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https://www.mitoglobal.org/index.php/MiPNet24.14_O2k-sV-Module_manual

O2k-sV-Module manual

O2k-sV-Module

Passrugger M, Gallée L, Krumschnabel G, Tindle-Solomon L, Gnaiger E, Doerrier C

Oroboros Instruments GmbH
Schoepfstrasse 18, A-6020 Innsbruck, Austria
Email: instruments@oroboros.at
www.oroboros.at
Mitochondria and Cell Research

Section Page

Overview

The **O2k-sV-Module** is a modular extension of the O2k specifically designed to perform high-resolution respirometry with reduced amount of biological sample and chemicals, leading to an overall decrease of running costs and expanding the application scope of samples with limited availability. The O2k-sV-Module exchanges the standard 2 mL O2k-Chamber for a smaller operation volume of 0.5 mL.
The O2k-sV-Module is compatible with all O2k-Series, however it cannot be used for simultaneous measurement of multiple parameters in the O2k-FluoRespirometer or in combination with the O2k-MultiSensor Modules. The use of O2k-sV-Module is recommended at normoxic range, in a narrow experimental range of oxygen concentration near air saturation to reduce technical problems of oxygen diffusion and instrumental O_2 background.

1. Components of the O2k-sV-Module

	O2k-sV-Module, containing:
(1)	2× O2k-Chamber Holders sV (black POM) for PVDF or PEEK stoppers, with O-ring\Viton\16x2 mm and V-ring\30-35-4.5 mm
(2)	2× O2k-Chambers sV: Duran glass chambers of 12 mm inner diameter and an operation volume of 0.5 mL
(3)	1x Stirrer Bar sV white PVDF\11.5x6.2 mm
(3)	1x box of 8 spare O-rings sV Viton\9.5x1mm
(4)	2x box of 8 spare O-rings sV Viton\9.5x1mm
(5)	2× OroboPOS-Holders sV (POS-Holder sV)
(6)	2× Stoppers sV black PEEK\conical Shaft\central port, with two Volume-Calibration Rings sV and two mounted O-rings (Viton\9.5x1mm)
(7)	1x Screwdriver Allen wrench

The components of the O2k-sV-Module replace the standard components of the O2k and may also be used in a mixed set-up (i.e. a parallel use of one standard 2 mL O2k-Chamber and one 0.5 mL O2k-Chamber sV) with adapted instrumental/SUIT protocols.

2. Assembly of the O2k-sV-Module

2.1. Instrumental overview

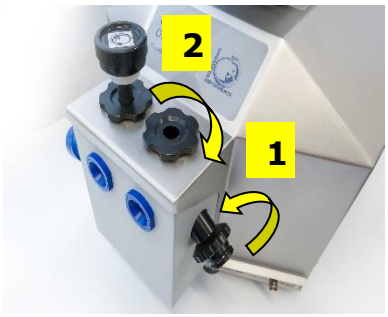
For general instructions on the set-up and installation of the O2k-FluoRespirometer:
MiPNet22.11 O2k-FluoRespirometer manual

The O2k-sV-Module is designed specially for respirometry, therefore only the O2 channel can be used. For simultaneous measurement of multiple parameters the standard 2 mL chamber should be used (MiPNet22.11 O2k-FluoRespirometer manual).

2.2. O2k-Chamber sV assembly

ClipA2

1. Switch off the O2k with the power switch at the rear side of the O2k. If the O2k-FluoRespirometer is not assembled with standard volume 2 mL O2k-Chambers, proceed to step 4.
2. Remove the Stopper and aspire the medium from the chamber.
3. Unscrew the O2k-Chamber Holder and the OroboPOS-Holder and gently pull out the O2k-Chamber by using your fingers.
4. Insert the glass O2k-Chamber sV into the copper block of the O2k-Main Unit and add the Stirrer Bar sV. The angular cut should be aimed towards the insertion point of the OroboPOS-Holder sV.



5. Screw the black OroboPOS-Holder sV 1 into a position so that the glass chamber is slightly lifted up at the angular cut.
6. Screw the black O2k-Chamber Holder sV loosely to the copper block (arrow 2). Loosen the OroboPOS-Holder sV stepwise (counterclockwise, not more than successive quarter turns; arrow 1) and follow any downwards movement of the glass chamber by screwing down the O2k-Chamber Holder sV (arrow 2). When a slight backwards movement of the OroboPOS-Holder sV 1 cannot be followed by any further downwards movement of the O2k-Chamber Holder sV 2, then screw the OroboPOS-Holder sV 1 clockwise (tight), thus securing the glass chamber in a fixed position.

