UNISENSE

pH AND REFERENCE ELECTRODE USER MANUAL





pH and reference electrode manual

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pH AND REFERENCE ELECTRODE MANUAL

UNISENSE A/S

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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 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.

Repair or 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.

CONGRATULATIONS WITH YOUR NEW PRODUCT!

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 variaty 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

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

REPLACEMENT OF SENSORS

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 the 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 note included in the sensor box.*

RECOMMENDED METERS FOR PH SENSORS

One-channel meter: pH/mV-Meter

Multi-channel meters for pH: Microsensor Multimeter

OVERVIEW

This manual covers all Unisense pH and reference electrodes

WARNING
Unisense sensors are neither intended nor approved for use on humans

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^{14} 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.

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 electrode.
Unisense offers selected versions of pH electrodes with internal reference, please view description under "pH combination electrode"

GETTING STARTED

Unpacking a new pH electrode

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 in order to empty the tube of storage liquid. This liquid can be saved for future storage.
3. Remove the clear tape covering the hole just below the screw. This will expose a small hole in the protection tube, and allow the liquid to run out. The sensor is now ready for calibration. NOTE: if you have a pH-MR electrode, calibrate in the protective tube and then carefully move the sensor from this tube into the provided blue guide.

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 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 the KCl-Ag 3M KCl solution, saturated with AgCl.

Unisense Reference electrode

1. Follow 1-2 under pH electrode
2. The tip of the Unisense reference electrode (not the Ref-RM electrode) should be kept immersed in water or an aqueous solution at all times, but can tolerate up to 10 minutes of

WARNING

Do not remove the seal and protective plastic tube before the following steps and calibration are successfully completed.

IMPORTANT

If you have a pH-MR electrode, calibrate in the protective tube and then carefully move the sensor from this tube into the provided blue guide.



exposure to air.

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 7 solution. Place the tip of a reference electrode in the same solution.

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

In the following, we assume that you are using Unisense pH/mV-Meter. If you are using another meter, perform analogous steps with the mV-meter in question. Also, please see note in the margin of this page.

Calibration

Please consult the software manual for instruction on how to calibrate in the software.
Connect the pH/mV-Meter to your computer and start your SensorTrace program. If you are using a strip chart recorder or other data acquisition device, connect this to the output connections of the pH/mV-Meter. Expose both the reference electrode and pH micro electrode tip to at least three different pH buffers covering the range in which measurements are to be made. Record the signal size in your software for each pH buffer. **The pH micro electrode should respond linearly between these points with a 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 SensorTrace software (please see the software manual for instructions).

IMPORTANT

As the pH micro electrode is sensitive to temperature, it is necessary to perform calibration and measurements at the same temperature.

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.

If the sensor functions according to the above description, carefully remove the black tape with the white seal and the protective plastic tube before making measurements.

MEASUREMENTS

Mounting of the electrode

To mount the electrode for measurements, unscrew the screw, breaking the white paper seal. When the screw is loose, the sensor can be drawn from the protective tube and used normally.

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 LS18 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.

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.

TEMPERATURE

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

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 causes for pH combination electrodes.

Special cautions 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 minutes) exposure to air
- Reference electrode is situated near the electrode tip about 1mm away, thus minimum immersion depth is 1mm.
- 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.

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.

STORAGE AND MAINTENANCE

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 (5-35°C).

WARNING

The tip of the pH electrode should be kept in aqueous solution at all times, it can however, tolerate up to 20 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 3M KCl solution.

Micro 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 1M KCl in the protective casing used for shipping. The room in which the electrodes are stored should be dry and not too hot (10-25°C).

IMPORTANT

Construction of the electrode with an internal reference involves the creation of a liquid junction approximately 3 cm from the electrode tip. During measurements this liquid junction must be submerged in the medium in order to complete the measurement circuit.

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 1M KCl.

