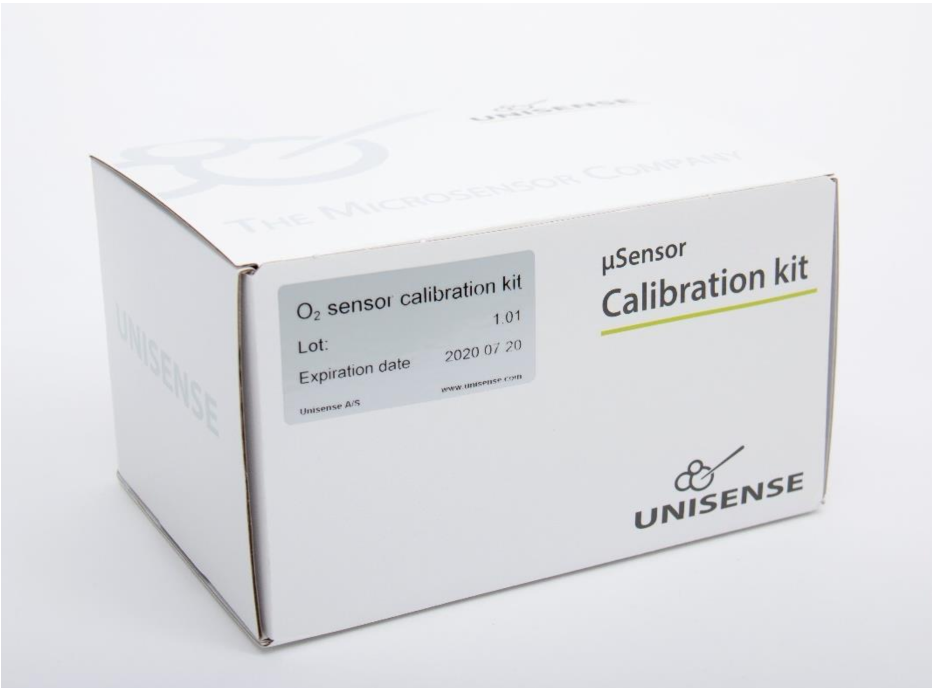
O_2 sensor calibration kit

For electrochemical and optical oxygen sensors

Manual



UNISENSE

Contents

O_2 CALIBRATION KIT USER MANUAL

Version: May 2023

1 Warranty and liability

1.1 Notice to Purchaser

This product is for research use only. Not for use in human diagnostic or therapeutic procedures.

1.2 Warning

Microsensors have very pointed tips and must be handled with care to avoid personal injury and only by trained personnel. Unisense A/S recommends users to attend instruction courses to ensure proper use of the products.

1.3 Warranty and Liability

The Oxygen Calibration Kit is guaranteed to give a calibration solution with zero oxygen until expiry as indicated on the package label. The warranty does not include replacement necessitated by accident, neglect, misuse, unauthorized repair, or modification of the product. In no event will Unisense A/S be liable for any direct, indirect, consequential or incidental damages, including lost profits, or for any claim by any third party, arising out of the use, the results of use, or the inability to use this product.

2 Support, ordering, and contact information

If you wish to order additional products or if you encounter any problems and need scientific or technical assistance, please do not hesitate to contact our sales and support team. We will respond to your inquiry within one working day.

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Further documentation and support are available at our website: www.unisense.com.

3 Content of the calibration kit

Item	Number
Exetainer with zero O_2 solution	10
Calibration cap with O-ring and 3 cm Viton tubing	1
10 ml syringe	1
80 x 2.1 mm needle (green)	1

Figure 1: Calibration kit contents: A: Calibration kit box with Exetainers, B: 80 x 2.1 mm needle (green), C: 10 ml syringes, D: Calibration Cap with tubing, E: O-ring.

4 Principle of calibration

Unisense electrochemical O_2 sensors respond linearly to O_2 concentrations within their linear range (see specifications for your sensor at https://www.unisense.com/O2). Likewise, the processed signal of Unisense optical oxygen sensors (optodes) respond linearly to O_2 concentrations (see https://www.unisense.com/MicroOptode). Therefore, a two-point calibration is sufficient for both types of sensors, one point is the signal for zero O_2 and the other point is the signal for a known O_2 concentration.

