O2k-Manual: Oxia

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Oxia – HyperOxia to Hypoxia

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Contents

1. Intended use

The Oxia generates gaseous oxygen and hydrogen by electrolysis of water using a proton exchange membrane (PEM). O_2 and H_2 gas can be used to control the O_2 regime in the Oroboros O2k. Low oxygen concentrations (<50 µM) are used to mimic tissue normoxia or hypoxia. Hyperoxic conditions above air saturation (250-600 µM O_2) are routinely used for high-resolution respirometry of permeabilized muscle fibers or to induce oxidative stress in cells and mitochondrial preparations.

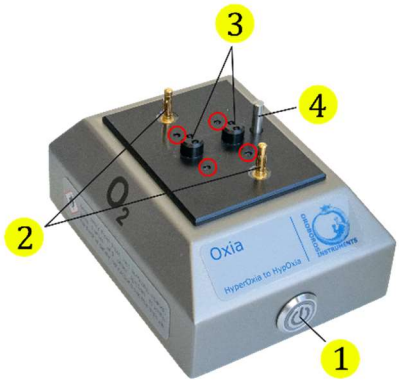
2. Safety

- Before operating the Oxia, read the user manual.
- Flammable gases, Category 1. Keep the Oxia away from heat, hot surfaces, sparks, open flames, and other ignition sources.
- The produced gas is for laboratory use only.
- Do not leave running unattended.
- Do not run overnight.
- Do not switch on the Oxia without the Oxia-Cell being connected to the Oxia-Base.
- Do not operate the Oxia without water in the H_2O-chamber.
- Do not use any liquid besides deionized or distilled H_2O to run the Oxia.
- Do not use grease on the valves.
- Do not run the Oxia in a space smaller than 9 m^3, such as a small cabinet.
- Unless there is a continuous air exchange, air the room for 5 min after 20 cycles of gas production.
- Do not cover the Oxia while it is running/during operation.
- The Oxia must only be operated with a power supply or USB-C to USB-C cable supplied or recommended by Oroboros Instruments.
- The Oxia-Base must not be exposed to liquids (IP Code 21).
- Use different syringes for the withdrawal of O_2 or H_2 gas, respectively.
- Do not disassemble the Oxia-Cell by loosening the hex head screws.
- Any servicing or repair must only be done by the manufacturer. In case of any problems please contact Oroboros Instruments (https://www.oroboros.at/index.php/o2k-technical-support/).

3. Components

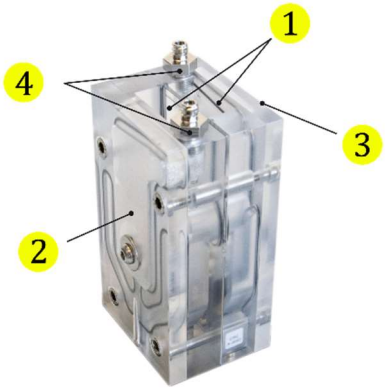
Oxia-Base

(1) Power button on the front, (2) electrical contact pins, (3) LEDs for gas chamber illumination, (4) stainless steel assembly guide, red circles: sensors to detect filling of the O_2- and H_2-chambers on top, and USB-C socket (not shown) on the rear.



Oxia-Cell

Proton exchange membrane (PEM) electrolysis cell and (1) separate H_2O-chambers A (left) and B (right), which are connected to the (2) O_2 or (3) H_2-chamber, respectively. (4) The Luer Lock connections with spring valves allow for gas withdrawal from the O_2- and H_2-chamber.



Cloth Cover to protect the Oxia-Cell from contamination during storage.

Gas syringes 10 mL syringes with Luer Lock connection for gas withdrawal from the O_2- and H_2-chamber and gas injection into the O2k-chamber.

O2k-Injection needle with spacer to obtain the correct insertion length for gas injection into the O2k-chambers.

Voltage supply USB-C to USB-C cable to connect the Oxia to the O2k (I-Series or higher; NextGen-O2k). Alternatively, the Oxia can be plugged to a power socket via an external power supply.

Do not use a USB-A to USB-C adapter.

Use only the power supply provided by the manufacturer.