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.
- Thomas, R.C. 1978. Ion-selective intracellular microelectrodes; how to make them and use them. Academic Press, London

TROUBLE SHOOTING

Problem	The sensitivity of the pH micro electrode is low, the signal is stable and does not change with pH
Possible cause	The pH sensitive tip has suffered physical damage and is most likely broken.
Solution	Replace the electrode

Problem	Sensitivity of the pH micro electrode is low or the signal is unstable
Possible cause 1	The pH meter is not suitable for pH micro electrodes
Solution	Change the pH meter to one with a sufficiently high impedance ($>10^{14}$ Ohm) (e.g. Unisense pH/mV-Meter)
Possible cause 2	The reference electrode is not properly connected or is malfunctioning.
Solution	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.
Possible cause 3	Insoluble compounds have been deposited on the pH sensitive tip.
Solution	Rinse the tip with ethanol, rinse with 0.01 M HCl and rinse with distilled water.
Possible cause 4	Air bubbles present at the tip of a micro reference electrode.
Solution	Remove bubbles with a fine brush and avoid entrapment of bubbles.
Possible cause 5	Contamination of micro reference half-cell with soluble compound e.g. sulfide (visible dark band approaching the half-cell through the gel)

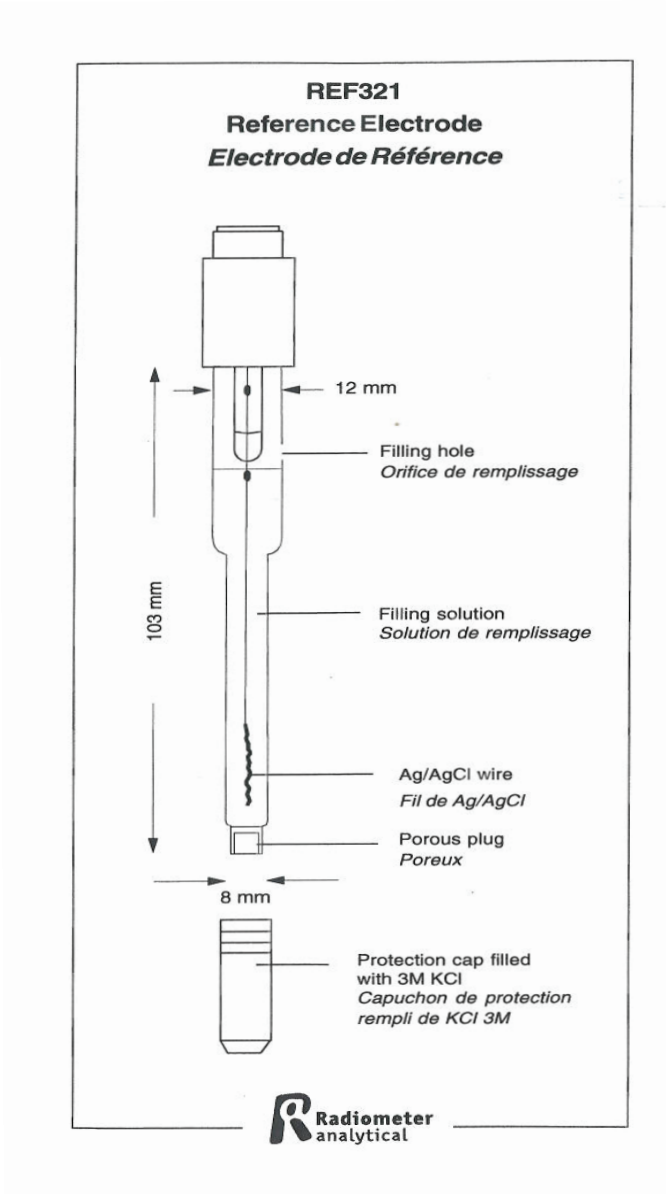
TROUBLE SHOOTING

Solution Replace reference electrode.

Possible cause 6 Insoluble compounds deposited at the gel/water junction of the micro reference electrode

Solution Replace 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)


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.

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_3): 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

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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