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O2k-cleaning SOP and Integrated Suction System (ISS)

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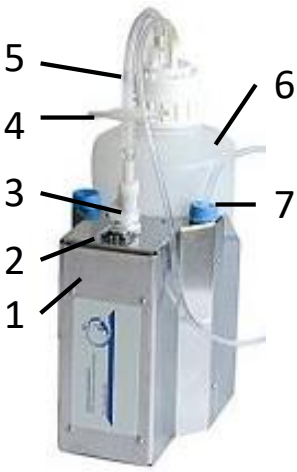
1. The Integrated Suction System (ISS)

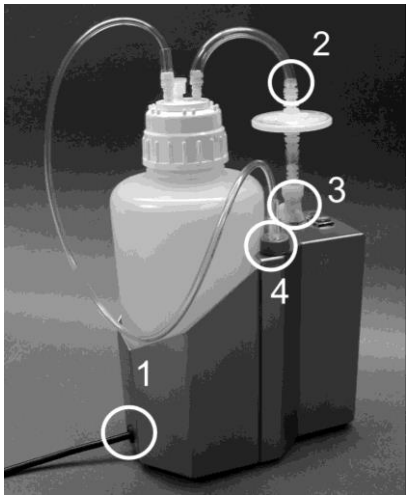


The ISS represents an integral component of the O2k-FluoRespirometer, for siphoning off medium and cleaning solutions from the O2k-chambers. Media containing living cells, tissues, microorganisms, various inhibitors, uncouplers, and mixtures of proteins and substrates are safely disposed of in the 2-litre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

The ISS (230 V or 120 V) is enclosed in a stainless steel housing (1), which holds a readily accessible mains 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 data and assembly - WGT

Power supply	AC
Power consumption	5 W
Dimensions: housing with bottle	220x125x360 mm
Weight with empty bottle	2.4 kg



Connect 1 - 4 (no tools required)

1.2. ISS-cleaning

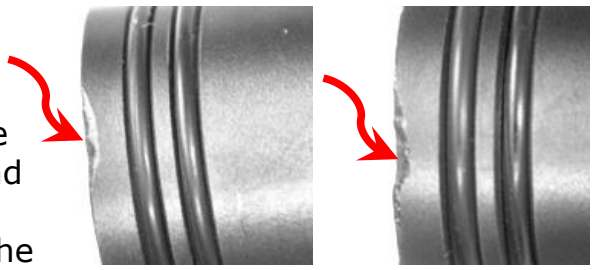
Remove the waste bottle with filter for emptying and cleaning, which should be done regularly. Empty the waste bottle immediately when the water rises above the level of the stainless steel housing. It is important to ensure the filter is kept dry, otherwise the airflow is blocked.

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

2. O2k-chamber cleaning

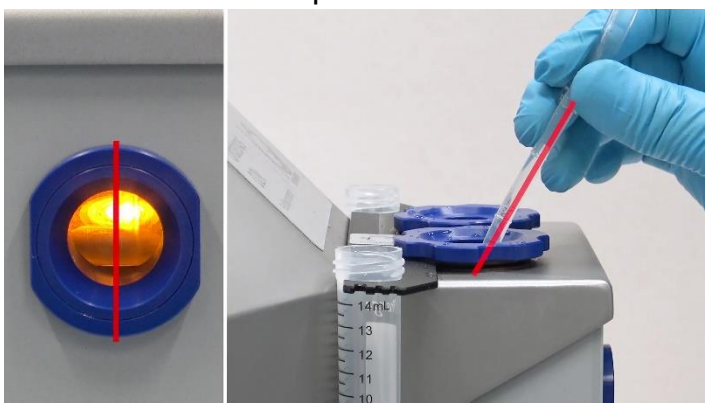
2.1. General

For O2k-chamber cleaning, 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 (arrows) and can influence the functionality.

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



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), to avoid damage of the membrane.

