

O2k-Protocols SOP: O2k-titration



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SOP for manual O2k-titrations with Hamilton microsyringes

Verena Laner, Alba Timón-Gómez, Eleonora Baglivo, Erich Gnaiger

Oroboros Instruments

High-Resolution Respirometry

Schoepfstrasse 18, 6020 Innsbruck, Austria

Email: instruments@orooboros.at

www.orooboros.at



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1. General Information

Hamilton microsyringes with volumes of 10, 25, 50 or 100 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, barrel with volume markings, flange, plunger.

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

[Microsyringes\10 mm³ 51/0.13 mm](#): 10 mm^3

[Microsyringes\25 mm³ 51/0.15 mm](#): 25 mm^3

[Microsyringe\50 mm³ 51/0.15 mm](#): 50 mm^3

[Microsyringe\100 mm³ 51/0.41 mm](#): 100 mm^3

[Plunger\10 mm³](#): for 10 mm^3 syringe

[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 8 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 using color-coded 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. One or two Syringe Racks for placing the Hamilton syringes in sequence of the SUIT protocol.
2. Hamilton syringes according to the specific [SUIT protocol](#).
3. A Tube Rack with 50-mL tubes for the washing procedure:



Washing step	Tube filled with	Use for
Pre-wash	H ₂ O	All syringes
H ₂ O	H ₂ O	Syringes used for chemicals with H ₂ O as solvent
EtOH	EtOH 100 %	Syringes used for chemicals with EtOH or DMSO as solvent
Additional	E.g. H ₂ O	Inhibitor syringes used for chemicals with H ₂ O as solvent, e.g. malonic acid

4. A beaker for waste.
5. Cleaning lint-free wipes for drying the needle and glass barrel.

2.2. Initial washing

A syringe cleaning cycle consists of (1) filling an empty syringe with a washing solution while immersing not only the needle itself but also the base of the barrel into the washing solution, without fully removing the plunger, and (2) emptying the syringe into the waste beaker. Be swift in pushing down the plunger to be sure that substances are washed out thoroughly.

1. After storage, perform three cleaning cycles with H₂O.
2. Wipe off any liquid from the syringe.
3. Perform three cleaning cycles with EtOH.
4. If the solvent of the chemical is different from EtOH, wipe off any liquid from the syringe, perform three cleaning cycles with that solvent, and wipe off any liquid from the syringe.
5. Put the cleaned syringe on the Syringe Rack.

2.3. Titrations

- Take care not to include gas bubbles when filling the syringe – this causes inaccuracies in titration volumes and might introduce gas bubbles to the chamber upon titration.
- If gas bubbles are forming, going up and down several times may help to eliminate bubbles.
- Fill the syringe with a small excess volume above the desired mark.
- Before titration into the chamber, press the plunger slightly until the desired mark and check if a small drop appears on top of the needle. Wipe off the drop on the wall of the chemical vial before titration.
- Introduce the needle completely into the stopper capillary until the base of the barrel resides on the stopper.
- Be **swift** when titrating into the chamber, especially when titrating with EtOH as solvent. During slow titrations, a fraction of the titration volume might attach externally to the needle and thus escape into the titration port of the stopper instead of being mixed into the chamber volume.



Caution: Take care to perform cleaning cycles especially with syringes used for ADP (with MgCl₂) and fatty acids **immediately after** use. Otherwise, a precipitate may form and block the needle.

2.4. Washing after titration

- After titration, discard residual chemicals into the waste beaker.
- Immerse the syringe into the 'Pre-wash' tube (H₂O) and wipe off any liquid. Rinse not only the needle itself but also the barrel base.
- Perform three cleaning cycles with H₂O or pure EtOH (depends on solvent H₂O or EtOH). For inhibitors (with solvent H₂O), you may consider using the additional tube (see above).

2.5. Handling after experiments and storage

- Washing sequence: (1) substrate-, (2) uncoupler- and (3) inhibitor-syringes.
- Immerse the syringe in the 'Pre-wash' tube, as described above.
- Perform five cleaning cycles with appropriate solvent (e.g. H₂O for substrates as P, M, G, etc., EtOH for uncouplers etc.).
- Perform three cleaning cycles with absolute EtOH if the solvent of the chemical is different from EtOH.
- Store syringes in the Syringe Storage box in dry condition protected from dust.



Caution: When using a syringe with a chemical different from the one usually assigned to the syringe, repeated washing steps and an overnight exposure filling the syringe with absolute ethanol may be crucial to prevent contamination by carry-over.

2.6. Faulty syringes

- **Clogged syringes:** To solubilize the clog in the needle, flush the syringe with an appropriate solvent ([O2k-Titrations](#)) more than 10 times and/or leave the syringe (without the plunger) overnight in a cup filled with Milli-Q® water/ distilled H₂O. It may be helpful to fill it from the back with absolute EtOH or

H₂O and try to press the plunger gently down. A cleaning wire (included in package) can be used to dislodge any clogging material in the needle.

- **Plunger:** If the plunger seems to scratch during titration or black residues appear on the plunger, take it out and clean it with H₂O and Kimwipe.
- **Needle burrs and surface irregularities:** Eliminate rough edges with 3M™ Wetordry™ Paper Sheet P1000 by gentle rubbing.
- **Broken plungers:** Syringe plungers are interchangeable and replaceable for 10 µL – 100 µL volume syringes.
- **Defective plungers:** Place the syringe filled to the maximum volume mark on the rack. If the plunger slides down over time (1 h), the plunger is not tight and must be discharged.

2.7. Further information

- For quality control of your washing procedure, use a dye such as Trypan blue to evaluate the washing efficiency.
- Visit also: [Hamilton](#) and [Hamilton care and use guide](#) for further information.
- Use less plastic and more glassware.



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

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