PreSens

PreSens Precision Sensing

Manual

OxoPlate® OP96U

OxoPlate® OP96C

TABLE OF CONTENTS

1 Preface 3
2 Measurement Principle of the OxoPlate® 4
3 Instrumentation 5
3.1 Fluorescence Reader 5
3.1.1 Important note for users of BMG fluorescence plate readers 5
3.2 Filter Pairs 5
4 Reader Calibration 6
4.1 Preparation of the Calibration Standards 6
4.2 Two-point Calibration of Your Reader 7
5 Measurement of Oxygen Concentration 9
6 Technical Data 11
6.1 Performance Specification 11
6.2 Storage of the OxoPlate 11
6.3 Cross Sensitivity 11
7 Contact Address 12

1 PREFACE

This manual will explain the use of the following types of OxoPlates®:

1. OxoPlate® OP96U
2. OxoPlate® OP96C

For most standard applications OxoPlate® OP96U is the best choice.

The OxoPlates® OP96U are round bottom microplates whereas OxoPlate® OP96C is a flat bottom microplate.

2 MEASUREMENT PRINCIPLE OF THE OXOPLATE®

The OxoPlate® is a **sterile microplate** in the common 96 well format with **integrated sensors**. A sensor is immobilized on the bottom of each well (figure 1). The sensor can be read out from the bottom like depicted in figure 1. This is done by a commercially available fluorescence reader.

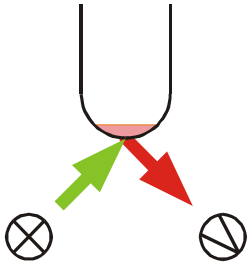

Figure 1 Measurement principle of OxoPlate®

Each well has a sensor on its bottom which can by read out from the bottom by a commercially available fluorescence reader

The sensor contains **two different dyes**. One is the oxygen indicator. Its phosphorescence intensity $I_{indicator}$ is dependent on the concentration of oxygen in the sample filled into the well. The other dye is the reference. Its fluorescence intensity $I_{reference}$ is independent of the oxygen concentration. From these two luminescences (intensities) the ratio I_R has to be calculated. This referenced signal I_R corresponds to the concentration of oxygen. Response times are very low because the extremely thin sensor is permeable for even non-gaseous permeates (It measures directly 'in' the solution.).

$$I_R = \frac{I_{indicator}}{I_{reference}} \qquad \textit{equation 1}$$

It is necessary to calibrate the reader once before the first measurement. 8 wells of a OxoPlate® are needed for this calibration procedure . Subsequent used OxoPlates are **calibration-free**.

Re-calibration of the reader from time to time (see chapter 3, reader calibration) can be necessary.

3 INSTRUMENTATION

3.1 Fluorescence Reader

The OxoPlate® can be read out by any commercially available fluorescence reader (fluorescence intensity reader) which can read out from bottom side. Because the sensor contains two different dyes the reader should be capable of measuring in the ***dual kinetic mode*** *(e.g. Labsystems Fluoroskan Ascent, BMG Fluostar or Wallac Victor 2…)*... This means, that two readings are taken several times from each measurement point (each well) using two different filter pairs. If you are not sure about the capability of your fluorescence reader, please contact your reader dealer.

3.1.1 Important note for users of BMG fluorescence plate readers

With BMG readers much better signals are observed, if the time-resolved modus is used. Set *Integration start* for both filter combinations to 0 µs and *Integration time* to 500 µs.

With this option the detector collects more light to receive higher resolution. For further questions: please contact our service team: *info@presens.de*.

3.2 Filter Pairs

Two filter pairs (4 filters) are required to measure the oxygen concentration in the OxoPlate®.

Filter pair 1: 540 / 650 nm

Filter pair 2: 540 / 590 nm

The first filter pair (Filter pair 1) detects the luminescence of the indicator dye $I_{indicator}$, the second filter pair (Filter pair 2) measures the luminescence of the reference dye $I_{reference}$.

Although this combinations are ideally suited, similar filter combinations may work as well. If you are in doubt, please contact our service team (*info@presens.de*).

