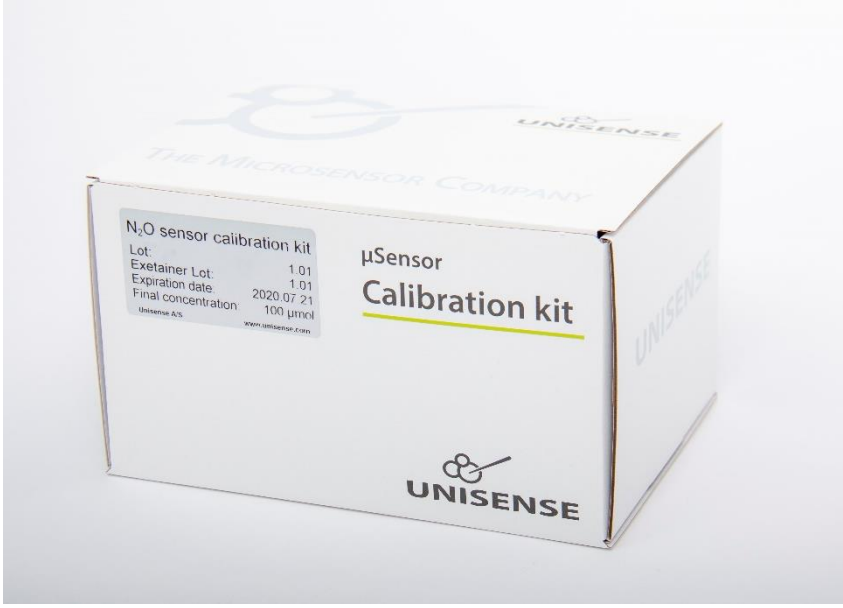
# $N_2O$ sensor calibration kit

# Standard concentration version

# Manual




UNISENSE

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# 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 Nitrous Oxide Calibration Kit is guaranteed to give the concentration indicated on the package label 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 |
|---|---|
| Ampoule with $N_2O$ equilibrated water | 10 |
| Labco Exetainer (empty) | 10 |
| Calibration cap with O-ring and 3 cm Viton tubing | 1 |
| 10 ml syringe | 1 |
| 1 ml syringe | 1 |
| 80 x 2.1 mm needle (green) | 1 |
| 30 x 0.6 mm needle (blue) | 1 |
| 50 x 1.2 mm needle (red) | 1 |
| Bag with glass beads (20-30 pieces) | 1 |

