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A Member of PHC Group

CELL CULTURE INCUBATORS CLEANING, DECONTAMINATION AND STERILIZATION METHODS

Basic care for optimum performance and care in the event of contamination

Models:

MCO-170AC-series
MCO-170AIC-series
MCO-171AICD-series
MCO-230AIC-series
MCO-170M-series
MCO-50AIC-series
MCO-50M-series

PHC Corporation of North America

Life Science Innovator Since 1966


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5.0
Buzzer
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Cell Culture Incubators
Cleaning and Decontamination Methods

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Cell Culture Incubators

Cleaning, Decontamination,
and Sterilization Methods

NB: Depending on the model, the appearance of parts in the manual may differ slightly.

Introduction to cell culture incubators

A cell culture incubator is designed to artificially replicate *in vitro* conditions essential to *in vivo* physiology typical of human and animal models. Cell growth outside of a natural environment presents a multitude of challenges associated with exposure to microorganisms that are not present in the *in vivo* state. Depending on the type of cell cultures being managed, several operating parameters must be carefully controlled with accuracy, repeatability and flexibility in setpoint choices. These include temperature and gas control.

- Cell culture incubators are designed to establish and maintain a controlled, stable environment by regulating temperature at a typical setpoint of 37°C or over a range from ambient to points above 37°C.
- Incubator gases typically include CO_2 and/or O_2.

CO_2 is controlled at a precise setpoint to maintain desired pH in the cell culture media, whether liquid or gel. The CO_2 concentration in the incubator functions to maintain this critical pH buffer.

Some biological materials may require different pH levels. Desired CO_2 setpoint concentrations may differ. Most media contain an indicator which helps detect the change in pH.

- Optimal cell culture environments must include humidification to prevent desiccation of cell culture media.

While some incubators have internal humidification systems with heated water reservoirs, most incubators include simplified, removable humidifying trays designed to hold sterile distilled water which evaporates to naturally increase the relative humidity within the chamber.

TYPES OF CELL CULTURE CONTAMINATION

Contamination of a cell culture in vitro is usually caused by the inadvertent introduction of one or more organisms that can damage or destroy the cell culture in progress.
These organisms include:

- Bacteria (including Thermophilic Bacteria) and Mycoplasma
- Molds and Yeasts
- Viruses

Other contaminants include dust, volatile organic compounds (VOC's) from adjacent instrumentation or processes, cross contaminants from other cultures in a shared incubator environment and particulates found in the natural environment. Regardless of the contaminant or its source, prudent laboratory techniques can help avoid the recurrence of contamination.

THE INCUBATOR BUBBLE

Unlike closed systems, such as hollow-fiber substrates, stirred tank or airlift bioreactors, the typical cell culture incubator is a conditioned chamber with a door that closes against a soft gasket. When the door is closed, the incubator creates an ideal environment for the cell culture process based on user defined setpoint parameters for temperature, CO_2 and O_2. Humidification is naturally evaporative from the humidifying tray, and an elevated relative humidity is sufficient to eliminate desiccation, especially in microplates with small media volumes. Some larger cell culture incubators use immersion heaters to supplement the natural humidification process.

When the incubator door is opened, however, the conditioned bubble is lost. Accessing cell culture labware for transport to a biological safety cabinet (BSC) or other processes is a normal part of laboratory workflow. Opening the door exposes the incubator interior walls, shelves, humidifying water tray and culture vessels to ambient conditions that carry the potential for contamination from molds, yeasts, fungi or other microorganisms such as mycoplasma and viruses. In a practical sense, unless the incubator is installed in a clean room, this exposure cannot be avoided. Proper technique can reduce the potential. The first consideration is to understand basic incubator systems and how they can harbor contamination.

INCUBATOR DESIGN PREREQUISITES

The first step in managing cell culture contamination is to consider the incubator design, specifically, the interior. All interior components exposed to the high humidity atmosphere should be constructed on high quality stainless steel and should be easily removable (preferably without tools) for manual cleaning or autoclaving. These include: shelves, shelf brackets, plenums, floors, humidifying trays, blower wheels, sensor housings, inner door gaskets and anything present in the chamber during cell culture. Control probes are often protected by stainless steel sheath housings. These must be cleaned according to the manufacturers' instructions.

Components manufactured from copper enriched or copper supplemented stainless steel contain an inherent germicidal property that resists airborne organisms introduced to the chamber during door openings. Such materials are considered "passive" contamination control insofar as the inability of organisms to sustain growth on these surfaces.

Important points for installation

There are many factors to consider when determining the permanent location of the cell culture incubator. It is desirable to locate the unit where there is minimal foot traffic and where air disturbance is of little consequence. This reduces the volatility of outside air entering the incubator during a door opening. Avoid installing the incubator near windows, air conditioners, ceiling or floor HVAC air diffusers and return air intakes, all of which are sources of airborne contamination.

INSTALLATION, LOCATION, AND CLEARANCES

It is important to consider the function of the biological safety cabinet when planning for incubator contamination mitigation.
If feasible, locate the incubator as close to the biological safety cabinet (BSC) as possible. This limits the exposure when removing or replacing cell cultures for processing.

Improper use of the BSC, wrong sash window height, blockage of downflow slots and use of instrumentation or equipment on the BSC work surface can create pathways for contaminants to attach to the cell culture labware when working in the hood. These contaminants are then returned to the incubator where they can migrate to other cultures via cross contamination or to interior surfaces exposed to a conditioned atmosphere ideal for cell growth. While BSCs are usually equipped with HEPA filters designed to trap particulates of 0.3 microns (0.12 microns for ULPA filters), smaller viruses can easily pass through these barriers. Although the cell culture lab may normally be under positive pressure, this can change to neutral or even negative pressure when a BSC is operating, especially when the BSC has an exhaust transition connected to or over the exhaust filter.

Other laboratory equipment such as centrifuges, stirrers, shakers and robotic plate readers can aggravate an otherwise calm air environment to create aerosols that are easily airborne.

It is important to establish clearances adjacent to and behind the incubator because this space is required to provide easy access to gas supply tubing, tubing filters, gas input ports, pass-thru ports and blanking plugs and any interior components such as blower motors, fans or sensors that must be removed for maintenance.

