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SOP for manual O2k-titrations with Hamilton microsyringes

Fontana-Ayoub M, Verena L, Gnaiger E, Dröscher S

OROBOROS INSTRUMENTS
Schöpfstr 18, A-6020 Innsbruck, Austria
Email: instruments@oroboros.at
www.oroboros.at

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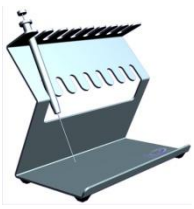
1. General information

Hamilton microsyringes with volumes of 10, 25 or 50 µl (mm^3) are used for manual O2k-titrations in substrate-uncoupler-inhibitor titration (SUIT) protocols, which are a hallmark of high-resolution respirometry (HRR). The components of a Hamilton microsyringe are: needle, termination, volume markings, barrel, flange, plunger.

A Hamilton syringe O2k-Titration Set is included in the purchase of an O2k-Core as a basic HRR-Accessory, which consists of:



6	Microsyringes\10 mm3 51/0.13 mm: 10 mm^3
6	Microsyringes\25 mm3 51/0.15 mm: 25 mm^3
2	Microsyringes\50 mm3 51/0.15 mm: 50 mm^3
3	Plunger\10 mm3: for 10 mm^3 syringe
1	Syringe\500 mm3 51/0.41 mm: 500 mm^3

1 Package of two Syringe Racks including 20 Syringe Collars.
1 Package of two Tube Racks including eight 50 ml tubes.
1 Syringe Storage Box including Syringe Labels.

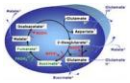
Hamilton microsyringes are specifically produced for O2k-titrations and can be ordered directly from OROBOROS INSTRUMENTS to assure exact fitting with the O2k-Stopper and O2k-Chamber dimensions.

2. Working with microsyringes

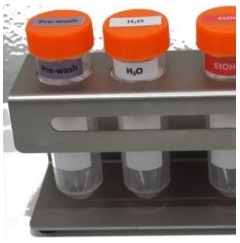
2.1. Start

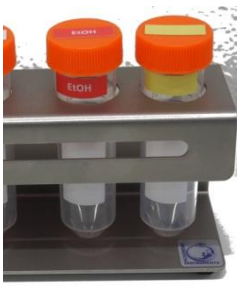
In order to minimize contamination by carry-over, the syringes should be labeled with the names of the substrates, uncouplers and inhibitors. We suggest to use coloured labeling according to O2k-Titrations: white labeling for substrates, blue for uncoupler, red for inhibitors and yellow for other chemicals.

Before an experiment, prepare:



1. Hamilton syringes according to the specific SUIT protocol.
2. One or two Syringe Racks for placing the Hamilton syringes in sequence of the SUIT protocol.
3. A Tube Rack with 50 ml tubes for the washing procedure:





Washing step	Tube filled with	Use for
Pre-wash	Distilled water	All syringes
H_2O	Distilled water	Syringes used for chemicals with H_2O as solvent
EtOH	EtOH 100%	Syringes used for chemicals with EtOH or DMSO as solvent
Additional	E.g. distilled water	Inhibitor syringes used for chemicals with H_2O as solvent, e.g. malonic acid

4. A beaker for waste.
5. Wipes for cleaning the needle and glass barrel.

2.2. Initial washing

- To be sure that the syringes are completely clean, wash them three times with H_2O and three times with EtOH

after storage. Take care to rinse not only the needle itself but also the needle base.
- Wash three times with the solvent of the chemical if it is different from H_2O or EtOH.
- Between each washing, clean the needle with a wipe.

2.3. Titrations

- Fill the syringe only shortly prior to titration to avoid warming of the chemicals which might lead to gas bubble formation.
- Hold the syringe on the top of the glass barrel (at the Syringe Collar) to prevent warming.
- Fill the syringe with a small excess volume above the desired mark.
- Take care not to include gas bubbles when filling the syringe – this causes inaccuracies in titration volumes.
- Fill the syringe not too fast but steady. If gas bubbles are forming, a continued pumping helps to extrude bubbles.
- Before titration into the chamber, press the plunger slightly and check if a small drop appears on top of the needle. Wipe off the drop before titration.
- Be **swift** when titrating into the chamber, especially when titrating with EtOH as solvent. During slow titrations, a fraction of the titration volume might remain attached to the needle and escape into the titration port of the stopper instead of being mixed into the chamber volume.

☞ **Caution**: Take care to rinse syringes used for ADP (with $MgCl_2$) and fatty acids **immediately** after use (on the surface, but more importantly inside the needles three times with distilled water). Otherwise a precipitate may form and block the needle.

2.4. Washing after titration

- After titration discard residual chemicals into the waste beaker.
- Plunge syringe in 'Pre-wash' tube (H_2O) and clean it with a small wipe. Rinse not only the needle itself but also the needle base to clean the barrel.
- Wash syringe three times in tube filled with H_2O or pure EtOH (depends on solvent H_2O or EtOH). For inhibitor syringes (with solvent H_2O) you may consider the additional tube (see above).

2.5. Handling after experiments and storage

- Exchange H_2O and EtOH in the tubes for final washing.
- Take care of the washing order: first substrate-, then uncoupler- and finally inhibitor-syringes.
- Plunge the syringe in the 'Pre-wash' tube as described above.
- Wash five times with appropriate solvent (e.g. water for substrates as P, M, G, etc., EtOH for uncouplers etc.). Take care again to also rinse the needle base.
- Rinse three times with EtOH 100%.
- Store syringes in dry condition protected from dust.

Caution:

☞ Be swift in pushing down the plunger to be sure that substances are washed out thouroughly.

☞ When using a syringe with a chemical different from the one usually assigned to the syringe, then repeated washing steps and a overnight exposure filling the syringe with pure Ethanol may be crucial to prevent contamination by carry-over.

3. Further information

- For quality control of your washing procedure use a strong dye such as Trypan blue to evaluate your washing.
- Visit also: Hamilton and Hamilton care and use guide for further information.

☞ **Caution**: If you use aceton for washing as mentioned in the Hamilton guide, please take care to wash it out carefully as aceton damages the POM, PVDF and PEEK parts of the O2k and the polarographic oxygen sensor when introduced into the glass chamber.

- **Plunger**: If the plunger seems to scratch during titration or black residues appear on the plunger take it out and clean it with water and Kimwipe.
- Needle burrs and surface : eliminate rough edges with 3M™ Wetordry™ Paper Sheet P1000 by gentle rubbing.
- Use a cleaning wire for the needle (included in package). If a syringe is clogged, it may be helpful to fill it from the back with pure EtOH or H_2O and try to press the plunger gently down. You can also soak the syringe for a few minutes or overnight and then press down the plunger carefully.
- Use less plastic and more glass ware.