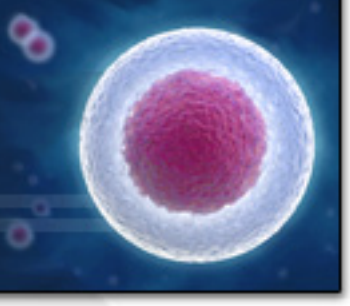
Application Note

MCQ Gas Blender 100 Series Application:

Dynamic gas mixtures with adjustable O_2 and CO_2 concentration in cell cultures study



Introduction

Starting from the pioneering studies made in the early 1900's, the cell culture process has been developed and refined to become one of the most important and widely used experimental method for the scientific research. The growth of cells is a complex process that requires appropriate and controlled conditions (such as pressure, temperature, and system pH) to be achieved. A crucial role is carried out by the gas mixture fluxed over the culture. The instrument for the gas mixtures management must secure high precision outcomes over the whole experiement duration; high accuracy, great stability and high repeatability are required. On the other hand it's also important to work with an instrument capable of creating gas mixtures dynamically adjustable since variation of the conditions for a particular cell type can result in gene/protein/enzyme expression changes. For all these applications we propose the use MCQ Gas Blender 100 Series, an instrument specifically made to work with 3 components gas mixtures that offers a quick and easy way to efficiently manage the experimental condition of your cell culture.

Common experimental setup

One of crucial aspect of the grown of cells in vitro is the complete control over the working parameters during the entire process. Even if the cell culture applications may vary widely from each other, growth medium represent one the most important of these parameter. The medium used is usually buffered with sodium bicarbonate/carbonic acid and for a proper cell grown the pH value must be strictly maintained constant. The amount of CO_2 in the culture atmosphere directly affects the medium pH, therefore the control over the gas mixture in which cells are grown becomes another fundamental process parameter. Due to the low content of CO_2 (<0,04%), the air isn't a valid option for cell culture. In most of the common applications the cells are grown in an atmosphere of 5-10% CO_2, inside special CO_2 incubators. The basic CO_2 incubators make use of gas mixture cylinders (pre-mixed by the supplier) in which the amount of CO_2 in the flow cannot be changed or adjusted by the user. Newer and more advanced incubators include a CO_2 sensor for a feedback regulation of the the amount of CO_2 inside the chambers but are less common and more expansive then the basic ones.

Dynamic gas mixtures applications

Working with static gas mixtures with fixed or adjustable amount of CO_2 represents now just the base of the cell culture applications. Since the last two decades until today many experiments have been successfully conducted with gas mixtures dynamically obtained and many more demands for instruments capable of controlling both O_2 and CO_2 relative amounts in the culture atmosphere. Fine control over the gas mixture allow those experiments, in which an induced hypoxic, hyperoxic or hypercapnic state is required, to be performed efficiently.

Dry air contains roughly 78.08% nitrogen, 20.95% oxygen, 0.92% argon, 0.04% carbon dioxide and other components in trace (mostly noble gases). An hypoxic gas mixture is by definition a mixture with a lower oxygen content compared with air. Basically the amount of O_2 in a hypoxic mixture goes from a minimum of 0% (this condition is called anoxia) to a maximum of 21%. On the contrary a gas mixture that exceeds 21% oxygen is defined hyperoxic. Inducing hypoxic condition over the cell cultures is a common experimental technique for the cardiovascular disease studies [1], for the tumor research [2,3], for the apoptosis [3,4], the gene expression [5] and for enzyme studies [6], while the hyperoxic condition is primary induced over the cells for the lung injuries study [7]. A hypercapnic mixture is prepared varying CO_2 concentration instead of O_2. Basically a hypercapnic mixture contains 21% oxygen and an increased amount of CO_2 compared with air. Studies conducted over cell cultures in hypercapnic condition have been useful for the collection of data about ischemia [8]. A combination between hypoxic and hypercapnic mixtures is also a common mixture configuration in many experiments [9,10].

