

O2k-Manual: sV-Module

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O2k-sV-Module manual

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1. Overview

The **O2k-sV-Module** is a modular extension of the O2k specifically designed to perform high-resolution respirometry with a 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 the normoxic range, in a narrow experimental range of oxygen concentration near air saturation to reduce technical problems of oxygen diffusion and instrumental O₂ background.

2. 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) 2× Stirrer Bar sV white PVDF\11.5x6.2 mm
- (3) 1× box of 8 spare O-rings sV Viton\9.5x1mm
- (4) 2× 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.

3. Assembly of the O2k-sV-Module

3.1. Instrumental overview

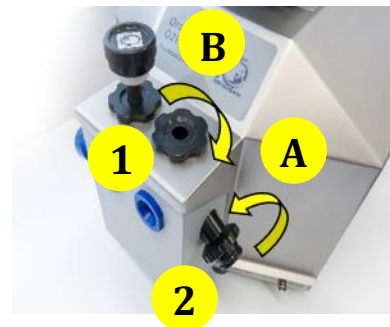
For general instructions on the set-up and installation of the O2k-FluoRespirometer:
 » [MiPNet22.11 O2k-FluoRespirometer manual](#)

The O2k-sV-Module is designed especially 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](#)).



3.2. O2k-chamber sV assembly

1. Switch off the O2k with the power switch at the rear 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 suction 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 gloved 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 (2) into a position so that the glass chamber is slightly lifted up at the angular cut.
6. Screw the black [O2k-Chamber Holder sV](#) (1) loosely to the copper block (arrow (B)). Loosen the OroboPOS-Holder sV stepwise (counterclockwise, slow successive quarter turns; arrow (A)) and follow any downwards movement of the glass chamber by screwing down the O2k-Chamber Holder sV (arrow (B)). When a slight backward movement of the OroboPOS-Holder sV (2) cannot be followed by any further downwards movement of the O2k-Chamber Holder sV (1), then screw the OroboPOS-Holder sV (2) clockwise (tight) following arrow (A), thus securing the glass chamber in a fixed position.



3.3. O2k OroboPOS assembly

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

Go to: POS-service

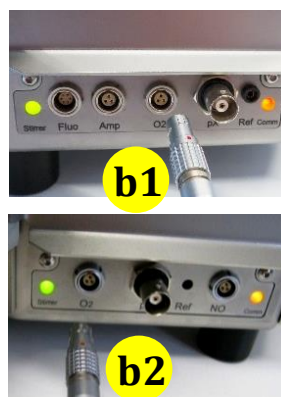
» [MiPNet19.18B POS-service](#)



1. Screw a POS head onto each [OroboPOS-Connector](#) (finger-tight). Push a *wetted* POS seal tip (black gasket) over the POS head (arrow **(a)**). 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 **(b)** into the female O2 plug (**(b1)**: O2k-Series H-I, **(b2)**: O2k-Series G). The red dot on the male plug **(b)** 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 **(c)** must be in an unstrained, undistorted position. Press the sleeve downwards (arrow **(d)**), insert the POS with the black gasket straight into the [OroboPOS-Holder sV](#) **(e)**, and release the sleeve to fix the POS in its final position.



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



4. O2k-chamber volume calibration

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 H₂O 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.
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 the capillary dry (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](#)



5. Start DatLab 7

5.1. 4.1. Overview

For general instructions on the start of DatLab 7:
» [MiPNet22.11 O2k-FluoRespirometer manual](#)

5.2. 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.

6. 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 2). The instrumental oxygen background correction can be performed in the O2k-sV-Module with the Titration-Injection microPump ([TIP2k-Module](#)) or with manual injections.



