

#### A Critical Research Tool

 $\mathrm{CO}_2$  incubators provide an optimum environment for tissue cell culture growth in clinical and life science research laboratories.

The parameters that contribute to optimum growth conditions are humidity, temperature control, cleanliness,  $CO_2$  gas control, and/or  $O_2$  gas control.

Operated effectively, these incubators can maintain cells for extended periods of time, allowing for research and other necessary activities related to the cell and tissue culture contained within. Yet to assure that the cells are maintained in an environment in which they are protected requires purchasing the right incubator for your specific needs. To achieve that requires a strong awareness of the different aspects and features of today's incubators. This buying guide covers the key considerations when making a purchase decision for a  $\mathrm{CO}_2$  incubator.

# Water-Jacket vs. Direct-Heat CO<sub>2</sub> Incubators

Both water-jacket and direct-heat  $CO_2$  incubators establish and maintain a uniform interior temperature of, typically,  $37^{\circ}C$  to assure proper growth of cells. To achieve this consistent temperature, the interior chamber is surrounded by water or heating elements; each technology has specific advantages and disadvantages. Before making decisions regarding whether to purchase a direct heat or water jacketed incubator several factors should be taken into consideration.



### Direct-Heat CO<sub>2</sub> Incubators

NuAire direct-heat  ${\rm CO_2}$  incubators provide a stable in-vitro growth environment with heating elements on all six sides of the chamber. High-density insulation stabilizes the interior chamber temperature, potentially lowering energy costs. Unique features such as dual decontamination cycles, humidity, and hypoxia control help ensure research needs are met.

Direct-heat incubators surround the interior chamber with heating elements, typically enclosed by insulation. Direct-heat  $\mathrm{CO}_2$  incubators are lighter and, because the interior chamber is heated directly, operating temperature can be achieved and recovered more quickly then their water-jacketed counterparts.

Some direct heat incubators rely on convection to keep the heat evenly distributed inside the chamber, while others maintain heat distribution via mechanical assistance such as a fan. A potential challenge with direct heat incubators is that forced air can lead to increased evaporation from cultures. In addition, a fan can create vibrations and, in some instances, create an environment more conducive to the growth of contaminants such as fungi and bacteria.

Some direct heat incubators are capable of heat decontamination, using humidified or dry-heat cycles. Water-jacketed models do not have this capability.



# Advantages:

- Faster Temperature Start Up and Recovery Time
- High-Heat Decontamination Cycle
- Lighter than Water-Jacket Models

#### **Drawbacks:**

More Affected by Power Outages

## Water-Jacket CO<sub>2</sub> Incubators

NuAire water-jacket  $CO_2$  Incubators provide a stable in-vitro growth environment by heating the growth chamber with a water jacket. Water circulates within the jacket walls producing a temperature uniformity of  $\pm 0.2^{\circ}$ C. The water jacket makes the chamber temperature less susceptible to fluctuations in the surrounding area. The additional water mass dampens vibrations, benefiting vibration-sensitive cells.

Water has a greater specific heat capacity than air, so it is frequently used to regulate the interior temperature of lab incubators. The growth chamber is surrounded by a jacket of water that is warmed by heating elements, which in turn warms the growth chamber.

Water circulates via convection, exchanging heat with the growth chamber, and acting as a thermal buffer. This buffer is especially important in the event of a power outage. The greater thermal stability of water vs. air allows the incubator to maintain internal temperature four to five times longer than a direct heat incubator.

Water-jacketed incubators, when filled, are very heavy and must be emptied before being moved. Once moved and refilled, it can take as long as long as 24 hours to achieve a stable operating temperature. An advantage of the additional water mass is the tendency to dampen vibration, which may aid the growth of vibration-sensitive cells.

# **Advantages:**

- Higher Level of Temperature Accuracy and Uniformity
- Lower Vibration
- · Less Affected by Power Outages

#### **Drawbacks:**

- Heavier than Direct-Heat Models
- Slower Start-Up and Recovery Times
- No Integrated Decontamination Cycle



#### **Maintaining Chamber Conditions**

While location and other physical factors can influence incubator performance, proper personnel training is of even greater importance in maintaining consistent operating temperature. If an incubator will be placed in an area subject to temperature instability, consider a water-jacketed model due to the greater thermal stability.

Each time an incubator door is opened, cooler air from the surrounding area can enter the growth chamber. This lowers the temperature, disrupts the gas mixture of the growth chamber, and potentially introduces contaminants such as mold spores or bacteria. There will be a period of suboptimal performance as the incubator restores the proper interior conditions.

Personnel should be trained to limit the number and duration of door openings. If frequent door openings are likely, consider the purchase of a direct-heat incubator which can restore growth chamber temperature more quickly than a water-jacketed model. CO<sub>2</sub> Incubators equipped with multiple interior doors can limit the exposure of contents when the main door is opened.

# **Humidity Control**

Maintaining the correct humidity within an incubator is essential. Without proper humidity control, airflow can lead to excessive evaporation, allowing cell culture dessication.

Preventing damage to stored cells depends both on humidity control and the volume and velocity of airflow that occurs within the chamber. Some manufacturers, including NuAire, reduce airflow within its incubators to avoid drying out cell cultures. NuAire's "Closed Loop HEPA Filtration System" achieves this, and is standard equipment on all NuAire  $CO_2$  incubators. The technology slows airflow to one air exchange per 30 minutes within the inner chamber on water-jacket models or one air exchange per 20 minutes on direct-heat models, which minimizes evaporation or desiccation of the cell samples.