4. Specifications

4.1. Technical specifications

Voltage supply:	USB-C Power supply with 1.5 A, 5 V, max. 7.5 W
Dimensions:	138x108x206 mm
Weight:	1.5 kg
Gas production rate:	O_2: 7 mL/min
	H_2: 14 mL/min
Volume of two H_2O-chambers:	37 mL
Volume of O_2-chamber:	32 mL
Volume of H_2-chamber:	32 mL

4.2. Ambient conditions

Operating temperature:	5-40 °C
Maximum height above sea level:	2000 m
Maximum relative air humidity:	80 % at 31 °C, 50 % at 40 °C; linear temperature dependence
Tolerance of voltage supply:	max ±10 %

4.3. Safety specifications

- Optical sensors for the O_2- and H_2-chamber stop electrolysis and thereby gas production as soon as the water is completely replaced by gas, indicated by illumination (blue) of the respective gas chamber.
- If gas production is not automatically stopped upon filling of one of the gas chambers, the excessive gas escapes to the H_2O-chamber. The H_2O-chambers are open to allow the gas to escape. The separator between the two H_2O-chambers prevents mixing of H_2 and O_2. Thus, there is no risk of oxyhydrogen combustion.
- The minimum cubature of the room (>9 m^3) and frequent air exchange (see above) prevents the formation of oxyhydrogen, as under atmospheric pressure, the volume fraction of hydrogen is kept below 4 %.

5. Operating instructions

5.1. Assembly

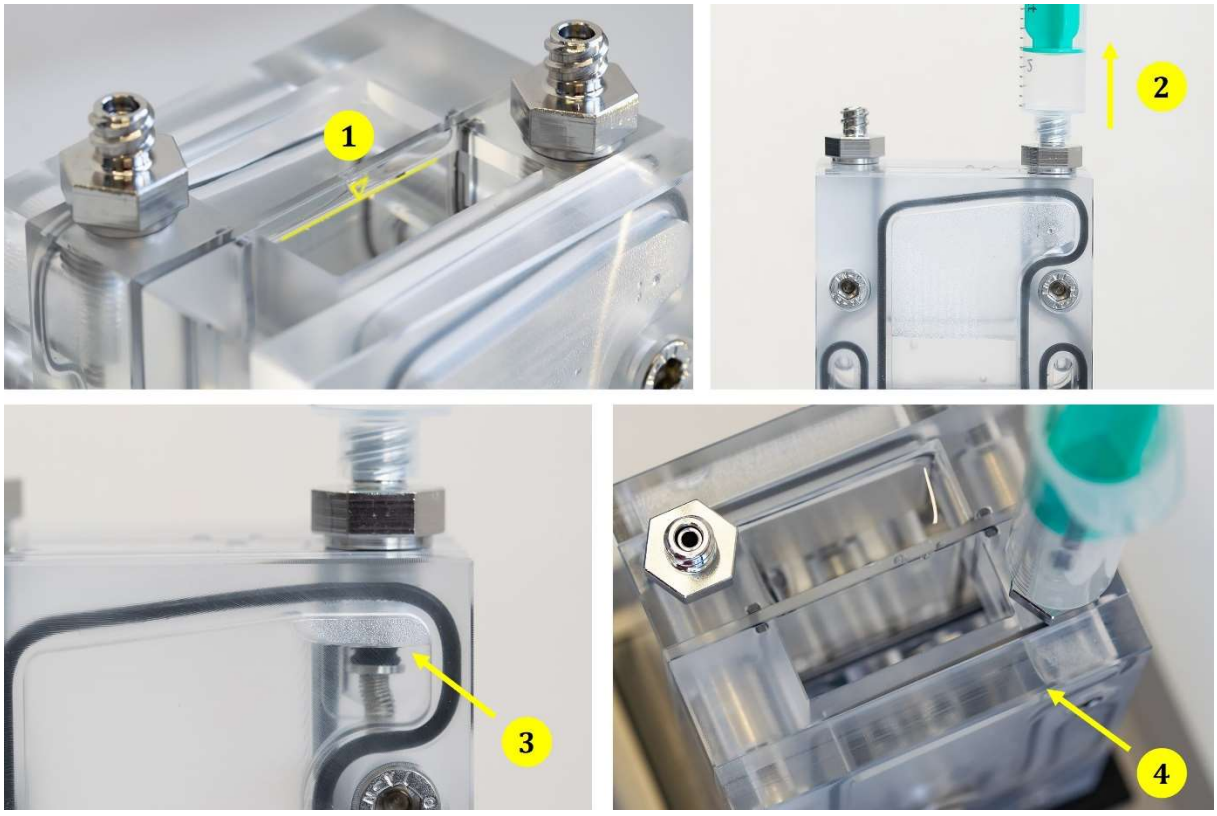
Ensure the Oxia power is switched off (power button is not illuminated) when the Oxia-Cell is not connected to the Oxia-Base.

