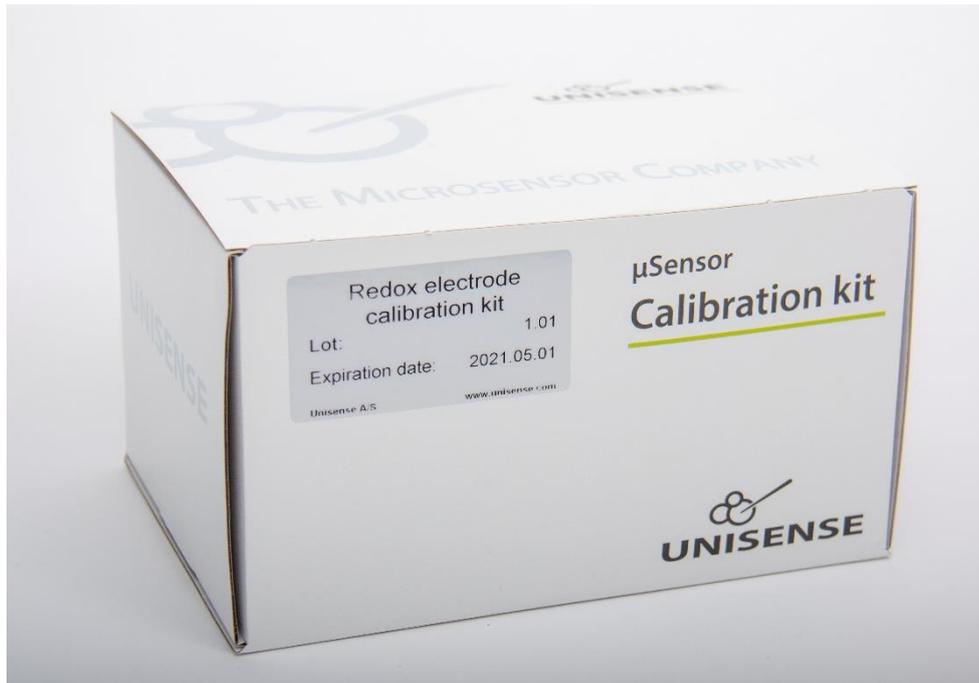


# Redox electrode calibration kit

## Manual



## 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>SAFETY</b> .....	<b>3</b>
<b>4</b>	<b>CONTENT OF THE CALIBRATION KIT</b> .....	<b>4</b>
<b>5</b>	<b>PRINCIPLE OF CALIBRATION</b> .....	<b>4</b>
<b>6</b>	<b>CALIBRATION PROCEDURE</b> .....	<b>5</b>
6.1	CALIBRATING A REDOX MICROELECTRODE WITH EXTERNAL REFERENCE ELECTRODE .....	5
6.1.1	<i>Estimating the offset</i> .....	5
6.1.2	<i>Verifying sensor response</i> .....	5
6.2	CALIBRATING A REDOX MICROELECTRODE WITH BUILT IN REFERENCE ELECTRODE .....	6
6.2.1	<i>Estimating the offset</i> .....	6
6.2.2	<i>Verifying sensor response</i> .....	6
6.3	CALIBRATING A REDOX MICROELECTRODE FOR THE MICRORESPIRATION SYSTEM .....	7
6.3.1	<i>Estimating the offset</i> .....	7
6.3.2	<i>Verifying sensor response</i> .....	8
<b>7</b>	<b>SPECIFICATIONS</b> .....	<b>8</b>
<b>8</b>	<b>REDOX POTENTIAL OF THE CALIBRATION SOLUTION AT DIFFERENT TEMPERATURES</b> .....	<b>9</b>

REDOX ELECTRODE CALIBRATION KIT USER MANUAL

Copyright © Unisense A/S

Version: February 2020

## 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 Calibration Kit is guaranteed to give the redox potential given in section 8 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

Tueager 1

DK-8200 Aarhus N, Denmark

Tel: +45 8944 9500

Fax: +45 8944 9549

Further documentation and support are available at our website: <https://www.unisense.com/>.

## 3 Safety

The calibration solution contains sodium hydroxide.



Signal Word

Warning

Hazard Statements

H319 - Causes serious eye irritation

H302 - Harmful if swallowed

H315 - Causes skin irritation

H360FD - May damage fertility. May damage the unborn child

See the full Materials Safety Data Sheet at: [https://www.unisense.com/calibration\\_kits/](https://www.unisense.com/calibration_kits/)

**NOTE:** The calibration solution is iodine based and strongly colouring. Wipe off any spills immediately!

## 4 Content of the calibration kit

Item	Number
Exetainer with Redox buffer	10
Exetainer with low Redox test solution	10
Calibration cap with O-ring and 3 cm Viton tubing	2
Y-connector 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 Exetainers, 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.

## 5 Principle of calibration

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.

The calibration solution in this calibration kit has a well-defined redox potential relative to the SHE (see table in paragraph 8) and this can be used to determine the offset between the measured redox potential and the redox potential relative to the SHE.