This calibration kit contains Exetainers with a zero O_2 solution. A solution with a known O_2 concentration can be made by bubbling water, at a known temperature and salinity, with atmospheric air. The O_2 concentration at equilibrium can be looked up in a table (Table 1) or by using the built in O_2 solubility function in SensorTrace Suite. This is available through the *O_2 table* button in the SensorTrace calibration routine and through the main menu in the software: *Tools -> O_2 calculator* (See the SensorTrace Suite manual for details: https://www.unisense.com/manuals/).

5 Calibration procedure

5.1 Important notes for calibrating all Unisense O_2 sensors

- The temperature of the two calibration solutions must be the same.
- The pre-polarization of electrochemical O_2 sensors must have been completed before doing the calibration. See the O_2 sensor manual for details: https://www.unisense.com/manuals/
- It is recommended to use the Unisense Calibration Chamber for obtaining the high calibration point (e.g., air saturation).
- If doing the high calibration point with the calibration cap, make sure to do this before the zero calibration to avoid carry over from the zero-calibration solution. Wash the calibration cap and the protection tube very well with water after exposing it to the zero-solution.

Figure 2:Oxygen microsensor with the calibration cap mounted. Calibration solution is injected with the 10 ml syringe.

5.2 Calibrating most electrochemical O_2 sensors

(All sensors except those in Flow Cells and for the MicroRespiration System - see 5.3 & 5.4)

For calibrating an electrochemical and optical O_2 sensors, a low and a high calibration point are needed.

5.2.1 Obtaining the high calibration point

5.2.1.1 Using the Unisense Cal300 Calibration Chamber

1. Place the sensor with the protection tube in air saturated water at a known temperature (see the O_2 microsensor manual (https://www.unisense.com/manuals/)
2. Allow the sensor to respond and stabilize and record the calibration value in SensorTrace (see the SensorTrace manual for details: https://www.unisense.com/manuals/)

5.2.1.2 *Using the calibration cap*

1. Mount the calibration cap on the protection tube with the O_2 sensor. Make sure that the O-ring is in place at the bottom of the calibration cap creating a seal between this and the protection tube.
2. Fill the 10 ml syringe with air saturated water.
3. Inject this water into the calibration cap until the sensor tip is immersed at least 2-3 cm.
4. Allow the sensor to respond and stabilize and record the calibration value in SensorTrace (see the SensorTrace manual for details: https://www.unisense.com/manuals/)

5.2.2 Obtaining the zero-calibration point

1. Mount the calibration cap, if not already mounted, as described above in 5.2.1.2
2. Open the Exetainer with the zero solution
3. Aspirate ca. 10 ml of the zero solution with the syringe and needle.
4. Keep the syringe vertical and avoid mixing of the zero solution with the air bubble inside.
5. Remove the needle and attach the 10 ml syringe to the calibration cap tubing.
6. Inject the calibration solution slowly until the sensor tip is immersed at least 2-3 cm.
7. Allow the sensor to respond and stabilize and record the calibration value in SensorTrace (see the SensorTrace manual for details: https://www.unisense.com/manuals/)
8. Remove the zero solution with the syringe.
9. Wash the calibration cap and protection tube carefully, removing all of the zero solution.

5.3 Calibrating electrochemical and optical O_2 sensors in flow cells

Sensors with flow cells cannot be calibrated using the Calibration Cap. Instead, it is recommended to follow the procedure outlined below and to pay attention to the general information in section 5.1.

5.3.1 Create a calibration setup

In order to calibrate a sensor in a flow cell, the sensor tip must be exposed to the calibration liquid. The optimal way to do this depends on the actual setup, however, it is recommended to make a setup that allows calibration of the sensor without removing it from the flow cell and from the setup. Generally, this could be a Luer connector connected to the flow cell, directly or via tubing, that allows injection of the calibration liquid into the flow cell. A three-way valve on either side of the flow cell will allow easy injection of the calibration liquid with the sensor and flow cell in place.

Connection of the syringe with calibration liquid to the flow cells:

- PEEK flow cells: The syringe may be attached directly to the flow cell via a Luer adapter that is mounted directly in the flow cell (Figure 3, left)
- Glass and Swagelok stainless steel flow cells: The syringe may be attached via rubber tubing. The syringe may be connected directly to the rubber tubing or via a barbed Luer adaptor (Figure 3, right)

Figure 3: Left: Luer adaptor for direct mounting in the flow cell (e.g., IDEX P-624). Right: Barbed Luer adaptor for tube connection

5.4 Calibrating electrochemical and optical O_2 sensors for the Microrespiration system

Sensors of the Microrespiration type (OX-MR) cannot be calibrated using the calibration cap. Instead, it is recommended to use the MR zero calibration chamber as described below.

