ThermoFisher SCIENTIFIC

$CO_2$ incubators

# Question: Which incubation parameters are most important for proper cell growth and expression?

## Answer:

All parameters are important. Proper temperature, gas tension ($CO_2$/$O_2$/$N_2$) and humidity work together to provide optimum growth. Speedy recovery to your set parameters is a critical consideration any time you want to mimic an *in vivo* state.

The goal of placing your cells in a $CO_2$ incubator at body temperature (37 °C/98.6 °F) is indisputable; mammalian cells will grow best at their native temperature. $CO_2$ gas serves to maintain *in vivo* pH, similar to $CO_2$ tension in the bloodstream. High humidity prevents evaporation of growth media. All these parameters work together for healthy cells which express proper protein profiles. Proper culturing is especially important for sensitive primary and stem cells.

But every time you open the incubator door, conditions inside the incubator rush to equilibrate with the conditions in your lab. Once you close the door, the incubator works to re-establish the required state. So how long your cells spend at your specified conditions depends on how long and how often you open the incubator door, and upon the design and engineering of the incubator itself. Different technologies and engineering can result in vastly different recovery times.

The Thermo Scientific™ THRIVE™ Active Airflow technology, combined with dual temperature probes, cutting edge $CO_2$ sensors and a unique covered, integral humidity reservoir, is designed to provide recovery of all parameters within only 10 minutes after a 30 second door opening.

thermo scientific

# Why consider all parameters?

## Different technologies produce different results

Although $CO_2$ incubators may appear similar from the outside, the engineering inside critically affects your project outcomes. Sensor design and in-chamber location, gas inlet positioning, airflow speed and path, and experienced engineering work together to provide the ideal environment for your cell growth and health.

## Temperature

Cellular functions are highly responsive to temperature. As one example, chick embryos show increased mortality with a difference of only 1 ° C[1]. Metabolism and growth slow down at lower temperatures. So fast temperature recovery after a door opening is important, but it's also important to not overshoot the set temperature, because higher temperatures are even more detrimental.

## $CO_2$ tension

$CO_2$ gas works with sodium bicarbonate in the growth medium to control pH to a neutral 7.4. This mimics bloodstream biochemistry. When the pH varies from neutral, cells will first stop growing and then lose viability. Take time in examining your cells, because improper pH may produce morphological changes such as vacuoles in the cytoplasm or granules around the nucleus.

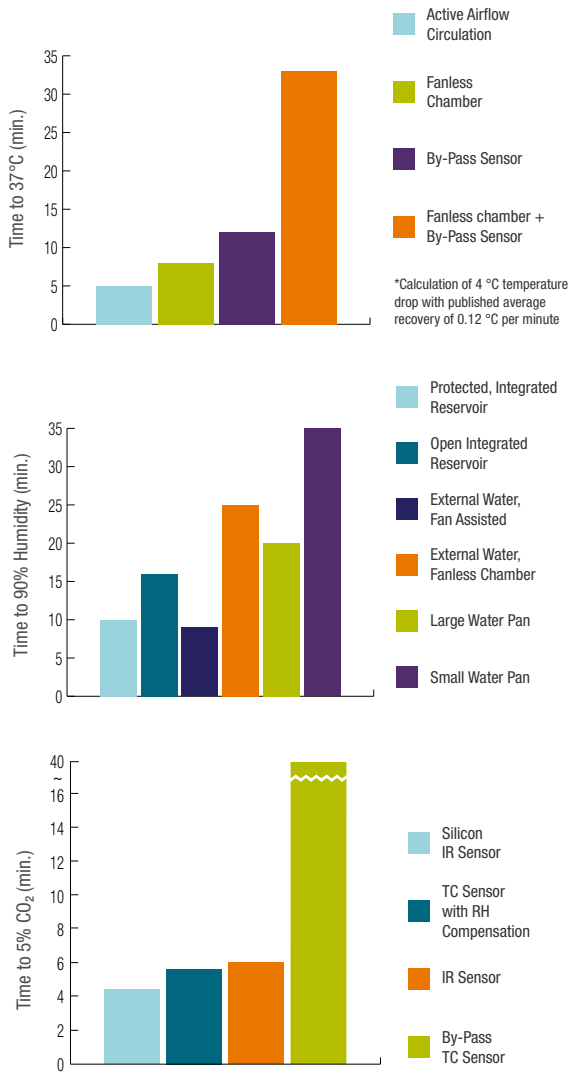
## Humidity

The human body is about 60% water, with internal organs 75-80% water[2]. In a cell culture incubator, balanced growth media provide moisture and nutrients for cells. Humidity of 85-95% limits evaporation of water from the media. Evaporation leaves too-high concentrations of salts, minerals, etc., resulting in toxicity and cell death. High humidity is the most difficult condition to reestablish but is critically important, as evaporation is 4 times faster at 80% humidity than at > 93%[3].

## Summary

Temperature, gas tension and humidity all work together to replicate in vivo conditions. Thermo Scientific THRIVE active airflow, cutting edge in-chamber sensors and design technologies recover all parameters in 10 minutes or less (30 sec. door opening).

**Figure 1: $CO_2$ incubator design affects recovery**
Comparison of (A) temperature (within 98% of 37 °C), (B) humidity (to >90%) and (C) $CO_2$ (within 98% of 5% $CO_2$) recovery after a 30 second door opening. Different technologies produce widely varied results. Data from published brochures.





**References:**

1. Poultry Hub: Physiology and Incubation. http://www.poultryhub.org/most-popular/incubation
2. Mitchell HH et al. The chemical composition of the adult human body and its bearing on the biochemistry of growth. J Biol Chem. 158:625, 1945.
3. Esser P and Weitzmann L. Evaporation from cell culture plates. Thermo Fisher Scientific TILSPNUNCBU02 0111, 2011.


Find out more at **thermofisher.com/co2**