Most CO_2 cylinders, for example, contain an industrial grade CO_2 supply in liquid form wherein the CO_2 gas evaporates and moves through the two-stage pressure regulator as a gas. It exits the regulator at a pressure of approximately 4.4 PSIG - 14.5 PSIG (CO_2) / 7.3 PSIG - 14.5 PSIG (N_2/O_2), sufficient to prevent the introduction of contaminants into the gas system. The CO_2 itself, however, often contains microscopic particles that may provide surfaces for contaminants.

Locations where there is a lot of foot traffic are not suitable for the unit.

- Locate the incubator in a clean room or location where few people enter.
- Choose a clean room that is safe or a place where there are as few people as possible.

Set up the unit as high off the floor as possible

- Since there are fewer airborne bacteria in the upper part of a room, the incubator should be placed on a laboratory table or a special stand.
- If stacking two or three units on top of each other, use a special roller base for that purpose.

Install the unit away from products that cause vibration and away from heat sources

- Do not install the incubator near products that cause vibration. Vibration may adversely affect cultures.
- Do not install the incubator near significant heat sources, such as heaters, boilers, ovens, or autoclaves. Heat will adversely affect the performance of the incubator

Place in a location that is not directly affected by outside air

- Avoid putting the unit in a location that will be directly affected by air from a window, door, or air conditioning/ heating vent.

Cleaning and decontamination methods

Most incubator manufacturers recommend a solution of ethanol and manual cleaning prior to initial start-up and regularly thereafter. The ethanol solution is intentionally diluted to give it time to kill the contaminant before the ethanol evaporates.

Why is an ethanol solution better than 100% ethanol in bacterial inhibition? 100% ethanol coagulates and dehydrates proteins so quickly that a layer of relatively impermeable denatured protein forms in the exterior parts of the bacterial cell (in and under the cell wall), and this prevents further diffusion of the alcohol into the cell. This protects the core of the cell from denaturation.

With an ethanol solution, the process is slower and the alcohol manages to diffuse throughout the cell denature proteins. In addition to conventional manual wipe down using ethanol, the incubator may be equipped with a sterilization cycle such as a high heat (180°C) system or a hydrogen peroxide vapor H_2O_2 system. The cycle should be performed prior to first use.

If commissioning and Current Good Manufacturing Practices (cGMP) criteria are in place, all contamination control efforts must be in compliance with previously approved best practices and facility protocol.

INSTRUMENTATION AND EQUIPMENT INTERFACE

Shakers, cell bottle rollers, magnetic stirrers and other devices are commonly used in the cell culture incubators. These must be free of contaminants before they are placed in the incubator.

Cell culture vessels usually include flasks with and without vent caps, petri dishes, roller bottles and multi-well plates. These are usually prepackaged and sterilized by gamma radiation prior to shipment. They should be opened only in a biosafety cabinet to preserve the integrity of the sterilization.

Other labware returned from a central sterilization room must be considered a source of contamination if exposed to ambient air during cart transit and shelf storage.

SUMMARY CONTAMINATION SOURCES

The following contamination points must be included in a regular schedule for cleaning in situ or removal and cleaning manually or by autoclaving.

INSIDE THE INCUBATOR
- Walls
- Ceiling
- Floor, shelves
- Chamber corners
- Ductwork, duct cover and plenums
- Humidifying tray
- UV light housing, if so equipped
- Temperature control probe and probe housing
- Probe wire to control panel
- Circulation fan and fan shaft
- Fan cover
- Humidifying tray cover
- Access port silicon cap
- Water-proof cap of the H_2O_2 generator connector

INCUBATOR CABINET
- Inner door gasket and feather surfaces
- Inner door latch
- Inner door glass
- Inner door hinges and fasteners
- Cool spots where condensation may accumulatedue to insufficient cabinet insulation

GAS SYSTEM
- CO_2 or O_2 sensor
- Sensor housing and connectors
- Injection tubing from control solenoid(s)
- Air pump
- Filters and housings
- Fan, shaft and seal

Basic care of cell culture incubators

Always puts on gloves before cleaning the unit.
As a basic rule, do not clean the incubator with bare hands.
Be sure to use rubber gloves.

Necessary materials
- Rubber gloves
- 70% Ethanol
- Gauze, non-woven cloth, or sterile paper

