



# pH & Reference Electrode

## User Manual

# pH & 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 pH 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

This microelectrode is a miniaturized conventional pH electrode based on selective diffusion of protons through pH glass. The small tip size facilitates rapid and precise pH measurements in a broad variety of micro environments.

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](mailto:sales@unisense.com)  
Unisense A/S  
Langdysen 5  
DK-8200 Aarhus N, Denmark  
Tel: +45 8944 9500

Further documentation and support is available at our website [www.unisense.com](http://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

This manual covers all Unisense pH and reference electrodes.

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

### 4.1 pH electrode

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

**Measurement principle:** The electric potential reflects the acidity of the solution. Thus the pH micro electrode must be used in combination with a reference electrode (internal or external) and connected to a high-impedance (>10<sup>13</sup> Ohm) millivolt-meter. When the electrode tip is immersed in an aqueous solution and connected via a millivolt-meter to a reference electrode immersed in the same solution, the pH electrode tip will develop an electric potential relative to the reference electrode, reflecting the pH of the solution.

### 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 micro electrode.

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 view description under "pH combination electrode"

## 5. GETTING STARTED

IMPORTANT: The pH electrode cannot be used with the tip pointing upwards. The sensor can be horizontal or the tip can point downwards. The electrode has a gas bubble inside and if the electrode points upwards, it will float into the narrow part of the sensor and block electrical connectivity in the electrolyte. This will result in a signal that will change randomly and will not stabilize. See Troubleshooting below for how to resolve this problem.

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

### 5.1 Unpacking a new sensor

1. When receiving a new micro electrode, remove the shock-absorbing grey plastic net.
2. Remove the lower piece of black tape and the rubber stopper from the protection tube in order to empty the tube of storage liquid. This liquid is pure water.
3. Remove the tape covering the hole on the side of the protection tube just below the nut. This will expose a small hole in the protection tube, allow the liquid to run out and the buffer to run in during calibration. The sensor is now ready for calibration.

*IMPORTANT: New pH-MR electrodes must be calibrated with the protection tube on. First when the electrode has been approved, it may be removed from the protection tube and inserted into the provided blue guide.*

### 5.2 Unpacking a new reference electrode

#### **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 with the 3 M KCl, saturated with AgCl, that is included in the box..

#### **Unisense Reference electrode:**

1. Follow steps 1-2 under "Unpacking a new pH electrode" above
2. The tip of the Unisense reference electrode should be kept 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 pH micro electrode to the amplifier.

Connect the reference electrode to the black box on the pH electrode cable (banana plug connection).

Immerse the pH electrode (still in its protective tube) in a buffer solution. Place the tip of a reference electrode in the same solution.

*NOTE: The software converts the raw pA sensor signal into mV.  
Standard setting is 1 mV = 1 pA.  
This may be modified by changing the Pre-Amp setting (mV/pA) in the software (UniAmp)  
or on the instrument (Multimeter, Monometer, and Field Multimeter).*

A Unisense pH 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. Also, please see the note below.

## 5.4 Calibration

It is recommended to use the Unisense pH Electrode Calibration Kit for calibrating the pH electrodes ([unisense.com/calibration\\_kits](http://unisense.com/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-pH Manual ([unisense.com/manuals](http://unisense.com/manuals))

*NOTE: Conventional pH-meters often have an automated calibration procedure. However, due to the very small area of pH-sensitive glass on the electrodes, this does not work well. Therefore Do NOT use the auto calibration, but read the signal in mV and convert it to pH afterwards.*

## 5.5 Alternative calibration

Please consult the software manual for instruction on how to calibrate in the software.

Connect the amplifier to your computer and start your uSense Solutions program. If you are using a strip chart recorder or other data acquisition device, connect this to the output connections of the amplifier. Expose both the reference electrode and pH micro electrode tip to at least two to three different pH buffers covering the range in which measurements are to be made. Record the signal in your software for each pH buffer. **The pH micro electrode should respond linearly between these points with a negative slope of 50-70 mV/pH-unit.** Millivolt readings during subsequent measurements can be converted to pH values with a linear conversion using the calibration values. This can be done directly in the uSense Solutions software (please see the software manual for instructions).

