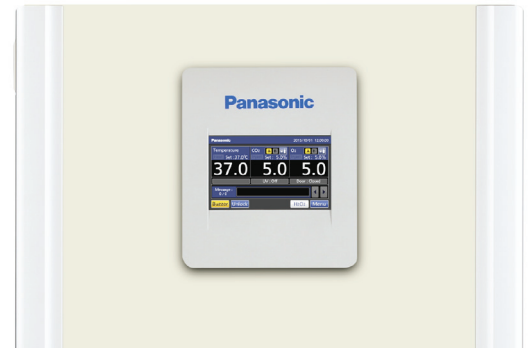


cellIQ™

Understanding Biological Oxygen Levels



LOW OXYGEN TECHNICAL BULLETIN

MCO-170M Series

In today's life science research sector, there are a variety of ways to grow, or "culture" cells. Depending on the organism or cell of interest, different incubation parameters are required for cell cultivation. Mammalian cell culture, a method of growing a particular tissue type out of its natural environment (or *in vitro*), has been an extremely valuable tool for the progress of science and medicine. To keep these delicate cells alive and healthy, CO₂ incubators are used to properly balance the temperature, pH level, and humidity of cells *in vitro*. Often overlooked, but very impactful to cellular health, is the level of oxygen at which mammalian cells are cultured. Multigas CO₂ incubators are one of several tools that lower oxygen within an incubator or chamber to bring cultured cells the oxygen levels that precisely mimic the tissue from which a particular cell is derived.

The descriptions utilized to describe the various levels of oxygen conditions within cell culture can get convoluted. "Normoxia", "physiological normoxia" (physoxia or physiological oxygen), "hypoxia" (physiological and pathological hypoxia), and "anaerobia" are all examples of common terms used to describe atmospheric or sub-atmospheric oxygen levels. This document seeks to elucidate the exact meaning of these terms and what biological applications are most commonly used in each setting.

"Normoxia" or Atmospheric Oxygen (approximately 20-21% O₂)

Normoxia is the term most often used to describe atmospheric levels of oxygen. This range generally resides between 20-21% O₂ (160 mmHg) [1]. Oxygen levels can vary depending on the altitude as well as how much CO₂ is being utilized within a given system [1]. Most cell culture today is performed at normoxic conditions with CO₂ maintained at 5%. In these conditions, cells are exposed to atmospheric air, which has a concentration of generally 20-21% O₂. However, the majority of cell lines grown by cell culture are derived from tissues that physiologically contain oxygen levels of 12% or lower. High oxygen concentrations, in respect to *in vivo* conditions, correlate with a higher generation of reactive oxygen species, which can be damaging to DNA [2]. Normoxic studies are most frequently performed in CO₂ incubators that do not have oxygen control. These incubators are able to maintain CO₂ at 5% while oxygen remains at atmospheric levels.

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Physiological Normoxia, Physoxia, or Physiological Oxygen (approximately 3-7% O₂)

Most tissues do not experience oxygen levels at 20-21%. In our lungs, oxygen levels are around 14.5% and in peripheral tissues oxygen can be as low as 3.4-6.8% [1]. Thus, a more accurate representation of physiological oxygen levels tends to be between 3-7%, or most commonly 5% (Table 1). The term physiological normoxia (or just physoxia) is used to define oxygen levels between 3-7%, the same as the normal oxygen levels seen physiologically in tissues. However, it is important to note that these conditions are considered hypoxic (lower oxygen) compared to normoxic conditions (20% O₂). Culturing cells at physoxia has become a more popular direction in order to achieve accurate and relevant results in lieu of the effects of normoxia on cells. In normoxia, excess oxygen creates reactive oxygen species, which cause molecular damage to DNA and proteins and can skew experimental results [2]. Normoxia also produces inaccurate *in vivo* conditions that do not take into account other factors, such as hypoxia-inducible factor (HIF), that play important roles in regulating transcription of certain genes for cell growth and proliferation [1]. Today, most researchers use multigas incubators that can maintain oxygen between 3 to 7% to simulate physoxia conditions. These incubators maintain a low concentration of oxygen by displacing the oxygen gas with nitrogen. Zirconia sensors that can detect the levels of oxygen in incubators facilitate rapid recovery of nitrogen gases.

Table 1

Tumor Type	Median % Oxygen Normal Tissue	Median % Oxygen Tumor
Brain (6)	3.4	1.7
Head and Neck Cancer (13)	5.9	1.3
	5.3	1.6
	5.8	1.9
	6.7	1.9
Lung Cancer	5.6	1.9
	5.6	2.2
Breast Cancer (10)	6.8	1.3
Cervical Cancer (12)	5.5	1.2
Liver	3.9	0.8
Pancreatic Cancer	6.8	0.4
	-	0.3
Prostate Cancer	3.9	0.3
	-	0.6
	3.4	1.2
Vulval Cancer	-	1.4
	-	1.7
	-	1.4
	-	1.3
Melanoma	5.3	1.5
Renal Cell Carcinoma	4.9	1.3
Rectal Carcinoma	6.8	4.2
	6.8	2.5
Sarcoma	6.7	1.8

Table 1: Summary of partial pressures of O₂ in select human tumors and related normal tissues. [Adapted from McKeown SR. Defining normoxia, physoxia and hypoxia in tumours-implications for treatment response. Br J Radiol. 2014 Mar; 87(1035). <http://europepmc.org/articles/PMC4064601>]

