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TECHNICAL NOTE

Proper Care and Maintenance for a Cell Culture Incubator

Keywords

CO_2 incubator maintenance, disinfectant, cleaning, calibration, HEPA filter

Introduction

There are many different CO_2 incubators today, in different labs around the world. They all share some similarities. You trust your work – your cells – to your incubator. Having a well designed, properly functioning incubator means that you will never have to worry about it. In such an incubator, 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 in order to keep any incubator working its very best. The information presented here will help you to avoid common mistakes that can result in project delays. Please note that while these recommendations are generally applicable for any CO_2 incubator, we suggest that you consult your user manual to ensure you are also following the manufacturer's best practices.

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Positioning the CO_2 Incubator

Where you place the incubator can alter its performance. Ventilation, air flow, room temperature, and direct sunlight can all affect the temperature and humidity functions.

When installing a CO_2 incubator, be sure to lift only by the sides of the bottom, never from front and back – and do not ever lift using the door. 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. If the incubator sits directly on the floor, the air movement that is created each time the door is opened will sweep dirt, dust and contaminants directly into the incubator chamber. When stacking incubators, you should only stack similar brands. If you stack dissimilar units, you risk the stack tipping over or the top unit slipping off, since there is generally no mechanism to secure incubators that don't match.

Next, ensure there is adequate clearance on all sides of the incubator. This allows for ventilation and access to power cords and connectors including gas hookup. Try to place the incubator away from traffic areas, but avoid damp, humid corners that may harbor fungal growth. Make sure the unit is sheltered from ventilation and other airstreams, because these air currents can direct contamination-carrying dirt and dust into the incubator. Do not position the incubator in direct sunlight, because fluctuating temperatures outside can affect the anti-condensation functions. Finally, ensure that the incubator is level – both front to back and side to side – by adjusting the leveling feet or stand.

Installation

Each incubator model is different regarding connections and programming, but there are some general guidelines that apply to all incubators. Properly installing the incubator from the beginning will help provide the best conditions for your cells going forward.

After the incubator is properly positioned, clean and disinfect the incubator interior, the shelves and shelf supports, and air ducts if applicable (see below for cleaning recommendations). After disinfection, install these and any other internal components, following the manufacturer's directions.

Note: If an automated heat decontamination/sterilization cycle is available, you can skip the manual disinfection step and simply run the automated program – assuming the program is proven to eliminate accepted biological indicator organisms. An automated high heat program also eliminates the need for autoclaving any internal parts. It's important to mention that while recent Thermo Scientific™ models with a high temperature cycle require no handling of parts to run the cycle, some earlier models and many from other manufacturers have sensors that must be removed first.

When the incubator is clean, disinfected, and the shelf parts are in place, you can install any additional optional parts such as an oxygen sensor or HEPA filter.

The 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].

Note: A HEPA filter which is not located inside the chamber will not experience the same conditions as your cells and cannot provide the same level of assurance. Handle HEPA filters and gas sensors very carefully, as they are easily damaged.

If you have a water jacketed incubator, fill the water jacket at this time. Also, fill the humidity pan with sterile distilled water, to ½ inch (1.25 cm) from the top, or fill only to the "max" indicator if available. Keeping the water pan full is important for good, consistent humidity and best growth conditions.

Now, using the electronics and following the manufacturer's directions, set the temperature, over-temperature, under-temperature, CO_2 and O_2 setpoints, and the appropriate alarms. If you have a Thermo Scientific incubator with the Thermo Scientific™ iCAN™ interface, you can also set the data logging parameters at this time. Depending on your settings, the iCAN will collect data to be stored onboard for up to a week, or exported to Microsoft™ Excel™. This information can often be very useful, not only for cGMP (current Good Manufacturing Practice) monitoring, but also in helping to pinpoint the start of any problems.

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. An IR (infrared) sensor is preferred for some applications including cGMP 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. Thus, these incubators will not respond quickly to changes that affect cell growth.

CO_2 Gas Hookups

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.

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, and be sure to use a hose clamp and to check for gas leaks, which could be dangerous to you and your lab mates. When the incubator has been operating normally for 24 hours, check the calibration of the temperature using a NIST (National Institute of Standards and Technology) or other certified thermometer, and check the CO_2 using a fyrite or external IR tester.

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 (revolutions per minute), 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 offers a roller bottle assembly, which does not produce heat. Each substitutes for a shelf, so you can add up to 4 of these units inside, giving you added culturing flexibility.