### CHECKLIST FOR CALIBRATION:

1. Connect the pH microelectrode to the amplifier
2. Connect the reference electrode to the connector on the cable
3. The hole on the side of the protection tube must be open so the buffer can enter the protection tube. The tip of the pH electrode must be immersed in buffer
4. Place the pH and reference electrodes in the same solution in the same beaker

## 5.6 Approval of new sensor

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

## 6. MEASUREMENTS

### 6.1 Use of glass-tip microsensors

To mount the electrode for measurements, loosen the nut on the protection tube, breaking the white paper seal. Leave the nut on the protection tube and pull the sensor out. The pH microelectrode is now ready for use.

*TEMPERATURE: Closely monitor the temperature, because the sensor signal will vary with temperature.*

Although the Unisense micro electrodes are 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 microsensor tip and to the steepness of gradients in many environments, even a displacement of the electrode tip of few microns may change its environment.

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.

### 6.2 Use of sensors without visible glass tip

Sensors with stainless steel tubes, stainless steel protection caps, 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.

For Piercing Needle pH electrodes (pH-NP) see Advanced pH electrodes below.

### 6.3 Electrical noise

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

## 7. pH COMBINATION ELECTRODE

For a limited number of pH electrodes we offer combination electrodes with internal reference as indicated by the product number ending with a C , eg pH-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 to use our standard pH electrode with external reference electrode.

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

### 7.1 Special precautions for pH 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 2 mm away, thus minimum immersion depth is 2-3 mm
- Through the 10  $\mu\text{m}$  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 pH ELECTRODES

Unisense can construct pH electrodes for customer requested applications at an additional cost. Unisense provides several options for customizations (e.g. tip size, response time, and stirring sensitivity) and adaptations (e.g. mounting in needle or flow-through cell), making accurate measurements possible for even more applications.

Please visit our website for more information.

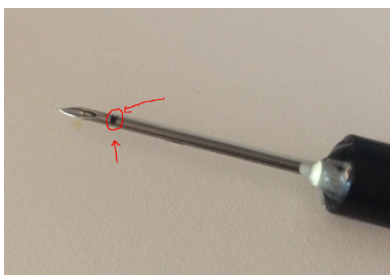
### 8.1 pH-NP electrodes

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

Before pushing the pH-NP electrode through the septum we recommend to test 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 pH-NP electrode. Instead use another (butyl) septum.

**Calibration and measurements:** When moving the pH-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 electrode needle through the small hole in the needle
2. Remove the water in the needle by gently patting the electrode on a tissue both in the front and near the hole of the needle until the space between the needle and the sensor is free of liquid.



3. Afterwards, flush the electrode needle with ethanol through the hole in the needle and dry the electrode with the tissue as described in item 2 – ethanol dries the electrode faster than water.

You can test if the electrode is treated correctly and is dry by placing it in 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

**pH-electrode:** The pH electrode should be stored upright in the protective tube. Always keep the tip moist. For long-term storage the electrode tip can be immersed in demineralized water in the tube. For short-term storage (<10 min) the electrodes can be stored in air.

The room in which the electrodes are stored should be dry and not too hot (10-30 °C).

Store pH-10 and pH-25 in the fridge (2 - 5°C) for longer shelf life.

pH electrodes with built in reference electrode must be stored in 2 M KCl.

*WARNING: The tip of the pH electrode should be kept in aqueous solution at all times, it can however, tolerate up to 10 minutes of exposure to air.*

**Ref-RM:** Between measurements, leave the electrode in 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 3 M KCl solution.

**Unisense reference electrode:** Store in the protective glass 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 2M KCl in the protective casing used for shipping. The room in which the electrodes are stored should be dry and not too hot (10-30 °C).

This covers all reference electrode purchased from Unisense except the REF-RM mentioned in the previous paragraph.

*IMPORTANT: Construction of the electrode with an internal reference involves the creation of a liquid junction approximately 2 mm from the electrode tip. During measurements this liquid junction must be submerged in the medium in order to complete the measurement circuit.*

### 9.2 Cleaning the electrode

The pH electrode can be cleaned by flushing it with 70 % ethanol, or 0.01 M HCl for a couple of minutes. After this, rinse with pH 4.01. 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 2 M KCl.