The offset is calculated as:

$$\text{Offset (mV)} = \text{Pot}_{\text{SHE}} \text{ (mV)} - \text{Pot}_{\text{CAL}} \text{ (mV)}$$

where  $\text{Pot}_{\text{CAL}}$  is the measured potential in the calibration solution using the redox electrode and the Ag/AgCl electrodes and  $\text{Pot}_{\text{SHE}}$  is the defined redox potential for the calibration solution relative to the SHE found in section 8. Once the offset is determined, redox potentials measured relative to a Ag/AgCl reference electrode can be recalculated relative to the SHE as:

$$\text{Pot}_{\text{TRUE}} \text{ (mV)} = \text{Pot}_{\text{MEAS}} \text{ (mV)} + \text{Offset (mV)}$$

where  $\text{Pot}_{\text{TRUE}}$  is the measured potential relative to the SHE and  $\text{Pot}_{\text{MEAS}}$  is the redox potential measured in the sample using the redox electrode and the Ag/AgCl reference electrode.

## 6 Calibration procedure

For calibration of redox electrodes it is only necessary to determine the offset relative to the standard hydrogen electrode and to verify that the redox electrode responds to a change in redox potential (see section 5). Note that electrodes with piercing needle will not fit the calibration cap and cannot be calibrated with this kit.

### 6.1 Calibrating a redox microelectrode with external reference electrode

#### 6.1.1 Estimating the offset

1. Connect the two calibration caps with the Y-connector.
2. Mount one calibration cap on the 10 cm transparent tube.
3. Mount the other calibration cap on the redox electrode protection tube.
4. Place the redox 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. Open the Exetainer with the Redox buffer and aspirate ca. 10 ml.
8. Remove the needle and attach the syringe to the black tubing on the Y-connector.
9. Inject the calibration solution into the calibration caps until the tips of both electrodes are immersed at least 1 cm.
10. Allow the redox electrode to respond and stabilize. Then record the calibration value in SensorTrace (see the SensorTrace manual for details: <https://www.unisense.com/manuals/>). The offset is now determined and SensorTrace will automatically apply this to the measurements
11. Remove the redox buffer from the calibration caps.
12. Rinse the 10 ml syringe and the calibration caps thoroughly with water.

#### 6.1.2 Verifying sensor response

1. Open the Exetainer with the Redox test solution and aspirate 10 ml.
2. Remove the needle and attach the syringe to the black tubing on the Y-connector.
3. Inject the Redox test solution into the calibration caps until the tips of both electrodes are immersed at least 1 cm.
4. Allow the redox electrode to respond. This response is much slower and more variable than for the Redox buffer.
5. The signal should now be at least 200 mV below the signal for the Redox buffer. If this is the case the redox electrode is working fine.

