

Oroboros® O2k: Sole Source Statement



Innsbruck, 17th of August 2023

The **Oroboros® O2k (O2k)** is developed by **Oroboros Instruments GmbH** (Oroboros Instruments GmbH, Innsbruck, Austria) in cooperation with WGT-Elektronik GmbH & Co KG (WGT, Kolsass, Austria). Since 2002, the O2k and its modular components have been manufactured exclusively by WGT, whereby Oroboros Instruments GmbH holds the proprietary **rights** for the O2k and modular components, being the exclusive Oroboros® O2k vendor worldwide, in collaboration with selected national distributors.

The O2k and NextGen-O2k are the only instruments worldwide with specifications for **high-resolution respirometry (HRR)** applied in mitochondrial and plastid physiology.

The O2k has been developed for high performance using isolated mitochondria, living and permeabilized cells, permeabilized tissue, and tissue homogenates from small biopsies, and is unique in its **sensitivity, reproducibility, and minimization of artefacts**.

The **Oroboros® O2k**

- is a two-chamber respirometer for monitoring oxygen consumption or production using small amounts of biological sample;
- provides an exact regulation of the temperature in both chambers (0.5- or 2.0-mL operation volume) by the built-in electronic Peltier thermostat with a stability of $\pm 0.002\text{ °C}$ in the range from 4 to 47 °C;
- contains two independently controlled electromagnetic stirrer systems, allowing regulation of stirring speeds between 100 and 900 rpm;
- enables monitoring of the barometric pressure;
- has been designed and built using specialized materials, thereby avoiding the use of oxygen-absorbing plastics (such as Teflon or Perspex). For example, PVDF-coated stirrer bars are used in the O2k-chamber for minimizing oxygen-back diffusion (which is high with conventional Teflon stirrer bars);
- has a limit of detection of **O₂ flux** as low as $1\text{ pmol O}_2\cdot\text{s}^{-1}\cdot\text{mL}^{-1}$ and the signal noise at zero oxygen concentration is $<0.02\text{ }\mu\text{M O}_2$, – two of the **outstanding sole-source features** of the O2k;
- has a high signal stability, thereby allowing the display of minimum respiratory rates in the full range of oxygen from (air) saturation to zero oxygen.

The specifications of the O2k are published in the scientific literature (see appended references) and until now, no literature or commercial leaflets have been published that provide specifications of an alternative instrument meeting the standards of the O2k.

Besides its **uniqueness in high-resolution-respirometry**, the Oroboros® O2k provides **high flexibility** as it is a modular system. The experimental system for basic high-resolution respirometry (HRR) can be extended with different add-on modules.

With the **O2k-Fluo Smart-Module**, the Oroboros® O2k can simultaneously measure oxygen consumption and, via real-time recording of fluorescence signals and their time-derivative, hydrogen peroxide production, ATP production, mitochondrial membrane potential, or

calcium uptake.

The **O2k-PB-Module** enables evaluation of the light-dependent net production of oxygen and light-enhanced dark respiration under controlled oxygen concentrations, extending the Oroboros® O2k capabilities from high-resolution respirometry to advanced state-of-the-art PhotoBiology.

Using ion selective electrodes ISE, the **O2k-TPP⁺ ISE-Module** and **O2k-pH ISE-Module** provide the flexibility to simultaneously measure additional parameters such as mitochondrial membrane potential and pH, alongside oxygen consumption.

The **NextGen-O2k** – the next generation of the Oroboros® O2k – can be further extended with the **O2k-Q-Module** and **O2k-NADH-Module**. These two modules enable the simultaneous measurement of oxygen flux and (1) real-time amperometric monitoring of the redox state of coenzyme Q or (2) real-time monitoring of NAD(P)H autofluorescence and redox state. In conjunction with **O2k-NO Amp-Module**, the **NextGen-O2k** allows for the simultaneous measurement of nitric oxide and oxygen flux. The NextGen-O2k can be used with all above-mentioned modules.



Data are recorded and analyzed with the O2k-software **DatLab**, which has been specifically developed for high-resolution respirometry and all other applications of the Oroboros® O2k and NextGen O2k. The software allows for the individual display of graphs, calculations, and the transfer of data to spreadsheet software. Furthermore, several Substrate-Uncoupler-Inhibitor-Titration (SUIT) protocols for in-depth analysis of mitochondrial function are included. These protocols can be easily adapted depending on the scientific question and there is no restriction on the number of titrations or reagents.

Additionally, the software-controlled **Titration-Injection microPump (O2k-TIP2k-Module)** allows the user to perform pre-programmed automatic titrations, steady-state injections and feedback-controlled maintenance of oxygen levels, pH, or other parameters in the O2k-chamber.

Taken together, the O2k and NextGen-O2k are not only unique in their specifications on **high-resolution-respirometry (HRR)** but also in their **flexibility** to combine the measurement of oxygen flux with other bioenergetic parameters.

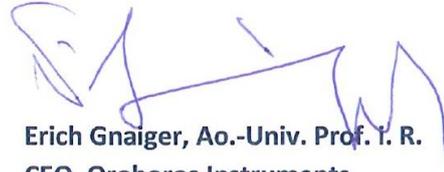
See:

- Sole source info: http://wiki.orooboros.at/index.php/MiPNet18.10_O2k_specifications
- Specifications:
- http://wiki.orooboros.at/index.php/MiPNet06.05_Specifications

Further information:

- O2k-Concept
<https://www.orooboros.at/index.php/o2k-concept/>
- O2k-Packages:
<https://www.orooboros.at/index.php/product-category/products/o2k-packages/>
- O2k-Modules:
- <https://www.orooboros.at/index.php/o2k-products/o2k-modules/>

- A unique training course on high-resolution respirometry with excellent international reputation virtual support tailored to user needs are offered by Oroboros Instruments:
- https://www.orooboros.at/index.php/product/o2k-virtual_support/
<https://www.orooboros.at/index.php/product/O2k-Workshops/>



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Appendix

Scientific references:

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- Zdrazilova L, Hansikova H, Gnaiger E (2022) Comparable respiratory activity in attached and suspended human fibroblasts. <https://doi.org/10.1371/journal.pone.0264496>
- Komlódi T, Sobotka O, Gnaiger E (2021) Facts and artefacts on the oxygen dependence of hydrogen peroxide production using Amplex UltraRed. <https://doi.org/10.26124/bec:2021-0004>
- Komlódi T, Cardoso LHD, Doerrier C, Moore AL, Rich PR, Gnaiger E (2021) Coupling and pathway control of coenzyme Q redox state and respiration in isolated mitochondria. <https://doi.org/10.26124/bec:2021-0003>
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- Lemieux H, Blier PU, Gnaiger E (2017) Remodeling pathway control of mitochondrial respiratory capacity by temperature in mouse heart: electron flow through the Q-junction in permeabilized fibers. Sci Rep 7:2840. <https://doi.org/10.1038/s41598-017-02789-8>
- Makrecka-Kuka M, Krumschnabel G, Gnaiger E (2015) High-resolution respirometry for simultaneous measurement of oxygen and hydrogen peroxide fluxes in permeabilized cells, tissue homogenate

and isolated mitochondria. <https://doi.org/10.3390/biom5031319>

Krumschnabel G, Eigentler A, Fasching M, Gnaiger E (2014) Use of safranin for the assessment of mitochondrial membrane potential by high-resolution respirometry and fluorometry. <https://doi.org/10.1016/B978-0-12-416618-9.00009-1>

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List of O2k-Publications:

<http://wiki.orooboros.at/index.php/O2k-Publications: Topics>