Redox electrode calibration kit

Manual

UNISENSE

Contents

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
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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/

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 solution	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

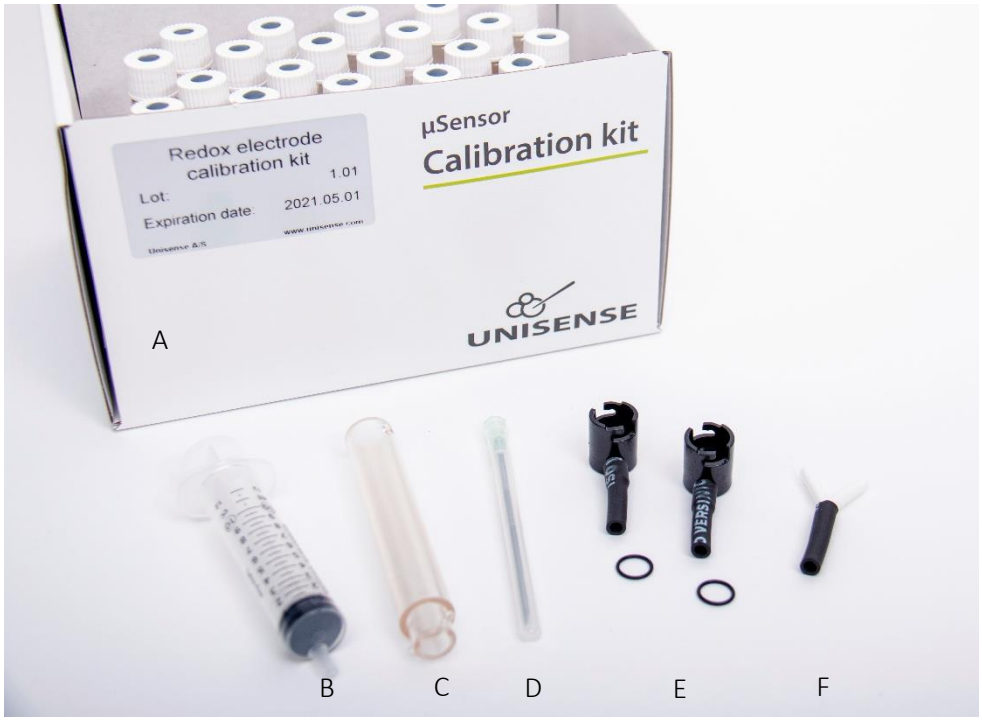

Figure 1: Calibration kit contents: A: Calibration kit box with Exetainers, B: 10 ml syringe, C: 10 cm tube for reference electrode, D: 80 x 2.1 mm needle (green), E: two Calibration Caps with tubing and O-rings, F: Y-connector with tubing.

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:

Offset (mV) = Pot_{SHE} (mV) - Pot_{CAL} (mV)

where Pot_{CAL} is the measured potential in the calibration solution using the redox electrode and the Ag/AgCl electrodes and Pot_{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:

Pot_{TRUE} (mV) = Pot_{MEAS} (mV) + Offset (mV)

where Pot_{TRUE} is the measured potential relative to the SHE and Pot_{MEAS} is the redox potential measured in the sample using the redox electrode and the Ag/AgCl reference electrode.

6 Calibration procedure

6.1 Notes regarding 1-point calibration

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: For calibration in the Unisense software, the *1 point calibration* box must be checked (Figure 2).

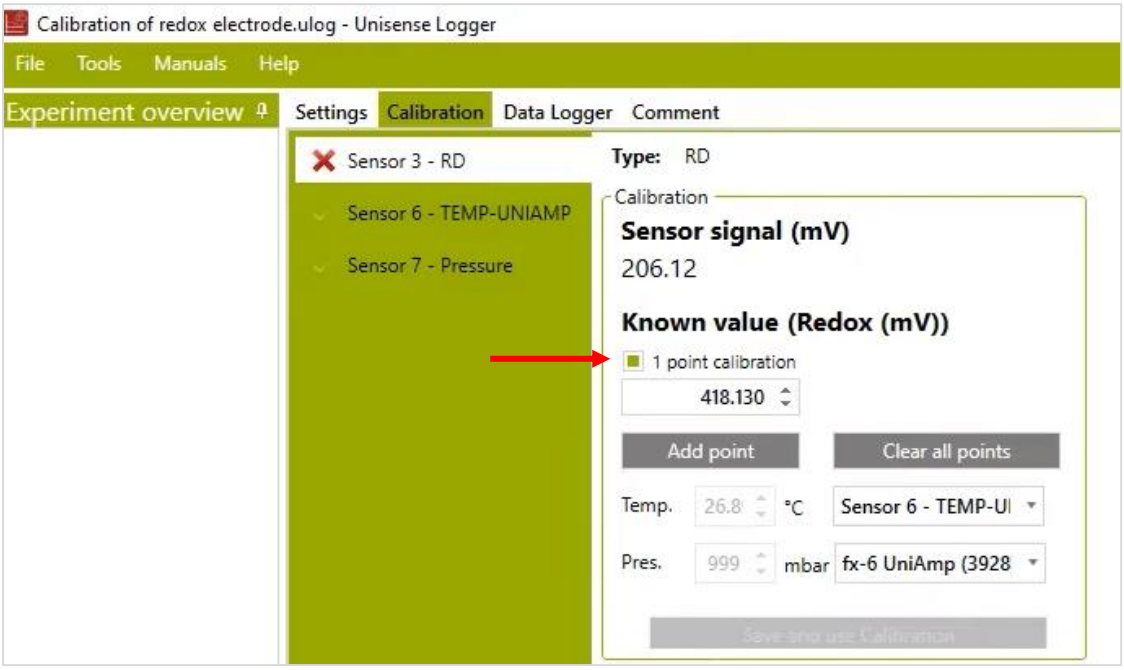

Figure 2: Calibration tab showing the 1 point calibration checked.

