
# Redox & Reference Electrode

## User Manual

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# Redox & Reference Electrode User Manual

UNISENSE A/S


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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 Redox electrode is covered by a 90 days limited warranty. Microsensors are a consumable. Unisense will only replace dysfunctional sensors if they have been tested according with the instructions in the manual within 14 days of receipt of the sensor(s).

The warranty does not include repair or 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.

Unisense mechanical and electronic laboratory instruments must only be used under normal laboratory conditions in a dry and clean environment. Unisense assumes no liability for damages on laboratory instruments due to unintended field use or exposure to dust, humidity or corrosive environments.

## 1.4 Repair and Adjustment

Sensors and electrodes cannot be repaired. Equipment that is not covered by the warranty will, if possible, be repaired by Unisense A/S with appropriate charges paid by the customer. In case of return of equipment please contact us for return authorization.

For further information please see the document General Terms of Sale and Delivery of Unisense A/S as well as the manuals for the respective products.

# 2. CONGRATULATIONS WITH YOUR NEW PRODUCT!

## 2.1 Support, ordering, and contact information

The Unisense Redox electrode is a miniaturized platinum electrode that facilitates reliable and fast measurements with a high spatial resolution designed for research applications.

If you wish to order additional products or if you encounter any problems and need scientific/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
Unisense A/S
Langdyssen 5
DK-8200 Aarhus N, Denmark
Tel: +45 8944 9500

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

# 3. REPLACEMENT OF SENSORS UNDER WARRENTY

Unisense will replace sensors that have been damaged during shipment provided that:

- The sensors were tested immediately upon receipt in accordance with the delivery note and this manual
- The seal is still intact
- The sensors are returned to Unisense for inspection within two weeks
- The sensors are correctly packed for return to Unisense, in accordance with the packing guide included in the sensor box

# 4. OVERVIEW

*IMPORTANT: Unisense sensors are neither intended nor approved for use in humans*

## 4.1 Redox electrode

The Unisense redox microelectrode is a miniaturized platinum electrode designed for research applications within physiology, biotechnology, environmental sciences, and related areas. With tip diameters down to around 10 microns, the Unisense redox microelectrode allows measurements of redox potential with a very high spatial resolution. The redox microelectrode can be used for measurements in salinities from 0 to full ocean strength.

**Measurement principle:** When the electrode tip is immersed in an aqueous solution and connected via a high-impedance millivolt-meter to a reference electrode (figure 1 and 2) immersed in the same solution, the Redox electrode tip will develop an electric potential relative to the reference electrode which reflects the tendency of the solution to release or take up electrons; also called the Oxidation-Reduction Potential (ORP). Thus, the redox microelectrode must be used in combination with a reference electrode (e.g. Unisense REF-RM) and connected to a high-impedance millivolt-meter.

## 4.2 Reference electrode

The Unisense reference electrode is a simple open-ended Ag-AgCl electrode with a gel-stabilized electrolyte. The reference electrode is used to establish a reference potential against the pH microelectrode.

For laboratory use, Unisense provides a macro reference electrode from Radiometer Analytical (please see separate manual in the back of this booklet).
Our micro-sized reference electrodes are glass electrodes manufactured at Unisense.

For in situ use, Unisense manufactures a pressure-compensated macro reference electrode. Unisense offers selected versions of pH electrodes with internal reference, please see description under the Redox combination electrode section.

# 5. GETTING STARTED

*WARNING: Do not remove the seal and protective plastic tube before these steps and calibration are succesfully completed.*

## 5.1 Unpacking a newredox electrode

1. When receiving a new microelectrode, remove the shock-absorbing grey plastic net.

## 5.2 Unpacking a new reference electrode

**Radiometer Ref-RM:**

1. Remove the protection cap from the electrode and any seals covering the filling hole.
2. Before starting a measurement, remove the clip which closes the electrode filling hole. Remember to put the clip back in place at the end of measurements.
3. Check the level of the filling solution. It should be approximately 0.5 cm below the filling hole. If necessary refill the KCl-Ag 3M KCl solution, saturated with AgCl.

**Unisense Reference electrode:**

1. When receiving a new electrode remove the shock-absorbing grey plastic net.
2. Remove the lower piece of tape and the rubber stopper in order to empty the tube of storage liquid (2 M KCl). This liquid can be saved for future storage.
3. The tip of the Unisense reference electrode should be kept immersed in a 2 M KCl solution at all times, but can tolerate up to 10 minutes of exposure to air.