The MCQ solution

Both the basic and the advanced CO_2 incubator are good instruments for standard cell culture applications but are inadequate when a dynamic gas mixture is required. Moreover neither the basic nor the advanced CO_2 incubators allow the control over the amount of O_2 in the culture atmosphere. For all those applications that require a dynamic gas mixture preparation and a complete control over the gas mixture composition, MCQ instruments suggests the use of the MCQ Gas Blender 100 Series. This instrument has been appositely designed for the management of 3 components dynamic gas mixtures and has been created to be versatile and adaptable to many lab-applications. MCQ calibrates its instruments with native gases. For cell culture applications the standard configuration is N_2 as the balance gas and O_2 and CO_2 as the solute gases but, under customer's request, the instrument can be set with other desired non-aggressive gas media. For each channel MCQ guarantees high accuracy (1.0% of set point), high repeatability (0.16% of reading value) and a fast response time for set point value

Dry Air

Composition		
	Nitrogen:	78.08%
	Oxygen:	20.95%
	Argon:	0.92%
	Carbon dioxide:	0.04%
	Others:	0.01%

Example of Hypercapnic miture

Composition		
	Nitrogen:	74%
	Oxygen:	21%
	Carbon dioxide:	5%

Example of Hypoxic mixture

Composition		
	Nitrogen:	95%
	Oxygen:	5%
	Carbon dioxide:	-

Example of Hyperoxic mixture

Composition		
	Nitrogen:	40%
	Oxygen:	60%
	Carbon dioxide:	-

Example of Hyperoic Hypercapnic mixture

Composition		
	Nitrogen:	76%
	Oxygen:	8%
	Carbon dioxide:	6%

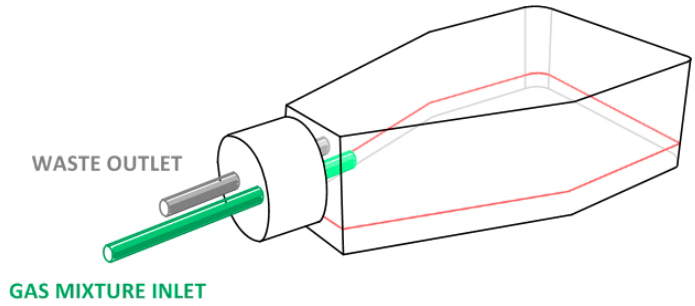
change (50ms). Along with the instrument a software for the gas mixture management, the MCQ Gas Mixer Manager, is also provided. The Gas Mixer Manager, manageable with any common desktop or laptop pc, has been designed to allow the user to take a complete control over the instrument and its functions. Easy and intuitive the Gas Mixer Manager allows to start working with dynamic mixtures immediately.