O2k-SOP:

- » [MiPNet06.03 POS-calibration-SOP](#)
- » [MiPNet14.06 Instrumental O2 background](#)
- » [MiPNet12.10 TIP2k-manual](#)

The use of the TIP2k to perform an instrumental O₂ background on the O2k-sV-Module can be done by adjusting:

- the dithionite concentration to 2.5 mM, using the "BG Feedback" settings.
- the TIP2k setup with specific settings for the O2k-sV-Module. The following parameters were optimized for the TIP2k setup file in the O2k-sV-Module:

Line	Mode	Start injection of O ₂ level (chamber A) is [μM]	Stop injection of O ₂ level (chamber A) is [μM]	Flow [μL/s]	Delay [s]	Interval [s]	Volume [μL]
1	FB	>130	<120	0.062	600	300	
2	FB	>70	<60	0.031	1200	300	
3	FB	>40	<30	0.012	1200	300	
4	D			50		120	50

TIP2k

Control | Chemicals | Configuration | Info

Delay [s] **600** 00:10:00

Mode Direct control Feedback control

Vol+Flow Volume [μL] **100.000** TIP backward
 Vol+Time Flow [μL/s] **0.062** TIP forward
 Flow+Time Time [s] **1612.90** Test start

Feedback control
 Stop and next program line
 after **300** seconds (0 for unlimited) or after **0** cycles (0 for unlimited).
 Pause **0** seconds

Select

Quantity	><	Value	DataN	Action
2A: O ₂ concentration [μM]	>	130	2	Start
2A: O ₂ concentration [μM]	<	120	1	Stop

Feedback line: Delete Insert

Program line: Append Insert Replace Delete Move up Move down

Line	Mode	Del [s]	Vol [μL]	Flow [μL/s]	Time [s]	Duration [s]	Cycles	Feedback quantity	><	Value	DataN	Action	Pause [s]
1	F	600	100.000	0.062	1612.90	300		2A: O ₂ concentration [μM]	>	130.00	2	start	0
2	F	1200	50.000	0.031	1612.90	300		2A: O ₂ concentration [μM]	>	70.00	2	start	0
3	F	1200	20.000	0.012	1666.67	300		2A: O ₂ concentration [μM]	>	40.00	2	start	0
4	D	1200	50.000	50.000	1.00	120	1						

Feedback control - cannot predict final volume

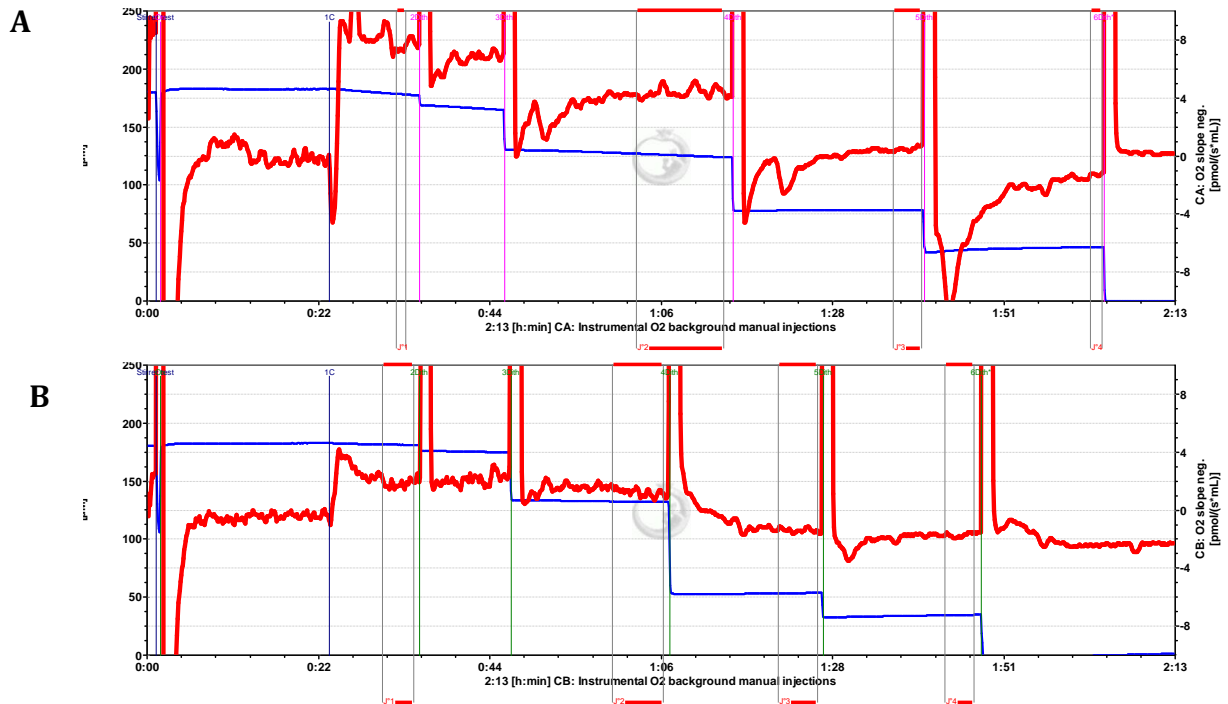
Suspend Repeat Next Stop immediately Start