## **Closed Loop HEPA Filtration**



Air is passed through a reservoir if moisture is needed\*

Room air enters the back and is routed to a front disk filter

Chamber air is recirculated through a capsule HEPA filter

Air is returned to the chamber to restart the cycle again

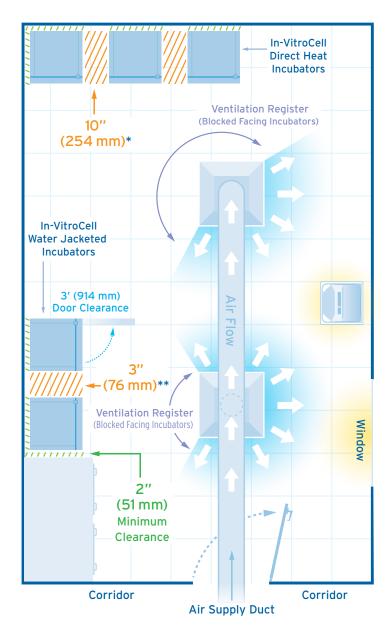
\*Available on select models

# CO<sub>2</sub> Incubator Location Considerations

Consider the planned location of a  $CO_2$  incubator when making a purchasing decision. The performance of an incubator can be adversely affected by temperature fluctuations in the surrounding environment.

Avoid placing incubators near sources of heat, such as direct sunlight, or next to an oven, shaker, or autoclave. Take into consideration the common patterns of heating or cooling when locating an incubator in the vicinity of an HVAC diffuser. It may be necessary to block heating / cooling airflow in the direction of an incubator.

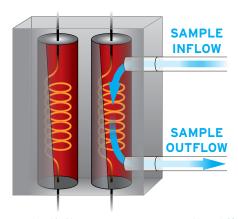
Manufacturers generally test their incubators for temperature fluctuations and follow up these tests by publishing temperature uniformity statistics. Consider these statistics when deciding where to locate an incubator.



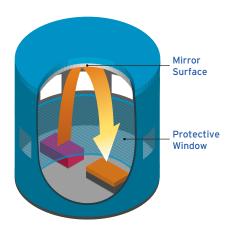
- \*Minimum recommended clearance for direct-heat models with decontamination cycle.
- \*\*Minimum recommended clearance for water-jacket models.

#### CO<sub>2</sub> Controls

Maintaining a healthy  $CO_2$  level within the incubator is important as  $CO_2$  interacts with the buffering system of the cell culture media to determine the media's pH. A key choice to make for  $CO_2$  control is what type of  $CO_2$  sensor the incubator will have. While many incubators use the more traditional thermal conductivity (TC) sensor, the newer type of infrared (IR) sensor is often more effective as it is not as sensitive to fluctuations caused by door openings.



Thermal Conductivity Sensors measure the difference in electrical resistance between a sealed reference cell and a cell open to the chamber atmosphere. CO<sub>2</sub> content is calculated based on the difference in resistance between the two cells.



**Infrared (IR) CO<sub>2</sub> Sensors** rely on the fact that each gas absorbs a distinct wavelength of light.  $CO_2$  absorbs the wavelength 4.3 $\mu$ m, within the infrared portion of the Electromagnetic spectrum.

#### **Contamination Considerations**

In addition to the many factors related to maintaining an ideal cell growth environment, another important concern when selecting a  $CO_2$  incubator is preventing contamination of cell cultures.

#### Normal Use

Contaminants can be introduced via normal incubator use. For example, airborne particulate such as mold spores may enter any time the door is opened. This risk can be minimized with good laboratory procedure, but the growth chamber will eventually be exposed to the surrounding environment.

## **User Error or Improper Procedure**

Contaminants can also be introduced via improper laboratory procedure, or personnel error. Touching the interior of the incubator chamber with a bare hand, or a contaminated glove, may introduce bacteria or viruses to your samples.

## Reducing the Risk of Contamination

HEPA filtration of the air inside the growth chamber is an effective means of removing airborne contaminants. NuAire's Closed Loop HEPA Filtration System continuously circulates growth chamber air through a HEPA filter to minimize risk from contaminants introduced by a door opening.

## **Decontamination and Cleaning**

Many  $CO_2$  incubators use decontamination cycles to clean interior surfaces. High-heat decontamination cycles require the incubator to be emptied before decontamination.



Direct-heat CO<sub>2</sub> incubators, such as **NuAire's In-VitroCell series**, offer two types of heat decontamination cycle. A 95°C humidified cycle or 145°C dry cycle can decontaminate the interior of the incubator.

# **Cleaning & Construction Considerations**

A CO<sub>2</sub> incubator growth chamber which is constructed with rounded corners offers contamination control advantages. Interior rounded corners eliminate tight crevices where contaminants may collect, and chemical disinfectants are able to contact more surface area of the chamber.

The main door gasket creates a seal around a  $CO_2$  incubator's inner door. This gasket is an area where humidity can accumulate, resulting in conditions more conducive to the growth of fungi. A gasket which can be removed for cleaning can be disinfected much more thoroughly. If using a V-gasket be aware of the direction of the flap. A flap that points outwards will collect particulate, preventing it from entering the growth chamber. A flap that points inward is an area for humidity to collect and a source of contamination.



Another way to obtain continuous protection is to select an incubator which makes use of copper alloy for interior surfaces. Copper has properties that may inhibit the growth of bacteria. NuAire offers an antimicrobial copper alloy for chamber shelving.



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