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An Inexpensive Incubator for Mammalian Cell Culture Capable of Regulating O₂, CO₂, and Temperature

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Abstract:

Mammalian cell culture is widely used for discovery and development. Recently, increasing attention has been paid to the importance of maintaining physiologically-relevant conditions in cell culture. Although oxygen level is a particularly important consideration, it is rarely regulated by experimentalists. The atmospheric O₂ levels commonly used in cell culture are significantly higher than those experienced by most mammalian cells *in vivo*, leaving cells susceptible to oxidative damage, senescence, transformation, and otherwise aberrant physiology. A barrier to incorporating O₂ regulation into most cell culture workflows has been the expense of investing in new equipment, as the vast majority of laboratory CO₂ incubators do not regulate O₂. Here, we describe an inexpensive (<CAD 1000), portable and user-friendly O₂/CO₂ incubator that can establish and maintain physiological O₂, CO₂, and temperature values within their physiological ranges. We used an Arduino-based approach to add O₂ and CO₂ control to a temperature-regulating egg incubator. Our incubator was tested against a commercial laboratory O₂/CO₂ incubator. Using PreSens OxoDish technology, we demonstrate that at a setpoint value of 5% gas-phase incubator O₂, media O₂ averaged 5.03 (SD = 0.03) with a range of 4.98–5.09%. MCF7, LNCaP and C2C12 cell lines cultured in the incubator displayed normal morphology, proliferation, and viability. Culture for up to one week produced no contamination. Thus, our incubator provides an inexpensive means of maintaining physioxia in routine mammalian cell culture.

Keywords: oxygen, physioxia, hypoxia, cell culture, incubator