



Environments For Science™

BAKER SCIENTIFIC APPLICATION NOTE

PhO₂x Box – a new hypoxia/physoxia cell culture system

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- Ambient room air contains approximately 21% of oxygen which is well above the physiological oxygen levels cells reside in.
- For instance, stem cells reside in hypoxic niches and growing them in vitro in ambient O₂ tension leads to loss of pluripotency.
- Excess oxygen leads to formation of free radicals inside the cells that damage biomolecules.
- Hypoxic responses are largely governed by transcription factor HIF. HIF is active in low oxygen conditions and rapidly (less than 5min) degraded in unphysiologically high oxygen concentrations.
- Disrupting HIF system disrupts the whole signalling cascade.
- Moving cells from hypoxia to ambient even for 5 minutes leads to reoxygenation damage that is irreversible, untraceable, and irreproducible.
- Untraceable and irreproducible experiments result in data that is not translational.

Oxygen is crucial for life, and maintaining correct levels is a fundamental requirement for cells to function properly. The atmospheric O₂ level at sea level is 21% but as oxygen diffusion distance through membranes is very short, the inhaled oxygen level rapidly declines to physiological levels. Even in lung epithelial cells oxygen concentration is around the level of 13% and declining all the way down to 0% in the bone marrow.

Hypoxia is a common phenomenon in solid tumours where functionally and structurally disturbed microcirculation as well as the deterioration of diffusion conditions leads to formation of hypoxic centres within tumours. Tumour hypoxia is strongly associated with tumour propagation, malignant progression, and resistance to therapy and as such it has become a central issue in tumour physiology and cancer treatment.

HIF-1 is a major regulator of the cell's response to a hypoxic microenvironment, which is strictly controlled through synthesis, and degradation. Hypoxia and overexpression of HIF-1 may be related to radiotherapy and chemotherapy resistance, increased risk of tumor invasion and metastasis, and poor clinical prognosis of most solid tumors. Therefore, the HIF-1 pathway is considered as a viable pharmacological target in the treatment of solid tumors. Hypoxia has been linked to cancer progression, recurrence, and metabolic reprogramming. Under hypoxic conditions, HIF-prolyl hydroxylases (PHDs) activity is inhibited, HIF-1α accumulates, and dimerizes with HIF-1β, thereby activating transcription of hundreds of genes.

The prevalence of hypoxia and the increase in HIF-1α have raised interest in targeting the HIF pathway for most solid tumors. Recent evidence from genetic and pharmacological research supports the view that inhibition of HIF-1 is beneficial for cancer treatment.

With all this said it is clear that in an experimental setting where the ultimate goal is to be able to mimic biology, attention to correct oxygen levels is of utmost importance. This precision is nowadays easily reached with the use of sophisticated gas-controlled systems from Baker.

PhO₂x Box – physiological oxygen box – is Baker's newest addition to our physoxia product range. It is comprised of a gas controller and cell culture chamber and is designed for *in vivo* cell cultures using plates and small flasks. The cell culture chamber module can be placed into any incubator, and the gas controller remains outside.

PHO₂X BOX APPLICATIONS

PhO₂x Box is a new, easy to use and economical physoxia/hypoxia system designed for *in vitro* cell culture experiments. PhO₂x Box offers a defined hypoxic or physoxic cell culture environment and is ideal for:

- Creating an oxygen controlled compartment inside your CO₂ incubator.
- Short term *in vitro* cell culture experiments
- Enables the use of multiple O₂ environments inside a workstation

PhO₂x Box can accommodate a range of multi-well plates and tissue culture flasks with a quick and easy set up time. This solution is ideal for a range of application fields, including HIF, hypoxia, stem cell research, cancer research, respiratory disease, sleeping disorders, altitude stress and genetic expression.



PHO₂X BOX USER EXPERIENCES

1. The Yilmaz laboratory at the Medical University of South Carolina, US does work using the PhO₂x Box system with 5% O₂/5% CO₂ conditions. The Yilmaz team utilizes multiple different dish types (multi-well plates, 100 mm dishes, different size tissue culture flasks (T-25, T-75, T-150, etc.) and the users appreciate PhO₂x Box's versatility. The lab reports that some cell types have grown better in PhO₂x Box conditions versus their typical normoxic conditions (20% O₂/5% CO₂). The laboratory is very happy with the product.
2. Preterm Birth and Prevention Center, Guang-Zhou Women and Children's Medical Center, China is culturing myometrium under 3-4% O₂ and 5% CO₂ and running trials to establish the best O₂ concentrations to use. PhO₂x Box allows the lab to use hypoxic cycling functionality that their existing trigas incubators do not have.
3. The University of Konstanz, Germany, works with human kidney cells under *in vivo* O₂ conditions (1-10% O₂) inside PhO₂x Box. Placing 2 PhO₂x Boxes inside a Baker Ruskin SCI-tive Hypoxia workstation, enables cells to be exposed to different oxygen concentrations in parallel.
4. The Institute of Biophysics, part of the Chinese Academy of Sciences, is using PhO₂x Box for pancreatic cancer cell research, on both cell lines and primary cells. The O₂% levels used is between 1.0% to 5.0%. PhO₂x Box supports this lab's research by offering a simple platform in which they can discover the optimal O₂% level and culture period for their cells.