4 READER CALIBRATION

The referenced signal I_R is correlated to the oxygen concentration in the well. Because the performance of each fluorescence reader is different, its characteristic has to be determined by a two-point calibration

4.1 Preparation of the Calibration Standards

The calibration of the used fluorescence reader is performed using a two-point calibration in ***oxygen-free water*** (cal 0) and ***air-saturated water*** (cal 100).

Preparation of calibration solution cal 0 (oxygen-free water)

1. Add one gram sodium sulfite (Na_2SO_3) to a suitable vessel and label it “cal 0”.
2. Dissolve Na_2SO_3 in 100 ml water

   Water becomes oxygen-free due to a chemical reaction of oxygen with Na_2SO_3. Additional oxygen, diffusing from air into water, is eliminated (removed) by surplus of Na_2SO_3.
3. Close the vessel after calibration with a screw top to minimize oxygen contamination.
4. Wait approximately one minute after all Na_2SO_3 is dissolved to ensure that water is oxygen-free.

The shelf life of “cal 0" is about 24 hours provided that the vessel has been closed with the screw top.

Preparation of calibration solution cal 100 (air-saturated water)

1. Add 100 ml water to a suitable vessel and label it “cal 100”.
2. Close the vessel using a screw top.
3. Shake this vessel rigorously 2 minutes to ensure that water is air-saturated

The shelf life of “cal 100" is about 3 days provided constant temperature.

Note: It is not recommended to prepare a cal 0 solution with cell culture media because cell culture media can contain substances which consume Na_2SO_3.

4.2 Two-point Calibration of Your Reader

The signal I_R for solution cal 0 and solution cal 100 filled in the OxoPlate® has to be measured with the fluorescence reader to receive the constants k_0 and k_{100}, respectively. If this has been measured once using 8 wells of one OxoPlate, subsequent OxoPlates can be measured (on the same reader and with equal settings) without recalibration.

OxoPlates are calibration-free.

Note 1: Perform the calibration measurement at the same temperature as your later measurements.

Note 2: While OxoPlate® shows constant behavior from plate to plate, commercially available fluorescence readers exhibit drift phenomena in their optics. Therefore we recommend to repeat the reader calibration every week.

Performing the measurement

1. Switch on your fluorescence reader and let the warm-up time pass by (see instructions of your fluorescence reader).
2. Prepare your reader to measure the whole first row (row 1) with the two filter pairs (filter pairs described in chapter 3.2 see instructions of your fluorescence reader).
3. Take one OxoPlate® and fill 200 µl of solution cal 100 in the wells A1, B1, C1 and D1.
4. Fill 200 µl solution cal 0 in the wells E1, F1, G1 and H1 of the same OxoPlate® as in the previous step.
5. Close the OxoPlate® with its cover.
6. Place the OxoPlate® inside the reader (or any other tempered containment) at the desired temperature (e. g. 37°C) at least 15 minutes to ensure constant temperature throughout the whole plate.
7. Measure the filled wells once with the recommended filter pairs (see 2.2. Filter Pairs).

Calculation of the constants k_0 and k_{100}

Calculate the reader-constants k_0 and k_{100} as shown below.

1. Calculate the referenced signal I_R of each well by using equation 1.
2. Calculate k_{100} by taking the average of the signals I_R of wells A1, B1, C1 and D1.

$$k_{100} = ¼ \cdot \left(I_R(A1) + I_R(B1) + I_R(C1) + I_R(D1) \right) \qquad \textit{equation 2}$$

3. Calculate k_0 by taking the average of the signals I_R of wells E1, F1, G1, H1.

$$k_0 = ¼ \cdot \left(I_R(E1) + I_R(F1) + I_R(G1) + I_R(H1) \right) \qquad \textit{equation 3}$$

Taking the average of four wells increases the accuracy of your later oxygen measurement.

Example

The values in table 1 represent a calibration result. The fluorescence reader measures the values $I_{indicator}$ and $I_{reference}$ (the values of your reader can be different). Calculate the signal I_R applying equation 1 and the constants k_{100} and k_0 applying equations 2 and 3, respectively. The results are shown below.

Table 1 Example: Reader Calibration.

