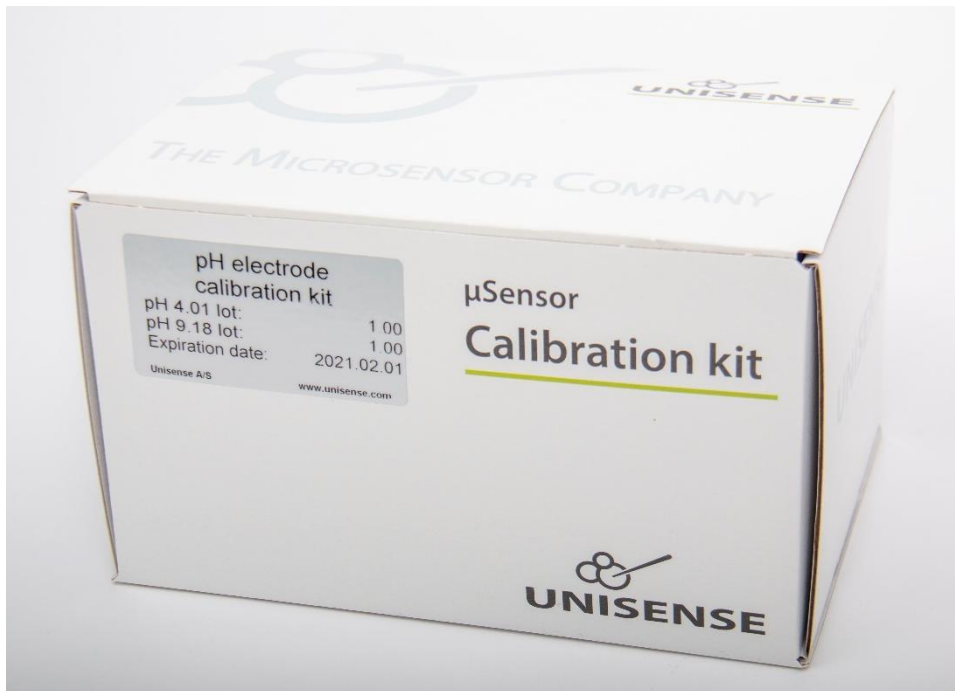


# pH electrode calibration kit

## Manual



**UNISENSE**   
SCIENTIFIC

## Table of Contents

<b>1</b>	<b>WARRANTY AND LIABILITY .....</b>	<b>3</b>
1.1	NOTICE TO PURCHASER .....	3
1.2	WARNING .....	3
1.3	WARRANTY AND LIABILITY .....	3
<b>2</b>	<b>SUPPORT, ORDERING, AND CONTACT INFORMATION .....</b>	<b>3</b>
<b>3</b>	<b>CONTENT OF THE CALIBRATION KIT .....</b>	<b>3</b>
<b>4</b>	<b>PRINCIPLE OF CALIBRATION .....</b>	<b>4</b>
<b>5</b>	<b>CALIBRATION PROCEDURE .....</b>	<b>5</b>
5.1	CALIBRATING MOST PH MICROELECTRODE WITH EXTERNAL REFERENCE ELECTRODE .....	5
5.2	CALIBRATING A PH MICROELECTRODE WITH BUILT IN REFERENCE ELECTRODE.....	5
5.3	CALIBRATING A PH MICROELECTRODE FOR THE MICRORESPIRATION SYSTEM.....	6
5.4	CALIBRATING PH ELECTRODES IN FLOW CELLS.....	7
5.4.1	<i>Create a flow cell calibration setup.....</i>	7
<b>6</b>	<b>SPECIFICATIONS .....</b>	<b>7</b>

## 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 Calibration Kit is guaranteed to give the pH values indicated on the pouches 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](mailto:sales@unisense.com)

Unisense A/S

Langdyssen 5

DK-8200 Aarhus N, Denmark

Tel: +45 8944 9500

Fax: +45 8944 9549

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

## 3 Content of the calibration kit

Item	Number
20 ml pouch with low pH (see pouch for exact pH value)	10
20 ml pouch with high pH (see pouch for exact pH value)	10
Calibration cap with O-ring and 3 cm Viton tubing	2
Y-piece with 3 cm Viton tubing	1
10 ml syringe	1
80 x 2.1 mm needle (green)	1
10 cm transparent tube for reference electrode	1



Figure 1: Calibration kit contents: A: Calibration kit box with pH buffer pouches, B: 10 ml syringe, C: 10 cm tube for reference electrode, D: 80 x 2.1 mm needle (green), E: two Calibration Caps with tubing and O-rings, F: Y-connector with tubing.

