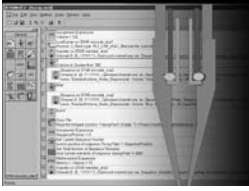


Technical Note



New Feature: Online pH Monitoring with the pH-Reader

Introduction

Hamilton and Global Cell Solutions recently launched the BioLevigator™ a revolutionary benchtop cell culture device which can grow four independent and high density cell cultures at once. The BioLevigator™ actively monitors and maintains the culture conditions, including temperature and CO₂. The Reader for pH monitoring is now available as an option to complement temperature and CO₂ control.

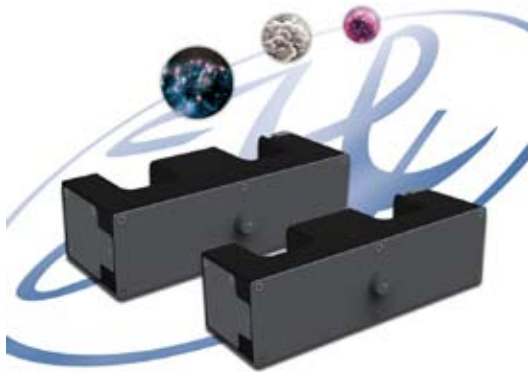


Figure 1: The pH-Reader of the BioLevigator™

Principle of pH Monitoring

Phenol red is a pH indicator present in most cell culture media. Its color exhibits a gradual transition from red to yellow over the pH range 8.4 to 6.6 (figure 2).

Acid yellow	Transition zone pH 6.6 - 8.4	Basic red
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Figure 2: The Colors of Phenol Red

The pH-Reader of the BioLevigator™ monitors the quality of the cell culture medium by measuring the absorbance at 560 nm of the cell culture medium in the Levi-Tube™. The absorbance at 560nm is used to determine the color of the medium and hence its pH.

Two Monitoring Modes

Two monitoring modes can be enabled in the BioLevigator™ user interface: a qualitative color mode and a semi-quantitative pH mode. In the color mode, the absorbance of the culture medium at 560 nm is directly reported. It increases when pH increases and the medium turns red.

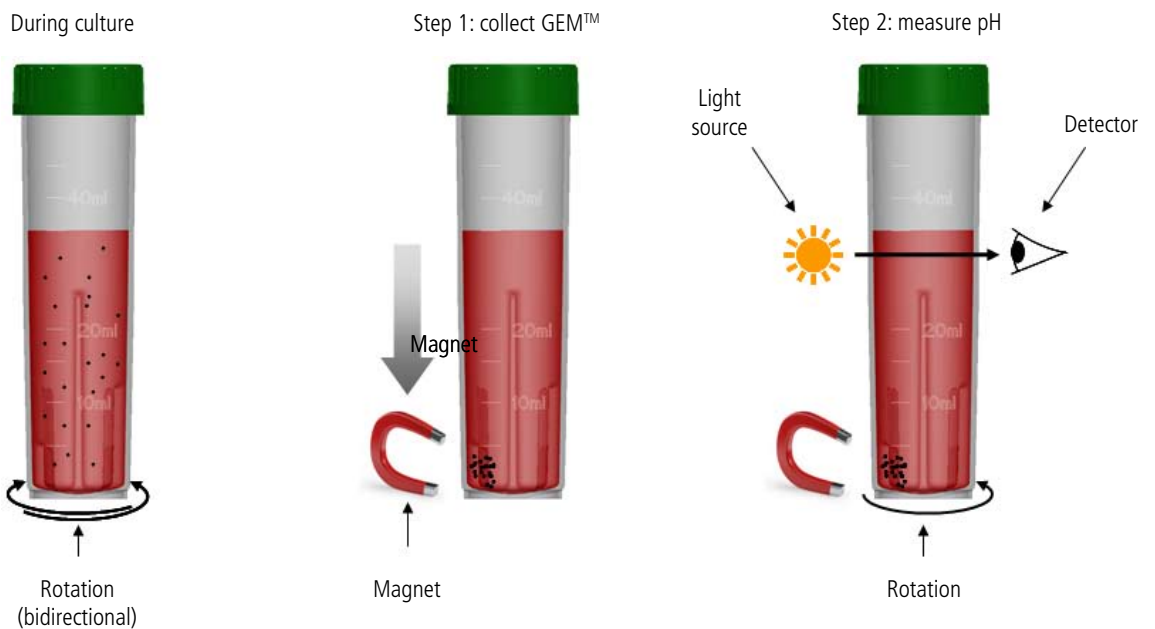


Figure 3: pH Measurement Step by Step

In the pH mode, the pH value is calculated from the measured absorbance using additional parameters, including the extinction coefficient, the acidity constant and the concentration of the pH indicator.