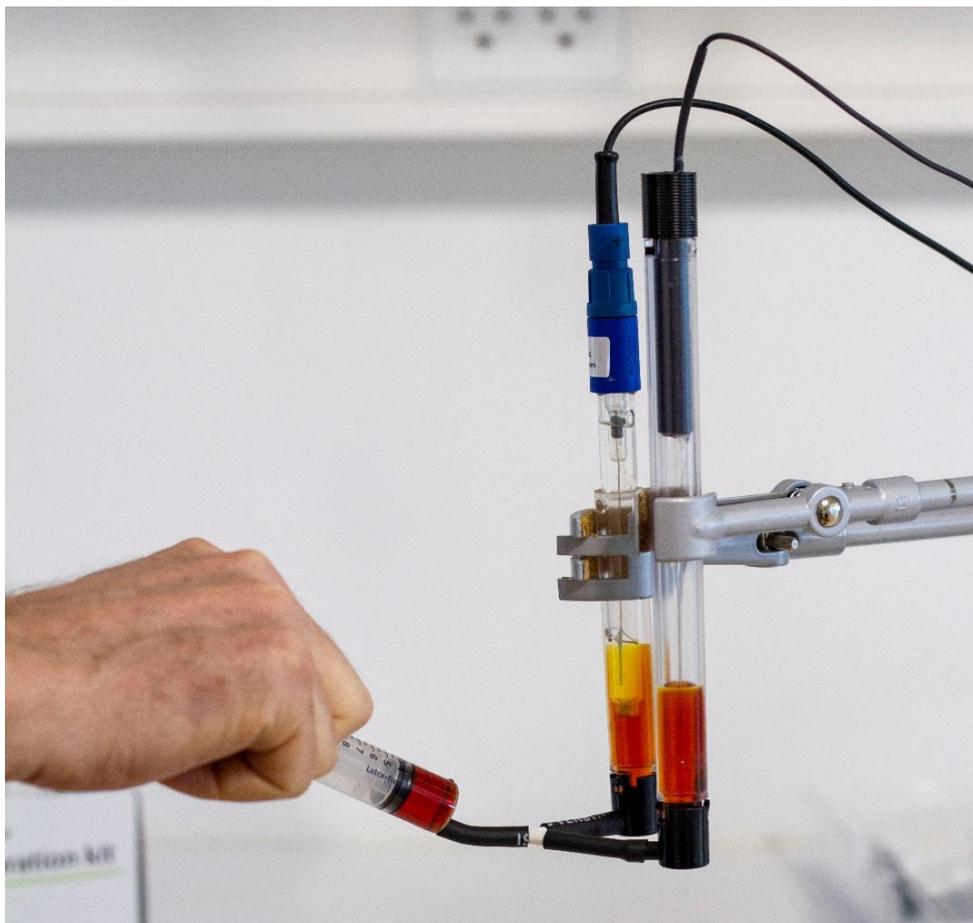


Figure 2: Redox electrode in the protection tube and reference electrode in the 10 cm transparent tube. The two Calibration Caps are mounted on the protection tubes and connected via the Y-connector. The redox calibration solution is injected using the 10 ml syringe.

## 6.2 Calibrating a redox microelectrode with built in reference electrode

### 6.2.1 Estimating the offset

1. Mount a calibration cap on the redox electrode protection tube (see note A below).
2. Place the redox electrode upright.
3. Mount the needle (green) on the 10 ml syringe.
4. Open the Exetainer with the Redox buffer and aspirate ca. 10 ml.
5. Remove the needle and attach the syringe to the black tubing on the calibration cap.
6. Inject the calibration solution into the calibration cap until the tip of the electrode is immersed at least 1 cm.
7. Allow the redox electrode to respond and stabilize. Then record the calibration value in SensorTrace (see the SensorTrace manual for details: <https://www.unisense.com/manuals/>). The offset is now determined and SensorTrace will automatically apply this to the measurements.
8. Remove the redox buffer from the calibration cap.
9. Rinse the 10 ml syringe and the calibration cap thoroughly with water.

### 6.2.2 Verifying sensor response

1. Open the Exetainer with the Redox test solution and aspirate 10 ml.
2. Remove the needle and attach the syringe to the black tubing on the calibration cap.

3. Inject the Redox test solution into the calibration cap until the tip of the electrode is immersed at least 1 cm.
4. Allow the redox electrode to respond. This response is much slower and more variable than for the Redox buffer.
5. The signal should now be at least 200 mV below the signal for the Redox buffer. If this is the case the redox electrode is working fine.

NOTES:

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

### 6.3 Calibrating a redox microelectrode for the Microrespiration System

#### 6.3.1 Estimating the offset

1. Fill the Redox 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 redox microelectrode in the stirrer rack with the plastic tip in the opening of the Microrespiration chamber lid.
5. Insert the redox microelectrode into the redox buffer.
6. Allow the redox microelectrode to respond and stabilize. Then record the calibration value in SensorTrace (see the SensorTrace manual for details: <https://www.unisense.com/manuals/>). The offset is now determined and SensorTrace will automatically apply this to the measurements.
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.



Figure 3: Redox microsensor in the Microrespiration guide.

### 6.3.2 Verifying sensor response

1. Fill the low Redox test solution into a Microrespiration chamber using the syringe and needle. Make sure that the chamber including the capillary opening in the lid is filled.
2. Repeat points 2 - 5 in “6.3.1 Estimating the offset” above.
3. Allow the redox microelectrode to respond and stabilize.
4. The signal should now be at least 200 mV below the signal for the Redox buffer. If this is the case the redox electrode is working fine.
5. Pull the redox microelectrode back into the blue shaft and remove it from the rack.
6. Clean the redox microelectrode by pushing it out of the blue shaft and dipping it into a beaker with water.

#### 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 redox buffer.
- B. There must be a continuous liquid connection between the redox electrode and the reference electrode. This is ensured by completely filling the Microrespiration chamber, including the capillary in the lid, with the redox buffer. The water in the water bath is then in contact with the redox buffer in the capillary opening of the lid, ensuring this continuous connection.

## 7 Specifications

- Volume of Redox buffer in Exetainer: 12.5 ml
- Volume of Redox test solution in Exetainer: 12.5 ml
- Lifetime of the calibration kit: See label on the calibration box
- Redox potential of the Redox buffer: See table in section 8 below.

## 8 Redox potential of the calibration solution at different temperatures

Redox potential of the Redox buffer solution relative to the Standard Hydrogen Electrode as a function of temperature				
Temp (°C)	Eh (mV)		Temp (°C)	Eh (mV)
0	438.0		26	418.9
1	437.4		27	418.0
2	436.8		28	417.2
3	436.1		29	416.3
4	435.5		30	415.4
5	434.8		31	414.5
6	434.2		32	413.6
7	433.5		33	412.7
8	432.8		34	411.8
9	432.1		35	410.9
10	431.4		36	409.9
11	430.7		37	409.0
12	430.0		38	408.0
13	429.2		39	407.1
14	428.5		40	406.1
15	427.7		41	405.1
16	427.0		42	404.1
17	426.2		43	403.1
18	425.4		44	402.1
19	424.7		45	401.1
20	423.9		46	400.1
21	423.1		47	399.0
22	422.2		48	398.0
23	421.4		49	396.9
24	420.6		50	395.9
25	419.8		51	394.8