## 4 Principle of calibration

Unisense glass pH sensors respond linearly to pH changes in the range from ca. 4 to 9 (for specifications see <https://www.unisense.com/pH>). Therefore, a two point calibration is sufficient for work in this range. The pH electrode works in the range from 2 to 10 but if working outside the linear range, it is necessary to use extra standards and calibrate manually.

This calibration kit contains pH buffers with low and high pH values. The actual pH values of the buffers can be seen on the individual pouches with the buffers. This pH value is valid for calibration at one specific temperature. The pH value for the buffers at other temperatures is given in a table on the pouches.

## 5 Calibration procedure

For calibration of pH electrodes, two calibration points are needed. These calibration points can be obtained using the two different pH buffers in this kit.

### 5.1 Calibrating most pH microelectrode with external reference electrode

1. Connect the two calibration caps with the Y-piece. 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. Mount one calibration cap on the 10 cm transparent tube.
3. Mount the other calibration cap on the pH electrode protection tube (see note A below).
4. Place the pH electrode and 10 cm transparent tube upright.
5. Place the reference electrode in the 10 cm transparent tube.
6. Mount the needle (green) on the 10 ml syringe.
7. Pierce a pouch with the low pH buffer and aspirate ca. 10 ml.
8. Remove the needle and attach the syringe to the black tubing on the Y-piece.
9. Inject the buffer into the calibration caps until the tips of both electrodes are immersed at least 1 cm.
10. Make sure that there are no air bubbles trapped in the tubing and Y-piece.
11. Allow the pH electrode to respond and stabilize. Then record the calibration value in uSense (see the uSense manual for details: <https://unisense.com/manuals/#software>)
12. Remove the pH buffer from the calibration caps.
13. Rinse the calibration caps thoroughly with water.
14. Repeat steps 7 through 12 using the high pH buffer.

### 5.2 Calibrating a pH microelectrode with built in reference electrode

1. Mount a calibration cap on the pH microelectrode protection tube (see note B below). 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. Place the pH electrode upright.
3. Mount the needle (green) on the 10 ml syringe.
4. Pierce a pouch with the low pH buffer and aspirate ca. 10 ml.
5. Remove the needle and attach the syringe to the black tubing on the calibration cap.
6. Inject this buffer into the calibration cap until the tip of the microelectrode is immersed at least 1 cm.
7. Allow the pH electrode to respond and stabilize. Then record the calibration value in uSense (see the uSense manual for details: <https://unisense.com/manuals/#software>).
8. Remove the pH buffer from the calibration cap.
9. Rinse the calibration caps thoroughly with water.
10. Repeat steps 4 through 9 using the high pH buffer.

#### NOTES:

- A. The protection tube of the pH microelectrodes without built in reference electrode is filled with demineralized water which keeps the pH sensitive tip hydrated. After use the pH microelectrode must be stored with the protection tube filled with water.

- B. The protection tube of pH microelectrodes with built in reference is filled with 2 M KCl which keeps the pH sensitive tip and the reference electrode hydrated. This liquid may be saved in a container and used for filling the protection tube after use.