2.3. O2k OroboPOS assembly

➲Clip A3

Before OroboPOS assembly, if the POS is dry without electrolyte, apply electrolyte and membrane, following the OroboPOS service instructions.

Go to: POS-service
» MiPNet19.18B_POS-service

1. Screw a POS head onto each OroboPOS-Connector (finger-tight). Push a *wetted* POS seal tip (black gasket) over the POS head (arrow 1). Position the pore centrally and do not stretch the gasket. Each OroboPOS head can be used on O2k-Chamber A or B. Note the POS number (marked on the cylindrical body of each POS) for each chamber.

2. Connect the POS connector cable to the O2k-Main Unit. Insert the male plug of the cable 2 into the female O2 plug (2A: O2k-Series H, 2B: O2k-Series G). The red dot on the male plug 2 must face directly upwards when inserting the plug.

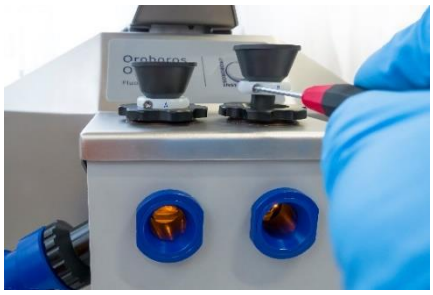
3. After inserting the plug of the OroboPOS-Connector cable to the O2k-Main unit the OroboPOS-Connector cable 3 must be in an unstrained, undistorted position. Press the sleeve downwards (arrow 4), insert the POS with the black gasket straight into the OroboPOS-Holder sV 5 and release the sleeve to fix the POS in its final position.

☞ **Do not rotate the POS connector** 6 after it is attached to the POS holder in order to prevent damage to the black gaskets. The gaskets provide a tight seal against sharp edges of the glass chamber.

3. O2k-Chamber volume calibration

Clip A1

O2k-SOP DatLab must be installed before calibrating the chamber volume. Connect the O2k to the Main Unit, connect the O2k with DatLab, and familiarize yourself with the O2k-control keys. The standard chamber volume of the O2k-sV-Module is 0.5 mL.



1. Dry the chamber. Remove all liquid droplets from the glass chamber. Place a dry white PVDF stirrer bar sV into each chamber.

2. Add accurately 0.54 mL of H_2O into the chamber for calibration of a chamber volume of 0.5 mL. The volume of the capillary of the stopper (0.040 mL or 40 µL when a tiny meniscus of water is seen on top of the capillary, black PEEK) is not part of the effective chamber volume. When titrating 0.54 mL, insert the tip of the pipette to the wall of the glass chamber to add the entire volume. Liquid must not be lost between the top of the glass cylinder and the black POM chamber holder.

ClipA4

3. Switch on the stirrer.

4. Loosen the Volume-Calibration Rings sV (A) and (B) with the screwdriver Allen wrench (OroboPOS-Service Kit and O2-sV-Module). Push them slightly downwards in the direction of the O-rings.

5. Insert the stopper with dry capillary (the O-rings should be moistened) and push it downwards until the gas phase fully extrudes through the capillary of the stopper, while the fixation ring is fully pushed onto the chamber holder.

6. Gently push the stopper further downwards until the capillary fills up and a small droplet appears on the top of the capillary of the stopper. This marks the volume calibration position.

7. Tighten the screws of the fixation rings (A) and (B) gently with the screwdriver Allen wrench.

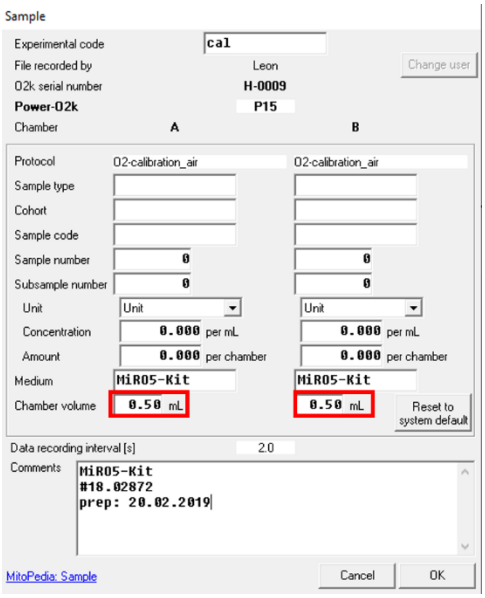
For maintenance or replacement of the glass chambers refer to MiPNet22.11 O2k-FluoRespirometer manual

4. Start DatLab 7

4.1. Overview

For general instructions on the start of DatLab 7:
MiPNet22.11 O2k-FluoRespirometer manual

4.2. Sample window - DatLab



After connecting to the O2k, the Sample window pops up. Enter the experimental code, sample information, medium, and any additional information. Be sure to change the chamber volume from the default of 2 mL to 0.5 mL. It is important to have the correct O2k-chamber volume entered for calculations of oxygen flux. Entries can be edited at any time during real-time or post-experiment analysis. All related results are recalculated instantaneously with the new parameters.

