



Getting Started in Cytocentric[®] Research: FAQ's

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Why does exposing my cells to room air, even for small amounts of time, matter?

HIF-1 alpha is downregulated at the protein level within minutes of oxygen elevation, which can in turn affect additional transcription factors and cellular behavior. Eliminating or minimizing pericellular oxygen changes is critical to avoid destabilizing HIF proteins.

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What is the oxygen level in the traditional open-room CO₂-controlled incubators I have been using?

When we measured oxygen levels in standard open-room incubators set to 5% CO₂, we found an average of $17.2 \pm 0.3\%$ O₂ after normal door access. Each door opening triggered another cycle of CO₂ loss and reinfusion, driving oxygen levels down again, with smaller incubators showing larger swings in gas levels even during normal use. Furthermore, in incubators with an open water pan, water vapor can occupy up to ~8% of the chamber's gas volume, displacing oxygen even further.

For researchers who assume their cultures experience stable atmospheric oxygen, these routine fluctuations may influence cell metabolism, gene expression, and experimental reproducibility.

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I have seen researchers use 1%, 2%, 5%, 8%, or 10% oxygen. What oxygen level should I use for my cells?

Oxygen levels in vivo vary widely depending on local physiology. Circulating venous blood is typically around ~5% O₂, while bone marrow, stem cell niches, and fetal tissues often exist in relatively low oxygen environments. Poorly vascularized regions, such as cartilage or the interior of solid tumors, can approach near-anoxic conditions. Because oxygen exposure strongly influences cell metabolism, signaling, and differentiation, we recommend consulting the scientific literature related to your specific model and performing preliminary experiments across a range of oxygen levels to determine optimal conditions for your cells.

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What about the oxygen levels outside in incubator outside of the C-Chamber?

Escaped gases used to control conditions inside the C-Chamber can escape to the interior of the incubator, which may reduce the partial pressure of oxygen. To prevent pressure buildup during gas infusion, C-Chambers include pressure-equalization features. A soft magnetic door seal allows the door to briefly "burp" and reseal if pressure rises above ambient levels. Additionally, chambers can be designed with an additional hose barb and tubing to vent excess gas to the outside of the incubator. For the most precise environmental control, we recommend a dedicated C-Chamber and controller for each oxygen or gas condition required.



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What oxygen level does my cell culture experience in the bottom of a cell culture vessel?

In addition to the oxygen concentration set in the incubation chamber, several factors determine the actual oxygen level at the cell layer:

- Depth of cell medium: Deeper media increase the diffusion distance, which can slow equilibration and reduce the oxygen available at the bottom of the vessel.
- Culture vessel design: Some specialized vessels use oxygen-permeable materials that allow oxygen exchange through the bottom of the plate or flask. In static culture, this can shorten the diffusion path and allow oxygen changes in the chamber to reach the cells more quickly.
- Cell density and metabolic activity: As cell numbers increase, oxygen consumption rises, which can significantly lower the pericellular oxygen concentration near the cell layer.

For more consistent and reproducible conditions, maintain controlled chamber oxygen levels, consistent media depth, and similar cell densities between experiments.

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How can I get the best results if I have to handle my cells in a standard room-air Biological Safety Cabinet (BSC)?

If cell handling must be done in room air, such as in a standard biological safety cabinet, the pre-equilibration of cell culture media and other solution can minimize undesirable pericellular oxygen level swings.

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What is the longest length of time I can have my cells out in the BSC?

The longer that cell media and other liquid components are mixed with HEPA-filtered room air in the BSC, the more they equilibrate with room air, which is supraphysiologic. Sensitivity to changes in oxygen levels differ based on cell type, though a general rule is that the longer that cells are out of their optimal physiologic range, the more likely they are to be affected by those conditions which, in turn, affects downstream applications.

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How does the Xvivo System® offer cells a better environment than a traditional laboratory or cleanroom setup?

Cells experience better growth and higher viability in the Xvivo System due to consistent and controlled physiologic conditions, including oxygen, carbon dioxide, temperature, humidity, and limit control measurements for particles, VHPs, and VOCs. The precise regulation of temperature mitigates edge effect, resulting in better confluence of cells, reduced cell stress, and decreased plastic and reagent waste. The Xvivo System also provides contamination control due to a patented self-sanitizing technology that reduces unwanted microbial growth. Learn more on our website at <https://biospherix.com/our-science/>.

Have a question not addressed here? Reach out to us!

