HOW TO CHOOSE BETWEEN

INFRARED AND THERMAL CONDUCTIVITY CO_2 SENSORS IN INCUBATORS

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Incubators, also called carbon dioxide (CO_2) incubators, are key equipment for biological and medical laboratories. They enable the necessary environmental control and isolate cell cultures from external conditions and contamination. Control focuses on three major factors:

1) Temperature

Normal temperature for the human body, 37 degrees Celsius, is an optimum temperature for most cell cultures, although some might require different temperatures. Cells must stay within a narrow temperature range – a few tenths of a degree – to avoid conditions that threaten the cell culture or create a significant delay in growth and impact on schedules.

2) Relative Humidity

Relative humidity (RH) levels in the incubator prevent growth medium desiccation. RH can be as low as 75 to 80 percent. More commonly, RH must remain above 90 percent.

3) Carbon Dioxide (CO_2)

Different cells grow best in environments with pH values that typically range from 7.0 to 7.7. Growth medium includes a pH buffer, often CO_2-bicarbonate-based, to keep conditions stable. Chemical reactions in the medium can alter the pH. Control of atmospheric CO_2 levels helps maintain steady growth-medium pH.

Measuring CO_2

There are two main technologies for measuring CO_2 – infrared (IR) and thermal conductivity. This paper compares them and examines which technology might be best for a given laboratory.

Incubator Structure

An incubator is essentially a box within a box. The outermost shell **[A]** is what remains visible when the door is closed and the system operating. Within that shell is the growth chamber **[B]**, in which temperature, relative humidity, and CO_2 are controlled.

All three conditions require control because they affect cell growth. Heating elements, whether directly applied to the growth chamber or indirectly through a water-filled jacket surrounding the chamber, maintain a temperature, typically 37 degrees Celsius, although, depending on the specific need, temperatures can range significantly higher or lower. A water reservoir or pan provides humidity. Both temperature and humidity are of secondary importance in this discussion.

An incubator also needs control of CO_2 content in the air, generally achieved through an exogenous source of CO_2, like an external tank **[C]** of gas attached through a hose to the incubator. A sensor, using either IR or TC technology, monitors the level of CO_2, activating a mechanism that adds more of the gas when levels are too low.

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Chemistry of pH and Cell Growth

The reason CO_2 control is critical in incubators is that it offers indirect control of pH levels.

Cells are grown in a container, whether a petri dish, a 96-well plate, or some other type. A liquid, solid, or semi-solid growth medium provides nutrients and a substance the cells can fasten to. Growth media typically include a pH buffer, often carbonate-based, to keep pH levels stable as cell processes throw off acids as byproducts.

As a reminder, pH, which stands for potential of hydrogen, is a measure of acidity on a scale from 0 to 14. Specifically, it is a measure of the number of hydrogen ions, symbolized by H^+, in a solution. Below is the formula for pH:

$$pH = -log10[H^+]$$

Cells that researchers study and cultivate tend to thrive in a relatively narrow pH range of about 7 (the "normal" level of pH found in unaltered water) to 7.7 (slightly alkaline). If the pH ranges too far above or below the optimum level for a type of cell, at best growth will slow and affect lab work and schedules. If the pH level is off by too extreme an amount, the culture will die.

Role of CO_2 in Incubator pH

Although growth media frequently include pH buffers, commonly used carbonate-based buffers depend on a balance between CO_2 and bicarbonate dissolved in the medium. If CO_2 escapes into the atmosphere, alkalinity increases and the pH level changes.

