

O2k-Manual: Sample Holder

Mitochondrial Physiology Network 27.07(01):1-4 (2022)

Version 01: 2022-08-09 ©2022 Oroboros

Updates: https://wiki.orooboros.at/index.php/MiPNet27.07_Sample_Holder



Sample Holder

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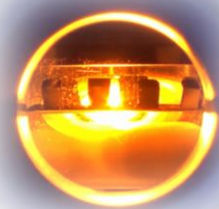
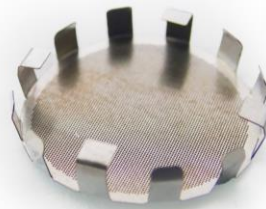
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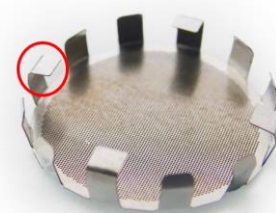
1. Introduction

Rigorous stirring is required for high-resolution respirometry but might damage samples that are susceptible to mechanical force, such as tissue slices, adherent cells grown on beads, 3D cell cultures, zooplankton, fish eggs, or plant samples. Placed in the 2 mL O2k-chamber, the Sample Holder protects such fragile samples from potential damage caused by stirring the medium. The Sample Holder creates a physical barrier between the sample and the stirrer. The 6000 pores in the stainless-steel mesh of the Sample Holder enable sufficient convection for homogenous distribution of dissolved oxygen and chemicals throughout the O2k-chamber.

2. Components



3 Sample Holders Sample Holder with stainless-steel mesh: 0.08 mm thickness, 6000 pores (diameter 0.01 mm); 4 stainless steel bent blades (red circle) to insert and remove the Sample Holder into or from the 2 mL O₂k-chamber with the Sample Holder Tool; 8 stainless steel side blades for stabilization in the O₂k-chamber.



Sample Holder Tool Stainless steel forceps with bent ends to insert and remove the Sample Holder into or from the 2 mL O₂k-chamber.

Sample Holder Brush to clean the stainless-steel mesh of the Sample Holder.

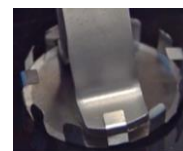
Sample Holder Box for storage of Sample Holders, Sample Holder Tool, and Sample Holder Brush.

3. Operating procedures

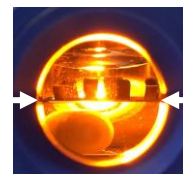
3.1. Insertion of the Sample Holder into the O2k-chamber



1. Before insertion, both the Sample Holder and the Sample Holder Tool must be clean and dry. Any moisture on the mesh increases the risk of air bubbles being trapped below the Sample Holder after closing the O2k-chamber.
2. Wash the O2k-chamber (Section 2.2 in MiPNet19.03 2021)
3. Fill the O2k-chamber with 2.3 mL respiration medium.
4. Stop the stirrer.
5. Place the bent ends of the Sample Holder Tool underneath the bent blades of the Sample Holder.



6. Insert the Sample Holder into the O2k-chamber in a horizontal position.
 7. Push the Sample Holder down until it is 3-5 mm above the stirrer bar. While pushing the Sample Holder down, take care to keep it in a horizontal position.
 8. Control via the front window that no air bubbles are trapped beneath the mesh of the Sample Holder.
- If you observe any air bubble remove, wash with H₂O, and dry the Sample Holder. Start again with step 5.



3.2. Calibration



Air calibration (MiPNet06.03 2022) and instrumental background (MiPNet14.06 2020) must be performed with the Sample Holder in place.

3.3. Experiment

1. Insertion of the sample must be optimized for each sample type (e.g. Dias et al 2018). The stirrer may be switched on before or after placing the sample in the O2k-chamber.
2. Close the O2k-chamber.

3.4. Removal from the O2k-chamber

1. Remove the stopper.
2. Place the bent ends of the Sample Holder Tool underneath the bent side blades of the Sample Holder.
3. Carefully pull out the Sample Holder keeping it in a horizontal position.
4. For some sample types, the Sample Holder may be blotted and used for determination of wet mass.

3.5. Cleaning

1. After use and removing the sample, rinse the Sample Holder immediately with deionized or double distilled H₂O to avoid clogging of the mesh. In case of any residual biological sample sticking to or clogging the mesh, carefully clean it with the Sample Holder Brush.
2. Rinse the Sample Holder Tool with deionized or double distilled H₂O water and clean mechanically with a paper cloth.
3. Rinse Sample Holder and Sample Holder Tool with 70 % EtOH.

4. Rinse Sample Holder and Sample Holder Tool with deionized or double distilled H₂O.
5. Let the Sample Holder dry on a lint free cloth. Store each Sample Holder in its box for protection of the blades.

4. Troubleshooting

- Air bubble(s) trapped under the Sample Holder after closing the chamber. ⇒ Remove the Sample Holder and start with step 5, Section 3.1.
- Sample Holder moves. ⇒ Carefully bend the blades slightly out using a pair of forceps.
- Sample Holder is hit by the stirrer (placed too far down). ⇒ Lift the Sample Holder up while keeping it in a horizontal position.
- The Sample Holder is twisted while pushing it down or it is not possible to keep it in a horizontal position. ⇒ Carefully bend the blades slightly in, using a pair of forceps.

Technical Support is provided by:

Oroboros Instruments

High-Resolution Respirometry

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<https://www.orooboros.at/index.php/support/>

5. References

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MiPNet06.03 (2022) O2k Quality Control 1: Polarographic oxygen sensors and accuracy of calibration. Mitochondr Physiol Network 06.03(20):1-8.

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<https://doi.org/10.1016/j.ab.2018.05.010>

6. Author contributions and acknowledgements

EG was responsible for the concept of the project based on open innovation in cooperation with Candida Dias. SS and EG prepared the manual.

We thank Phillip Gradl and Michael Walter-Vracevic (WGT Elektronik) for mechanical development, Lisa Tindle-Solomon for proofreading and Paolo Cocco for graphics.