## 5.3 Connecting the electrodes

Connect the Unisense redox microelectrode to the amplifier.
Connect the reference electrode to the black box on the pH electrode cable (banana plug connection).
Immerse the Redox electrode (still in its protective tube) in a buffer solution. Place the tip of a reference electrode in the same solution.

*IMPORTANT: As the redox microelectrode is sensitive to temperature, it is necessary to perform calibration and subsequently measurements at the same temperature.*

A Unisense redox electrode with internal reference has to be submerged sufficiently (about 2-3 mm) in the medium to cover the liquid junction during all measurements.

In the following, we assume that you are using a Unisense amplifier. If you are using another meter, perform similar steps with the mV-meter in question.

## 5.4 Calibration principles

The redox potential must always be reported relative to the Standard Hydrogen Electrode (SHE). However, during measurement it is not possible to use the SHE and another type of reference electrode must be used. This can be any

reference electrode (Ag/AgCl, Calomel, etc.) which all have a different half-cell potential than the SHE. The measured redox potential must be corrected for this difference. This difference is estimated during a calibration. Once a calibration is performed, the uSense software will give the measured redox potential relative to the SHE.

NOTE: The calibrated signal now shows the redox potential relative to a Standard Hydrogen Electrode. The uncalibrated signal shows the redox potential relative to the reference electrode used. The unit of both calibrated and uncalibrated signals is mV.

*IMPORTANT: There must always be a liquid, and thus electrical, continuity between the tips of the redox and reference electrodes.*

## 5.5 Calibration

It is recommended to use the Unisense Redox Electrode Calibration Kit for calibrating the redox electrodes (unisense.com/products/calibration-kits). This kit ensures accurate and simple calibration both in the lab and in the field. The calibration kit can be shipped as normal cargo and does not require dangerous goods shipping. Therefore, it is ideal also for shipping to field work, research cruises etc. The detailed calibration procedure can be found in the Calkit-Redox Manual (unisense.com/manuals).

This calibration is a 1 point calibration for determination of the difference in potential between the applied reference electrode and the SHE. In the uSense software the 1 point calibration checkbox must be checked.

*NOTE: The 1-point calibration requires that "Sensor Type" is set to RD in the "Settings window". This is automatically done with UniAmp instruments but with all other instruments, this must be done manually.*

## 5.6 Alternative calibration

It is also possible to do a two point calibration as described below (the 1 point calibration checkbox in SensorTrace must be left unchecked). This is based on the fact that the redox potential of a saturated quinhydrone solution varies with pH. The redox potential relative to the SHE is well defined and given for pH 4 and 7 in the table below.

Redox potential of saturated quinhydrone at different pH and temperature relative the the Standard Hydrogen Electrode:

| Temperature(°C) | pH 4 buffer | pH 7 buffer |
|---|---|---|
| 20 | 470 | 295 |
| 25 | 462 | 285 |
| 30 | 454 | 275 |

*NOTE: The Calibration Kit cannot be used for calibration directly on the Field Multimeter. This must be done through the Unisense software. Calibration directly on the Field Multimeter can be done using the Alternative Calibration described in this manual.*

Do the following:

1. Connect the amplifier to your computer and start your SensorTrace program. If you use a strip chart recorder or other data acquisition device, connect this to the output connections of the amplifier.

2. Prepare quinhydrone redox buffer solutions: Mix 100 ml of pH 4 buffer solution with 1 g of quinhydrone. Do the same with a pH 7 buffer solutions.
   Crystals of quinhydrone should be present indicating that the solution is saturated.

3. Expose both the reference electrode and the redox microelectrode tips to both quinhydrone redox buffers (pH 4 and 7).

With use of Unisense software do the following:

1. Start your uSense software with a new experiment, choose the redox sensor. Press **Start experiment**.

2. The calibration tab is now shown. Put the redox electrode and the reference electrode into the pH 4 buffer. The signal is shown in "signal". In "Value (Redox pot. (mV))" you manually enter the correct value from the table above (e.g. 470 at 20oC). Press **add point** when the signal is stable (watch the Live view signal at the bottom of the display).

3. Now move the electrodes to the pH 7 buffer and do the same, entering the value from the table above (e.g. 295 at 20oC). Press **add point**. You now have the correct calibration.

4. Press **Apply calibration** and the calibration is shown in the table below the graph.

5. Then you tick the box "Calibrated" in the lower part of the window. The electrode signals are now shown as calibrated values and the electrodes are ready for use.