BG_Feedback_sV-Module Load setup Save setup Hide details

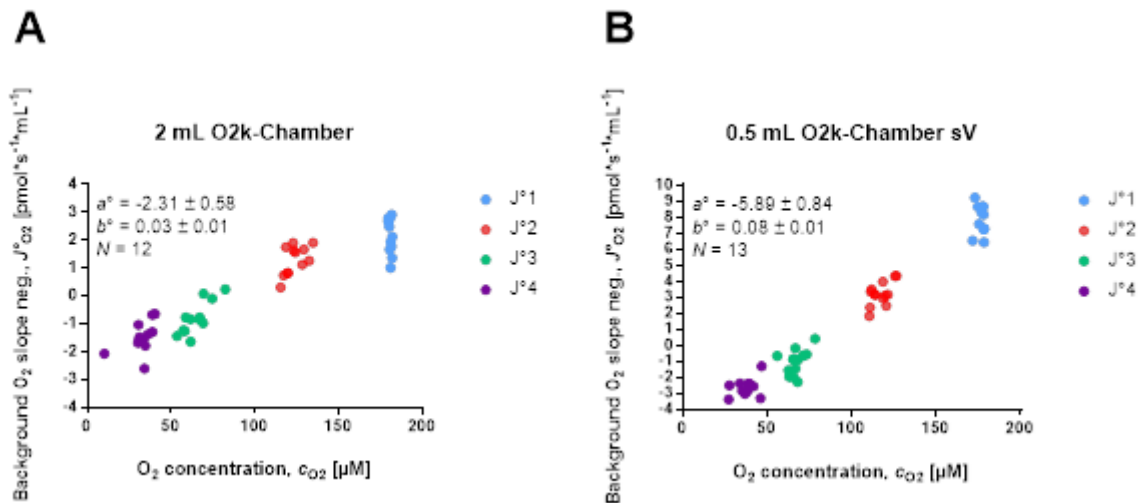
MitoPedia: Titration-Injection microPump Cancel / Close

TIP2k Setup
for the O2k-sV-Module

In the DatLab main menu select **TIP2k** and **TIP2kcontrol**. Introduce the parameters for the O2k-sV-Module ([MiPNet12.10 TIP2k-manual](#)) and save the TIP2k setup with a new name (e.g. "BG Feedback_sV-Module").



O2k Quality Control 2. O₂k traces showing an instrumental background oxygen flux 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



Instrumental background oxygen flux, $J^{\circ}O_2$, as a function of oxygen concentration, c_{O_2} [μM], in the O₂k (37 °C; MiR05-Kit with an oxygen solubility factor of 0.92 relative to pure water). Measurements in **(A)** 2-mL O₂k-chambers ($N = 12$), and **(B)** 0.5-mL O₂k-chambers sV ($N = 13$) 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.

Due to physical constraints (i.e. an altered ratio of POS surface to chamber volume), there is an increase in the instrumental background oxygen flux per volume, wherefore 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 the figure above.

7. 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 the addition of larger volumes of a 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 in the 0.5-mL O2k-chamber can be found in the [MiPNet09.12 O2k-Titrations](#).

8. DL-Protocols in DatLab 7.4

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 \(*.DLPU\)](#)).



9. Author contributions

Passrucker M and Gnaiger E were responsible for the project and instrumental development. Garcia-Souza LF adjusted the settings for the TIP2k. Passrucker M, Krumschnabel G, Gnaiger E, Tindle-Solomon L and Doerrier C prepared the MiPNet.

10. Acknowledgements

Gallée L as former members of the Oroboros Instruments performed the experiments. Contribution to K-Regio project MitoFit and SME phase 2 project NextGen-O2k. The project MitoFit is funded by the Land Tirol within the program K-Regio of Standortagentur Tirol. www.mitofit.org



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