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O2k-Chamber cleaning SOP and Integrated Suction System (ISS)

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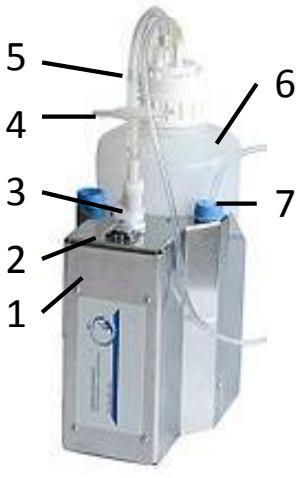
Contents

1. The Integrated Suction System (ISS)

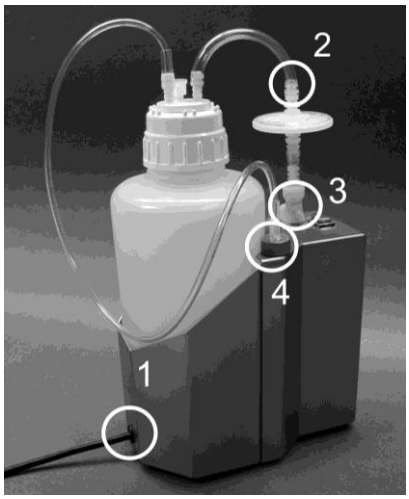
The ISS represents an integral component of the O2k-Core, for siphoning off medium and cleaning solutions from the O2k-Chambers. Media containing living cells, tissues or microorganisms, various inhibitors, uncouplers and mixtures of proteins and substrates are safely disposed off in the 2-litre waste 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 secure that the filter is maintained dry, otherwise the airflow gets blocked.

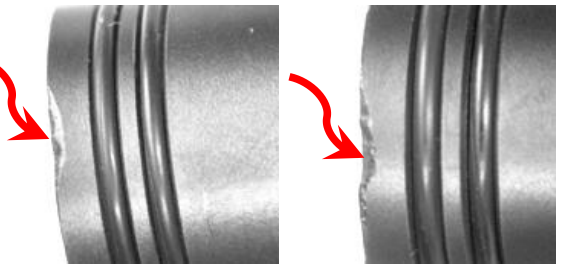
The receptacles for the tip of the tubing have to be cleaned periodically.

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 have to be placed safely 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.



Hold the stopper at the receptacle, not at the shaft that fits into the O2k-Chamber, to avoid contamination. 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.

Siphon off medium from the chamber by inserting the tip of the ISS suction tubing to the bottom of the O2k-Chamber while the stirrer 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. Move the tip of the ISS suction tubing up and down to the bottom of the chamber.

Remove the stirrer from the chamber for mechanical cleaning.

Do not exchange stoppers and stirrers between O2k-Chambers, except for O2k-Troubleshooting.

2.2. O2k-Chamber cleaning-steps

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Water wash: always before an ethanol wash

1. Fill 50-ml Falcon tubes in the rack with c. 48 ml H_2O, for each O2k-Chamber.
2. **O2k-Stopper**: Remove the O2k-Stopper, rinse Cover-Slip and stopper with H_2O, clean the stopper mechanically with a paper towel, rinse it to avoid a transmission of paper-particles. Insert the stopper into the H_2O in the Falcon tube and put on the Cover-Slip (Figure: O2k-Chamber B).
3. **Pre-wash**: Follow specific precautions if the sample is to be collected quantitatively. Siphon off medium with sample from the O2k-Chamber. Lift the stopper with Cover-Slip (holding the stopper on the volume calibration ring), take the Falcon into the other hand to fill a few ml H_2O into the chamber (45 ml should remain in the Falcon). Put the stopper with Cover-Slip into the Falcon (Figure: O2k-Chamber B).
4. Siphon off the H_2O from the chamber.
5. **O2k-Stirrer**: When working with potentially sticky tissue, stop the stirrer, remove it with a magnetic bar and place the stirrer into the cover of the falcon for cleaning. Clean the stirrer bar with a paper towel and rinse it with H_2O. Add it into the same chamber.
6. **1st H_2O wash:** Remove the stopper from the Falcon and fill the chamber with H_2O from the Falcon to the top of the chamber holder (33 ml remain). Lift the Cover-Slip and let the stopper slide into the chamber while siphoning off excess water. Pour H_2O from the Falcon into the top of the receptacle of the stopper and add the Cover-Slip (25 ml remain). Stirr for 5 min (while cleaning the other chambers).
7. **2nd H_2O wash:** Remove the stopper with Cover-Slip in place from the O2k-Chamber and insert it into the Falcon. Remove the Cover-Slip to allow water to rinse down through the capillary. During this time, siphon off the H_2O from the O2k-Chamber.
8. Fill the chamber (20 ml remain in the Falcon), insert the stopper without Cover-Slip, fill the receptacle (12 ml remain), add the Cover-Slip and continue stirring.
9. **3rd H_2O wash:** Repeat step 8. After filling the chamber, 7 ml remain, sufficient to finally fill the receptacle.

