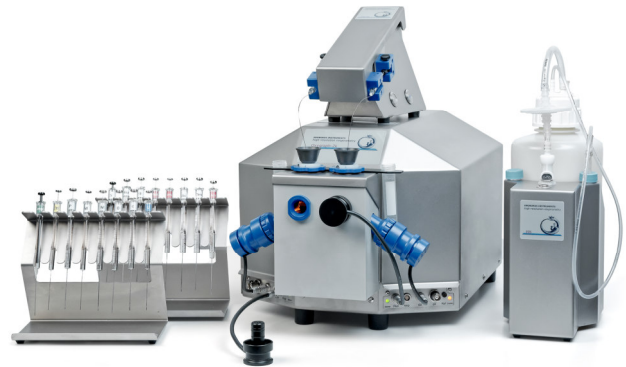


Oroboros® O2k: Sole Source Statement



Innsbruck, 3rd April 2019

The **Oroboros® O2k (O2k)** is developed by **Oroboros Instruments GmbH** (Oroboros Instruments Corp., 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 Corp. holds the proprietary rights for the O2k and modular components, being the exclusive Oroboros® O2k vendor worldwide.



The O2k is the only instrument worldwide with specifications for high-resolution respirometry (HRR) for applications in mitochondrial physiology. The O2k has been developed for high performance using isolated mitochondria, intact and permeabilized cells, permeabilized tissue, and tissue homogenates from small biopsies, and it is widely used for biomedical applications. The specifications of the O2k are published in the scientific literature (see appended references) and to this day, no literature or commercial leaflets have been published that provides specifications of an alternative instrument meeting the standards of the O2k.

The Oroboros® O2k is a modular system for high-resolution respirometry (Startup O2k-Respirometer) extended to fluo respirometry (O2k-FluoRespirometer). The O2k is a two chamber (2 mL) respirometer for monitoring oxygen consumption using small amounts of biological material, with the O2k-FluoRespirometer offering the additional possibility to simultaneously measure hydrogen peroxide production, ATP production, and mitochondrial membrane potential.

The modular concept of the O2k provides high flexibility for high resolution respirometry by offering MultiSensor modules such as the O2k-TPP⁺ ISE-Module, the O2k-pH ISE-Module and the O2k-NO Amp-Module. This provides the flexibility to simultaneously measure additional parameters such as TPP⁺, pH, nitric oxide and mitochondrial membrane potential. Additionally, the **Titration-Injection microPump (TIP2k)** allows the user to perform pre-programmed automatic titrations, steady-state injections and feedback-controlled maintenance of oxygen levels, pH or other signals recorded through DatLab in the O2k chamber.

The exact regulation of the temperature in both chambers by the built-in electronic Peltier thermostat with a stability of **<±0.002 °C** in the range from 4 to 47 °C is another technical characteristic. Furthermore, the limit of detection of **O₂ flux** is as low as **0.5 pmol O₂·s⁻¹·cm⁻³** and the signal noise at zero oxygen concentration is <0.02 μM O₂, which are some of the **outstanding sole-source features** of the O2k. Two independently controlled electromagnetic stirrer systems are integral parts

of the O2k, allowing regulation of the stirring speed between 100 and 900 rpm. Basic features of HRR are

- real-time recording of oxygen concentration and respiratory rate (oxygen flux; time-derivative of oxygen concentration),
- real-time recording of fluorescence signals and their time-derivative and
- monitorization of the barometric pressure.

All these measurements are performed using our specialized software for high-resolution respirometry (DatLab). The high signal stability allows the display of minimum respiratory rates in the full range of oxygen from (air) saturation to zero oxygen.

The O2k is unique in its **sensitivity, reproducibility, and minimization of artefacts**. It has been designed and built using specialized material, thereby avoiding the use of oxygen-absorbing plastics (such as Teflon or Perspex) that can seriously interfere with the function and reliability of conventional equipment. 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). In reviewing the specifications of other manufacturers of similar equipment, it is apparent that no available other system presents specifications that come close to the HRR features of the O2k.

See:

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

A past paradigm for the achievement of reproducible and sensitive measurements of respiratory rates was to minimize the chamber volume in order to maintain high concentrations of sample and to obtain high rates of oxygen consumption per volume. The advantage appears to be obvious, whereas the drawbacks are conventionally overlooked. Advancements of electronics, data acquisition and analysis, polarographic oxygen sensor specifications and chamber design made possible an alternative and superior approach, allowing for **respirometric measurements at high dilution** (reviewed by Gnaiger E 2001 Respir Physiol 128:277-97). In specifically designed mitochondrial respiration media, respiration is stable at high dilution, **complex substrate-uncoupler-inhibitor titrations** are possible without oxygen depletion, and a **low-oxygen regime** may be chosen to prevent induction of oxidative stress at air-level oxygen saturation. Micro-chambers on the other hand are characterized by a high surface-to-volume ratio which hinders optimum stirring, increases unfavourable surface effects and oxygen-back diffusion, and poses problems with accurate titrations and dilution effects of the sample. The potential artefacts of high back-diffusion of oxygen and leakage of lipid soluble inhibitors and uncouplers are avoided in HRR, using glass chambers, PVDF stoppers, and avoiding teflon-coated stirrers or perspex.

Further information:

- O2k-FluoRespirometer: <http://wiki.orooboros.at/index.php/O2k-FluoRespirometer>
- Startup O2k-Respirometer: http://wiki.orooboros.at/index.php/Startup_O2k-Respirometer
- O2k-Modules: http://wiki.orooboros.at/index.php/O2k-Catalogue:_O2k-Modules

A unique training course is offered by Oroboros Instruments on high-resolution respirometry with excellent international reputation:

http://wiki.orooboros.at/index.php/OROBOROS_Events.



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Appendix

Scientific references:

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

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. *Biomolecules* 5:1319-38.

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. *Methods Enzymol* 542:163-81.

Pesta D, Gnaiger E (2012) High-resolution respirometry. OXPHOS protocols for human cells and permeabilized fibres from small biopsies of human muscle. *Methods Mol Biol* 810: 25-58.

Scandurra FM, Gnaiger E (2010) Cell respiration under hypoxia: Facts and artefacts in mitochondrial oxygen kinetics. *Adv Exp Med Biol* 662: 7-25.

Gnaiger E (2008) Polarographic oxygen sensors, the oxygraph and high-resolution respirometry to assess mitochondrial function. In: *Mitochondrial Dysfunction in Drug-Induced Toxicity* (Dykens JA, Will Y, eds) John Wiley: 327-52

List of O2k-Publications:

[http://wiki.orooboros.at/index.php/O2k-Publications: Topics](http://wiki.orooboros.at/index.php/O2k-Publications:_Topics)