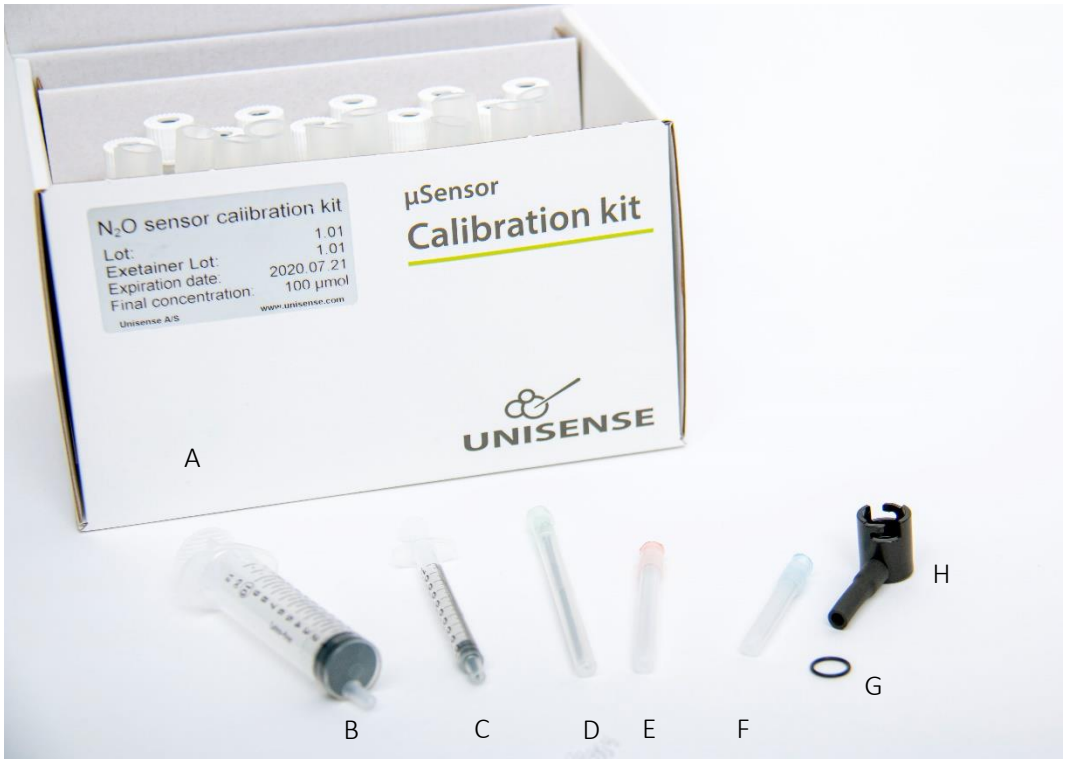


*Figure 1: Calibration kit contents: A: Calibration kit box with Exetainers and ampoules, B: 10 ml syringe, C: 1 ml syringe, D: 80 x 2.1 mm needle (green), E: 50 x 1.2 mm needle (red), F: 30 x 0.6 mm needle (blue), G: O-ring, H: Calibration Cap with tubing.*

# 4 Principle of calibration

Unisense $N_2O$ sensors respond linearly to $N_2O$ concentrations within their linear range (see specifications for your sensor at https://www.unisense.com/N2O). Therefore, a two-point calibration is sufficient. One calibration point is the signal for zero $N_2O$, which is water equilibrated with atmospheric air, and the other calibration point is the signal for one known $N_2O$ concentration. Note that the atmospheric concentration of $N_2O$ is approximately 330 ppb which corresponds to a concentration of around 9 nM in water. When working with the low-range sensor, with a detection limit of approximately 25 nM $N_2O$, this should be considered. This can be done by entering 9 nM as the concentration for air saturated water. For normal range sensors it can be assumed that such water is free of $N_2O$.

This calibration kit contains an ampoule with water equilibrated with a gas with a certified $N_2O$ content around 45000 ppm (vol) $N_2O$ in $N_2$, giving a concentration in the water in the ampoule of approximately 1200 µM. One ml of this water is diluted in approximately 12 ml of the water or medium in which the measurements will be performed. This gives a final concentration of approximately 100 µM.

## 4.1 The actual concentration

The actual concentration of $N_2O$ in the ampoule is calculated from the certified $N_2O$ content in the $N_2O$ gas mixture used and the temperature at equilibration (see *7 Specifications*). This, and the actual volume of the Exetainers, is used for calculation of the final concentration after dilution and this is shown on the sticker on the calibration kit box.

The concentration is given both in µmol $N_2O$/l and mg $N_2O$/l. The concentration in mg $N_2O$-N/l can be obtained by multiplying the $N_2O$ concentration in mg $N_2O$/l by the ratio of the N content in $N_2O$ to the molar mass of $N_2O$:

Concentration in mg $N_2O$-N/l = Concentration in mg $N_2O$/l x (28.0136 (g N/mol $N_2O$) / 44.013 (g/mol))

Concentration in mg $N_2O$-N/l = Concentration in mg $N_2O$/l x 0.6365

The empty vials (Exetainers) in the calibration kit should be filled with the water or medium in which the measurements will be done. This will ensure that the calibration and measurements are performed in the same environment, i.e., same salinity, composition of salts, proteins etc. This is important because the sensor reacts to the partial pressure of the gas, not the concentration as such, and in the software, this is recalculated into a concentration. The relationship between concentration and partial pressure depends on the salinity and temperature.

# 5 Standard calibration procedure

For calibration of $N_2O$ sensors a high and a low calibration point are needed.