STEP 1

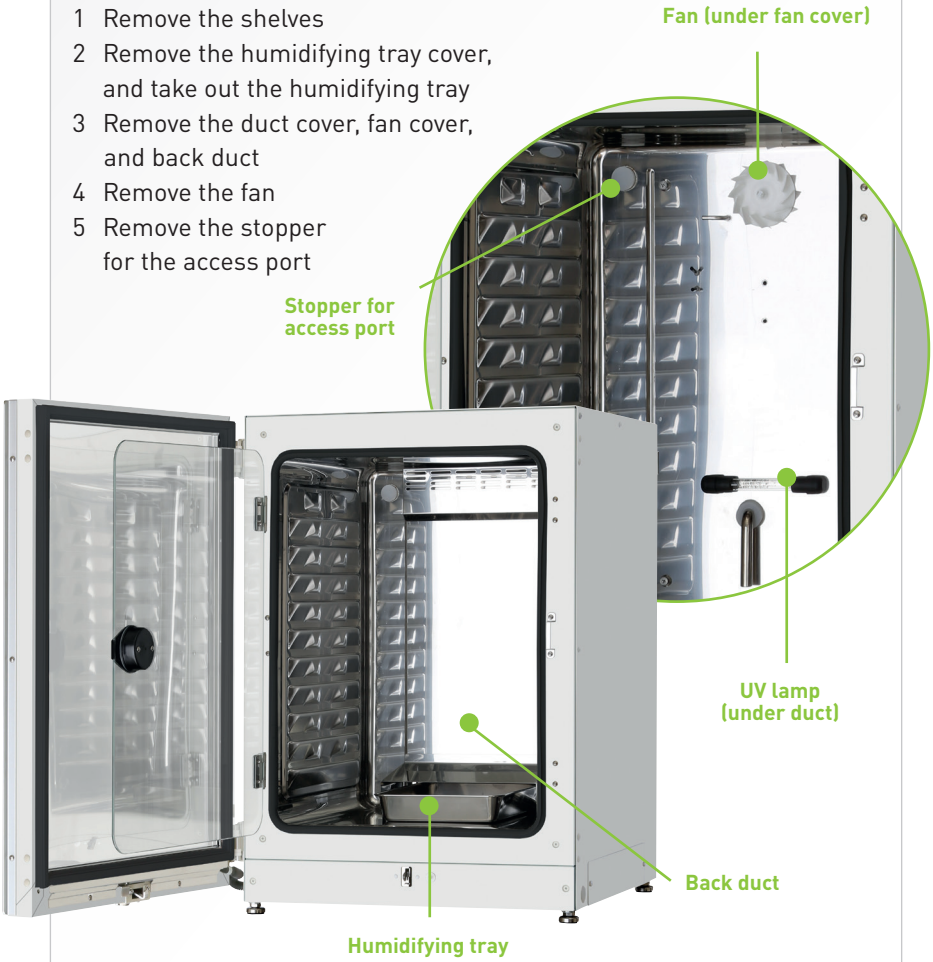
Turn off the power

STEP 2

Remove the interior components.

Remove the components in the correct order.

1 Remove the shelves
2 Remove the humidifying tray cover, and take out the humidifying tray
3 Remove the duct cover, fan cover, and back duct
4 Remove the fan
5 Remove the stopper for the access port

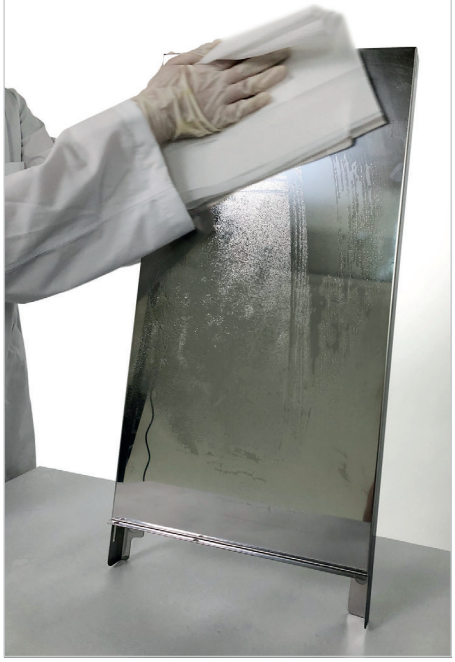


STEP 3

Clean the interior components

Use the correct cleaning procedure.

1 Wash with a neutral detergent (soap)
2 Rinse well with distilled water
3 Wipe with gauze, non-woven cloth, or sterile paper



Basic care of cell culture incubators

STEP 4

Spray disinfecting alcohol inside the unit and wipe (ethanol).

Do not spray ethanol into the CO_2 density measurement port directly! Just wipe off with gauze, non-woven cloth, or sterile paper which is sprayed with ethanol.

Picture 1

Picture 2

STEP 5

Disinfect all interior surfaces, internal components, shelves and water tray with ethanol.

STEP 6

Please make sure to spread the ethanol for disinfection to all corners of the inner door gasket and sufficiently remove stains while wiping it clean (picture 1)**.**

If it is operated with the inner door gasket not in place, the humidified air will leak and will cause condensation between the unit and outer door. After wiping, confirm that the inner door gasket is securely in place and doesn't have any creases.

Adjust shape of inner door gasket after wiping (picture 2)**.**
Adjust the shape of inner door gasket by sliding fingers from each corner in direction of arrows. Specifically, insert fingers behind fin of inner door gasket and slide.

The inner door gasket has an important role to maintain the chamber humidity. If it is operated with the inner door gasket not in place, the humidified air will leak and will cause condensation between the unit and outer door. After wiping, confirm that the inner door gasket is securely in place and doesn't have any creases. If the inner door gasket is not in place, please refer to the back side and adjust the shape of the inner door gasket.

STEP 7

Replace the interior components.

Replace the components in the reverse order of [STEP 2], and put sterilized distilled water in the humidifying tray.

1 Replace the stopper for the access port
2 Replace the fan, check to see if the fan spins smoothly by turning it with your hand.
3 Replace the back duct, duct cover and fan cover
4 Replace the humidifying tray and put back the humidifying tray cover.
5 Replace the shelves

STEP 8

Let it dual with the door ajar.

Before turning the power back on (restarting) let the inside dry out, check that there is no alcohol smell remaining.
If you turn the power on while it is still damp inside, the 0_2 and $C0_2$ sensors may be damaged.

STEP 9

If using a unit that is equipped with either a dual heat sterilization function or H_2O_2 (hydrogen peroxide) decontamination function, performing sterilization/decontamination before use will make prevention of contamination (bacterial contamination) more effective.

Cell-IQ™ CO_2 Incubator with heat sterilization:
MCO-171AICUVD-PA series

Cell-IQ™ CO_2 / Multi-Gas Incubator with H_2O_2 decontamination:
MCO-170AICUVHL-PA, MCO-230AICUVHL-PA
MCO-50AICL-PA*

* These models come with optional accessories for H_2O_2 decontamination

Basic care of cell culture incubators

CLEANING PRECAUTION

Get into the habit of wiping carefully

- Be sure to wear gloves to avoid cutting your hands on the interior component
- Do not use acid, alkali, or chlorine-based cleansers, disinfectants, or sanitizers

IMPORTANT POINT

Do not wipe with the same portion of the sterile non-woven cloth more than once.

If you wipe another area with same portion of the cloth, you will be spreading bacteria around.
Don't forget to wipe the gasket and the inside of the door.

CLEANING THE HUMIDIFYING WATER

When replacing the water, also clean the humidifying tray. Do this at least once every week.

- Lift the humidifying tray cover and pull out the humidifying tray.
- Wash the humidifying tray with neutral detergent, rinse it thoroughly with distilled water and wipe it off with gauze, non-woven cloth, or sterile paper.
- Spray ethanol to the humidifying tray and wipe off thoroughly.
- Wipe any moisture on the bottom of the chamber.
- Place the humidifying tray under the humidifying tray cover and pour sterile distilled water (preferably pre-heated to 37°C) into it.

IMPORTANT POINT

Do not use ultrapure water, tap water, deinoized water or reverse osmosis water as these are not suitable for incubators.

