Oroboros® O2k:
Sole Source Statement

The **Oroboros 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 modular components have been manufactured exclusively by WGT, whereby Oroboros Instruments Corp. holds the proprietory rights for the O2k and modular components, and is the exclusive Oroboros O2k vendor world-wide.

The O2k is the only instrument world-wide with specifications qualifying for 'high-resolution respirometry' (HRR) and High-Resolution FluoRespirometry' (HRFR) for applications with isolated mitochondria, intact and permeabilized cells, permeabilized tissue and tissue homogenates from small biopsies, and corresponding biomedical applications. The specifications of the O2k are published in scientific literature (see appended references) and to this day, no literature or commercial leaflets have been published to provide specifications of an alternative instrument meeting the standard of the O2k.

The Oroboros® O2k is a modular system for High-Resolution Respirometry (Startup O2k-Respirometer) extended to High Resolution FluoRespirometry' (O2k-FluoRespirometer). The O2k is a two-chamber High Resolution FluoRespirometer for monitoring oxygen consumption with 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. Additionally, the included **Titration-Injection microPump (TIP2k)** allows the user to do pre-programmed automatic titrations, steady-state injections, and feedback control of oxygen levels, pH or other signals recorded through DatLab in the O2k chamber.

The modular concept of the O2k yields a high flexibility for HRFR, with O2k-Modules supported by the O2k-FluoRespirometer. O2k-Modules are the O2k-TPP^+ ISE-Module, the O2k-pH ISE-Module, and the O2k-NO Amp-Module. Thes O2k-Modules offer the flexibility to run MultiSensor applications and measure additional parameters such as TPP^+, pH, nitric oxide, mt-membrane potential and

others simultaneously to oxygen consumption.

The temperature for both chambers is regulated by the built-in electronic Peltier thermostat at a stability of **<±0.002 °C** in the range of 4 to 47 °C. The limit of detection of **O_2 flux** is as low as **1 pmol $O_2 \cdot s^{-1} \cdot cm^{-3}$**, which is **one of the vital sole-source features** of the O2k.

The O2k is unique in its **sensitivity, reproducibility, and elimination of artifacts**. It has been designed to avoid 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-backdiffusion (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 and HRFR features of the O2k.

Signal noise at zero oxygen concentration is <0.02 µM O_2, which is another outstanding sole-source feature. Two independently controlled electromagnetic stirrer systems are integral parts, individually regulated at stirring speeds between 100 and 900 rpm (rotations per minute). A basic feature of HRFR is the real-time recording of oxygen concentration and respiratory rate (oxygen flux; time-derivative of oxygen concentration) as well as fluorescence signals and their time-derivative by the software DatLab, together with barometric pressure, temperature and Peltier power. The high signal stability allows the display of minimum respiratory rates, in the full range of oxygen (air) saturation to zero oxygen. The inclusion of these components into a compact two-chamber O2k yields an economical system.

Further information from:

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

A past paradigm for the achievement of measurable respiratory rates was minimization of the chamber volume to maintain high concentrations and 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 elevation 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-backdiffusion, 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.oroboros.at/index.php/O2k-FluoRespirometer
- http://wiki.oroboros.at/index.php/O2k-Catalogue:_O2k-Modules

A unique training course is offered by Oroboros Instruments on High-Resolution FluoRespirometry with excellent international reputation: http://wiki.oroboros.at/index.php/OROBOROS_Events.

Innsbruck, 7th August 2017

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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 biopisies 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.oroboros.at/index.php/O2k-Publications:_Topics