WARNING: Low Range $N_2O$ sensors must never be exposed to more than 50 µmol/L $N_2O$. Therefore, the dilution in 5.1 below must be different (0.5 ml instead of 1.0 ml).

Note that Microrespiration sensors and sensors with flow cells will not fit the calibration cap and must be calibrated as described in sections 5.4 and 5.5. For calibrating High Range $N_2O$ sensors that work in the mM range, use the *$N_2O$ Calibration kit - High Range*.

## 5.1 Preparation of the calibration solution

1. Mount the 50 x 1.2 mm needle (red) on the 1 ml syringe and mount the 80 x 2.1 mm needle (green) on the 10 ml syringe.
2. Open the Exetainer and put two glass beads into the vial.
3. Fill the Exetainer with water of the same composition as the water where the measurements will be done.
4. Close the Exetainer with the lid, avoiding any bubbles or headspace.
5. Adjust the Exetainer temperature to the sample/measuring temperature.
6. Insert the 30 x 0.6 mm needle (blue) needle through the septum so the tip is just below the septum.
7. Open the ampoule with the $N_2O$ containing water by breaking the top off (see note A below). Make sure that you don't shake or mix the content of the ampoule.
8. Fill the 1 ml syringe with 1.0 ml of the water from the ampoule (see note B below).
9. Inject the 1.0 ml $N_2O$ containing water into the Exetainer by inserting the needle fully. The excess water from the Exetainer will be expelled through the open needle (see note C below). For Low Range sensors, inject only 0.5 ml.
10. Remove first the open needle and then the 1 ml syringe from the Exetainer.
11. Shake the Exetainer vigorously to ensure an even distribution of the $N_2O$.
12. The calibration solution is now ready and should be used within a couple of hours.

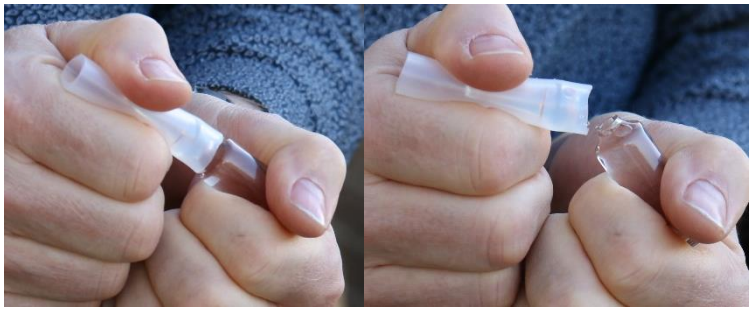

*Figure 2: Open the ampoule. Leave the tubing on for protection.*

## 5.2 Preparation of the $N_2O$ sensor

IMPORTANT:

- The pre-activation and polarization of the $N_2O$ sensor must have been completed before doing the calibration. See the $N_2O$ sensor manual for details: https://www.unisense.com/manuals/
- The temperature of the two calibration solutions must be the same.
- Perform the calibration at the same temperature as the measurements if possible. The UniAmp series of amplifiers has a built-in temperature compensation within ±3°C of the calibration temperature.
- It is recommended to obtain the low calibration point first to avoid carry over from the $N_2O$ standard.

## 5.3 Calibrating most $N_2O$ sensors

(All sensors except those in Flow Cells and for the Microrespiration System - see 5.4 and 5.5)

### 5.3.1 Obtaining the low calibration point

1. Mount the calibration cap on the protection tube with the $N_2O$ 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. Use the 10 ml syringe to inject $N_2O$ free water until the sensor tip is immersed at least 2-3 cm.
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. Remove the $N_2O$ free water with the syringe.