Figure 4: Oxygen sensor in the Microrespiration guide.

5.4.1 Obtaining the high calibration point

5.4.1.1 Using the Unisense Cal300 Calibration Chamber

1. Place the sensor in the Cal300 Calibration Chamber containing air saturated water at a known temperature (see the O_2 microsensor manual (https://www.unisense.com/manuals/).
    - The O_2 sensor must be mounted in the blue Microrespiration guide, and the tip must be retracted.
    - Temperature and salinity of the water must be the same as where the measurements are done.
2. Allow the sensor to respond and stabilize and record the calibration value in SensorTrace (see the SensorTrace manual for details: https://www.unisense.com/manuals/)

5.4.1.2 Using a Microrespiration chamber

1. Equilibrate water with atmospheric air by bubbling. The temperature and salinity of this must be the same as where the measurements are done.
2. Transfer the air saturated water to a MicroRespiration chamber and put the lid on the chamber.
3. Place the MicroRespiration chamber in the stirrer rack. It is recommended to place this in a water bath to keep control over the temperature.
4. Place the sensor in the stirrer rack with the plastic tip in the opening of the Microrespiration chamber lid and insert the O_2 sensor tip into the MicroRespiration chamber.
5. Allow the sensor to respond and stabilize and record the calibration value in SensorTrace (see the SensorTrace manual for details: https://www.unisense.com/manuals/).

5.4.2 Obtaining the zero-calibration point

1. Fill the silicone tubing, attached to the zero-calibration chamber lid, with water. Make sure all bubbles are removed.
2. Fill the 4 ml zero calibration chamber with the zero O_2 solution.
3. Mount the lid on the zero-calibration chamber taking care to avoid bubbles (see note A below).
4. Place the zero-calibration chamber at the single position, closest to the cable in the stirrer rack.
5. Place the sensor in the stirrer rack with the plastic tip in the opening of the Microrespiration chamber lid and insert the O_2 sensor tip into the MicroRespiration chamber.
6. Allow the sensor to respond and stabilize and record the calibration value in SensorTrace (see the SensorTrace manual for details: https://www.unisense.com/manuals/).

NOTE:
The water filled silicone tubing is now placed in the zero O_2 solution and O_2 from inside the silicone tubing will diffuse into the ascorbate solution and be consumed. It is recommended to leave the zero-calibration chamber over night for complete removal of O_2 from the water inside the silicone tubing.

Figure 5: Zero calibration chamber (MR-Ch 0-calibration). The zero solution is outside the silicone tubing and pure water is inside. The oxygen in the water inside the tubing diffuses through the silicone tubing into the zero solution where it is consumed.

5.5 Calibrating O_2 MicroOptodes - Retractable Needle design

NOTE: Before calibrating optodes, the optical fibre must be extended maximally out of the needle. This is done by pushing the inner steel cylinder into the black shaft.

Figure 6: Optical oxygen sensor, Optode, in the Retractable Needle design.

5.5.1 Obtaining the high calibration point

5.5.1.1 Using the Unisense Cal300 Calibration Chamber

1. Place the sensor with the protection tube in air saturated water at a known temperature (see the MicroOptode - Opto Series - Manual (https://www.unisense.com/manuals/)
2. Allow the sensor to respond and stabilize and record the calibration value in SensorTrace (see the SensorTrace manual for details: https://www.unisense.com/manuals/)

5.5.1.2 Using the calibration cap

1. Mount the calibration cap on the protection tube with the O_2 sensor. Make sure that the O-ring is in place at the bottom of the calibration cap creating a seal between this and the protection tube.
2. Fill the 10 ml syringe with air saturated water.
3. Inject this water into the calibration cap until the sensor tip is immersed.
4. Allow the sensor to respond and stabilize and record the calibration value in SensorTrace (see the SensorTrace manual for details: https://www.unisense.com/manuals/).