1. Both H_2O-chambers and both gas chambers of the Oxia-Cell must be empty before assembly.
   a. To remove residual water from the H_2O-, O_2-, and H_2-chambers, switch off the Oxia and unplug the Oxia-Cell from the Oxia-Base.
   b. Remove water from the H_2O-chambers by turning the Oxia-Cell upside down.
   c. Withdraw residual water from the O_2- and H_2-chambers with a gas syringe via the Luer Lock connection with the Oxia-Cell upside down.
2. Plug the Oxia-Cell onto the Oxia-Base. The stainless-steel assembly guide on the Oxia-Base must line up with the corresponding hole on the bottom of the Oxia-Cell to ensure it is aligned correctly. The Oxia-Cell is flush against the Oxia-Base if inserted correctly.

5.2. Production and withdrawal of O_2 and H_2 gas

Before switching on the device, ensure that the Oxia-Cell is correctly connected to the Oxia-Base.

1. Fill both H_2O-chambers with deionized or distilled H_2O to the fill mark (1).
2. Screw an empty gas syringe with the piston fully inserted onto the Luer connector of the O_2-chamber and withdraw air from the O_2-chamber by suction (2). Thereby, the water is sucked from the corresponding H_2O-chamber into the O_2-chamber. Repeat until the water level is right beneath the spring valve, as seen from the side (3) or on top (4).
3. Repeat step 2 for the H_2-chamber.