Please avoid adding any chemicals to the humidifying tray.

Tips for the incubator which is prone to be contaminated easily

TIPS TO MINIMIZE CONTAMINATON

- Increase the frequency of cleaning and humidifying water replacement.
- Spray Biocidal ZF to the inside of chamber once every week.

Replacement of humidifying water
Recommended frequency: Once every week (depending on frequency/ environment of use)

1 Lift the humidifying tray cover and pull out the humidifying tray.
2 Wash the humidifying tray with neutral detergent, rinse it thoroughly with distilled water and wipe it off with gauze, non-woven cloth, or sterile paper.
3 Wipe any moisture on the bottom of the chamber.
4 Spray ethanol to the humidifying tray and wipe off thoroughly.
5 Place the humidifying tray under the humidifying tray cover and pour sterile distilled water (preferably pre-heated to 37°C) into it.

IMPORTANT POINT

Do not replenish the humidifying water.
The humidifying tray area is a major air way which is prone to collect dust and/or dirt that UV sterilization can't remove.

The use of biodegradable Biocidal ZF spray-dis-infectant in incubators will help protect cultures against bacteria, fungi and enveloped viruses.

Non-volatile:
The active microbiocidal ingredients of Biocidal ZF are non-volatile. These protect cell cultures from microbial contamination and do not invade cell cultures via air. Thus cell cultures are protected against contamination and the disinfectant itself.

TIPS TO MINIMIZE THE RISK OF CONTAMINATION

- Locate the incubator in a clean room or a site where there are few people.
- Install the incubator some distance above floor level (The higher you go, the less floating bacteria are found). Use a roller base to facilitate cleaning around and under the incubators.
- Install the incubator in an area away from draughts and easy air intrusion when opening and closing the incubator doors. Beware of air dust and the air flow direction of any air conditioning.
- Ensure that there is no condensation inside chamber.
- Always keep the interior of an incubator clean and free from culture medium and/or water and fingerprints. They must be wiped off immediately if ever spilled or smeared (When there is a film or foreign matter foamed or placed on the surface of the copper alloy, sterilization effect will be lost).
- Always maintain and handle culture vessels under the maximum aseptic conditions possible. It is recommended to wipe off the bottom and periphery of the culture vessels with ethanol for sterilization when taking them into or out of an incubator.
- Minimize the door opening and closing frequency.
- Use sterile distilled water for the humidifying pan. Do not use ultrapure water, which may cause red rust-like particles in the humidifying tray. Change the water in the humidifying tray once a week.
- Take care when putting items that emit heat into the chamber. Putting items such as shakers which emit heat into the chamber may cause condensation and lead to possible contamination.

Care in the event of contamination

Get into the habit of wiping carefully

- Be sure to wear gloves to avoid cutting your hands on the interior component
- Do not use acid, alkali, or chlorine-based cleansers, disinfectants, or sanitizers

Necessary materials

- Rubber gloves
- Ethanol
- Gauze, non-woven cloth, or sterile paper

24 HOUR UV DECONTAMINATION

STEP 1

Turn off the power

STEP 2

Remove the interior components.

Remove the components in the correct order.

1 Remove the shelves
2 Remove the humidifying tray cover, and take out the humidifying tray
3 Remove the back duct
4 Remove the fan
5 Remove the stopper for the access port

STEP 3

Clean the interior components

Use the correct cleaning procedure.

1 Wash with a neutral detergent (soap)
2 Rinse well with distilled water
3 Wipe with a gauze, non-woven cloth, or sterile paper

NOTE: AVOID TOUCHING THE UV LAMP GLASS WITH YOUR BARE HANDS. Oils on your hands can cause hot spots on the UV lamp and shorten its life. Use a soft clean cotton cloth or gloves to handle the UV lamp.

STEP 4

Spray ethanol inside the unit and wipe

STEP 5

Disinfect all interior surfaces, internal components, shelves and water tray with ethanol

SEE PAGE 9/10 FOR MORE DETAILS ABOUT STEP 1 TO 5

STEP 6

Turn on the power and (activate the UV lamp for 24 hours)*

Once the interior components and UV lamp cover have been removed, carry out UV sterilization for 24 hours. It is not necessary to wipe the interior with ethanol afterwards.

STEP 7

Replace the interior components.

Replace the components in the reverse order of [STEP 2], and put sterilized distilled water in the humidifying tray.
Before replacing all the components, check to see if the fan spins smoothly by turning it with your hand.

* Models equipped with the 24 hour UV decontamination system:

STEP 8

Let it dry with the door ajar.

Before turning the power back on (restarting) let the inside dry out, check that there is no alcohol smell remaining.
If you turn the power on while it is still damp inside, the 0_2 and $C0_2$ sensors may be damaged.

PHCbi Cell-IQ™ CO_2 Incubators with Safecell UV™ Lamp:
MCO-170AICUVL-PA, MCO-170AICUVHL-PA,
MCO-230AICUVLG-PA, MCO-230AICUVHL-PA

PHCbi Multigas Incubator with Safecell UV™ Lamp:
MCO-50M-PA*, MCO-170MP-PA*

* Each unit has optional accessories required for the SafeCell UV™ Lamp

NB: Model MCO-171AICUVD does not include the 24 hour UV decontamination system.

Care in the event of contamination

ACTIVATION UV LAMP 24H FOR MCO-170AIC, MCO-230AIC AND MCO-170M SERIES (OPT.)

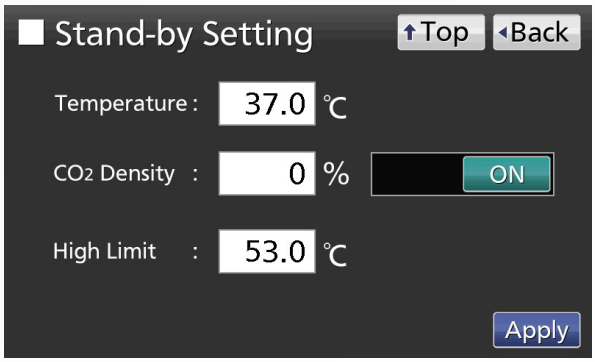
Lighting the UV lamp for 24 hours

If the chamber has been contaminated by dirt or by spilling the medium, use the following procedure to decontaminate it by lighting the UV lamp for 24 hours.