### 5.3.2 Obtaining the high calibration point

1. Open the Exetainer with the $N_2O$ calibration solution, prepared as described in 5.1 above.
2. With the 10 ml syringe, aspirate slowly, avoiding bubble formation, 10 ml of the $N_2O$ containing water.
3. Keep the syringe vertical and avoid mixing of the water with the air bubble inside.
4. Remove the needle and attach the 10 ml syringe to the calibration cap tubing.
5. Inject the calibration solution slowly until the sensor tip is immersed at least 2-3 cm.
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/)

NOTES:

A. Opening the ampoule with $N_2O$ containing water: Hold the bottom of the ampoule firmly while grabbing the tubing on the top. Break the top off the ampoule (Figure 2).
   IMPORTANT: Once an ampoule is opened it must be used immediately. It is only possible to prepare one calibration vial from one ampoule.
B. Pull up around 0.3 ml of $N_2O$ containing water from the ampoule. Point the needle upwards and knock the syringe gently to get all air bubbles to the top. Press the piston to eject these bubbles. Once the syringe is free of bubbles, empty the syringe. Pull up 1.1 ml of water from the ampoule.
   IMPORTANT: Insert the needle fully in the ampoule and pull up water slowly to avoid bubble formation.
   Adjust the volume to exactly 1.0 ml.
C. Injection of the $N_2O$ containing water is done with the needle inserted fully while the open needle in inserted just below the septum. Thereby the injected $N_2O$ containing water will not be lost.

## 5.4 Calibrating $N_2O$ sensors for the Microrespiration system

Sensors of the Microrespiration type ($N_2O$-MR) cannot be calibrated using the calibration cap. Instead, it is recommended to follow the procedure outlined below.

### 5.4.1 Obtaining the low calibration point

#### *5.4.1.1 Using the Unisense Cal300 Calibration Chamber*

1. Place the sensor in the Cal300 Calibration Chamber containing $N_2O$ free water (see the $N_2O$ microsensor manual (https://www.unisense.com/manuals/).
   - The $N_2O$ sensor must be mounted in the blue Microrespiration guide, and the tip must be retracted (Figure 3).
   - Temperature 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. Prepare a volume of $N_2O$ free water at the same temperature as the $N_2O$ calibration solution used in 5.4.2.
2. Transfer this water to a MicroRespiration chamber and mount the lid.
3. Place the MicroRespiration chamber in the stirrer rack
4. Place the $N_2O$ sensor in the stirrer rack with its plastic tip in the opening of the lid.
5. Insert the sensor into the 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/).
7. Retract the sensor tip and remove the sensor from the stirrer rack.

*Figure 3: Microsensor mounted in the Microrespiration guide.*

### 5.4.2 Obtaining the high calibration point

1. Prepare the $N_2O$ calibration solution as described in section 5.1.
2. Aspirate a suitable volume of the $N_2O$ calibration solution with the syringe and needle. Do this slowly to avoid bubble formation.
3. Dispense the $N_2O$ calibration solution into a MicroRespiration chamber. Place the needle at the bottom of the chamber, filling slowly from below, to avoid bubbles and splashing.
4. Mount the lid in the Microrespiration chamber making sure that no air bubbles are trapped.
5. Place the Microrespiration chamber in the stirrer rack.
6. Place the $N_2O$ sensor in the stirrer rack with its plastic tip in the opening of the lid.
7. Insert the sensor into the chamber.
8. 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/).
9. Retract the sensor tip and remove the sensor from the stirrer rack.

## 5.5 Calibrating $N_2O$ sensors in flow cells

Sensors with flow cells cannot be calibrated using the Calibration Cap. Instead, it is recommended to follow the suggestions outlined below. Pay attention to the general information in section 5.1 and 5.2 and follow the procedure below.

### 5.5.1 Create a calibration setup

In order to calibrate a sensor in a flow cell, the sensor tip must be exposed to the calibration liquid prepared in the Exetainer (described in section 5.1). 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 4, 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 4, right)

*Figure 4: Left: Luer adaptor for direct mounting in the flow cell (e.g., IDEX P-624). Right: Barbed Luer adaptor for tube connection*

# 6 Calibrating at other concentrations

The calibration kit may be used to calibrate at other concentrations than described in section 5.

NOTE: $N_2O$ sensors must not be exposed to concentrations higher than they are designed for as this may destroy the sensor.