## 5.7 Approval of new sensor

If the sensor functions according to the criteria given in the delivery note, carefully remove the seal and the protective plastic tube before making measurements.

# 6. MEASUREMENTS

## 6.1 Use of glass-tip microsensors

Although the Unisense Redox microelectrode is made of glass, the tip is flexible and can bend slightly around physical obstacles. However, large obstacles like stones or coarse lateral movements of the electrode when the tip is in contact with a solid substrate may cause the tip to break.
Also, due to the small size of the microelectrode tip and to the steepness of redox gradients in many environments, even a displacement of the electrode tip of few microns may change its immediate redox environment.

Therefore measurements should be performed only in a stabilized set-up fixed free of moving or vibrating devices. We recommend the Unisense lab stand LS18 and the Unisense micromanipulator MM-33 (MM33-2 or MMS) for laboratory use. For in-situ use we recommend our in situ stand (IS19) and a micromanipulator.

Therefore **measurements should be performed only in a stabilized set-up fixed on a sturdy table free of moving or vibrating devices**. We recommend the Unisense lab stand LS and the Unisense micromanipulator MM-33 (MM33-2 double) for laboratory use. For in-situ use we recommend our in situ stand (IS19) and a micromanipulator.

*IMPORTANT: Construction of the electrode with an internal reference involves the creation of a liquid junction approximately 3 mm from the electrode tip. During measurements this liquid junction has to be submersed in the medium in order to complete the measurement circuit.*

## 6.2 Use of sensors without visible glass tip

Sensors with stainless steel tubes, needles, flow through cells, etc. do not have an exposed glass tip and are, therefore, less fragile. However, these sensors still contain a glass sensor inside which can be damaged by physical shock. To protect the sensor, do not let the sensor drop onto the table or floor. If the sensor has a needle, make sure that the needle does not bend or flex. This will break the glass sensor inside.

## 6.3 Electrical noise

The electrical current generated by the high-impedance microelectrode is very small. Although the Unisense redox microelectrodes are very resistant to electrical noise from the environment, electrical fields may interfere with the electrode signal. Therefore we recommend that unnecessary electrical/mechanical equipment is switched off and electrode or wires are not touched during operation.

# 7. REDOX COMBINATION ELECTRODE

For a limited number of redox electrodes we offer combination electrodes with internal reference as indicated by the product number ending with a C , eg RD-500C. These are made for selected applications like small volume measurements or small space applications, measuring inside an applied electrical field or crude profiling. But for most applications we recommend using our standard redox electrode with external reference electrode.

Please test, calibrate and measure as described for standard redox electrodes, but with attention to the special cases for redox combination electrodes.

- Reference electrode is built-in – do not use external reference electrode as well
- Electrode must be stored in 2M KCl solution
- Do not allow electrode to dry out – only allow short time (less than 10 minutes) exposure to air
- Reference electrode is situated near the electrode tip about 1-2mm away, thus minimum immersion depth is 1-2 mm
- Through the 10 μm opening for the reference electrode, small amounts of KCl will diffuse out and give a small contamination of the sample
- Reference electrolyte cannot be refilled but the included electrolyte is expected to last for the lifetime of the electrode

# 8. ADVANCED REDOX ELECTRODES

Unisense can construct redox electrodes for customer requested applications at additional costs. The most frequently construction options are described at our web page under redox electrode specifications unisense.com/products/redox-microelectrode/ (click "Adaptations" below the picture)

The options include for instance customer specified dimensions, pressure tolerance, internal reference and cable length. If your specifications for a special redox electrode are not described at our web page please contact sales@unisense.com for further options and prices.

# 9. REDOX ELECTRODES WITH PIERCING NEEDLE - THE NP-TYPE

**Piercing rubber septa**: The RD-NP electrode is made to penetrate a rubber septum. The way this occurs depends on the material of the septum. In some stoppers the material from the septum will be pushed into the needle, instead of being pushed to the side, when the needle penetrates the septum, and this can damage the electrode. We recommend using septa made from butyl rubber and NOT from silicone.

Before pushing the RD-NP electrode through the septum we recommend testing the septum. Use a needle with the same diameter as the NP-needle (1.6 mm) and push it through the septum to see if the septum material enters the needle. If you find septum material in the needle, you should not use this kind of septum material for the RD-NP electrode. Instead use another (butyl) septum.

**Calibration and measurements:** When moving the RD-NP electrode from one solution to another e.g. from one calibration buffer to another, from calibration buffer to sample or sample to sample, the space between the needle and the electrode should be clean and dry. This is done in the following way.

