OROBOROS® Oxygraph-2k: Sole Source Statement

The **OROBOROS Oxygraph-2k (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 Oxygraph-2k vendor world-wide.

The Oxygraph-2k is the only instrument world-wide with specifications qualifying for 'high-resolution respirometry' (HRR) for applications with isolated mitochondria, intact and permeabilized cells, permeabilized tissue and tissue homogenates from small biopsies, and corresponding biomedical applications. The O2k-specifications are published in the 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® Oxygraph-2k is a modular system for high-resolution respirometry (HRR). The O2k is a two-chamber high-resolution respirometer for monitoring oxygen consumption with small amounts of biological material. The modular concept of the O2k yields a high flexibility for HRR, with the O2k-Core as the basis, and O2k-Modules supported by the O2k-Core. O2k-Modules are the Titration-Injection microPump (TIP2k-Module) and O2k-MultiSensor Modules (i.e. are the O2k-Fluo LED2-Module the O2k-TPP^+ ISE-Module, the O2k-pH ISE-Module, and the O2k-NO Amp-Module). The O2k-Core, the O2k-Fluo LED2-Module and the TIP2k form together the O2k-Fluorometer. The O2k-MultiSensor Modules offer the flexibility to measure simultaneously with oxygen consumption additional parameters such as TPP^+, pH, nitric oxide, ROS production, mt-membrane potential and others.

The temperature for both chambers is regulated by the built-in electronic Peltier thermostat at a stability of **<±0.002 °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 Oxygraph-2k 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 specifically available for the Oxygraph-2k chamber, for minimizing oxygen-backdiffusion

(which is high when using 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 Oxygraph-2k.

Signal noise at zero oxygen concentration is <0.05 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 HRR is the on-line recording of oxygen concentration and respiratory rate (oxygen flux; time-derivative of oxygen concentration) by the software DatLab. 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/MiPNet09.01_O2k-ParadigmShift
- 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: 277-297). 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. These potential artefacts are avoided in HRR, using glass chambers, PVDF stoppers, and avoiding teflon-coated stirrers or perspex (yielding high back-diffusion of oxygen and leakage of lipid soluble inhibitors and uncouplers).

In addition, entirely unique is the possibility offered by upgrading the Oxygraph-2k with the **Titration-Injection microPump (TIP2k)** for pre-programmed automatic titrations, for steady-state injections, and for feedback control of oxygen levels, pH or other signals recorded through DatLab in the O2k chamber. The DatLab 6 software is fully upgraded to monitor simultaneously the 6 channels (two amperometric oxygen with variable polarization voltage, two pX, two additional amperometric with variable polarization voltage), and the barometric pressure, temperature and Peltier power.

Further information:

- http://wiki.oroboros.at/index.php/O2k-Catalogue:_O2k-MultiSensor
- TIP2k: http://wiki.oroboros.at/index.php/TIP2k-Module

A unique training course is offered by OROBOROS INSTRUMENTS on high-resolution respirometry with excellent international reputation: http://wiki.oroboros.at/index.php/OROBOROS_Events.

Innsbruck, 2nd December 2016

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Appendix

Scientific References:

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.

Gnaiger E (2001) Bioenergetics at low oxygen: dependence of respiration and phosphorylation on oxygen and adenosine diphosphate supply. Respir Physiol 128: 277-97.

Websites:

O2k specifications:
http://wiki.oroboros.at/index.php/MiPNet06.05_Specifications

List of O2k-Publications:
http://wiki.oroboros.at/index.php/O2k-Publications:_Topics