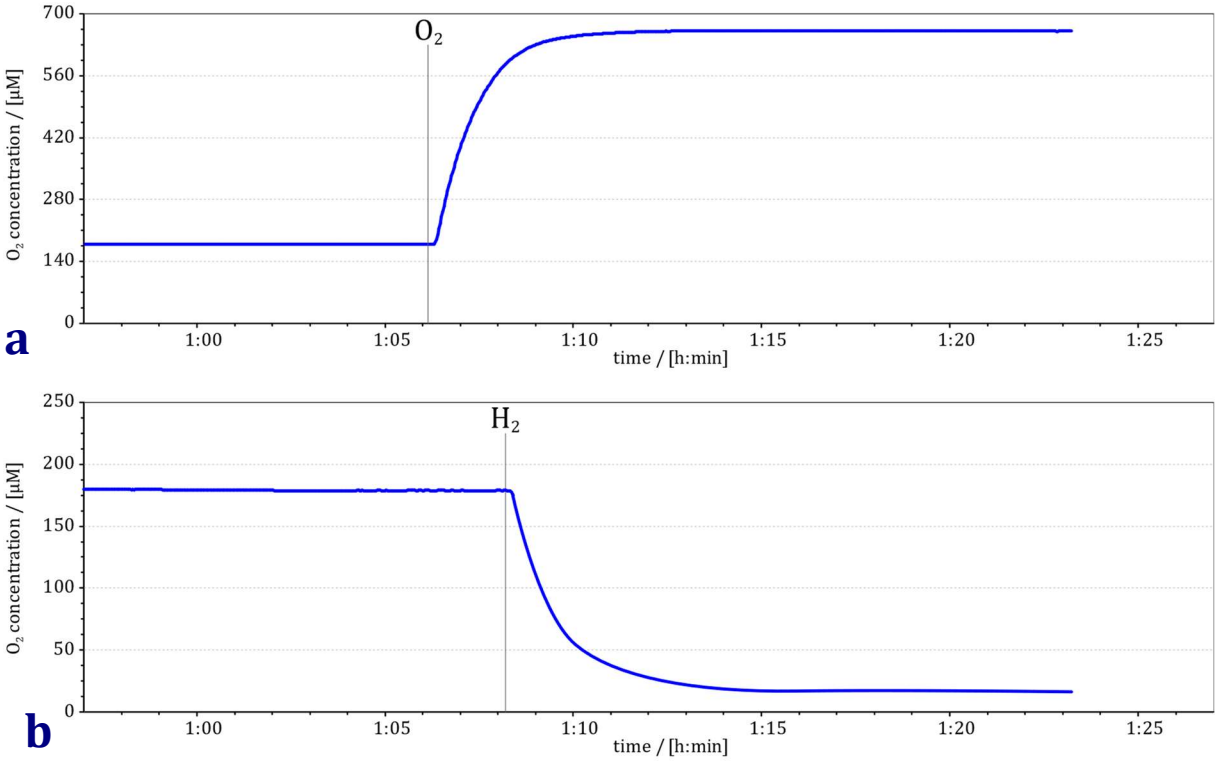


4. Connect the Oxia (USB-C socket on the rear of the Oxia-Base) to the USB-C socket on the rear of the O2k (I-Series or higher; NextGen-O2k) via the supplied USB-C to USB-C cable, or to a power socket via the external Power Supply.
5. Start gas generation by pressing the power button on the front of the Oxia-Base. The power button is illuminated in blue indicating that the Oxia is on.
6. Let the Oxia run until the H_2-chamber is filled with gas or until enough O_2 or H_2 gas is produced for your experiment. Water electrolysis and thereby gas production stops automatically when the O_2- or H_2-chamber is filled with gas. The blue illumination is automatically switched on in the respective chamber.
7. If only one type of gas (O_2 or H_2) is needed, the other gas can be continuously released by screwing an open syringe onto the respective Luer Lock connector.
8. It is recommended to withdraw the gas right before injection to the O2k-chamber as it mixes with air when stored in the open gas syringe. The gas can be stored for up to one hour in the gas chamber of the Oxia-Cell.
9. For gas withdrawal, screw a gas syringe to the Luer Lock connector of the Oxia-Cell. Pull out the amount of gas needed for the experiment. Screw the O2k-Injection needle immediately onto the gas syringe. Use only the provided needle and spacer to obtain the correct insertion length for gas injection into the O2k-chambers.
10. One filling of the H_2O-chambers is sufficient for about 25 cycles.
11. Before refilling a H_2O-chamber the respective gas chamber must be filled completely with gas. Otherwise H_2O may spill over upon gas production.

5.3. Setting O_2 concentrations in the O2k-chamber using O_2 or H_2 gas

1. Lift the stopper of the O2k-chamber into the open position using the Stopper-Spacer. Gas injection into aqueous phase must be strictly avoided.
2. Insert the injection needle into the titration port and gently inject the gas.
3. Remove the injection needle.
4. When the targeted O_2 concentration is approached, gently insert the stopper fully to close the O2k-chamber.

Further details:
https://wiki.oroboros.at/index.php/Setting_the_oxygen_concentration



Traces of O_2 concentration in the O2k-chamber upon injection of (a) O_2 and (b) H_2 gas: The O2k-chambers were not closed to illustrate the time courses of the O_2 regime after injections of (**a**) 2 mL O_2 or (**b**) 8 mL H_2. 2 mL experimental O2k-chamber volume with MiR05 at 37 °C.

5.4. Storage

- Remove residual water from the H_2O-, O_2-, and H_2-chambers as described in 5.1, Step 1.
- Cover the Oxia-Cell with the Cloth Cover to protected from contamination. There is no need to clean the Oxia-Cell internally. Never use an alcohol-based cleaner or any strong acidic or alkaline solutions as this can damage the material of the Oxia-Cell.

6. Troubleshooting

- Air is sucked into the O_2- or H_2-chamber if the H_2O-chambers are empty during gas withdrawal. ⇒ Switch off the Oxia. Unplug the Oxia-Cell and repeat steps of section 5.1 and steps 1 and 2 of section 5.2.
- Water spills over during gas generation if too much water is added to the H_2O-chambers. ⇒ Switch off the Oxia. Unplug the Oxia-Cell and repeat steps of section 5.1 and steps 1 and 2 of section 5.2.
- Water is sucked into the Luer Lock connector. ⇒ Let the Oxia run for about 5 min. Carefully suction gas thereby removing residual water from the Luer Lock connector. Repeat if necessary.

- Power button flashes. ⇒ Ensure that the Oxia-Cell is correctly plugged onto the Oxia-Base (see section 5.1, step 2) and that the H_2O-chamber contains enough water (repeat steps from section 5.1).
- Power button is still flashing although the Oxia-Cell is correctly inserted. ⇒ Contact Oroboros Technical Support.
- Gas escapes to the H_2O-chambers. ⇒ Contact Oroboros Technical Support
- Gas production does not stop automatically when the gas chambers are filled with gas. ⇒ Contact Oroboros Technical Support.

Technical Support is provided by:

Oroboros Instruments

High-Resolution Respirometry

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https://www.oroboros.at/index.php/support/

7. Author contributions and acknowledgements

Gnaiger E was responsible for the concept of the project. Walter-Vracevic M, and Merth A were responsible for electronic and mechanical development of the Oxia. Schmitt S and Gnaiger E prepared the manual and all coauthors contributed to the final version.

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