1. Remove all attachments from the chamber, including the trays, the fan cover, the duct, the fan, the humidifying tray, and the humidifying tray cover. Disinfect all the attachments in an autoclave or with ethanol.

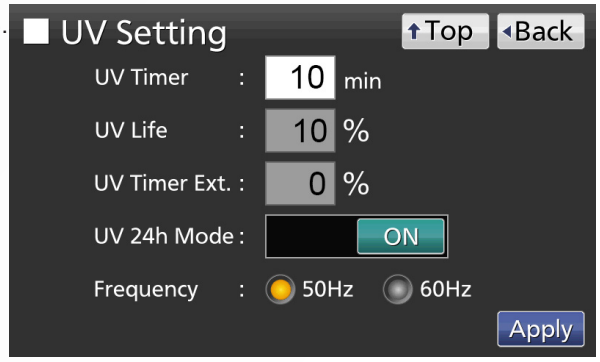
2. Clean and wipe down the inside of the chamber with ethanol.

3. Set the CO_2 density to 0 %.
   Go to the Stand-by-Setting screen (Menu > Set). enter 0 % at CO_2 density . Press "Apply" to save the entered values.



4. Go to the Tools #1 screen (Menu > Tools#1).
   Press "UV Setting" to display the UV Setting screen.

5. Turn the UV 24h Mode to ON and press "Apply".

6. 

   The UV lamp will now light continuously for 24 hours. "UV 24h Mode ON" is displayed on the UV lamp condition display.

Notes:
- The UV 24-hour mode may activate the automatic set temperature alarm because of rising chamber temperature.
- If the outer door is opened when the UV lamp is lit, the UV lamp is turned OFF and UV 24-hour mode is cancelled. Repeat procedures **4** to **6** to restart the UV 24-hour mode.

7. After 24 hours, the UV lamp turns OFF automatically. Reinstall all the attachments removed in procedure 1.

ACTIVATION UV LAMP 24H FOR MCO-170AC, MCO-50AIC, AND MCO-50M SERIES (OPTIONAL)

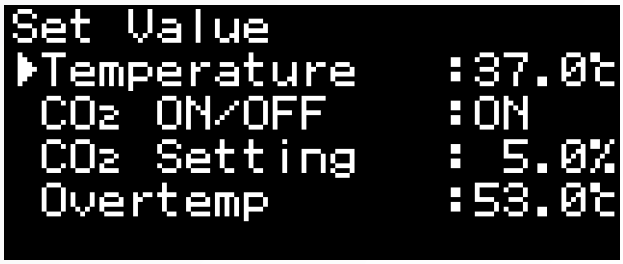
Lighting the UV lamp for 24 hours

If the chamber has been contaminated by dirt or by spilling the medium, use the following procedure to decontaminate it by lighting the UV lamp for 24 hours.

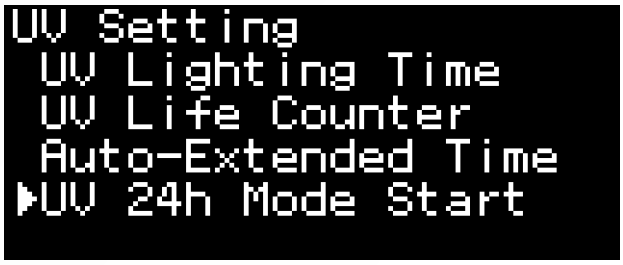
1. Remove all internal items from the chamber (racks, fan cover, duct, fan, humidifying tray and humidifying tray cover).
   Disinfect all the attachments in an autoclave or with ethanol.

2. Clean and wipe down the inside of the chamber with ethanol.

3. Set the CO_2 density to 0 %.

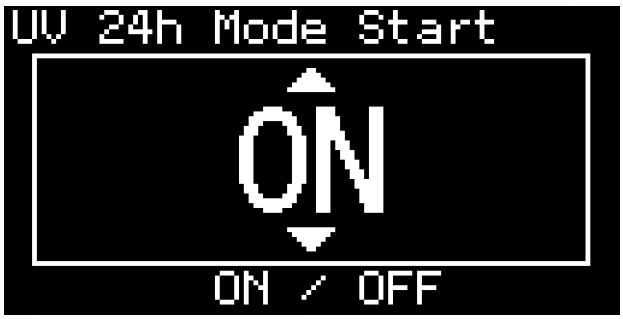


4. On the home screen press MENU. The left side of the display will change to Menu screen.
- Move the cursor to Alarms & Controls by using the up/down keys and press ENTER.
- Move the cursor to UV Setting by using the up/ down keys and press ENTER.



5. Move the cursor to UV 24h Mode Start by using the up/down keys and press ENTER.
   The right side of the display will change to the UV 24h Mode Start setting screen, the current setting (OFF) will be displayed.

6. Use the up/down keys to change the UV 24h lighting mode setting to ON and press ENTER.



7. Press the MENU key to display the home screen. The UV lamp will now light continuously for 24 hours.

Notes:
• The UV 24-hour mode may activate the automatic set temperature alarm because of rising chamber temperature.
• If the outer door is opened when the UV lamp is lit, the UV lamp is turned OFF and UV 24-hour mode is cancelled. Redo procedures from **4** to restart the UV 24-hour mode.

8. After 24 hours, the lamp UV turns OFF automatically. Reinstall all the attachments removed in procedure 1.

Care in the event of contamination

H_2O_2 DECONTAMINATION MCO-170AIC, MCO-230AIC, AND MCO-170M SERIES

No need to remove UV lamp and inner parts
It doesn't use a heater, so it conserves energy

STEP 1

15 MIN

Preparation Time: 10 - 15 minutes

1 Remove all interior components
2 Wipe down the inside of the incubator
3 Reposition interior components to specified locations for in situ decontamination
4 Set up the H_2O_2 generator (MCO-HP)*
*Optional Accessory. H_2O_2 reagent is required for this process.

