O2k-Manual: ISS

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Integrated Suction System (ISS) and O2k-cleaning SOP

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1. The Integrated Suction System (ISS)

The ISS represents an integral component of the O2k-FluoRespirometer, for siphoning off medium and cleaning solutions from the O2k-chambers. Media containing living cells, tissues, microorganisms, various inhibitors, uncouplers, and mixtures of proteins and substrates are safely disposed of in the 2-liter waste bottle.

The ISS: enclosed in a stainless-steel housing (1), which holds a readily accessible main switch (2), an easily removable and safe connection (3) to the gas filter (4) which further connects (5) to the fully stabilized waste bottle (6), and two removable receptacles for the tip of the tubing (7).

1.1. Technical Specifications

ISS-Series E is equipped with a USB-C plug and can be connected for power directly to the O2k-Series I. For use with O2k-Series A to H or independently of the O2k, the ISS can be connected to an external Power Supply (see section 1.2).

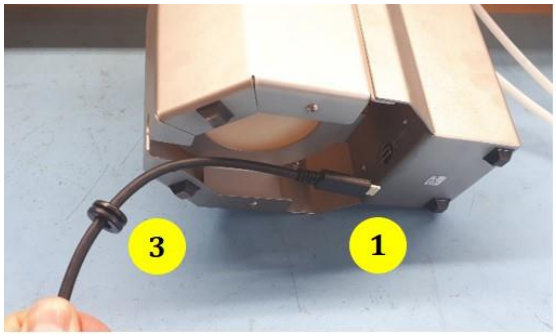
Power supply	USB-C (5V, 1.5 A)
Power consumption	7.5 W
Dimensions: housing with bottle	220x125x360 mm
Weight with empty bottle	2.4 kg

Do not use a USB-A to USB-C adapter.

Previous ISS-Series A to D: are specified for 120 V or 230 V.

Power supply	AC, complete with AC Heads for Euro, AUS, or USA, or an adaptor for UK.
Power consumption	5 W
Dimensions: housing with bottle	220x125x360 mm
Weight with empty bottle	2.4 kg

1.2. Assembly of the ISS.



For ISS-Series E, connect the USB cable to the hub on the bottom of the stainless-steel housing of the ISS (1) and to the USB-C hub on the back of the O2k-Series I* (2). Put the rubber on the cable to protect from damage caused by the edges of the stainless-steel housing (3). Connect the filter tube to the lid (4), screw the lid onto the waste bottle (5), connect the tip with the tube to the lid (6) and plug in the connector to the stainless-steel housing (7).

*An optional power supply with interchangeable AC Heads for UK, Euro, USA and AUS – Worldwide Approvals CE(EN55032) & UL/CUL (UL62368) – is recommended if the ISS-Series E is used with previous O2k-Series A to H or independently of the O2k.

1.3. ISS-cleaning

Unplug the safety connector from the stainless-steel housing and remove the waste bottle with filter for emptying and cleaning. This should be done regularly. Empty the waste bottle when the liquid rises above the level of the stainless steel housing.

It is important to ensure the filter is kept dry, otherwise the airflow is blocked.

The receptacles for the tip of the tubing need to be cleaned periodically.

1.4. Troubleshooting

The ISS is not running (no humming sound): Check the connection of the power supply: either USB-C hub of the O2k-Series I or the optional power supply provided with the ISS. If the power supply is connected properly but there is still no humming sound, try to connect to an alternative USB-C power supply with at least 1.5 A.

The ISS is running (humming sound) but there is no suction: Check the connection of the power supply as described above. If the ISS is properly connected, check for and remove any blockages in the tubes and the filter. A wet filter blocks the suction pressure and can be dried overnight at 50 °C.

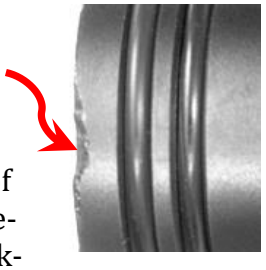
If the above steps do not solve the problem, please contact our technical support.

2. O2k-chamber cleaning

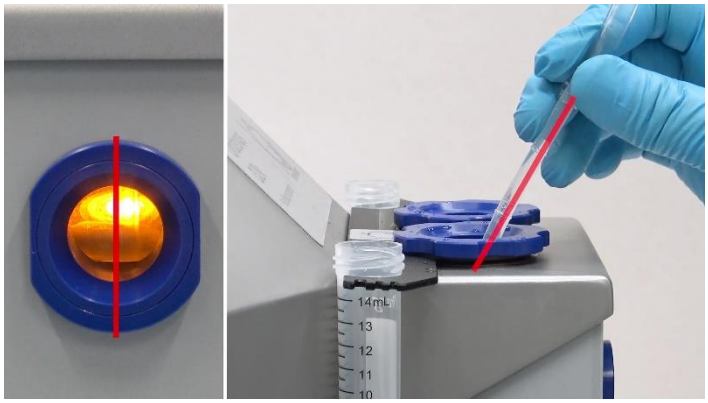
2.1. General

For regular cleaning of the O2k-chambers, remove the stoppers but keep the OroboPOS and O2k-chambers in place. Wash off aqueous salt solutions with water before using ethanol (EtOH).