6.2 Calibrating most redox microelectrode with external reference electrode

6.2.1 Estimating the offset

1. Connect the two calibration caps with the Y-connector. 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 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.
    NOTE: The *1 point calibration* box must be checked (Figure 2).
11. Remove the redox buffer from the calibration caps.
12. Rinse the 10 ml syringe and the calibration caps 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 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.

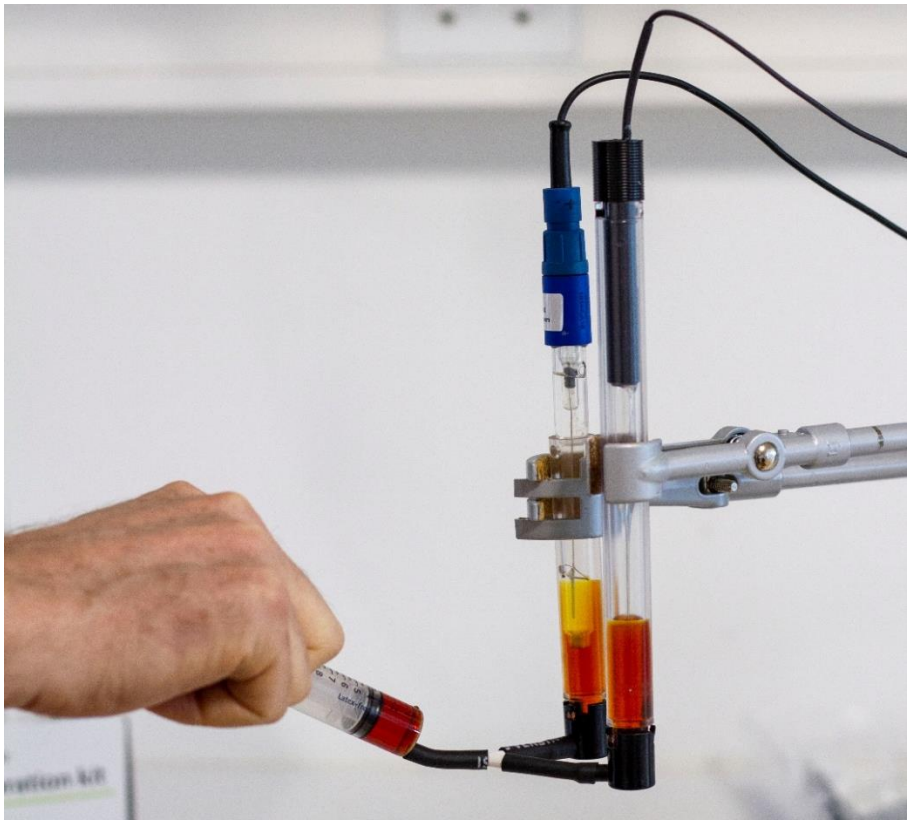

Figure 3:Redox electrode in the protection tube and reference electrode in the 10 cm transparent tube. The two Calibration Caps are mounted on the protection tubes and connected via the Y-connector. The redox calibration solution is injected using the 10 ml syringe.

6.3 Calibrating a redox microelectrode with built in reference electrode

6.3.1 Estimating the offset

1. Mount a calibration cap on the redox electrode protection tube (see note A 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 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.
   NOTE: The *1 point calibration* box must be checked (Figure 2).
8. Remove the redox buffer from the calibration cap.
9. Rinse the 10 ml syringe and the calibration cap thoroughly with water.

6.3.2 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. This type of redox electrode must always be stored with the tip immersed in 2 M KCl solution.

6.4 Calibrating a redox microelectrode for the Microrespiration System

6.4.1 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.
   NOTE: The *1 point calibration* box must be checked (Figure 2).
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 4: Redox microsensor in the Microrespiration guide.

6.4.2 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 "6.4.1 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 µl 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.

6.5 Calibrating redox electrodes in Flow Cells

Redox electrodes mounted with flow cells cannot be calibrated using the Calibration Cap. Instead, it is recommended to follow the suggestions outlined below, the notes in section 6.1 above and the general calibration procedure in section 6.2 above.

6.5.1 Create a flow cell calibration setup

The redox 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 redox electrode in a flow cell, the tips of both the redox 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 redox 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 adapter that is mounted directly in the flow cell (Figure 5, 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 5, right)

Figure 5: Left: Luer adaptor for direct mounting in the flow cell (e.g., IDEX P-624). Right: Barbed Luer adaptor for tube 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