Placement H_2O_2 generator

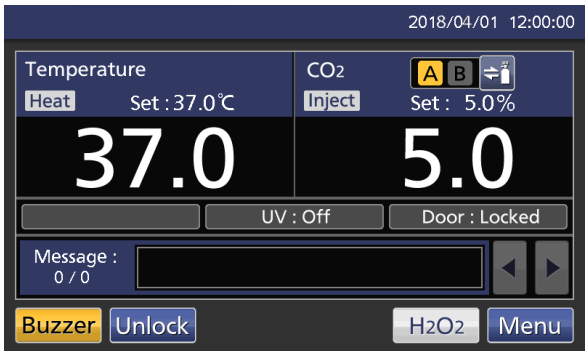
STEP 2

135 MIN

Decontamination Time: ~135 minutes

Decontamination of the chamber can be completed by pushing just 2 buttons on the control panel

1 Press "H_2O_2" for 3 seconds to open the Setting Position window. Enter the password if prompted. Press "Next" to display the H_2O_2 Decontamination Step1 screen.
2 If the system is normal, the H_2O_2 Decontamination Step2 screen appears. Press "OK" to start H_2O_2 decontamination.
3 The chamber will warm up to 45°C for optimum results
4 H_2O_2 vapour generation starts
5 Interior fan circulates vapour
6 UV lamp reduces H_2O_2 to water and oxygen



STEP 3

10 MIN

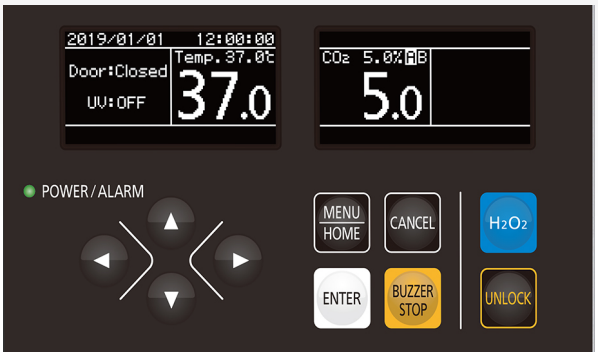
Finish Time: ~10 minutes

1. Open the outer and inner doors. Disconnect the connector, cable, and H_2O_2 generator, and remove the H_2O_2 generator and cable from the chamber. **Note: Put on protective glasses and rubber gloves to do this.**
2. Wipe off remaining liquid with sterile non-woven cloth
3. Reposition interior components to normal positions

H_2O_2 DECONTAMINATION MCO-50AIC AND MCO-50M SERIES (OPTIONAL)

FOLLOW STEPS 1 TO 3 FROM PAGE 18

(SCREEN APPEARANCE MAY SLIGHTLY VARY DEPENDING ON THE MODEL.PLEASE REFER TO THE USER MANUAL)



H_2O_2 generator MCO-50 series (MCO-50HP)

HOW DOES IT WORK?

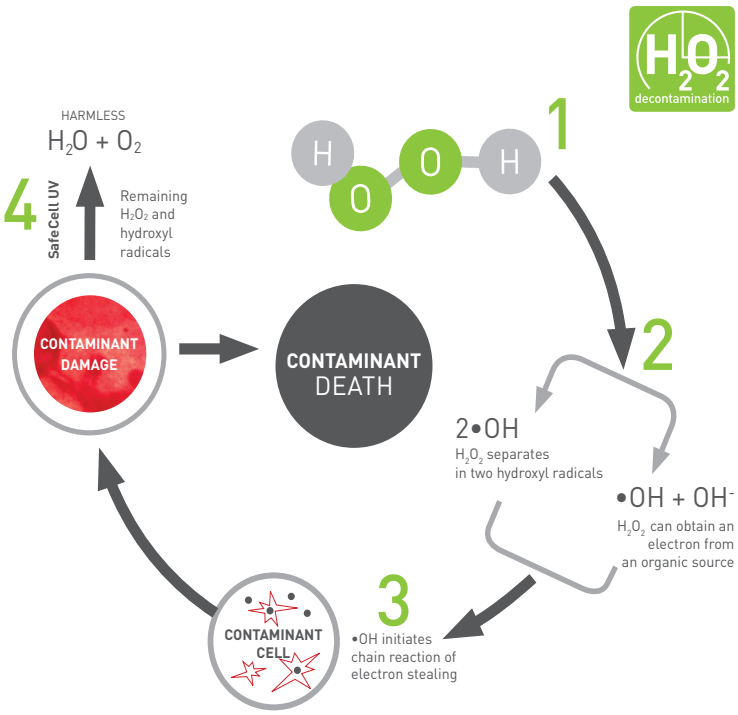
1 Hydrogen peroxide (aqueous) is converted to vapour using high frequency ultrasonics. During this process, the fan motor remains active, ensuring H_2O_2 vapour accesses every point of the chamber and the tubing to and from, and the inside of the CO_2 sensor.

2 The H_2O_2 vapour breaks down into hydroxyl radicals naturally.

3 The hydroxl radicals initiate a chain reaction of electron stealing.

4 This unstable internal environment leads to death of contaminants. Remaining hydroxyl radicals and H_2O_2 are resolved to H_2O (aqueous) & O_2 (gas).

PHCbi's H_2O_2 decontamination achieves at least a 6 log reduction of major contaminants. The full decontamination process takes less than three hours.



DNA is very susceptible to oxidative damage. Since most bacteria have a single chromosome controlling all their life functions, this kind of effect can be detrimental to their normal function. Prokaryotic organisms often lack repair mechanisms to limit such damage, making them more prone to change.

Care in the event of contamination

HEAT sterilization

HEAT STERILIZATION Convenient approc. 11 hrs. / 180°C heat sterilization

STEP 1 Preparation time: 10 - 15 minutes

15 MIN

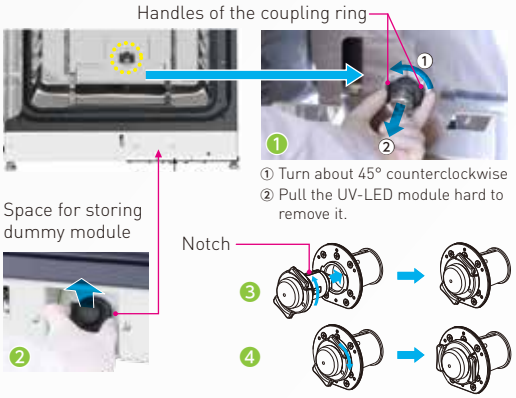
1 Take out the trays, humidifying tray, humidifying tray cover, fan cover, duct, duct cover, and fan from the chamber.
2 Dispose of the water in the humidifying pan, and wipe the inner attachments removed from the chamber with a gauze containing ethanol.
3 Wipe the inner walls of the chamber with a gauze containing ethanol.
4 Attach the dummy module (for the models with UV-LED).