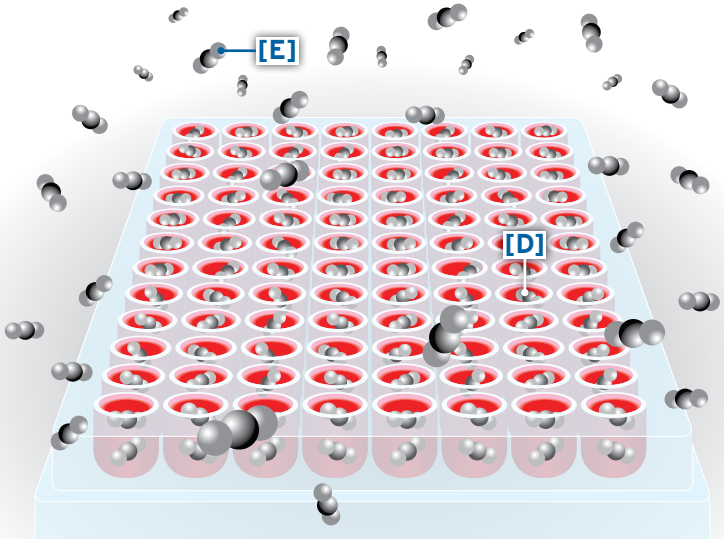
The most common method to prevent release of CO_2 into the atmosphere of the growth chamber is to ensure a sufficiently high percentage of the gas in the air. This is due to the physics of gases, including the concept of partial pressures, each separate gas in a mixture exhibiting a portion of the overall pressure of the mixture, and a principle called Henry's Law. It states that the concentration of a gas that is dissolved in a liquid, like the CO_2 in the growth medium **[D]**, is directly proportionate to the partial pressure of that same gas in the atmosphere above the liquid **[E]**.

In other words, for a particular medium and type of buffer, a sufficient partial pressure of CO_2 in the growth chamber atmosphere prevents more CO_2 from escaping the medium. As a result, the pH of the medium stays controlled.

In practical terms, given normal atmospheric pressure, the incubator only needs to keep the CO_2 level sufficiently high as a percentage of the overall atmosphere. A commonly used value is 5 percent, compared with the normal roughly 0.3 percent of CO_2 found in the atmosphere at sea level. The exact percentage needed will vary with the type of growth medium.

If too little CO_2 is in the atmosphere, CO_2 will be able to escape from the growth medium and the mixture will become too alkaline. Too much CO_2 in the atmosphere will enable more of the gas to be absorbed by the medium, ultimately turning it too acidic.

No matter what the necessary value, the incubator must be able to calculate the amount of CO_2 in the atmosphere accurately and quickly.



Henry's Law *states that the concentration of a gas that is dissolved in a liquid is directly proportionate to the partial pressure of that same gas in the atmosphere above the liquid.*

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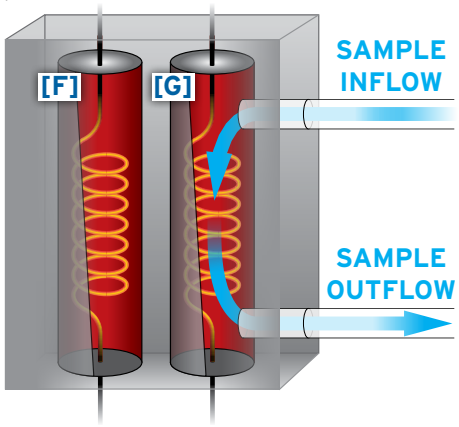
CO_2 Sensor Technologies

There are two technologies in use that measure the percentage of CO_2 in the atmosphere of the growth chamber.

Thermal Conductivity (TC)

TC sensors work through measuring electrical resistance through the air. Typically, a sensor is composed of two cells, each containing a thermistor, a "thermal resistor" in which the resistance changes with atmospheric conditions, including gas composition, temperature, and humidity. A sealed reference cell **[F]** encloses reference atmosphere at a controlled temperature while the other cell **[G]** can be filled with the growth chamber's atmosphere.



Thermal Conductivity Sensors *measure the difference in electrical resistance between a sealed reference cell and a cell open to the chamber atmosphere. CO_2 content is calculated based on the difference in resistance between the two cells.*

Circuitry measures the difference in resistance between the two cells. When the chamber atmosphere is in a steady physical state and the system is calibrated to known temperature and humidity conditions, the difference in resistance between the reference cell and the other is the result of the difference in CO_2 concentration.