Figure 3 describes every steps required for pH monitoring. The culture protocol is shortly interrupted for the Reader to monitor pH. The GEM™ are collected at the bottom of the tube with the integrated magnet to avoid interference with the absorbance measurement. A slight rotation is applied to monitor pH at different angle of the Levitube™. A minimum medium volume of 35 mL is required for pH monitoring.

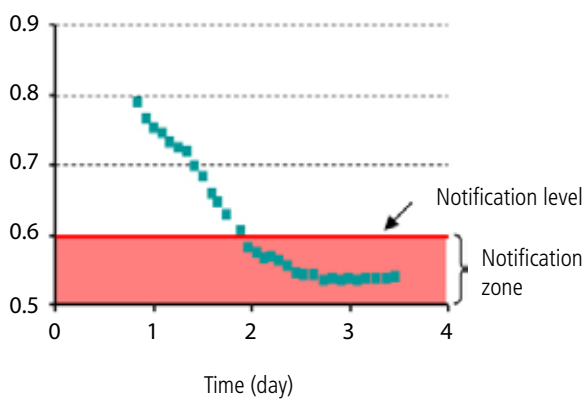
When cultures are complete, all data are saved in a culture report and can be transferred to a computer for analysis using the BioLevigator's USB port.

pH Monitoring during CHO Culture

6 million CHO cells were inoculated in the BioLevigator™ with 0.5 mL GEM™ in 10mL DMEM/F12. After 4 hours inoculation, the volume of culture medium was increased to 35 mL and the culture protocol started. The pH of the cell culture medium was monitored using the qualitative color mode (Figure 4A) and the semi-quantitative pH mode (Figure 4B).

A CHO Culture

Color (absorbance)



Both absorbance and pH decrease as cells are growing. The user can be notified when the measured absorbance or pH drops below the defined notification level. In the example, the color notification level has been set at 0.6 and the pH notification level at 6.8.

Analysis of the systematic errors shows that media with indicator concentrations in the range of 0.02mM to 0.04 mM give acceptable results. The overall precision of this measurement is estimated to be $\Delta\text{pH} = \pm 0.1$ in the range of $6 < \text{pH} < 8$. It is recommended to check the results of a particular cell culture composition against a standard pH glass electrode as a reference.

Conclusion and Outlook

The BioLevigator™ is a convenient fully-equipped cell culture incubator which actively monitors and maintains the culture conditions, including temperature and carbon dioxide. The new pH-Reader monitors the quality of the cell culture medium via a non-invasive optical absorption measurement. The pH Reader enables medium change on need rather than on schedule and hence optimizing the consumption of culture medium.

Further information on the BioLevigator @ www.biolevigator.com

B CHO Culture

pH

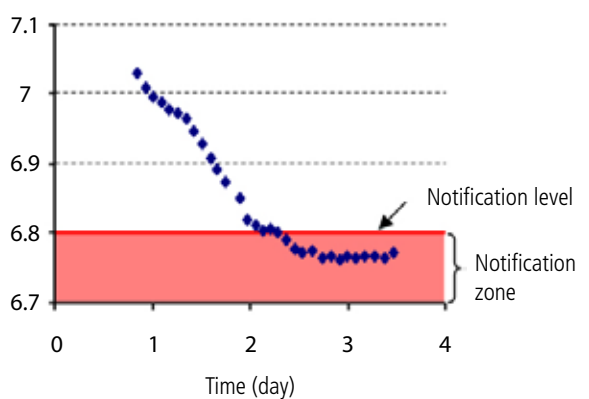


Figure 4: Online monitoring of the culture of CHO cells in the BioLevigator™ in the color mode (A) and in the pH mode (B).

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