## 6.1 Higher concentrations

It is possible to inject more than 1 ml of the ampule-solution into the Exetainer which will create a higher concentration than that obtained using the standard procedure in section 5. The concentration is directly proportional to the amount injected:

Final conc. (µM) = Inj. vol (ml) x Certified conc. (µM)

where *Final conc. (µM)* is the concentration obtained in the Exetainer, *Inj. vol (ml)* is the volume injected into the Exetainer and *Certified conc. (µM)* is the $N_2O$ concentration obtained when following the standard procedure in section 5, which is the concentration shown on the label on the calibration kit box.

The dilution of the stock solution into the Exetainer requires that the injected $N_2O$ containing water is not spilled out of through the open needle. Therefore, it is important that the tip of the open needle is right below the septum and the injection needle is as deep as possible. Furthermore, if injecting more than approximately 3 ml, there is a risk that some of the $N_2O$ is mixed with the liquid in the exetainer and lost through the open needle. Injection should only be done once. Therefore, a larger syringe is needed for injecting more than 1 ml.

Please note that when injecting less than the full volume of a syringe, the accuracy of the injection will become lower. Therefore, always use a syringe with a full volume that is close the amount to be injected. E.g., if 3 ml is injected with a 10 ml syringe the accuracy is low.

## 6.2 Lower concentrations

It is possible to obtain a lower concentration than that obtained using the standard procedure in section 5. This may be done either injecting less than 1.0 ml or by diluting the calibration solution obtained using the standard procedure in section 5.

When injecting less than 1.0 ml it is important to use a precision syringe. The resulting concentration can be calculated using the formula in section 6.1.

Lower concentration may also be obtained by dilution of the solution made in section 5.1.

1. Fill two Exetainers with water of the same composition as the water where the measurements will be done.
2. Inject the 1.0 ml $N_2O$ containing water into first Exetainer as described above in section 5.1.
3. After shaking the Exetainer vigorously to obtain a homogenous distribution of $N_2O$, open the Exetainer and aspirate a volume with a syringe.
4. Inject a known volume into the second Exetainer with the blue needle inserted just below the septum to allow excess liquid to run out.
5. Shake the Exetainer vigorously.

Now the sensor may be calibrated as described in section 5.2 using the calibration solution in the second Exetainer.

The concentration of $N_2O$ in the second Exetainer may be calculated as:

Final conc. (µM) = Inj. vol. (ml)/Exetainer vol. (ml) x Certified conc. (µM)

where *Final conc. (µM)* is the concentration obtained in the second Exetainer, *Inj. vol (ml)* is the volume injected transferred from the first to the second the Exetainer, *Exetainer vol. (µM)* is the volume of the Exetainer, and *Certified conc. (µM)* is the $N_2O$ concentration obtained when following the standard procedure in section 5. The *Certified conc.* and the *Exetainer volume* are shown on the label on the calibration kit box.

Please note that when injecting less than the full volume of a syringe, the accuracy of the injection will become lower. Therefore, always use a syringe with a full volume that is close the amount to be injected. E.g., if 3 ml is injected with a 10 ml syringe the accuracy is low.

The Exetainers may be cleaned and re-used for serial dilutions.

# 7 Specifications

- Volume of calibration solution in one ampule: 5 ml
- Content of the ampoule: Slightly acidic water with $N_2O$
- Lifetime of the calibration kit: See label on the calibration kit box
- $N_2O$ concentration in final calibration solution*: See label on the calibration kit box
- Certificate of $N_2O$ gas concentration: See label on the calibration kit box

*The ampoule contains water equilibrated with a gas with a certified $N_2O$ content. The concentration is calculated according to Weiss R.F. & Price B.A. 1980. Nitrous oxide solubility in water and seawater. Marine Chemistry 8:347-359. The concentration of $N_2O$ in the calibration solution is specified on the label on the calibration kit box. The certificate of the $N_2O$ gas mixture used to produce the ampoule water can be requested from Unisense. See the ID of this certificate on the label on the calibration box.