#### WHITE PAPER No. 56

# CO<sub>2</sub> Incubator Temperature Control: What Is the Best Place For Your Cell Culture Vessels?

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#### **Executive Summary**

Does it make a difference if cell cultures are incubated on the top shelf in the incubator or on the bottom shelf? Well, it certainly should not! But depending on the incubator you are using, it may not be a good idea to put sensitive cells near a circulating fan. Even if your incubator does not have a fan causing vibrations or increased evaporation of the culture medium, the incubator should provide a homogeneous temperature distribution throughout the complete chamber. A CO<sub>2</sub> incubator is the safe haven for your cells providing optimal atmospheric conditions. Sensitive primary or stem cells are especially susceptible to temperature increases and will react in adverse ways. When you are planning a complex experiment, or use the cells for a certain application, you want to be sure that the location of the culture vessel inside the incubator has no impact on the cells and experimental result. Just imagine if the temperature on the upper shelf is 37.5°C while it is 36.5°C on the lowest shelf.

### Temperature homogeneity in CO<sub>2</sub> incubators (according to German DIN 12880:2007-05 norm)

In CellXpert<sup>®</sup> CO<sub>2</sub> incubators, the temperature homogeneity, or the spatial temperature deviation, was verified based on a test method that is defined in a German standard for ovens and incubators (DIN 12880:2007-05). In this test, a set-up with not less than 27 temperature probes is used for measuring the temperature at different locations inside the incubator (Figure 1).



**Fig. 1:** Measurement set-up according to DIN 12880:2007-05 – 27 temperature probes placed at different locations on three shelves inside the CO<sub>2</sub> incubator

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The results of the test according to the standard DIN12880:2007-05 proved that CellXpert  $CO_2$  incubators provide a reliable and homogeneous temperature distribution throughout the complete chamber. Applying the same 27-point temperature measurement to other incubators

revealed much higher variations in temperature depending on the location in the incubator (Figure 2).

Taken together, your cells are exposed to the same stable atmospheric conditions in CellXpert  $CO_2$  incubators no matter where the culture vessels are located.

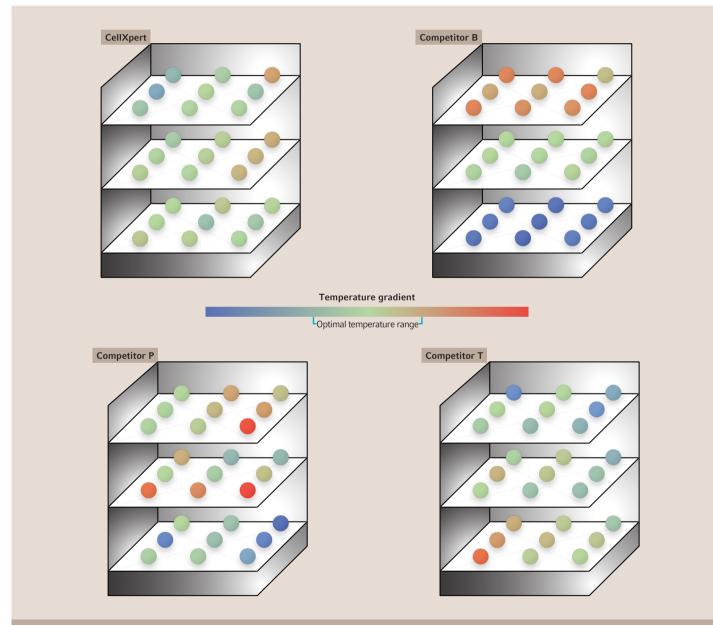
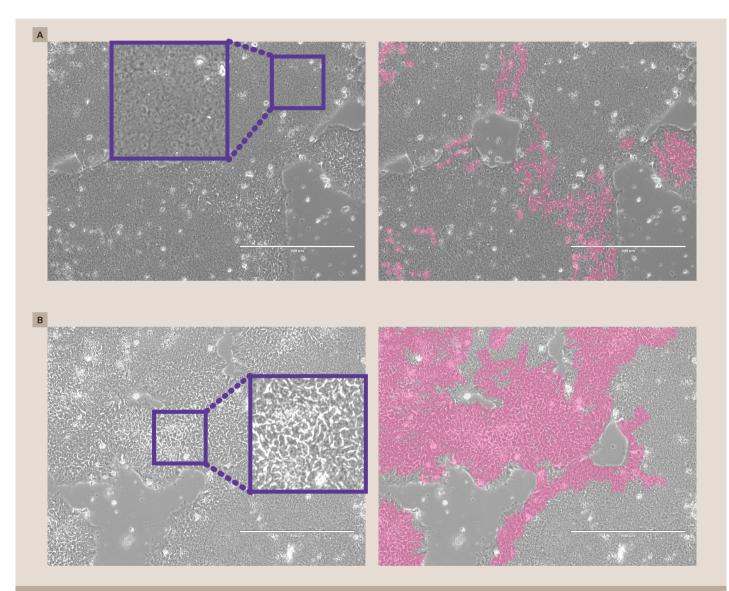