Attaching the dummy module

When performing dry heat sterilisation, the UV-LED module in the chamber needs to be exchanged with the dummy module to protect it from the heat.

❶ Remove the UV-LED module in the chamber by holding the handles of the coupling ring, turning it counterclockwise 45°, and then pulling it hard.
❷ Remove the dummy module stored in the space for storing dummy module at the bottom front of the incubator by pulling it out. Then, insert the UV-LED module removed in step 1) into the space and store it.
❸ Turn the coupling ring of the dummy module counterclockwise 45° so that the notch of it faces upward. Then, insert the dummy module into the place where the UV-LED module was placed so that the notch on the dummy module fits the projected part in the sleeve.
❹ Turn the coupling ring of the dummy module clockwise 45° by pushing it to fix the dummy module. Then, check that the dummy module is securely fixed by pulling the coupling ring.

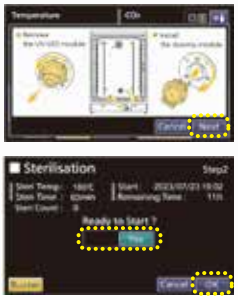
5 Attach the fan, duct, duct cover, and fan cover.
6 Set the trays on the 3rd, 5th, 6th, and 7th tray runners from the top.



STEP 2 Sterilisation time: approx. 11 hours

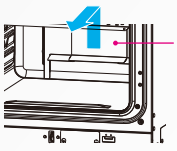
11 HRS

1 On the Top screen, long tap the Sterilisation button.
2 Check that the dummy module is correctly inserted and fixed in the position shown in the screen and tap the Next button.
3 Check that the inner attachments are properly set by looking at the description on the screen. Then, tap the Next button.
4 In Sterlisation STEP 1, a system check is performed automatically.
5 In Sterlisation STEP 2, move slide button to the right, select "Yes" and tap the OK button. STEP 3 to STEP 5 are automatically performed.
6 In STEP 3 the chamber is warmed up to 180°C.
7 STEP 4 The chamber is kept at 180°C or higher for 60 minutes to sterilise the chamber.
8 STEP 5: The chamber is cooled down to 45°C.
9 STEP 6: Tap the OK button.



STEP 3 Finish time: Approx. 10 minutes

10 MIN



1 Outer door is unlocked upon completion.
2 Open the outer and inner door
3 Take out the duct cover from the chamber by lifting it to disengage the protruded parts from the duct.
4 Then, put the UV-LED module (for the models with UV-LED) and dummy module in the original place. Attach the dummy module." after wiping the UV-LED module with a gauze containing ethanol.
5 Return the duct cover
6 Reposition interior components to normal positions

Both chambers can be in use at the same time even when double stacked.

One unit in heat sterilisation mode the other in incubation mode.

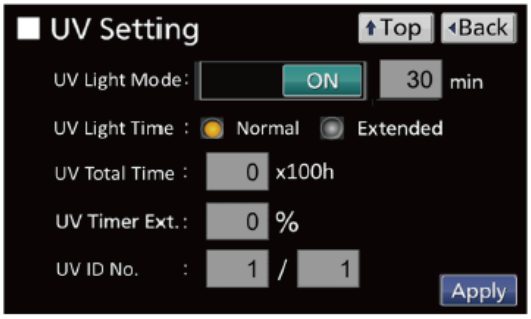
To prevent burning during the heat sterilization cycle, the outer door is electronically locked. The top surface temperature of the MCO-171AICUVD during heat sterilisation is approximately 60°C. 60°C is within the tolerance described in the International Safety Standard IEC6101010.1 Surface temperature limits or the burn prevention. Safety limit for outer metal is 65°C.

DISINFECTION BY UV-LED : MCO-171AICUVD

The water in the humidifying pan can be disinfected by UV-LED light. You can set if the UV-LED is turned on or not each time the outer door is closed. When set to ON, in order to reduce the heat impact on the chamber by the UV light, the UV-LED is automatically turned on or off during the automatically calculated time.

In this setting, you can select if the UV-LED is turned on or off after the outer door is closed, and select the period for which the UV-LED is ON (Normal or Extended).
The UV light ON period is automatically calculated based on the set chamber temperature set point, selected "UV Light Time" (Normal or Extended), and the value automatically calculated and displayed at "UV Timer Ext.".

1. **On the Top screen, tap the Menu button.**
   ▶ The Menu screen is displayed.
2. **Tap the Tools #1 button on the Menu screen.**
   ▶ The Tools #1 screen is displayed.
3. **On the Tools #1 screen, tap the UV Setting button.**
   ▶ The UV Setting screen is displayed
4. **Slide the ON/OFF buttons for "UV Light Mode,"** and select Normal or Extended for "UV Light Time" if you turned ON the UV Light Mode.



Select the duration of the UV-LED light ON period:

Normal: For usual use.

Extended: When longer disinfection time is needed. Based on this selection, actual ON period is calculated and displayed in the box at the UV Light Mode.

5. **Tap the Apply button.**
   ▶ The values are saved and the screen returns to the Tools #1 screen.

Cleaning the exterior, interior, and inner attachments

Clean the exterior and interior of the incubator and inner attachments once a month. Regular cleaning keeps the incubator in good condition. When the chamber is contaminated or when cleaning the chamber prior to starting a culture, you can perform dry heat sterilisation.

