


# UNISENSE 2023.05 O2 Calibration Kit User Manual

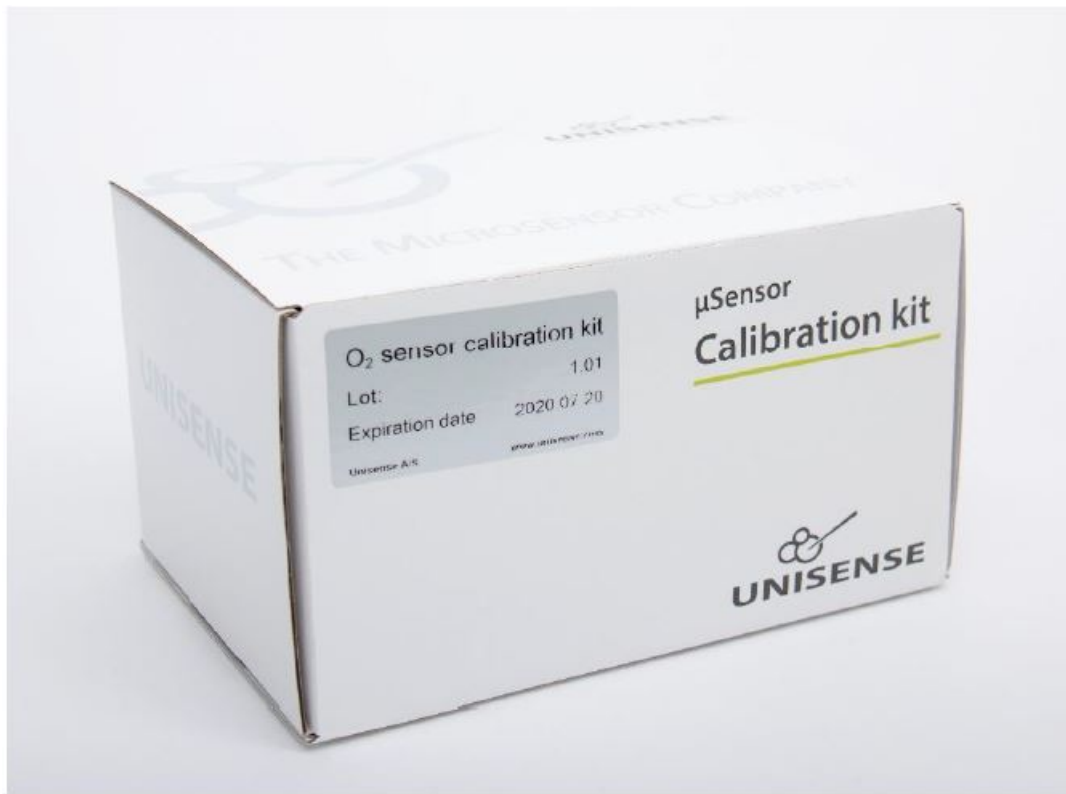
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**UNISENSE 2023.05 O2 Calibration Kit**



## Warranty and liability

### Notice to Purchaser

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

### 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.

## 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.

### 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.

- **E-mail:** [sales@unisense.com](mailto:sales@unisense.com).
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- DK-8200 Aarhus N, Denmark

- **Tel:** +45 8944 9500
- **Fax:** +45 8944 9549
- Further documentation and support are available at our website: [www.unisense.com](http://www.unisense.com).

### Content of the calibration kit

Item	Number
Exetainer with zero O <sub>2</sub> 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.

### Principle of calibration

Unisense electrochemical O<sub>2</sub> sensors respond linearly to O<sub>2</sub> 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) responds linearly to O<sub>2</sub> 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<sub>2</sub> and the other point is the signal for a known O<sub>2</sub> concentration. This calibration kit contains Exetainers with a zero O<sub>2</sub> solution. A solution with a known O<sub>2</sub> concentration can be made by bubbling water, at a known temperature and salinity, with atmospheric air. The O<sub>2</sub> concentration at equilibrium can be looked up in a table (Table 1) or by using the built-in O<sub>2</sub> solubility function in SensorTrace Suite. This is available through the O<sub>2</sub> table button in the SensorTrace calibration routine and through the main menu in the software: Tools -> O<sub>2</sub> calculator (See the SensorTrace Suite manual for details: <https://www.unisense.com/manuals/>).