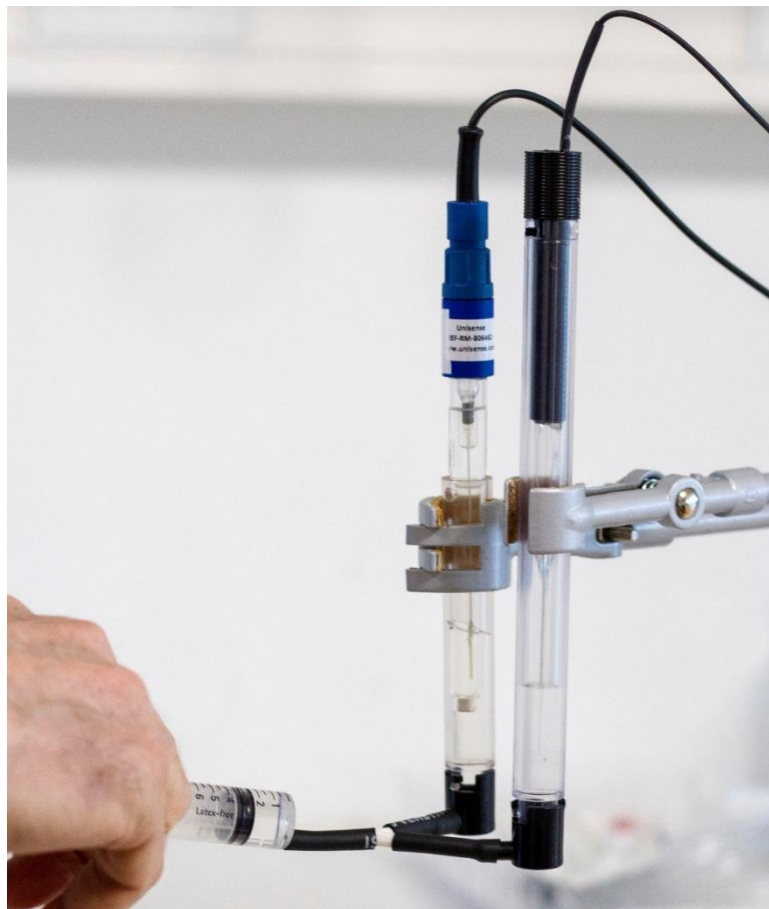


Figure 2: pH electrode in the protection tube and reference electrode in the 10 cm transparent tube. The two Calibration Caps are mounted on the protection tubes and connected via the Y-connector. The pH calibration solution is injected using the 10 ml syringe.

### 5.3 Calibrating a pH microelectrode for the Microrespiration System

1. Fill the low pH buffer into a Microrespiration chamber using the syringe and needle. Make sure that the chamber including the capillary opening in the lid is filled (see note A below).
2. Place this chamber in the Microrespiration stirrer rack.
3. The stirrer rack with chamber must be immersed in a water bath and the reference electrode must be dipped into the water bath (see note B below).
4. Place the pH microelectrode in the stirrer rack with the plastic tip in the opening of the Microrespiration chamber lid.
5. Insert the pH microelectrode into the pH buffer.
6. Allow the pH microelectrode to respond and stabilize. Then record the calibration value in uSense (see the uSense manual for details: <https://unisense.com/manuals/#software>).
7. Pull the redox microelectrode back into the blue shaft and remove it from the rack.
8. Clean the redox microelectrode by pushing it out of the blue shaft and dipping it into a beaker with water.
9. Repeat steps 1 through 8 using the high pH buffer.

NOTES:

- A. Microrespiration chambers from 400  $\mu$ l to 4 ml may be used. Fill the chamber and mount the lid making sure that the capillary opening in the lid is filled with pH buffer.
- B. There must be a continuous liquid connection between the pH electrode tip and the reference electrode. This is ensured by completely filling the Microrespiration chamber, including the capillary in the lid, with the pH buffer. The water in the water bath is then in contact with the pH buffer in the capillary opening of the lid, ensuring this continuous connection.

## 5.4 Calibrating pH electrodes in Flow Cells

pH electrodes mounted with flow cells cannot be calibrated using the Calibration Cap. Instead, it is recommended to follow the suggestions outlined below and the general calibration procedure in section 5.1 above.

### 5.4.1 Create a flow cell calibration setup

The single pH electrodes in flow cells require an external reference electrode. This is typically also placed in a flow cell, so the two electrodes can be placed in line with each other. In order to calibrate a pH electrode in a flow cell, the tips of both the pH electrode and the reference electrode must be exposed to the calibration standard, and there must be a continuous liquid connection between the two electrode tips. The optimal way to do this depends on the actual setup, however, it is recommended to make a setup that allows calibration of the electrode without removing it from the flow cell and from the setup. Generally, the pH electrode and the reference electrode could be connected with tubing and a Luer connector connected to these, directly or via tubing, will allow injection of the calibration standard into the flow cell. A three-way valve on either side of the two flow cells will allow easy injection of the calibration standard with the electrodes and flow cells 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 adaptor that is mounted directly in the flow cell (Figure 3, 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 3, right)



Figure 3: Left: Luer adaptor for direct mounting in the flow cell (e.g., IDEX P-624). Right: Barbed Luer adaptor for tube connection

## 6 Specifications

- Volume of pH buffer pouches: 20 ml
- Lifetime of the calibration kit: See label on the calibration box or the buffer pouch
- pH of the calibration solutions: See label on the calibration box or the buffer pouch
- Temperature effect on calibration pH: See table on the buffer pouch