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

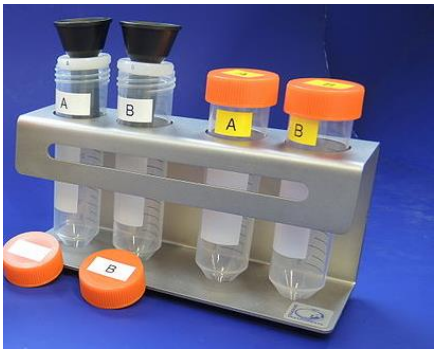
2.2. O2k-chamber cleaning before experimental use

(O2k-cleaning_BeforeUse.DLP)

- 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.
- Siphon off ethanol from the O2k-chamber and rinse the chamber with distilled water 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. Stirr 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 step 2.

2.3. O2k-chamber cleaning after experimental use

(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. In order to place safely and avoid further contamination, put the stoppers into the 50-mL tubes labelled A and B in the tube rack.
2. **Pre-wash**: Follow specific precautions if the sample is to be collected quantitatively. 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 the 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.
6. **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 stirr for 5 min.
7. **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.
8. **3rd 70 % EtOH wash:** Repeat step 7.
9. **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 at the top of the stopper. Place the Cover-Slip on the top of the stopper. Continue stirring for 15 min. (In the case of **Instrumental O2 Background** or when only **substrates were used**: water wash (3x) is sufficient; use 70 % EtOH for storage.)
10. Siphon off the pure EtOH keeping the stirrer on. Prepare for chemical sterilization and storage (**2.4**) 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

(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). *The chamber can be filled with 2.3 mL of suspension only. 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).

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 immerse in a beaker with HCl (10 N) overnight under a hood, with the O2k-Stirrers removed. 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, bleachers or strong acids.
- Do not use scourers or hard objects.
- The frequent use of commercial stainless steel care products is recommended.

Full version: *go Bioblast*
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Acknowledgements

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Supplement

A) Types of O2k-chamber contaminations and following washing procedures

- In specific cases (Steininger et al 2002), 3 % formaldehyde may be applied for 10 min.

B) Storage in 70 % ethanol

EtOH storage saves time and chemicals, and improves wash-out of ethanol-soluble inhibitors from the O2k-chamber. Experimental tests were performed on storage of the polarographic oxygen sensors (OroboPOS) in chambers of the O2k filled with 70 % ethanol (EtOH) over periods extended up to several weeks. Based on these results, we recommend filling the O2k-chambers with 70 % ethanol for storage overnight and over extended periods of time, instead of using distilled water. Storage with EtOH thus replaces the time-consuming procedure described previously, and improves experimental reliability in high-resolution respirometry.

Tests and recommendations: extended storage with 70 % ethanol: Intensive tests were carried out which show that the OroboPOS remains fully functional after storage for several days (weeks) in 70 % EtOH. The following considerations led to a new recommendation on using 70 % EtOH for short-term storage (20 days) and washing five times with distilled water immediately before addition of mitochondrial respiration medium (experimental salt solution). The test runs have been performed with the PEEK stirrer bars and with new PVDF stirrer bars, and with our titanium stoppers and with the new PVDF stoppers.

1. 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 at the subsequent experimental day. Before the next experiment, the ethanol is simply siphoned off from the chamber (ISS), and a chemically sterilized chamber is available.
2. Save ethanol: Instead of washing with EtOH in the evening and before the next experiment, a single filling of the chamber is sufficient for the O2k-chambers and stoppers.
3. Washout of ethanol-soluble inhibitors and uncouplers: Long-term storage with 70 % ethanol ensures an extensive solution of trace amounts of inhibitors from the materials of the chamber and stopper into the large volume of EtOH (>5 mL). The stopper is loosely inserted into the chamber, then the receptacle of the

stopper is filled up completely and is sealed with the cover slips put on top of the stoppers.

The following observations provide the basis for the recommendation on ethanol storage:

1. Over a period of 20 days, the calibration factor of the OroboPOS changed by <2 % when measured intermittently in salt solution after storage in 70 % EtOH.
2. The OroboPOS signal stability at 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 or 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.