## Calibration procedure

### Important notes for calibrating all Unisense O2 sensors

- The temperature of the two calibration solutions must be the same.
- The pre-polarization of electrochemical O2 sensors must have been completed before doing the calibration. See the O2 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 carryover from the zero-calibration solution. Wash the calibration cap and the protection tube very well with water after exposing it to zero-solution.



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

## Calibrating most electrochemical O2 sensors

- (All sensors except those in Flow Cells and for the MicroRespiration System – see 5.3 & 5.4)
- For calibrating electrochemical and optical O2 sensors, low and high calibration points are needed.

### Obtaining the high calibration point

Using the Unisense Cal300 Calibration Chamber

1. Place the sensor with the protection tube in air-saturated water at a known temperature (see the O2 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/>)

### Using the calibration cap

1. Mount the calibration cap on the protection tube with the O2 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/>)

### 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 solutions.

### Calibrating electrochemical and optical O2 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.

### 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 adaptor 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

### Calibrating electrochemical and optical O<sub>2</sub> 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.

### Obtaining the high calibration point

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<sub>2</sub> microsensor manual (<https://www.unisense.com/manuals/>)).
  - The O<sub>2</sub> sensor must be mounted in the blue Microrespiration guide, and the tip must be retracted.
  - The 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/>)

### 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<sub>2</sub> 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/>).

### 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> solution and O<sub>2</sub> from inside the silicone tubing will diffuse into the ascorbate solution and be consumed. It is recommended to leave the zero calibration chamber overnight for the complete removal of O<sub>2</sub> from the water inside the silicone tubing.



*Figure 5: Zero calibration chamber (MR-Ch O-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.*

### Calibrating O<sub>2</sub> MicroOptodes – Retractable Needle design

**NOTE:** Before calibrating optodes, the optical fiber 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.

## Obtaining the high calibration point

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/>)

## Using the calibration cap

1. Mount the calibration cap on the protection tube with the O<sub>2</sub> sensor. Make sure that the Oring 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/>).

## 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 fiber 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 solutions.

## Calibrating O<sub>2</sub> MiniOptodes – Opto-3000

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/>)

### 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.

### Specifications

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



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

### Equilibrium O<sub>2</sub> concentration at known temperature and salinity

- Detailed tables are available at: [http://www.unisense.com/technical\\_information/](http://www.unisense.com/technical_information/).
- At 20°C and 1 atm.: 1  $\mu\text{mol O}_2/\text{l}$  = 0.032 mg O<sub>2</sub>/l = 0.024 ml O<sub>2</sub>.



Table 1: Concentrations of oxygen ( $\mu\text{mol O}_2/\text{liter}$ ) 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
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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	360.9	318.3	283.9	255.7	232.2	212.5	195.7	181.1	168.2
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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## Documents / Resources

 <p>O<sub>2</sub> sensor calibration kit For electrochemical and optical oxygen sensors Manual</p> 	<p><b>UNISENSE 2023.05 O2 Calibration Kit</b> [pdf] User Manual 2023.05 O2 Calibration Kit, 2023.05, O2 Calibration Kit, Calibration Kit, Kit</p>
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## References

- [!\[\]\(6302aad5aed157b291fddf37b4870784\_img.jpg\) Microsensors, instruments, and meters for microscale measurements](#)
- [!\[\]\(a9ca2c237943a6d0a9f22252f295b6f3\_img.jpg\) Microsensors, instruments, and meters for microscale measurements](#)
- [!\[\]\(9a01a64e0b4ff865df7d32ee7991fe8b\_img.jpg\) Technical Information - Unisense](#)
- [!\[\]\(6aefe9a3d997eb8b55c40ecd5fa7053f\_img.jpg\) Manuals - Unisense](#)
- [!\[\]\(baa8f8ba8c970db55300f5bb45bb3460\_img.jpg\) O2 MicroOptode - Optical oxygen sensor technology](#)
- [!\[\]\(a6e28495607b2299466d3d5d3193848c\_img.jpg\) O2 Microsensor for Research Applications - Unisense](#)

Manuals+.