Getting Started with VisiSens™ -

Notes on O_2 & pH in Cell Culture

1. Sensor foils are usually not delivered sterile. Beta-irradiated sensor foils are available upon request.
   O_2 sensor foils can be treated with 70 % ethanol or ethylene oxide.
   pH sensor foils can be treated with 10 % ethanol.
   Autoclavation, steam sterilization or sterilization via UV-light is **NOT** possible.

2. Please use black well plates with a flat, clear bottom for your experiments. Clear well plates do not shield background light which may disturb your image recording. The images are taken through the transparent bottom of the plates. For exclusion of ambient light, please cover the lid of the well plates or perform your experiments in the dark (e. g. in an incubator).
   Beta-irradiated well plates with pre-integrated sensors are available upon request.

3. Sensor foils can be cut into desired size or shape using scissors.

4. Please do not use colored buffer or media!

5. pH Sensor foils have to be equilibrated in medium for at least 3 hours before coating, cell seeding, calibration or measurement.

6. Although the sensor foils are highly biocompatible not all cell types adhere and grow on the sensor foils spontaneously. Recommended coatings are:
   - 25 µg/mL fibronectin diluted in water (millipore, sterilized), apply for 1 h at room temperature
   - 100 µg/mL poly-L-lysine diluted in water, apply for 1 h at room temperature
   - fetal calf serum, apply for 1 h at room temperature
   - 0.5 % gelatine in water, apply for 1 h at 37 °C

7. A two point calibration is required for the quantitative image analysis of O_2 sensor foils. Please follow the links for a video tutorial and a description on how to prepare the calibration solutions:
   https://www.presens.de/support-services/videos/video/visisens-analytical-1-calibration-936.html
   https://www.presens.de/support-services/faqs/question/how-can-i-prepare-the-calibration-solutions-cal0-and-cal100-for-oxygen-sensors-35.html

8. The foils for pH imaging require at least a 6-point calibration. The calibration is ideally performed in the experiment set-up. At least, the calibration set-up should be as similar as possible to the final measurement set-up (identical temperature, ambient light conditions, camera settings, distance to the sample, ionic strength,…). As an accessory you can make use of the CaliPlate. (https://www.presens.de/products/detail/caliplate-for-sf-hp5r.html or https://www.presens.de/products/detail/caliplate-for-sf-lv1r.html).

9. Do not use pH sensor foils in tap or distilled water! Minimum ionic strength for the pH sensors is 50 mM, and minimum buffer capacity is 2 mM.

10. Store the sensors in the dark. Once the sensors have been placed in liquid for equilibration, and you would like to reuse them later, please store them in buffered medium.

11. Avoid any contact of the sensitive layer with glue or oily substances like hand cream.

12. Examples for suitable calibration buffers for pH sensor foil calibration (please adjust the ionic strength to the ionic strength of your sample medium):

Buffer recipe for PBS buffer (usually used for calibration of **SF-HP5R**):

Preparation of 1 L Stock Solution					
	buffer capacity	total ionic strength	NaH_2PO_4 * 1 H_2O	Na_2HPO_4 * 2 H_2O	NaCl
Solution A	40 mM	140 mM	5.5 g		5.8 g
Solution B	40 mM	140 mM		7.1 g	1.2 g

Buffer recipe for citrate buffer (usually used for calibration of **SF-LV1R**):

Preparation of 1 L Stock Solution					
	buffer capacity	total ionic strength	$Na_3C_6H_5O_7$ * 2 H_2O	$C_6H_8O_7$	NaCl
Solution A	40 mM	140 mM	11.8 g		1.2 g
Solution B	40 mM	140 mM		7.7 g	8.2 g

Solution A and solution B are mixed respectively to create calibration buffers of different pH values but constant ionic strength.

Webinar

O2 & pH in Cell Cultures, Engineered & Native Tissue: https://www.presens.de/support-services/videos/video/visisens-webinar-o2-ph-in-cell-cultures-engineered-native-tissue-1050.html

Related Application Notes

https://www.presens.de/knowledge/publications/application-note/monitoring-po2-in-cell-culture-to-improve-directed-differentiation-641.html

https://www.presens.de/knowledge/publications/application-note/cellular-oxygen-consumption-in-microfluidic-devices-612.html

https://www.presens.de/knowledge/publications/application-note/imaging-the-oxygen-consumption-of-microbial-cultures-640.html

https://www.presens.de/knowledge/publications/application-note/monitoring-skin-tissue-oxygenation-in-mice-618.html

https://www.presens.de/knowledge/publications/application-note/monitoring-oxygen-in-a-mouse-model-of-renovascular-hypertension-623.html