

# SOP for manual O2k-titrations with Hamilton microsyringes

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## 1. General information



Hamilton microsyringes with volumes of 10, 25, 50 or 100  $\mu\text{l}$  ( $\text{mm}^3$ ) are used for manual O2k-titrations in substrate-uncoupler-inhibitor titration (SUIT) protocols, which are a hallmark of High-Resolution FluoRespirometry (HRFR). 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-FluoRespirometer as a basic HRFR-Accessory, which consists of:

- 6 [Microsyringes\10 mm<sup>3</sup> 51/0.13 mm](#): 10  $\text{mm}^3$
- 6 [Microsyringes\25 mm<sup>3</sup> 51/0.15 mm](#): 25  $\text{mm}^3$
- 1 [Microsyringe\50 mm<sup>3</sup> 51/0.15 mm](#): 50  $\text{mm}^3$
- 1 [Microsyringe\100 mm<sup>3</sup> 51/0.41 mm](#): 100  $\text{mm}^3$
- 3 [Plunger\10 mm<sup>3</sup>](#): for 10  $\text{mm}^3$  syringe
- 1 [Syringe\500 mm<sup>3</sup> 51/0.41 mm](#): 500  $\text{mm}^3$

- 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](#).

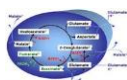
Hamilton microsyringes are specifically produced for O2k-titrations and can be ordered directly from Oroboros Instruments to assure exact fitting with the O2k-Stopper and O2k-Chamber dimensions.

## 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 <sub>2</sub> O	Distilled water	Syringes used for chemicals with H <sub>2</sub> 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 <sub>2</sub> 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 H<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>) 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<sub>2</sub>O) 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<sub>2</sub>O or pure EtOH (depends on solvent H<sub>2</sub>O or EtOH). For inhibitor syringes (with solvent H<sub>2</sub>O) you may consider the additional tube (see above).

## 2.5. Handling after experiments and storage


- Exchange H<sub>2</sub>O 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 thoroughly.
-  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 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 O<sub>2</sub>k 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<sub>2</sub>O 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.