1. Use a squeeze bottle and flush the inside of the electrode needle tip
2. Remove the water in the needle by gently wiping the electrode with a tissue until the space between the needle and the sensor is free of liquid. Always wipe from the shaft towards the tip!
3. Afterwards, flush the electrode needle tip with ethanol and dry the electrode with the tissue as described above – ethanol dries the electrode faster than water.

You can test if the electrode is treated correctly and if it is dry by inserting it into the same buffer before and after the treatment and control if the signal is the same (remember also to place the reference electrode in the same solution).

# 9. STORAGE AND MAINTENANCE

## 9.1 Storage

**Redox electrode:** Store the electrode in the protective plastic tube used for shipping. The room in which the redox microelectrode is stored should be dry and not too hot (10-30°C).

Redox electrodes with built in ref must be stored in 2 M KCl

**Ref-RM:** Between measurements, leave the electrode in 3 M KCl solution. Overnight or longer: seal the filling hole with paraffin film or with the electrode clip and mount the plastic protection cap filled with the 3 M KCl solution. The room in which the electrodes are stored should be dry and not too hot (10-30°C).

**Micro-reference electrode:** Store in the plastic protection tube used for shipping. For short-term storage (<10 min) the electrodes can be stored in air. For long-term storage the electrode tip must be immersed in 2 M KCl in the plastic protection tube used for shipping. The room in which the electrodes are stored should be dry and not too hot (10-30°C).

## 9.2 Cleaning the electrode

The redox electrode can by cleaned by immersing it in nitric acid (1:1 $H_2O$) Keep the electrode in the nitric acid solution for about 30 minutes and allow it to cool and rinse with water afterwards.

The Ref-RM electrode should be rinsed with distilled water after measurements. Check the level of filling solution often. In case of deposits, please consult the separate manual in the back of this manual.

The micro reference electrodes can be cleaned by flushing or submergence in 70% ethanol for a couple of minutes. After this, rinse with 1M KCl.

# TROUBLESHOOTING

| | |
|---|---|
| **Problem** | Drift in the calibration potential values |
| **Possible cause** | Contamination of the platinum surface |
| **Solution** | Clean the electrode tip as described above |

| | |
|---|---|
| **Problem** | Sensitivity of the redox microelectrode is low or the signal is unstable |
| **Possible cause 1** | Contamination of the platinum surface |
| **Solution 1** | Clean the electrode tip as described above |

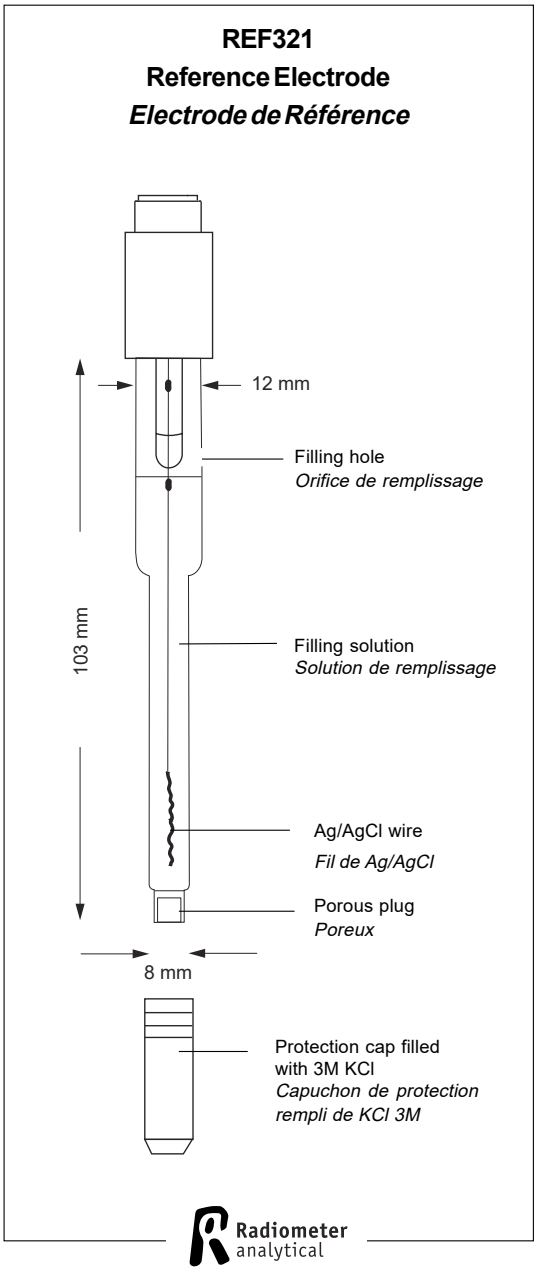
| | |
|---|---|
| **Possible cause 2** | The reference electrode is malfunctioning |
| **Solution 2** | Repair or change the reference electrode |

If you encounter other problems and need scientific/technical assistance, please contact sales@unisense.com for online support (we will answer you within one workday).