1. **Take the humidifying pan out of the incubator, dispose of the water in it, and turn off the power to the incubator.**
2. **Take the inner attachments out of the chamber.**
3. **Clean the exterior, inner attachments, and chamber.**
   If the outside panels are dirty, clean them by wiping them with a gauze moistened with a diluted neutral detergent (using an undiluted solution of detergent may cause the unit's plastic areas to crack. Follow the directions on the detergent for details of dilution). After that, be absolutely sure to wipe the surfaces using a cloth moistened with clean water to remove traces of the detergent and wipe the surfaces with a dry cloth. After that, wipe the surfaces with a cloth moistened with ethanol.
4. **Put back the inner attachments to the original positions.**
5. **Open the doors for a while to dry the chamber.**
6. **Wipe the control panel with a dry cloth.**
   If the stain will not come out, wipe it using a cloth moistened with a proper amount of water (the amount that cannot form droplets) and then, wipe thoroughly with a dry cloth. If the water enters the control panel, it may cause the panel to malfunction. (Do not apply spray directly, it may cause damage.)

Care in the event of rust

Always put on gloves before cleaning the unit.
As a basic rule, do not clean the incubator with bare hands.
Be sure to use gloves.
Be careful as hands may get cut on the interior components.

Necessary materials
- Rubber gloves
- 70% Ethanol
- Gauze, non-woven cloth, or sterile paper

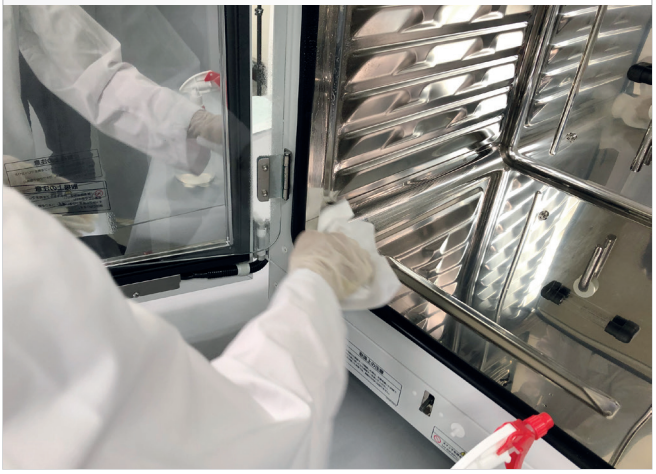
FIRST FOLLOW STEPS 1 TO 5 FROM PAGE 9/10

STEP 6

Remove the rust with a cream cleanser.
Use a suitable amount of fine-grained cream cleanser, and carefully remove the rust.

STEP 7

Wipe with ethanol.



STEP 8

Activate the UV lamp for 24 hours.
*If it is a UV-lamp models

Once the interior component and UV lamp cover have been removed, carry out UV sterilization for 24 hours. It is not necessary to wipe the interior with ethanol afterwards.

STEP 9

Replace the interior components.

Replace the components in the reverse order of [STEP 2], and put sterile distilled water in the humidifying tray.
Before replacing all the components, check to see if the fan spins smoothly by turning it with your hand.

KEEP IN MIND

These are the conditions that promote rust
- Insufficient wiping after the use of acid, alkali, or chlorine-based cleansers, disinfectants, or sanitizers
- Scratches on the surface of the unit interior or interior components
- The incubator is used with foreign matter left stuck to the inside of the unit or interior components
- Sodium dodecyl sulfate (SDS) is added to the humidifying water
- Using ultrapure water, deinoized water or reverse osmosis water in the humidifying tray
- Adding chemicals to the humidifying water

- **If an autoclave is used**
  If articles are stored wet in an enclosed place after coming out of an autoclave, rust can easily occur.

- **If a dual heat sterilizer is used**
  After coming out of a heat sterilizer, once oxide scale appears on the surface that turns yellow or black, rust can easily occur.

Best practices and good laboratory techniques

The most obvious approach to contamination-free incubator operation is to keep the incubator clean. A combination of manual cleaning and automatic (if equipped) decontamination processes managed on a regular schedule help protect cultures in situ and minimize loss of work due to contamination and downtime. Predictive maintenance is analogous to preventive maintenance, whereby cleaning processes can be documented for standardization and compliance, scheduled in advance, and assigned to laboratory staff as required. There is no substitute for aseptic technique when handling cell cultures. Both personal and laboratory hygiene are essential to a holistic contamination management program.

ACTIVE VS. PASSIVE DECONTAMINATION

Active decontamination, whether by manual wipe down, high heat sterilization, H_2O_2 vapor or other method, must be initiated by the user. Design attributes inherent to a properly engineered cell culture incubator offer an additional layer of protection by working in the background to inhibit and destroy contaminants as they occur.

Active Decontamination

High Heat. A high heat process utilizes time and temperature, typically 160°C to 170°C for a two-hour period, for a proven method of decontamination. The PHCbi brand, new thermal decontamination system operates at a higher temperature. It is the fastest and most effective active method of decontamination in a cell culture incubator reaching 180°C for a two-hour dwell before returning to ambient temperature. To minimize downtime, total cycle time is less than 12 hours. This energy-efficient process does not require the removal of the CO_2 sensor and UV light in the PHCbi brand incubator.

Hydrogen Peroxide (H_2O_2) Vapor. PHCbi brand incubators permit the use of active hydrogen peroxide (H_2O_2) vapor decontamination with complete safety and zero impact on the surrounding environment. Hydrogen peroxide starts in aqueous form and is converted to vapor using a nebulizer; this exposes all interior surfaces to the H_2O_2 vapor which ultimately resolves to water and oxygen at less than 1 ppm when catalyzed by a UV lamp.

Passive Decontamination

Copper Enriched Stainless Steel (marketed as inCu-saFe® under the PHCbi brand) is a stainless steel and copper composite alloy that forms a germicidal barrier to prevent growth of organisms on surfaces. All interior surfaces, shelves and brackets are comprised of the inCu-saFe composite. This material is a hybrid of Type 304 stainless steel. It is 100% corrosion-proof and will not corrode or discolor like conventional C100 copper surfaces.

Ultraviolet Light (marketed as SafeCell UV™ under the PHCbi brand) consists of a concealed UV lamp that creates a serial exposure of 257.3 nm wavelength to destroy DNA of any organism passing through the airflow system as well as surface water contaminants in the removable humidity tray. The UV lamp initiates automatically upon a door opening/closing event. SafeCell UV inhibits the growth of mycoplasma, bacteria, molds, spores, viruses, yeasts and fungi without costly HEPA filter air scrubbers which accumulate contaminants in the filter media. Additionally, the UV lamp can be programmed for a timed 100% ON cycle for a supplemental chamber decontamination.

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