Well	Measured values		Calculated value	Average value
	$I_{indicator}$	$I_{reference}$	I_R	
A1	328	245	1.34	
B1	360	267	1.35	
C1	308	235	1.31	**k_{100}**
D1	273	210	1.30	**1.325**
E1	892	224	3.97	
F1	592	151	3.91	
G1	945	240	3.93	**k_0**
H1	990	250	3.96	**3.943**

5 MEASUREMENT OF OXYGEN CONCENTRATION

Performing the measurement

Perform the measurement according to the instructions of your reader. Remember to measure the two filter combinations.

Evaluation of the measurement

1. Calculate for each measurement point the signal I_R by using equation 1.
2. The oxygen concentration **pO_2** as [%] air saturation is calculated for each measurement point by using equation 4

$$pO_2 = 100 \cdot \left(\frac{k_0}{I_R} - 1\right) / \left(\frac{k_0}{k_{100}} - 1\right) \qquad \textit{equation 4}$$

This value can be transferred to other units like pO_2 [Torr], pO_2 [hPa] or cO_2 [ppm]. Therefore you have to multiply the pO_2 from equation 4 by a factor f. For T = 25 °C and p = 1013 hPa you find this factor f in table 1. For other temperatures and atmospheric pressures PreSens delivers an Excel sheet to calculate the factor f. Please contact our service team.

Table 2 Multiplication factors f to convert measured air saturation to other units
Valid for: 1013 hPa, T = 20 °C

Unit	Multiplication factor f
[%] oxygen saturation	0.209
pO_2 [hPa]	2.074
pO_2 [Torr]	1.556
cO_2 [mg/l]	0.0905
cO_2 [ppm]	0.0905
cO_2 [µmol/l]	2.8295

Example

In one well you have taken a kinetic measurement with 7 subsequently measured points. An oxygen consuming process is going on (i. e. more oxygen is consumed than entered into the well by diffusion from air to water, typical for bacterial growth or many enzymatic reactions).

The signals $I_{indicator}$ and $I_{reference}$ are measured using a fluorescence reader (the values of your reader can be different). The value I_R is calculated using equation 1. Thereafter the oxygen concentration is calculated applying equation 4. The reader constants k_0 = 3.943 and k_{100} = 1.325 are taken from the example in chapter 3.

The figures of this example are shown in Table 3.

Table 3 Example for a kinetic measurement of a oxygen consuming process.

Well xx	**Measured values**		**Calculated values**	
time [min]	$I_{indicator}$	$I_{reference}$	I_R	pO_2 [%] air saturation
0	325	245	1.327	100
10	405	244	1.660	70
20	504	246	2.049	47
30	600	243	2.469	30
40	712	246	2.894	18
50	852	245	3.478	7
60	899	244	3.684	4

6 TECHNICAL DATA

6.1 Performance Specification

Measuring range	0 % to 150 % air saturation
Resolution (at 37 °C)*	up to 1 % air saturation*
Accuracy (at 37°C)*	up to 3 % air saturation*
Temperature range	15 – 45 °C
pH drift per hour*	< 1 % air saturation
Types of plates offered	96 well round bottom microplates
Response time (t_{90}) (at 37°C)	< 30 sec
Indicator filters	540 / 650 nm
Reference filters	540 / 590 nm

* performance dependent on used reader

6.2 Storage of the OxoPlate

OxoPlates should keep dark and cool (under 20°C). To obtain best results open the package just before use.

6.3 Cross Sensitivity

OxoPlate® sensors are not affected by pH, salinity, carbon dioxide (CO2), hydrogen sulfide (H2 S), ammonia (NH3) or amino acids. Gaseous sulfur dioxide (SO2) and gaseous chlorine (Cl2) interfere.

For self fluorescing or highly scattering media a different calibration method may be necessary. If you are in doubt, please contact our service team (*info@presens.de*).

7 CONTACT ADDRESS

PreSens
Precision Sensing GmbH
Josef-Engert-Straße 9
D-93053 Regensburg

Phone	+49 (0)941 942720
Fax	+49 (0)941 9427227
Email	info@presens.de
Internet	www.presens.de