5.5.2 Obtaining the zero-calibration point

1. Mount the calibration cap, if not already mounted, as described above in 5.5.1.2
2. Open the Exetainer with the zero solution
3. Aspirate ca. 10 ml of the zero solution with the syringe and needle.
4. Keep the syringe vertical and avoid mixing of the zero solution with the air bubble inside.
5. Remove the needle and attach the 10 ml syringe to the calibration cap tubing.
6. Inject the calibration solution slowly until the sensor fibre tip is well immersed and stop before the solution reaches the needle. If the ascorbate solution gets into the needle, it is difficult to wash out.
7. Allow the sensor to respond and stabilize and record the calibration value in SensorTrace (see the SensorTrace manual for details: https://www.unisense.com/manuals/)
8. Remove the zero solution with the syringe.
9. Wash the calibration cap, sensor tip and protection tube carefully, removing all of the zero solution.

5.6 Calibrating O_2 MiniOptodes - Opto-3000

5.6.1 Obtaining the high calibration point

1. Place the sensor in air saturated water at a known temperature (see the MicroOptode - Opto Series - Manual (https://www.unisense.com/manuals/)
2. Allow the sensor to respond and stabilize and record the calibration value in SensorTrace (see the SensorTrace manual for details: https://www.unisense.com/manuals/)

5.6.2 Obtaining the zero-calibration point

1. Open the Exetainer with the zero solution.
2. Insert the Opto-3000 sensor into the zero solution at least 5 mm. Use a clamp or other holder to keep the sensor steady. Take care that air bubbles are not trapped at the front of the sensor.
3. Allow the sensor to respond and stabilize and record the calibration value in SensorTrace (see the SensorTrace manual for details: https://www.unisense.com/manuals/)
4. Wash the sensor carefully, removing all of the zero solution.

6 Specifications

- Content of the zero-calibration solution: Slightly alkaline ascorbate
- Volume of the zero-calibration solution: 12 ml
- Lifetime of the calibration kit: See label on the calibration kit box

Figure 7: Optical oxygen sensor, Optode, in the Opto-3000 design.

7 Equilibrium O_2 concentration at know temperature and salinity

Detailed tables are available at: http://www.unisense.com/technical_information/

At 20°C and 1 atm.: 1 µmol O_2/l = 0.032 mg O_2/l = 0.024 ml O_2

Table 1: Concentrations of oxygen (µmol O_2/litre) in water equilibrated with moist atmospheric air at a partial pressure of 0.21 atm. as a function of temperature and salinity.

˚C ‰	0.0	5.0	10.0	15.0	20.0	25.0	30.0	35.0	40.0
0.0	456.6	398.9	352.6	314.9	283.9	257.9	235.9	217.0	200.4
2.0	450.4	393.6	348.1	311.1	280.6	255.0	233.3	214.7	198.3
4.0	444.2	388.5	343.7	307.3	277.3	252.1	230.8	212.4	196.3
6.0	438.1	383.3	339.4	303.6	274.0	249.3	228.3	210.2	194.3
8.0	432.1	378.3	335.1	299.9	270.8	246.5	225.8	207.9	192.3
10.0	426.1	373.3	330.8	296.2	267.6	243.7	223.3	205.7	190.3
12.0	420.3	368.4	326.7	292.6	264.5	240.9	220.9	203.6	188.4
14.0	414.5	363.5	322.5	289.1	261.4	238.2	218.5	201.4	186.5
16.0	408.8	358.7	318.4	285.5	258.3	235.5	216.1	199.3	184.6
18.0	403.2	354.0	314.4	282.1	255.3	232.8	213.7	197.2	182.7
20.0	397.7	349.3	310.4	278.6	252.3	230.2	211.4	195.1	180.8
22.0	392.2	344.7	306.5	275.2	249.3	227.6	209.1	193.0	179.0
24.0	386.8	340.2	302.6	271.9	246.4	225.0	206.8	191.0	177.1
26.0	381.5	335.7	298.7	268.5	243.5	222.5	204.5	189.0	175.3
28.0	376.2	331.2	294.9	265.3	240.6	219.9	202.3	187.0	173.5
30.0	371.0	326.9	291.2	262.0	237.8	217.4	200.1	185.0	171.7
32.0	365.9	322.5	287.5	258.8	235.0	215.0	197.9	183.0	170.0
34.0	3609	3183	2839	2557	2322	2125	1957	1811	1682
36.0	355.9	314.1	280.3	252.5	229.5	210.1	193.6	179.2	166.5
38.0	351.0	309.9	276.7	249.5	226.8	207.7	191.4	177.3	164.8
40.0	346.2	305.8	273.2	246.4	224.1	205.4	189.3	175.4	163.1
42.0	341.4	301.8	269.4	243.4	221.5	203.1	187.3	173.6	161.5

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