Fig. 2: Spatial temperature distribution in different CO<sub>2</sub> incubators verified by measurement according to DIN12880:2007-05.

#### Does 1 °C difference matter?

**Example: Effects on induced pluripotent stem cells (iPSCs)** One example is the atypical morphology developed by human iPS cells grown at 38 °C compared to optimal temperature of 37 °C (Figure 3). But even if you set the temperature to the 37 °C desired in most cases, not all incubators provide uniform spatial distribution of temperature. So, placing the culture vessels on different shelves or different positions on the shelf can make a difference depending on the incubator you are using.

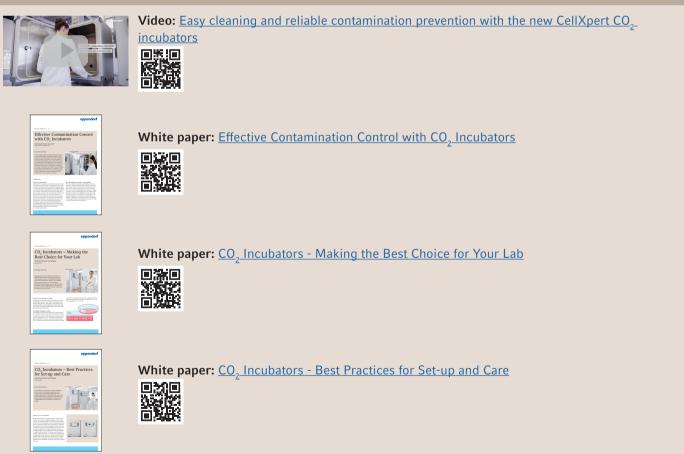


**Fig. 3:** Human induced pluripotent stem cells incubated to illustrate differences in morphology. Appearance of atypical cell morphology highlighted in pink. hiPS cell incubated at 37° (A) predominantly show typical morphology of high nuclear-to-cytoplasm ratio, prominent nucleolus and densely packed colonies. Incubation at 38 °C (B) increases the appearance of less densely packed and more shiny cell colonies with less pronounced nucleoli.

## The technology behind – multiple sensors, individually controlled heating circuits, and no fan

Most incubators are equipped with one temperature probe mounted on the back wall of the inner chamber. Thus, temperature is measured locally at one spot inside the incubator. A second temperature probe is often included to avoid harmful over-temperature, but not to control for spatial homogeneity. A quick recovery of temperature and gas levels after door opening is often achieved by an internal fan. This fan-assisted heating technology leads to a forced airflow inside the incubator which may cause multiple issues, like spreading of air-borne contaminants, vibration on the shelves, and evaporation of the culture medium. CellXpert CO<sub>2</sub> incubator offer three-dimensional regulation of the temperature achieved by several sensors placed directly at the different heating circuits surrounding the chamber and the door. This 3D temperature control and the direct heating technology result in highly uniform temperature distribution throughout the whole incubator. In addition, the temperature control system leads to quick temperature recovery after door opening (under 5 min without temperature overshoot). Thus, CellXpert CO<sub>2</sub> incubators provide optimal conditions for cell cultures independent from their location on the shelves.

## Want to learn more about the new family of Eppendorf CO<sub>2</sub> Incubators and compare with other manufacturers?



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Eppendorf was founded in Hamburg, Germany in 1945 and has more than 3,300 employees worldwide. The company has subsidiaries in 26 countries and is represented in all other markets by distributors.

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