



O2k-SOP

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SOP for manual titrations with Hamilton microsyringes

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1. General information

Hamilton microsyringes in different sizes and volumes are used for substrate-uncoupler-inhibitor titration (SUIT) protocols in high-resolution respirometry. The components of a Hamilton Microsyringe are: Needle, Termination, Volume markings, Barrel, Flange, Plunger.

A Hamilton syringe [titration set](#) is included in the purchase of an O2k-Core as a basic HRR-Accessory and consists of:

- 6 [Microsyringes\10 mm³ 51/0.13 mm](#): 10 mm³
- 6 [Microsyringes\25 mm³ 51/0.15 mm](#): 25 mm³
- 2 [Microsyringes\50 mm³ 51/0.15 mm](#): 50 mm³
- 3 [Plunger\10 mm³](#): for 10 mm³ syringe

Recommendation:

Hamilton microsyringes are especially produced for O2k titrations. In order to assure exact titration, please purchase them directly from OROBOROS INSTRUMENTS.

2. Working with microsyringes

2.1. Start

In order to avoid contamination, 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 and red for inhibitors.

Before every experiment, make sure to:

- Prepare the syringes according to [SUIT protocol](#)
- Prepare a rack with three 50 ml falcons for the washing procedure:
 - First rinse (distilled water)
 - Distilled water
 - Pure EtOH
- Prepare a beaker for waste
- Prepare a Kimwipe for wiping the needle

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.
- Wash three times with the solvent of the chemical if it is different from H₂O or EtOH.
- Between each washing, wipe the needle also with a Kimwipe.

2.3. Titration

- Hold the syringe on the top to prevent warming of the glass.
- Fill the syringe above desired mark in order to have a little bit of excess.
- Fill the syringe only shortly prior to titration to avoid warming of the chemicals.
- Take care not to produce bubbles when filling the syringe – this causes inaccuracies in measurement.
- Fill the syringe not too fast but steady. If bubbles are forming, a continued pumping helps to avoid this.
- Before titrating the chemical into the chamber: carefully press the plunger and check if a small drop appears on top of the needle. Wipe drop before titration.
- Be **swift** when titrating into the chamber, especially when titrating EtOH due to its volatile nature.

Caution: take care to specifically rinse the syringe used for ADP (with MgCl₂) and fatty acids IMMEDIATELY after use (on the surface, but more importantly inside three times with distilled water) as otherwise a precipitate may form and cause blocking of the needle.

2.4. Washing after titration

- After titration discard residual chemical into the waste beaker.

- Plunge syringe in 'first rinse' falcon (H₂O) and wipe it with a small kimwipe. Take care to rinse not only the needle itself but also the needle base to clean the barrel.
- Wash syringe three times in falcon filled with distilled water or pure EtOH (depends on whether reagents were dissolved in distilled water or EtOH).

2.5. Handling after experiment and storage

- Exchange H₂O and EtOH in the falcon tubes for final washing.
- Take care of the washing order: first substrate-, then uncoupler- and finally inhibitor syringes.
- Wash five times with the last used solvent, take care again to also rinse the needle base, rinse three times with EtOH 100%.
- Store syringes in dry condition protected from dust.

Caution:

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

- Speed in pushing the plunger is crucial for an accurate washing of the syringes. 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. pdf](#) 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 O2k and the polarographic oxygen sensor when introduced into the glass chamber.
- **Plunger:** If the plunger seems to scratch during titration 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 is sometimes also helpful to fill it from the back with pure EtOH or H₂O 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!