5. O2k Quality Control

The maintenance and accurate calibration of the polarographic oxygen sensors and the correction for instrumental background oxygen flux are standard in high-resolution respirometry. Both are components of the MitoFit Quality Control System. In the O2k-sV-Module, the standard operating procedures for the O2k Quality Control 1 (polarographic oxygen sensors and accuracy of calibration), and O2k Quality Control 2 (instrumental oxygen background correction and accuracy of oxygen flux) are comparable to the ones applied for the standard 2.0 mL chamber (figure 1). The instrumental oxygen background correction should be performed in the O2k-sV-Module with manual injections.

O2k-SOP:

» MiPNet06.03 POS-Calibration-SOP
» MiPNet14.06 Instrumental Background

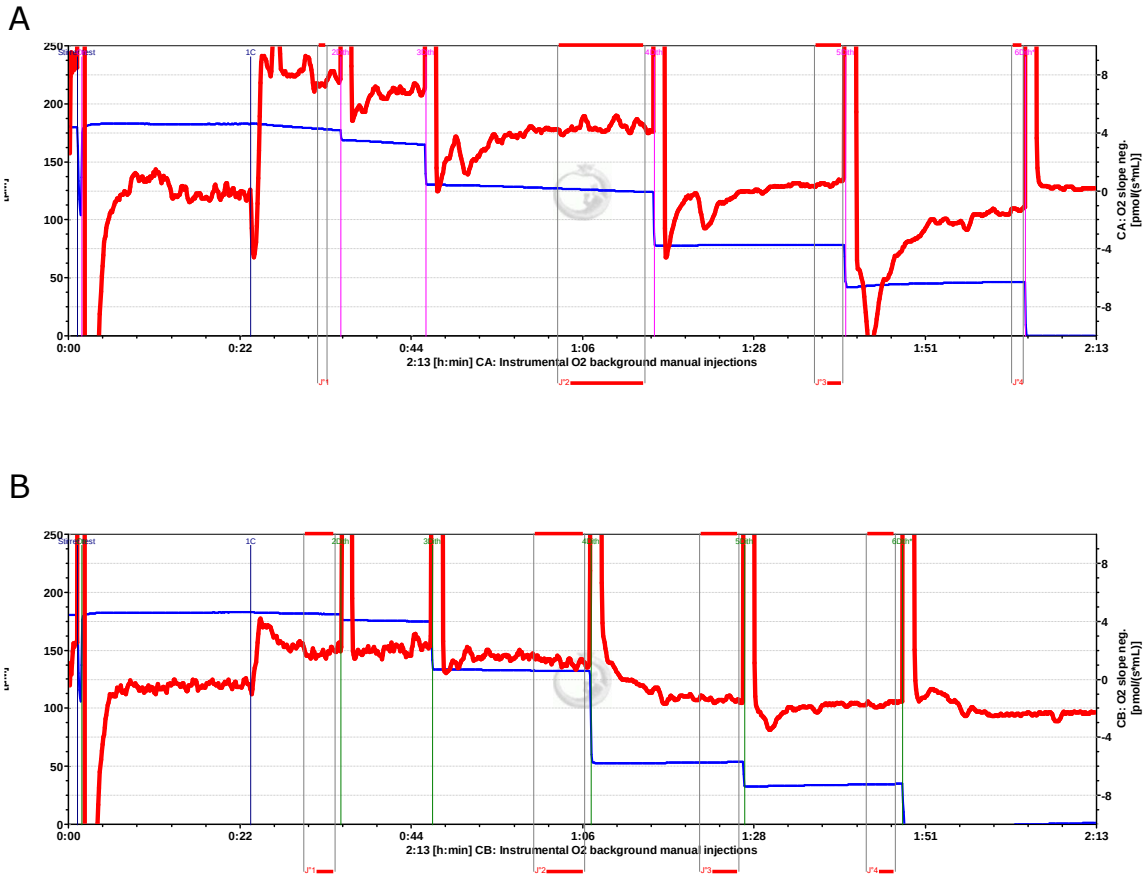


Figure 1. O2k Quality Control 2. O2k traces showing an instrumental background oxygen flux determination ran in parallel in one O2k with **(A)** 0.5 mL chamber volume (O2k-sV-Module) in chamber A and **(B)** 2.0 mL chamber volume in chamber B. File: 2019-03-22 PC-02.BG.to0.DLD

