

N₂O sensor calibration kit

High concentration version

Manual



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N₂O SENSOR CALIBRATION KIT - HIGH CONCENTRATION VERSION - USER MANUAL

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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.

E-mail: sales@unisense.com

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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 water equilibrated with a N ₂ O gas*	10
1 ml syringe	1
5 ml syringe	1
50 x 1.2 mm needle (red)	2

4 Principle of calibration

Unisense N₂O sensors respond linearly to N₂O 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 N₂O, which is water equilibrated with atmospheric air, and the other calibration point is the signal for one known N₂O concentration.

This calibration kit contains ampoules with water equilibrated with pure N₂O gas. This gives a N₂O concentration of approximately 27 mM. The actual concentration of N₂O in the ampoule is determined for each batch of calibration kits and is shown on the sticker on the calibration kit box.

The content of the ampoule should be diluted into an appropriate amount of water to obtain the desired calibration concentration. This calibration water should be of the same composition of salts, proteins etc. as the water where the measurements will be done. This will ensure that the calibration and measurements are performed in the same environment. 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.

WARNING: N₂O sensors must never be exposed to higher concentrations of N₂O than they are designed for. This will result in bubble formation in the tip of the sensor which will then be destroyed.

5 Calibration procedure

For calibration of N₂O sensors a high and a low calibration point are needed.

5.1 Preparation of the calibration solution

The N₂O stock solution in the ampoule must be diluted to the desired concentration in water with the same composition and temperature as where the measurements will be done. Calculation of volume for dilution water and N₂O stock solution is described in 5.1.1.

1. Prepare an appropriate volume of water for the dilution, in a flask with a narrow opening (see note A below).
2. Make sure this water has the same composition and temperature as where the measurements will be done.
3. Open the ampoule with the N₂O containing water by breaking the top off (see note B below). Make sure that you don't shake or mix the content of the ampoule.
4. Use a syringe to aspirate the amount from the ampoule needed to create the desired concentration (see note C below).
5. Inject the N₂O containing water into the flask prepared in step 1 (see note D below).
6. Mix the water in the flask gently to obtain a homogenous distribution of N₂O and avoid mixing air into the water. This will cause N₂O to escape from the water.



Figure 1: Open the ampoule. Leave the tubing on for protection.

5.1.1 How to obtain the desired concentration

The concentration of N₂O in the final calibration solution depends on the volume of N₂O equilibrated water and dilution water used.

$$\text{Final conc. (mM)} = \text{Vol N}_2\text{O Ampoule (ml)} / \text{Vol cal solution (ml)} \times \text{Conc Ampoule (mM)}$$

where *Final conc. (mM)* is the final concentration of N₂O after dilution, *Vol N₂O Ampoule (ml)* is the volume of water transferred from the ampoule to the dilution water, *Vol cal solution (ml)* is the final volume of the calibration solution, and *Conc Ampoule (mM)* is the N₂O concentration in the ampoule as indicated on the calibration box label.

Example:

Vol. N₂O Ampoule = 3.0 ml

Vol. cal. solution 300 ml

Conc. Ampoule = 27 mM

$$\text{Final conc. (mM)} = 3.0 \text{ ml} / 300 \text{ ml} \times 27 \text{ mM} = 0.27 \text{ mM} = 270 \mu\text{M}$$

5.1.2 Conversion of molar concentrations to mg/l

$$\text{Concentration (mg N}_2\text{O/l)} = \text{Concentration } (\mu\text{mol/l}) \times 44.013 (\mu\text{g}/\mu\text{mol}) \times 0.001 (\text{mg}/\mu\text{g})$$

Where *Concentration (μmol/l)* is the molar concentration calculated as shown in 5.1.1, *44.013 (μg/μmol)* is the molar mass of N₂O and *44.013 (mg/μg)* is a conversion factor from μg to mg.

Example:

Molar concentration = 270 μmol/l

$$\text{Concentration (mg N}_2\text{O/l)} = 270 (\mu\text{mol/l}) \times 44.013 (\mu\text{g}/\mu\text{mol}) \times 0.001 (\text{mg}/\mu\text{g}) = 11.88 \text{ mg/l}$$

5.2 Calibrating the N₂O sensor

IMPORTANT:

- The pre-activation and polarization of the N₂O sensor must have been completed before doing the calibration. See the N₂O 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 N₂O standard.

5.2.1 Obtaining the low calibration point

1. Put the sensor, mounted in the protection tube, into a beaker with N₂O free water at the same temperature as used for the high calibration point.
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/>)

5.2.2 Obtaining the high calibration point

1. Put the N₂O sensor, mounted in the protection tube, into the N₂O calibration solution prepared in section 5.1.
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/>)

NOTES:

- A. It is important to minimize the area of the calibration solution that is exposed to the atmosphere in order to minimize the loss of N₂O to the atmosphere. Therefore, it is recommended to use a narrow mouth bottle, e.g. a conical flask, Erlenmeyer flask.
- B. Opening the ampoule with N₂O containing water: Hold the bottom of the ampoule firmly while grabbing the tubing on the top. Break the top off the ampoule (Figure 1).
IMPORTANT: Once an ampoule is opened it must be used immediately.
- C. Pull up N₂O 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 water from the ampoule and adjust the volume to that needed.

IMPORTANT: Insert the needle fully in the ampoule and pull up water slowly to avoid bubble formation.

- D. Injection of the N₂O containing water is done with the needle inserted well into the water to avoid splashing and bubble formation. Thereby the injected N₂O containing water will not be lost.

6 Specifications

- Volume of calibration solution in one ampule: 5 ml
- Content of the ampoule: Slightly acidic water with N₂O
- Lifetime of the calibration kit: See label on the calibration kit box
- N₂O concentration in the ampoule*: See label on the calibration kit box
- Certificate of N₂O gas concentration: See label on the calibration kit box

*The ampoule contains water equilibrated with a gas with a certified N₂O 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 N₂O in the ampoule is specified on the label on the calibration kit box. The certificate of the N₂O 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.