# TROUBLESHOOTING

<b>Problem</b>	The sensitivity of the pH micro electrode is low, the signal is stable and changes only little with pH
<b>Possible cause</b>	The pH sensitive tip has suffered physical damage and is most likely broken
<b>Solution</b>	Replace the electrode
<b>Problem</b>	Sensitivity of the pH micro electrode is low or the signal is unstable
<b>Possible cause 1</b>	The pH meter is not suitable for pH micro electrodes
<b>Solution 1</b>	Change the pH meter to one with a sufficiently high impedance (>1013 Ohm) (e.g. Unisense pH/mV-Meter)
<b>Possible cause 2</b>	The reference electrode is not properly connected or is malfunctioning
<b>Solution 2</b>	Connect the reference electrode and make sure that it is properly submerged in the same solution as the pH electrode. Alternatively, repair or change the reference electrode
<b>Possible cause 3</b>	Bubble in the narrow parts of the sensor, often not visible to naked eye
<b>Solution 3</b>	Shake the electrode gently like an old mercury fever thermometer as shown in this video: <a href="https://www.unisense.com/video-guides/#troubleshooting">unisense.com/video-guides/#troubleshooting</a>
<b>Possible cause 4</b>	Insoluble compounds have been deposited on the pH sensitive tip
<b>Solution 4</b>	Rinse the tip with ethanol, rinse with 0.01 M HCl and rinse with distilled water.
<b>Possible cause 5</b>	Air bubbles present at the tip of a micro reference electrode
<b>Solution 5</b>	Remove bubbles with a fine brush and avoid entrapment of bubbles
<b>Possible cause 6</b>	Contamination of micro reference half-cell with soluble compound e.g. sulfide (visible dark band approaching the half-cell through the gel)
<b>Solution 6</b>	Replace reference electrode
<b>Possible cause 7</b>	Insoluble compounds deposited at the gel/water junction of the micro reference electrode
<b>Solution 7</b>	Replace reference electrode

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

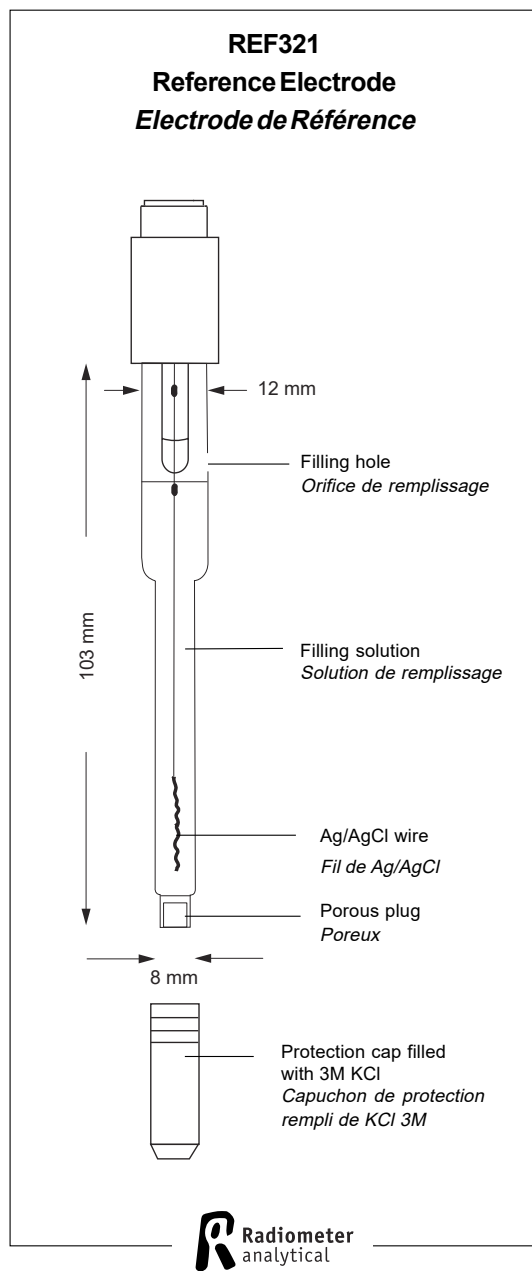
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# MANUAL FOR REF-RM



# REF321 REFERENCE ELECTRODE

## Introduction

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

## Preparation 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 HNO<sub>3</sub>): 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 Normal Cleaning Solution, 250 ml	S16M002
Xtra Strong Cleaning Solution, 250 ml	
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 <sub>2</sub> Cl <sub>2</sub> elec.	-35	-36	-36	-36	-37	-38

Temperature range: 0 to 80 °C

**Note:** an electrode chain comprising pHG301 or XG100/200 glass electrode and a REF321 filled with 3M KCl will give a zero pH of approx. pH 7.25.

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