However, due to physical constraints (i.e. an altered surface-to-volume ratio), there is an increase in the oxygen consumption by the POS and, the oxygen background parameter values (intercept, *a*°, and slope, *b*°) differ from those determined for the standard 2.0 mL chamber. A comparison of instrumental background oxygen flux in 0.5 mL and 2.0 mL chambers is shown in figure 2.

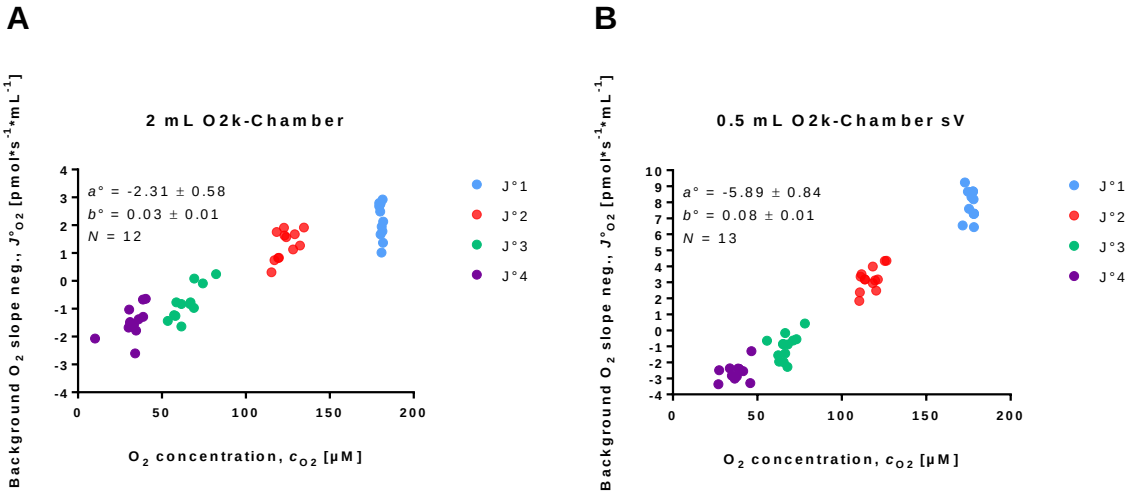


Figure 2. Instrumental background oxygen flux, $J°_{O2}$, as a function of oxygen concentration, c_{O2} [µM], in the O2k (37 °C; MiR05-Kit with an oxygen solubility factor of 0.92 relative to pure water). Measurements in **(A)** 12 chambers (O2k-Chamber, 2 mL), and **(B)** 13 chambers (O2k-Chamber sV, 0.5 mL) of three different O2ks. In all tests, four oxygen ranges were evaluated in declining order. Averages and SD were calculated for the intercept, *a*°, and the slope, *b*°, by linear regression for each individual chamber.

6. Titrations

With the reduction of the chamber volume it is also necessary to adjust either the titration volumes or stock concentrations of injected chemicals. Wherever possible the injection volume should be reduced to keep the concentration of the carrier to a minimum. However, since volumes below one microliter are very difficult to inject with accuracy, where required the concentration of the stock solution should be adjusted rather than the injection volume. For addition of larger volumes of carrier, we recommend separate test runs to evaluate carrier effects. A list of recommended concentrations of stock solutions and injection volumes for substrates, uncouplers, and inhibitors used in SUIT protocols into the 0.5 mL O2k-chamber can be found in the MiPNet09.12 O2k-Titrations.

7. DL-Protocols in DatLab 7.4

ClipDL

Editions of concentrations and titration volumes of injections in a specific DL-Protocol can be edited and saved as user-specific DL-Protocol (Export DL-Protocol User (*.DLPU).

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Author contributions
Passrugger M and Gnaiger E were responsible for the project and instrumental development. Passrugger M and Gallée L performed the experiments. Passrugger M, Krumschnabel G, Gnaiger E, Tindle-Solomon L and Doerrier C prepared the MiPNet.