# REFERENCES

Kühl, M., Revsbech, N. P. 2001. Biogeochemical microsensors for boundary layer studies. In: The Benthic boundary layer, Boubreau, B. P, Jørgensen, B. B. (eds.). New York, Oxford University Press. pp 180-210.

Revsbech, N. P., and B. B. Jørgensen. 1986. Micro electrodes: Their Use in Microbial Ecology, p. 293-352. In K. C. Marshall (ed.), Advances in Microbial Ecology, vol. 9. Plenum, New York.


REF321
Reference Electrode
Electrode de Référence
12 mm
Filling hole
Orifice de remplissage
103 mm
Filling solution
Solution de remplissage
Ag/AgCl wire
Fil de Ag/AgCl
Porous plug
Poreux
8 mm
Protection cap filled
with 3M KCl
Capuchon de protection
rempli de KCl 3M
Radiometer
analytical

# REF321 REFERENCE ELECTRODE

## Introduction

The REF321 is a general purpose Ag/AgCl reference electrode, fitted with a screw cap.

## Preperation for measurement

1. Remove the protection cap from the electrode and any seals covering the filling hole.
2. Before starting a measurement, remove the clip which closes the electrode filling hole. Remember to replace the clip at the end of measurements.
3. Check the level of the filling solution. It should be approximately 0.5 cm below the filling hole. If necessary, refill with KCl•Ag 3 M KCl Solution, saturated with AgCl.

   If desired, the concentration of KCl can be changed. However, it is advisable to use a high concentration. Remember always to saturate the solution with AgCl. For nonaqueous applications such as measurements in acetic acid. Empty the electrode and fill it up again with a saturated KCl solution in acetic acid. Saturate the solution with AgCl.

4. In order to remove air bubbles trapped inside the electrode, shake the electrode holding it at its head with the porous plug down.

## Maintenance

1. Electrode contamination is a major cause of faulty measurements.
2. The electrode should be rinsed with distilled water after measurements.
3. Check frequently the level of filling solution.
4. In case of deposits which cover the electrode, clean the electrode with:

• a solution of acid (0.1M HCl, 0.1M HNO3): mineral salt deposits, etc...
• KS400 Pepsin in HCl Solution or RENOVO•X Xtra Strong Cleaning Solution: protein deposits (milk, cheese, serums...). Duration of treatment 1 to 2 hrs.
• KS410 Thiourea Solution: for porous plugs contaminated with sulphides or blocked by an AgCl precipitate. Duration of treatment, a few hours until the porous plug turns white.
• RENOVO•N Normal Cleaning Solution: greasy or oily deposits...

The porous plug of the electrode can be cleaned using a fine abrasive paper.

## Storage

Between measurements: leave the REF321 in KS110 3M KCl Solution.
Overnight or longer: seal the filling hole with paraffin film or with the electrode clip and fit back in place the protection cap filled with the KS110 3M KCl Solution.

## Accessories

| | |
|---|---|
| KS110 3M KCl Solution, 500 ml | C20C320 |
| KCl•Ag 3M KCl Solution saturated with AgCl, 100 ml | S21M004 |
| RENOVO•N Normal Cleaning Solution, 250 ml | S16M001 |
| RENOVO•X Xtra Strong Cleaning Solution, 250 ml | S16M002 |
| KS400 Pepsin in HCl Solution, 250 ml | C20C370 |
| KS410 Thiourea Solution, 250 ml | C20C380 |
| CL111 Electrode cable with banana plug | A94L111 |

## Specifications

| Reference potential when filled with 3M KCl (in mV) | | | | | | |
|---|---|---|---|---|---|---|
| Temperature (C °) | 0° | 10° | 20° | 25° | 30° | 40° |
| vs. std. hydrogen elec. | 225 | 219 | 212 | 208 | 204 | 196 |
| vs. sat. $Hg/Hg_2Cl_2$elec. | -35 | -36 | -36 | -36 | -37 | -38 |

Temperature range: 0 to 80 °C

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