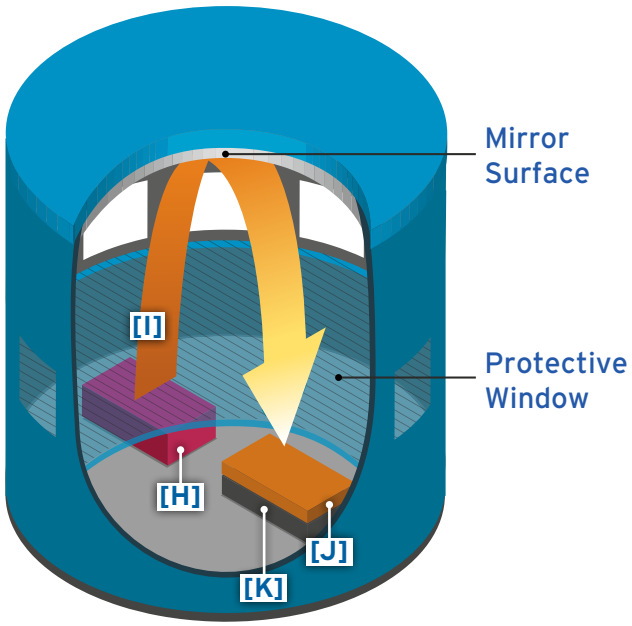
TC sensors offer a substantial price savings by as much as 20 percent of the device cost. However, they have a significant drawback. Because thermistor resistance varies with temperature and relative humidity, as well as gas composition, any time the door to the growth chamber is opened, the temperature and humidity change from what the calibrated sensor expects, which means the readings are no longer accurate.

Once the door is closed again, regaining a steady state of temperature and relative humidity can take as long as 40 minutes for the typical incubator. Until then, any reading is unreliable. Some vendors begin to add CO_2 after a door opens to regain levels, but because they don't know what the existing levels are, the percentage may be incorrect, affecting the pH balance of the medium. In some cases, like medical and pathology uses, this may not matter. But for many types of research, the inaccuracy can lead to poor reliability and consistency in measurement and in results.

Infrared (IR)

IR sensors rely on the fact that each gas absorbs a distinct wavelength of light. CO_2 absorbs the wavelength 4.3µm, within the infrared portion of the Electromagnetic spectrum.

An IR emitter **[H]** directs infrared light **[I]** through a sample of the growth chamber's atmosphere, then through a Fabry-Perot Interferometer **[J]** filter which isolates the proper wavelength, and finally into a sensor **[K]**. Periodically calibrated circuitry measures the amount of 4.3µm light that strikes the sensor and calculates the difference between it and what was emitted by the source.



Infrared (IR) CO_2 Sensor

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The more CO_2, the less light passes. The difference allows the circuitry to calculate the percentage of CO_2.

Because the light absorption does not depend on temperature or humidity, the sensor is accurate any time, including shortly after opening and closing the door of the growth chamber.

Making the Choice

An incubator with a TC sensor is financially tempting to any lab, as the price difference can be substantial although in recent years the cost of IR sensors have decreased making them more affordable. If the lab's work is of a type that will feel little effect from CO_2 reading inconsistency and inaccuracy every time the incubator door is open, the choice might make sense.

However, for labs that either do more sensitive work now or might in the future, an IR sensor-equipped incubator is the right choice. Aside from the loss in accuracy in work product with TC, which could affect quality, there is a subtle but substantive financial argument for IR.

If personnel cannot trust the readings of the CO_2 sensor for even half an hour after a door is opened, they lose productive time. You can calculate the effective cost to the organization. Multiply the typical number of times the door is opened a day by a half-hour. Determine the fully loaded cost of a researcher's time. Add the expected margin for an employee's time. The total is the total cost to the organization. For example, say the door is opened four times a day and a researcher's time is effectively worth $150 an hour. The resulting two hours a day is worth $300, or $1,500 of lost productivity a week. That would be $6,450 for the average 4.3-week month and $77,400 a year.

The money "saved" by using a TC sensor-equipped incubator quickly evaporates. Within just a month or two, the total cost of the IR sensor-equipped model is lower.

For most labs, especially ones looking to expand either their type or amount, of work IR sensors make sense, both financially and qualitatively.

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