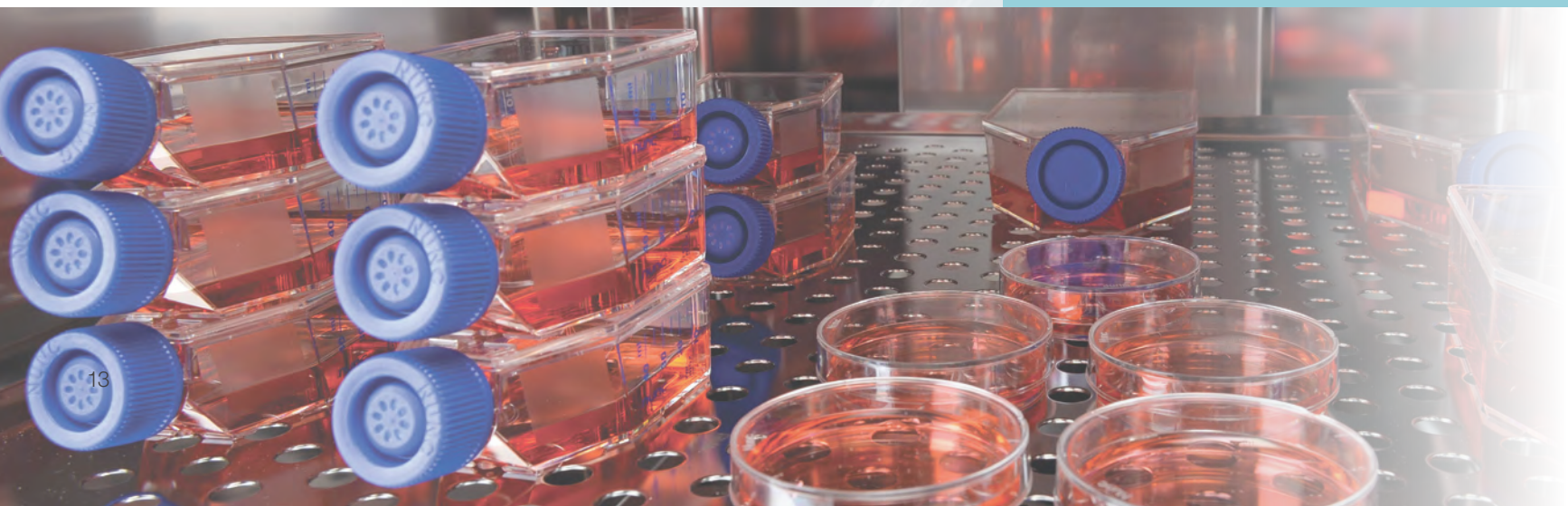
How often should you run the heat sterilization cycle?

The answer depends on the cleanliness of your lab, generally, how many people use the same incubator; how often the door is opened; and how convenient it is for you to shut down the incubator overnight. Most users use this function from one time per month to once every six months.

One of the most critical aspects of maintaining your incubator is ensuring that the water pan remains adequately filled. If the water pan becomes dry, it can severely impact your cell cultures.

Humidity is a crucial factor for cell health. A dry water pan causes a drop in humidity, which in turn leads to the evaporation of water from your culture medium. This evaporation results in the concentration of essential salts, minerals, amino acids, and other components in the growth medium, potentially causing toxicity and cell death.[5]

Beyond the danger to your cells, too low humidity can also damage the CO_2 sensor, as explained earlier.



The effective functioning and longevity of a CO_2 incubator hinges significantly on proper care, informed maintenance, and adherence to recommended operational protocols. By adhering to these guidelines, researchers can be confident in optimal incubator performance, thereby facilitating successful cell culture experiments.

Thermo Scientific™ Heracell™ VIOS™ CO_2 Incubators in electropolished stainless steel and copper can be easily cared for and maintained using the information provided in this guide.

References:

1. Thermo Scientific. Importance of Class 100 Air in a CO_2 Incubator. Thermo Scientific 2009, WPCO2Class100 1109.
2. Thermo Scientific Smart Note (2022) :Why does the location of sensors in my CO_2 incubator affect responses from my cultured cells? Thermo Fisher Scientific
3. Freshney, RI. Culture of Animal Cells. Hoboken, NJ: John Wiley & Sons, Inc., 2010.
4. United States Department of Labor, Occupational Safety & Health Administration. OSHA Technical Manual (OTM) Section III: Chapter 2. TED 01-00-015, 1999.
5. Esser, P and Weitzmann, L. Evaporation From Cell Culture Plates. Thermo Scientific 2011, TILSPNUNCBU02 0111.

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