MCQ Gas Mixer Manager

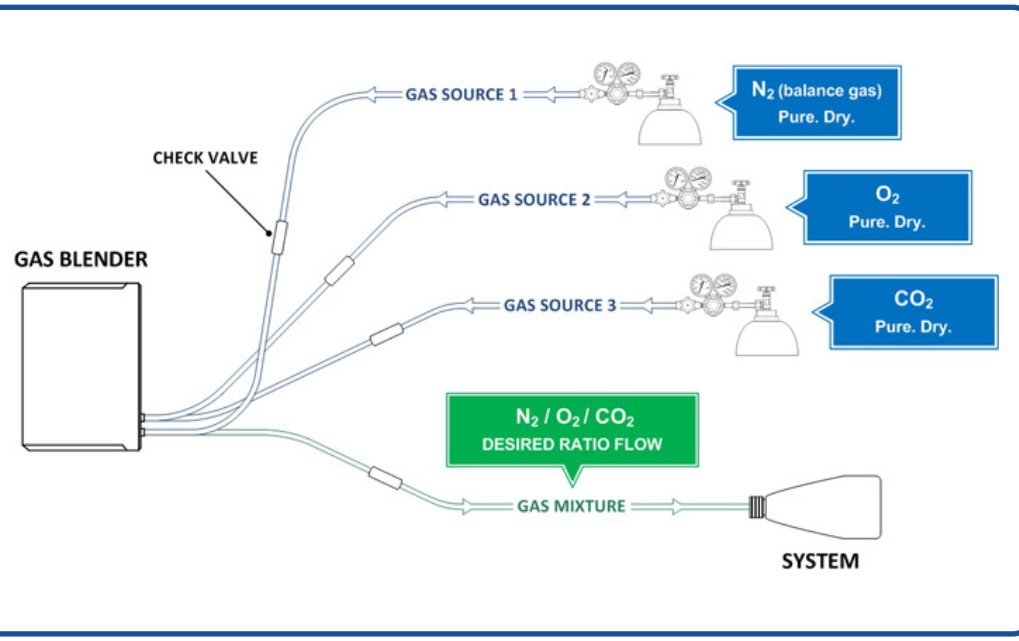
Hardware configuration

A simple example of MCQ Gas Blender 100 Series hardware configuration is represented in the image below. The instrument works with dry gases only. The gas sources can be both pure or mixtures (in our example pure gases have been chosen for simplicity). The gas cylinders are connected to the instrument through 6 mm diameter tubes and a check valve is installed along each line as backflow prevention device. Each gas media is connected and controlled by one of the 3 channels of the Gas Blender 100. Another 6 mm tube finally connects the instrument to the working system in which the cells are grown. The image below shows an example of working system composed by a typical cell culture flask with an inlet for the gas mixture and an outlet for the waste that prevents a pressure increase inside the system itself. The relative amount of O_2 and CO_2 in the outcoming mixture can be easly adjusted, monitored and modified by the user thanks to the MCQ Gas Mixer Manager software.

SYSTEM: Cell culture flask



Experimental Set-up



References

[1] J. G. Murphy, T. W. Smith and J. D. Marsh, *Calcium flux measurements during hypoxia in cultured heart cells* – J. Mol. Cell. Cardiol. 19, 3 (1987) 271-279.

[2] I. Ginis, D. V. Faller, *Hypoxia affects tumor cell invasiveness in vitro: the role of hypoxia-activated ligand HAL1/13 (Ku86 autoantigen)* – Cancer Lett. 154, 2 (2000) 163-174.

[3] M. Weinmann, P. Marini, V. Jendrossek, A. Betsch, B. Goecke, W. Budach and C. Belka, *Influence of hypoxia on TRAIL-induced apoptosis in tumor cells* - Int. J. Radiat. Oncol. Biol. Phys. 58, 2 (2004) 386-396.

[4] S. M. Wang and W.-L. Yang, *Circulating hormone adrenomedullin and its binding protein protect neural cells from hypoxia-induced apoptosis* – BBA General Subjects 1790, 5 (2009) 361-367.

[5] L. Flamant, S. Toffoli, M. Raes and C. Michiels, *Hypoxia regulates inflammatory gene expression in endothelial cells* - Exp. Cell Res. 315,5 (2009) 733-747.

[6] J. Fan, H. Cai, S. Yang, L. Yan and W. Tan, *Comparison between the effects of normoxia and hypoxia on antioxidant enzymes and glutathione redox state in ex vivo culture of CD34+ cells* - Comp. Biochem. Physiol. B Biochem. Mol. Biol. 151,2 (2008) 153-158.

[7] K. Y. Bhakta, W. Jiang, X. I. Couroucli, I. S. Fazili, K. Muthiah and B. Moorthy, *Regulation of cytochrome P4501A1 expression by hyperoxia in human lung cell lines: Implications for hyperoxic lung injury* - Toxicol. Appl. Pharmacol. 233, 2 (2008) 169-178.

[8] A. L. Russ, K. M. Haberstroh and A. E. Rundell, *Experimental strategies to improve in vitro models of renal ischemia* - Exp. Mol. Pathol. 83, 2 (2007) 143-159.

[9] J. M. Kramer, P. C. Nolan and T. G. Waldrop, *In vitro responses of neurons in the periaqueductal gray to hypoxia and hypercapnia* - Brain Res 835, 2 (1999) 197-203.

[10] K. Vaporidi, C. Tsatsanis, D. Georgopoulos and P. N. Tsichlis, *Effects of hypoxia and hypercapnia on surfactant protein expression proliferation and apoptosis in A549 alveolar epithelial cells* - Life Sci. 78, 3 (2005) 284-293.