O2k-stoppers should be handled carefully when taken out from the chambers; dropping from a table height range is likely to cause splintering at the edges (arrow) and can influence the functionality.



To avoid contamination, hold the stopper at the receptacle, not on the shaft that fits into the O2k-chamber. To avoid displacement of the volume calibration position, do not hold the stopper on the volume-calibration ring when pulling it out from or inserting it into the O2k-chamber.

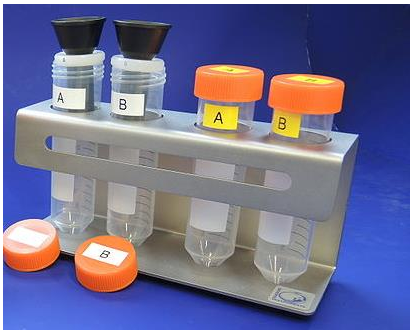


When using the ISS to siphon off solution from the chamber, insert the tip of the ISS to the bottom of the O2k-chamber, pointing it away from you while the stirrer bar is rotating. Do not point the tip towards the oxygen sensor (left and right side in chambers A and B, respectively), to avoid damage of the membrane.

Do not exchange stoppers and stirrers between O2k-chambers, except if advised to by O2k-technical support.

2.2. O2k-chamber cleaning before experimental use

DatLab Protocol DLP-file:
O2k-cleaning_BeforeUse.DLP



1. Make sure the stirrer is rotating. Remove the Cover-Slip and the stopper from the O2k-chamber. Rinse the surface and capillary of the stopper with H_2O. Put the stoppers into the 50-mL tubes labelled A and B in the tube rack.
2. Siphon off ethanol from the O2k-chamber and rinse the chamber with H_2O three times:

   1st H_2O wash: Fill the chamber with H_2O to the top of the chamber holder. Let the stopper slide into the chamber while siphoning off excess water. Pour H_2O into the top of the receptacle of the stopper and add the Cover-Slip. Stir for 5 min.

   2nd H_2O wash: Remove the Cover-Slip and the stopper from the O2k-chamber, siphon off water and repeat 1st H_2O wash.
   3rd H_2O wash: Repeat 2nd H_2O wash.

2.3. O2k-chamber cleaning after experimental use

DatLab Protocol DLP-file:
O2k-cleaning_AfterUse.DLP

1. **O2k-stopper**: Remove the O2k-stopper, rinse the Cover-Slip and the stopper several times with H_2O. Clean the stopper mechanically with a paper towel, rinse it to avoid a transmission of paper-particles. To store safely and avoid further contamination, put the stoppers into the 50-mL tubes labelled A and B in the tube rack.
2. **Pre-wash**: Siphon off medium with sample from the O2k-chamber. Fill the O2k-chamber with H_2O. Siphon off the H_2O from the chamber.
3. **O2k-stirrer**: When working with potentially sticky tissue, stop the stirrer, siphon off H_2O, remove it with a magnetic bar and place the stirrer into the cover of the tube. Clean the stirrer bar with a paper towel and rinse it with H_2O. Add it into the same chamber.
4. Rinse the chamber with distilled water three times: see section 2.2.
5. Siphon off H_2O from the receptacle, remove the stopper and shake off water. Put the stopper into the 50-mL tube and siphon off H_2O from the O2k-chamber. Wash the stopper with 70% EtOH and allow EtOH to rinse down through the capillary.

   1st 70 % EtOH wash: Fill the O2k-chamber with 70 % EtOH. Let the stopper slide into the chamber while siphoning off excess EtOH, fill the receptacle with 70 % EtOH, add the Cover-Slip and stir for 5 min.

   2nd 70 % EtOH wash: Remove the Cover-Slip and the stopper. Then, siphon off the EtOH from the chamber. Fill up the chamber again with 70 % EtOH, insert the stopper, fill up the receptacle, add the Cover-Slip and continue stirring for 5 min.

   3rd 70 % EtOH wash: Repeat 2nd EtOH wash.

6. **Pure EtOH wash:** Siphon off the 70 % EtOH from the O2k-chamber. Fill chamber and stopper receptacle with absolute EtOH (99.6 %). Make sure that the EtOH fills up the receptacle to the top of the stopper. Place the Cover-Slip on the stopper. Continue stirring for 15 min. For cleaning after an **Instrumental O2 Background** or when only **substrates were used**: water wash (3x) is sufficient; use 70 % EtOH for storage.
7. Siphon off the pure EtOH keeping the stirrer on. Prepare for chemical sterilization and storage (**2.5**) or immediate use (**2.2**).