Table 2: Various O₂ levels and their associated terminology [Adapted from McKeown SR. Defining normoxia, physoxia and hypoxia in tumours-implications for treatment response. Br J Radiol. 2014 Mar; 87(1035). <http://europepmc.org/articles/PMC4064601>]

Table 2

mmHg	% Oxygen	Comments
760	100.0	Standard atmospheric pressure
160	21.0	Oxygen in air at normal atmospheric pressure
100	13.5	Inspired oxygen pO ₂ in alveoli; O ₂ level affected by in/outflow of gases and water vapor
70	9.5	Arterial blood oxygen concentration
50	6.5	Approximate pO ₂ at venous end of circulation
		Suggested definitions
38	5.0	Physoxia: physiological O ₂ level in peripheral tissues with an average of approximately 6% (ranging from approximately 7.5% to 4% depending on the tissue). For experimental studies, 5% is the proposed compromise since this is often used.
15	2.0	Physiological hypoxia: i.e. the lower level at which normal hypoxic responses are elicited (range: lower limit approximately 1%; upper limit possibly ≤5%)
8	1.0	Pathological hypoxia: shows persistence of poor oxygenation suggesting disruption to normal homeostasis. Below this level pathological hypoxia applies.
3	0.4	Radiobiological hypoxia: the O ₂ level at which the cytotoxic effect of radiation is half maximal.

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Hypoxia (Pathological hypoxia: ~0.1% to 4.2% O₂)

Pathological hypoxia exists when the mechanisms to reverse O₂ depletion fail or become insufficient. Pathological hypoxia may occur in certain instances of loss or occlusion of blood vessels or, in such cases as cancer, leaky and inadequate vasculature (Table 1) [1]. In these examples, O₂ levels tend to fall below 2%, but can range from 0.3-4.2% [1]. In many tumors, the medium tumor O₂ levels tend to be less than 2% [1]. Cancer research highly relies on maintaining hypoxic conditions in order to truly simulate the *in vivo* environments of tumors. Equipment most often used in this research include hypoxic chambers that can be placed into incubators or are stand-alone.

Anaerobic Conditions (No Oxygen)

In the life science realm, the lowest level of O₂, or being oxygen-free, is referred to as an anaerobic environment. Many microorganisms, including bacteria within the digestive tract of humans and at the bottom of the ocean are considered anaerobic species [4]. Since many of these species would be killed off by any trace of O₂, these microorganisms must be studied by scientists within an environment completely devoid of O₂. Typically, mammalian cell culture is not conducted within an anaerobic chamber, as these mammalian cells would not survive in an environment that is oxygen-free. Anaerobic chambers provide a 100% anaerobic environment by displacing the O₂ within the chamber with a vacuum and number of injected gases.

Equipment You May See in the Field

Multigas Incubator Chambers

Multigas incubators offer physiological mammalian conditions ideal for replicating *in vivo* environments. These incubators regulate O₂ levels by injecting nitrogen gas into the incubator chamber to displace excess atmospheric oxygen within the chamber space. To precisely regulate the nitrogen/oxygen balance, a zirconia sensor will detect the oxygen levels within the incubator and facilitate rapid recovery of nitrogen gas.

cell/Q™ Multigas Incubators MCO-170M Series



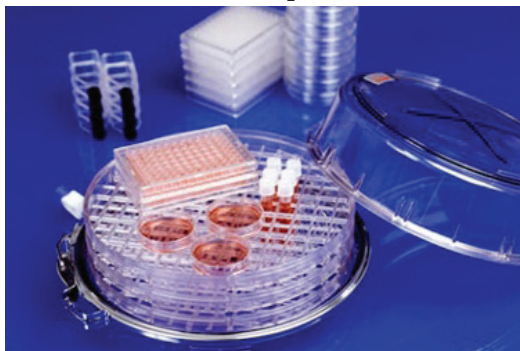
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Hypoxia Incubator Chambers

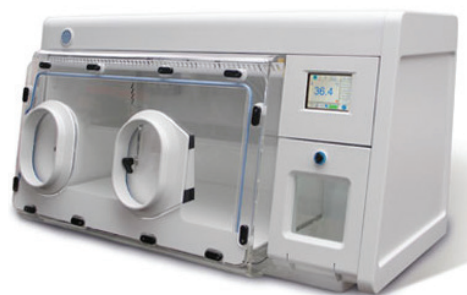
There are many types of hypoxic chambers. A hypoxic incubator chamber is a common tool used by scientists seeking to study cells at a lower oxygen level from 0.1-2%, but most offer the capability to adjust oxygen from 0.1-20%. These are most often used for pathological hypoxia studies.

**Multi-Plate Hypoxia Chamber
used within a CO₂ Incubator**



<http://www.brincubator.com/incubatorchamber.htm>

**Reach-In "Glove Box" Hypoxia Work Station
for Cell Handling Between Incubation Sessions**



<http://www.biocompare.com/Lab-Equipment/19290-Hypoxia-Hyperoxia-Incubators/>

Anaerobic Chamber

Anaerobic chambers are used for applications, which require zero oxygen. Anaerobic chambers provide a 100% anaerobic environment by displacing the oxygen within the chamber with a vacuum and number of injected gases. Tightly sealed interiors keep contaminants and outside air from affecting samples. These are most often used for microbiological studies which require anaerobic conditions.



http://www.tmaapr.com/pdf/COY_Product_Catalog_Anaerobic_911.pdf

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