SENSOLUX® stand-alone version: noninvasive determination of pH and DO in shake flasks

The determination of growth kinetic parameters during early process development is of crucial importance. The more information output is obtained, the more optimization strategies can be applied to generate highly productive cell lines. The new SENSOLUX® technology enables an optical and noninvasive measurement of pH and dissolved oxygen saturation in shake flasks. This technology provides a simple detection method suitable for effective final clone screening and medium optimization.

Sartorius Stedim Biotech now introduces the SENSOLUX® technology, which enables an optical and noninvasive measurement of the pH value and the dissolved oxygen saturation (DO) during the cultivation of animal and human cells. The first member of this product line is the SENSOLUX® stand-alone, an intelligent shaker tray with an integrated sensor system. Used in combination with the new single-use SENSOLUX® Erlenmeyer Flasks (EF), it facilitates the easy, safe and highly informative online measurement of these crucial process parameters in incubation shakers. SENSOLUX® EF are equipped with two precalibrated sensor patches that are sensitive to pH and DO, respectively. The sensor system integrated into the tray monitors both parameters optically and noninvasively from outside the SENSOLUX® EF.

The SENSOLUX® technology is based on the principle of fluorescence. The sensor patches contain fluorescent dyes that can be excited with light of a given wavelength. The SENSOLUX® shaker tray contains nine independent optical sensors. Optical fibers integrated into the shaker tray transmit light of a particular wavelength to the sensor patches; at the same time, they also transmit the luminescence response from the patches to a measuring amplifier. The characteristics and intensity of the light emitted is influenced by changes in the concentration of the parameters pH or DO, respectively (Fig. 1).

We evaluated the SENSOLUX® technology under different conditions. Here we compare the accuracy of the pH and DO patches to that of standard pH and DO electrodes. We also analyze the cultivation of Chinese hamster ovary (CHO) cells by using the SENSOLUX® EF.

SENSOLUX® versus standard technology

We evaluated the SENSOLUX® pH patch technology in comparison to the standard electrochemical pH electrode by implementing the patch into a BIOSTAT® A plus 1-liter glass vessel. A pH profile (6.05–8.60)

was induced by a controlled titration (0.4 M NaOH, 0.4 M HCl) in a phosphate-based buffer system (0.05 M per liter, pH 8.6, 0.15 M ion strength with NaCl). The temperature was controlled via the control unit at 37 °C, and data were detected with an interval of 6 s.

We achieved a maximum deviation smaller than ± 0.1 pH units with a

Figure 1 | SENSOLUX® stand-alone version: product picture and schematic.

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APPLICATION NOTES

Figure 2 | SENSOLUX[®] patch technology versus standard electrochemical pH and DO electrodes. **(a)** A pH profile (6.05–8.6) was induced by titration (detection interval, 6 s). **(b)** Under a controlled gas atmosphere (50% DO air saturation), the DO was detected every 15 s.

detection interval of 6 s during a 19-h experiment (**Fig. 2a**). This signifies that 11,600 measurements can be carried out with this low deviation range. For real-time cultivation, this means, for example, a cultivation time of 16 d with a detection interval of 2 min. During our studies, the pH showed a drift of 0.01 units per day.

We also evaluated the SENSOLUX[®] DO patch technology in comparison to the standard Clark electrode by using a 250-ml Erlenmeyer flask equipped with both technologies. We used the same phosphate buffer system as described above and ensured a controlled environment by carrying out the experiment in the CERTOMAT[®] IS incubation shaker (37 °C, 40 r.p.m., orbit 50 mm). Using a sparger implemented in the E-Flask, nitrogen was added with a gassing rate of 0.5 liter per min. The DO drift of both technologies was monitored over 35 h at an interval of 15 s. The initial DO was 100% air saturation. To achieve a constant gas environment in the flasks, nitrogen and air were added (both 0.5 liter per min) during the complete test. After 10 h, a constant gas condition could be achieved in the system, and the drift study commenced.

The comparison between the DO patch technology and a standard Clark electrode showed similar curve progressions over the course of the experiment (**Fig. 2b**). We detected a deviation of 0.2% at a lower (1%) air saturation and 1.0% at a higher (100%) air saturation. Up to 8,600 light spots, the SENSOLUX[®] DO patches had an average drift of 0.08% per hour (data not shown; detection interval, 15 s). For a real-time cultivation, this implies a drift of 0.02% per day at 50% air saturation with a detection interval of 5 min.

Figure 3 | Growth of CHO cells in single-use SENSOLUX[®] EF. CHO cells were batch-cultivated in three single-use SENSOLUX[®] EF 250 ml (EF1, EF2 and EF3) at 37 °C, 200 r.p.m. (25 mm orbit), 5% CO₂ and 85% humidity. The graph shows the pH value during the cultivation time as determined by SENSOLUX measurements (online) and standard measurement (offline).

Cultivation CHO cells in single-use SENSOLUX[®] EF

CHO cells were grown in SENSOLUX[®] EF 250 ml (proCHO5 medium, 4 mM L-glutamine, 1× HT) at 37 °C in an incubation shaker (200 r.p.m., 25 mm orbit) with a CO₂ saturation of 5% and a humidity of 85%. The working volume was 50 ml; starting cell density was 5×10^5 cells per ml. Samples for subsequent analyses were collected once a day. Beside the online, fluorescence-based measurement of pH and DO, samples were also collected for external pH measurement by using a standard pH electrode and amplifier. The sample vials were prewarmed (37 °C) and were closed immediately after sampling to avoid temperature and CO₂ shifts.

In the course of the cultivation in the SENSOLUX[®] EF, CHO cells achieved a maximum viable cell density of 5×10^6 cells per ml (data not shown). The cells were not oxygen limited during the total cultivation time (DO = 90–100%). A maximum deviation 0.04 units was detected when comparing the external measured pH with the online fluorescence-based detection method, which was in the described deviation range of 0.1 pH units (**Fig. 3**).

Conclusion

The SENSOLUX[®] patch technology produced similar results to those of the standard pH and DO electrodes, indicating that this technology is a useful alternative to the standard electrochemical pH and DO measurement. Both accuracy and drift of the new technology are comparable to the commonly used electrodes. Thus, the SENSOLUX[®] stand-alone version is a suitable tool for providing conclusive results in the early process-development phase—for example, advanced clone screening and medium optimization.

ACKNOWLEDGMENTS

I thank D. Riechers, J. Lueders, M. Hantke and Y. Stark of Sartorius Stedim Biotech GmbH, Goettingen, Germany, for their support and for providing their data.

This article was submitted to *Nature Methods* by a commercial organization and has not been peer reviewed. *Nature Methods* takes no responsibility for the accuracy or otherwise of the information provided.