2.4. O2k-chamber cleaning after use, if there are indications of carry-over of inhibitor and uncouplers dissolved in ethanol

DatLab Protocol DLP-file:
O2k-cleaning_AfterUse_inhibitors.DLP, O2k-cleaning_AfterUse_stirrer.DLP

1. Remove the O2k-Stopper with Cover-Slip and place it into the 50-mL tubes labelled A and B in the tube rack.
2. Siphon off medium with sample from the O2k-chamber.
3. Fill chamber with suspension (cells, tissue or isolated mitochondria).
   Optionally, the chamber can be filled with just 2.3 mL of suspension. In this case, the stopper needs to be pushed into the chamber down to the calibration ring instead of just sliding it in.
4. Stir for 30 min.
5. Continue with the section 2.3.

2.5. Storage and chemical sterilization

1. Fill the chamber with 70 % EtOH. Insert the stopper loosely and fill 70 % EtOH up to the rim of the receptacle.
2. Place the Cover-Slip onto the stopper to minimize evaporation and leakage of EtOH.
3. For overnight storage and chemical sterilization keep EtOH in the chamber and switch off the O2k. You can use this method for storage up to several months, with the OroboPOS in place, ready for use (more information: Supplement B).

2.6. O2k-chamber cleaning with HCl

Turbidity on the glass wall may be caused by precipitated protein. Remove the chambers from the O2k and with the O2k-stirrers removed, immerse in a beaker filled with HCl (10 M) overnight under a hood. If necessary, clean with chromic acid overnight. After reassembly of the O2k-chambers, instrumental test runs should be performed.

3. Cleaning the O2k stainless-steel housing

For cleaning the O2k-housing, general guidelines for the maintenance of commercial stainless-steel surfaces should be applied.

- Do not use chlorinated detergents, bleaches or strong acids.
- Do not use scourers or hard objects.

- The frequent use of commercial stainless-steel care products is recommended. The conditioning cloth, supplied the O2k-Series I, is to be used after cleaning the stainless-steel housing with warm water and washing up liquid.

4. Acknowledgements

Supported by the NextGen-O2k project.

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Supplement A: Types of O2k-chamber contaminations and corresponding cleaning procedures

For further information on different types of contamination of the O2k-chamber please see: Cleaning the O2k-chamber.

Supplement B: Storage in 70 % ethanol

Intensive tests were performed on the storage of the polarographic oxygen sensors (OroboPOS) in O2k-chambers filled with 70 % ethanol (EtOH) over periods extending up to several weeks. The test runs have been performed with both PEEK and PVDF stirrer bars, with titanium stoppers and PVDF stoppers giving the following results.

1. Over a period of 20 days, the calibration factor of the OroboPOS changed by <2 % when measured intermittently using a salt solution after storage in 70 % EtOH.
2. The OroboPOS signal stability during air calibration with salt solution corresponded to a slope of 0.1 ± 0.3 pmol·s^{-1}·mL^{-1} (mean ± SD) in 18 test runs with 6 different sensors over a 20-day period of ethanol storage.
3. Oxygen consumption by the OroboPOS at air saturation in salt solution was 2.0 ± 0.2 pmol·s^{-1}·mL^{-1} (mean ± SD) in 18 test runs (25 °C; 2 mL chamber volume; 6 different chambers) over a 20 day period of EtOH storage.
4. Air equilibration for estimation of the calibration factor was equally rapid after storage in 70 % EtOH compared to storage in distilled water.
5. The exponential time constant of the OroboPOS remained constant over a 20-day period of EtOH storage.
6. The zero current of the OroboPOS remained stable over a 20-day period of EtOH storage, when measured in salt solution after oxygen depletion by isolated mitochondria.

These results provide the basis for the recommendation on ethanol storage, which is further supported by the following considerations:

Save time: At the end of an experimental day, the chambers are washed with water and then filled completely with 70 % EtOH, which remains in the chamber until the next experiment. Then it is not necessary to (1) wait for 15 min upon addition of ethanol, (2) wash the chambers with water, and (3) repeat the 15-min ethanol incubation on the subsequent experimental day. Instead, before the next experiment, the ethanol is simply siphoned off from the chamber (ISS), and a chemically sterilized chamber is available.

Save ethanol: Instead of washing with EtOH in the evening and before the first experiment the next day, a single filling of the chamber is sufficient for the O2k-chambers and stoppers.

Washout of ethanol-soluble inhibitors and uncouplers: Long-term storage with 70 % ethanol ensures that trace amounts of inhibitors on the chamber and stopper are diluted into the large volume of EtOH (>5 mL) and washed out.

Based on these considerations and the experimental results mentioned above, we recommend filling the O2k- chambers with 70 % ethanol for storage overnight and over extended periods of time. Wash five times with distilled water immediately before addition of mitochondrial respiration medium or experimental salt solution. Storage with EtOH thus replaces the time-consuming procedure described previously and improves experimental reliability in high-resolution respirometry.