

O2k-Protocols SOP: O2k-titration



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Updates: http://wiki.orooboros.at/index.php/MiPNet19.14_SOP_Hamilton_microsyringes

SOP for manual O2k-titrations with Hamilton microsyringes

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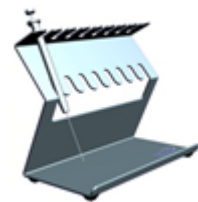
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1. General Information

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-FluoRespirometer 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
- 1 [Microsyringe\50 mm³ 51/0.15 mm](#): 50 mm^3
- 1 [Microsyringe\100 mm³ 51/0.41 mm](#): 100 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.

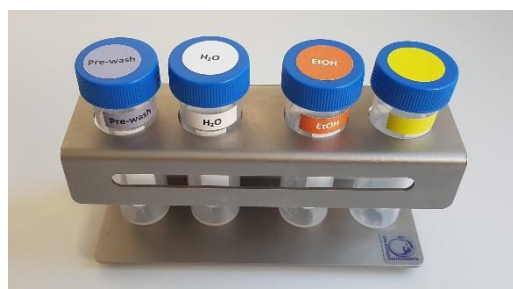
2. Working with microsyringes

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 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. 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 ₂ O	Distilled water	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. distilled water	Inhibitor syringes used for chemicals with H ₂ O 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 H₂O 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 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 and might introduce gas bubbles to the chamber upon titration.
- Fill the syringe at a steady rate, but not too quickly. If gas bubbles are forming, continued aspiration may help to eliminate 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 MgCl₂) 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 the 'Pre-wash' tube (H₂O) and clean it with a wipe. Rinse not only the needle itself but also the needle base to clean the barrel.
- Wash syringe three times in the tube filled with H₂O or pure EtOH (depends on solvent H₂O or EtOH). For inhibitor syringes (with solvent H₂O) you may consider using the additional tube (see above).

2.5. *Handling after experiments and storage*

- Exchange H₂O 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.
- Wash three times with absolute EtOH if the solvent of the chemical is different from EtOH.
- Store syringes in dry condition protected from dust.

Caution:



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



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 foreign 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 water and Kimwipe.
- **Needle burrs and surface irregularities:** eliminate rough edges with 3M™ Wetordry™ Paper Sheet P1000 by gentle rubbing.
- **Broken plungers:** syringes plungers are easily interchangeable and replaceable for 10 µL – 100 mL volume syringes.

2.7. 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.
- 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.

3. Acknowledgements



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