Ethanol wash: after H_2O wash

10. Remove H_2O from the Falcon and fill it with 48 ml 70% EtOH.
11. Siphon off H_2O from the receptacle, remove the stopper and shake off the water. Insert the stopper into the Falcon (70% EtOH) and siphon off the H_2O from the O2k-Chamber.
12. **1st 70% EtOH wash:** Fill the O2k-Chamber with 70% EtOH from the Falcon (>35 ml remain).

13. Insert the stopper while lifting the Cover-Slip, fill the receptacle with EtOH (>30 ml remain), add the Cover-Slip and stirr on for 5 min.
14. **2nd 70% EtOH wash:** Remove the stopper with Cover-Slip in place and insert it into the Falcon. Remove the Cover-Slip to allow EtOH to rinse down through the capillary (note: for ultra-pure cleaning use a new Falcon to avoid recycling of the used EtOH). During this time, siphon off the EtOH from the chamber. Fill up the chamber (25 ml remain), insert the stopper, fill up the receptacle (18 ml remain), add the Cover-Slip and continue stirring for 5 min.
15. **3rd 70% EtOH wash:** Repeat step 14. After filling the chamber, >12 ml remain, sufficient to fill the receptacle; continue stirring for 5 min.
16. Close the Falcon with the remaining 70% EtOH for later use.
17. **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. Incubate with stirring for 15 min.
18. Siphon off the pure EtOH keeping the stirrer on. Prepare for chemical sterilization and storage (**2.3**) or immediate use (**2.4**).

2.3. 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).

2.4. Preparations before an experiment

1. Make sure the stirrer is rotating. Remove the stopper, rinse the surface and cannula of the stopper with H_2O. Place the stopper clean and securely (in the Falcon tube with distilled water).
2. Rinse the chamber with distilled water three times (steps 6 to 9).

2.5. 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. Use teflon stirrers and cover the beaker during the procedure. If necessary clean with chromic acid overnight. After reassembly of the O2k-Chambers instrumental test runs should be performed.

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Supplement

A) Types of contaminations and following washing procedures

- **Instrumental Background/ only substrates were used**: water wash (3x) is sufficient; use 70% EtOH for storage.
- Experminets with **biological sample** and/or use of **hydrophobic inhibitors dissolved in ethanol or DMSO** (such as oligomycin, rotenone, or antimycin A): procedure as described in Sect. **2.2**.
- 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 Oxygraph-2k filled with 70% ethanol (EtOH) over periods extended up to several weeks. Based on these results, we recommend to fill the O2k chambers with 70% ethanol for storage over night 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.

Until August 2006: 70% ethanol for 20 min: For many years, we recommended to maintain distilled or deionized water in the O2k chamber during short-term storage over a few days or weeks. This made it necessary to fill the chamber with 70% ethanol for a minimum of 20 min, to obtain a chemically sterilized system.

Tests and recommendations: extended storage with 70% ethanol: Intensive tests were carried out which show that the oxygen sensor remains fully functional after storage for several days (weeks) in 70% EtOH. The following considerations led to a new recommendation on using 70% ethanol 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 20 min upon addition of ethanol, (2) wash the chambers with water, and (3) repeat the 20-min ethanol incubation at the subsequent experimental day. Before the next experiment, the ethanol is simply siphoned 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.