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[2], 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. So 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.

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. Some strong disinfectants may give off fumes that enter the incubator and then affect cell growth. These fumes contain VOCs (volatile organic compounds) that can induce expression of heat shock and other stress proteins. Common laboratory chemicals such as phenol, isoamyl alcohol and beta-mercaptoethanol 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. Recognizing that VOCs are an increasing concern for cell culturists, some Thermo Scientific incubators with HEPA filtration will accept specialized HEPA filters that also capture VOCs.

Thermo Fisher Scientific's Technology Team investigated and tested a number of different types of disinfectants. We looked for a disinfectant that fulfilled the following criteria: broadly effective against a range of microorganisms; and harmless (non-corrosive) to incubator components. Of all those tested, the compound that best fulfilled our requirements is a quaternary ammonium disinfectant. This basic type is available in all regions from different manufacturers. Some examples include Lysol No Rinse (formerly Roccall-D), Conflikt (North America) and Fermicidal-D (Europe). It is important to check the MSDS (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 cleaners. Chlorine bleach and its derivatives with oxidizing activity corrode stainless steel and copper. In addition, these chemicals are very toxic to your cells!

Procedure for Cleaning the Incubator

Regular cleaning of the incubator, while sometimes a chore, is a necessity to help protect your cells from contamination and to keep the incubator functioning properly. Carefully following these simple steps will keep your incubator clean, help reduce chances of contamination becoming established, and help keep your cells growing well. If you use an automated heat decontamination/sterilization cycle that is proven to eliminate bacteria and fungi, you should not need to use a disinfectant, but you should still periodically clean the incubator to eliminate spilled liquids and dirt.

1. **Move all the cultures to a different incubator.** Or if the cleaning will not take very long, you can store them in a clean, disinfected plastic box that will keep them warm. Then, turn off the incubator, including turning off the gas supply.

2. **Remove all the shelves, the shelf supports, and any brackets or air ducts.** Empty the water reservoir and wipe it dry with a clean, lint-free cloth.

3. **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. An incubator with coved corners makes this task easier.

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

5. **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. Again, be sure to reach all the corners, and remember to treat the door gasket as well. Replace the internal parts.

6. **Now you can turn the incubator heat back on and allow the incubator to dry completely.** This should only take a moment or two, so do not leave the door open – that would only reintroduce new dust and contaminants!

7. **If you have an automated decontamination/sterilization cycle, you can run it now.** After that cycle is complete, fill the water reservoir with sterile distilled water, and turn on the gas supply.

8. **Remember to clean the incubator exterior to eliminate dirt and microorganisms that could find their way inside.** If the top of your incubator is very dusty, it's likely that dust is passing into the incubator when you open the door, so it's important to clean the top as well. Use a lint-free cloth dampened in mild soapy water. Then 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. Do not use any liquids or spray cleaners to clean a touch sensitive surface or display. Instead, use a dry microfiber cloth to clean these.

That's it, you're done! When the incubator has reached the set conditions, return your cells inside.

We are often asked "how do I clean my 100% pure copper incubator?" 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.

Importance of Correct Water

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.

Thermo Scientific Barnstead Classic Still

DI (de-ionized) 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. RO (reverse osmosis) 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.

Our Technology Team has done extensive testing on water quality. Based on these tests and for long life of the incubator, we recommend sterile distilled water with a pH of 7-9 and a conductivity of 1-20 microSiemens/cm (resistivity of 50 K-1 M ohm-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 Mega ohms/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.

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

If you have a Heracell incubator, the AutoStart cycle will automatically zero the sensors. In addition, the Heracell incubator will automatically compensate for pressure changes that can affect the concentration of the gases inside the chamber.

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 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.

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 between 6 months to 1 year. The life of a filter will depend on the number of people using the incubator and 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 into your CO_2 incubator, 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.

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You should also replace the gas inlet filters (where the CO_2 gas enters the incubator) between 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 an incubator overnight. Most users use this function from one time per month to once every six months.

Probably the most important thing you can do to maintain your incubator is to maintain a high level of water in the water pan. If the pan runs out of water, it is very bad for your cell cultures because when the water pan dries out, the humidity drops and that leads to evaporation of water from your culture medium. Thus, without proper humidity, the carefully balanced salts, minerals, amino acids, etc. in your growth medium become too concentrated, which can lead to toxicity and cell death[4]. But beyond the danger to your cells, too low humidity can also damage the CO_2 sensor, as explained earlier.

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