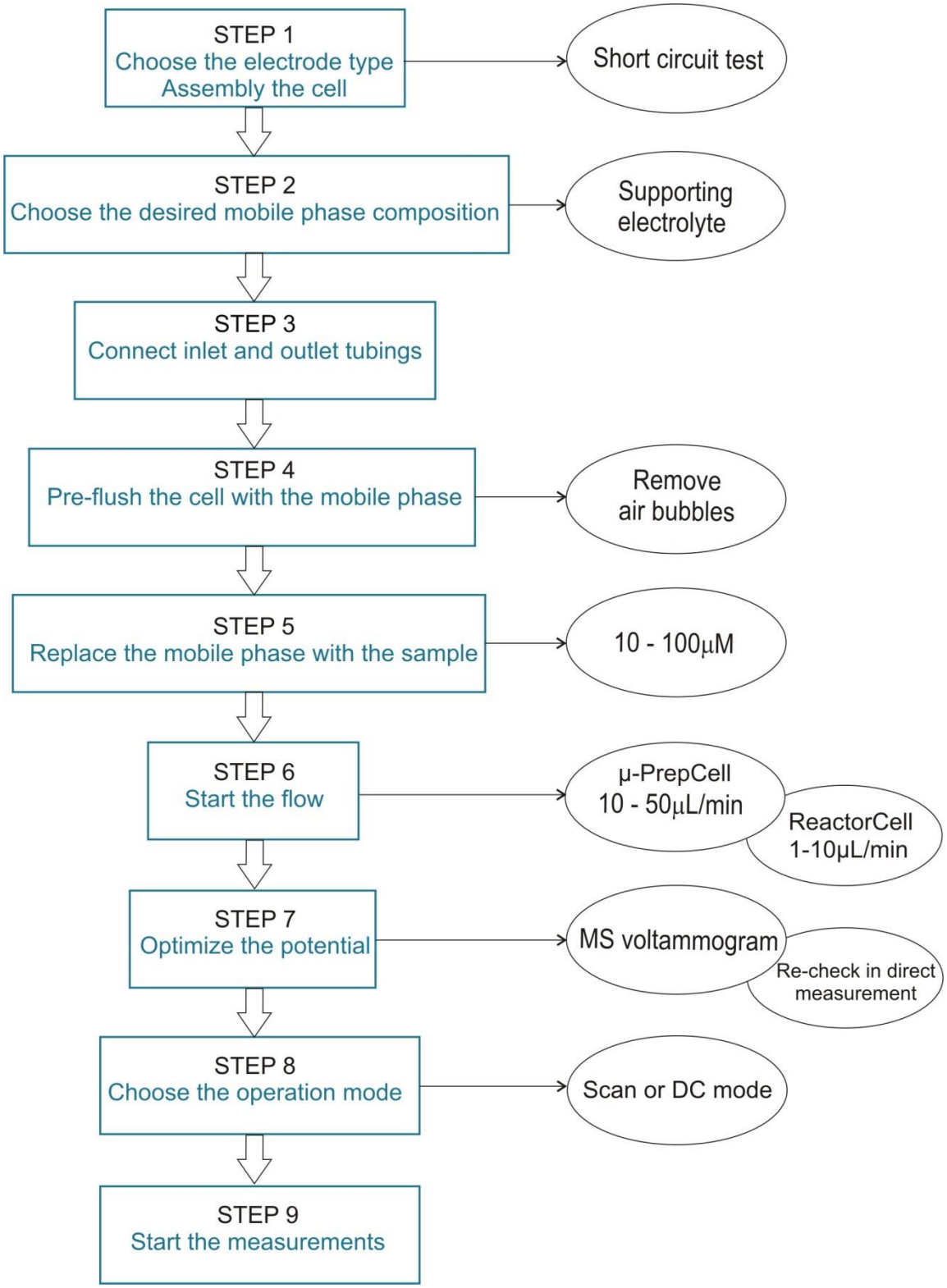
T +31 71 5813333 | F +31 71 5813334 | info@myAntec.com | www.myAntec.com

Antec
Industrieweg 12
2382 NV Zoeterwoude
The Netherlands

ROXY™ EC system Quick Start Up



ROXY™ EC system Quick Start Up

This Quick Start Up card covers the most important aspects about the use of the ROXY EC system and assure optimal performance for your experiments. It is recommended to read the µ-PrepCell or ReactorCell user manual before performing the experiments. Note: this guide does not replace the µ-PrepCell user manual (p/n 204.0010) or ReactorCell user manual (p/n 210.7014).

Step 1 – Choice of electrode & assembling the cell: Samples can show electrode-dependent oxidation patterns and it is recommended to test samples with both the MD and GC electrode. The MD electrode is more inert (less absorption) and has a wider working potential range for electrochemical oxidation in aqueous solution. Select the electrode and mount the cell as described in the corresponding manuals. µ-PrepCell: Do not forget to insert the metal spacers (at least thickness of 150 µm) and O-ring. The WE electrode contact pin should be screwed in completely (be aware of locking mechanism) to assure proper contact. It is recommended to check if none of the electrodes are short-circuited using a voltmeter.

Step 2 – Mobile phase considerations: The µ-PrepCell requires the use of an electrolyte at concentrations of 10 mM or higher (e.g. ammonium formate, ammonium acetate) in the mobile phase. The supporting electrolyte will assure the stable working conditions and conversion. Alternatively, formic acid at concentration 0.1 – 1 % can be used as supporting electrolyte.

- *pH of the mobile phase*: In many cases pH of 7.4 can be used, but optimal value for synthesis of required metabolite needs to be experimentally confirmed. Change pH to lower value, especially when MD electrode is used could increase the yield in metabolite formation.

- *Organic solvent content:* It is recommended to add organic solvent (e.g., acetonitrile) to minimize the adsorption of the compounds on the electrode surface. Up to 80% of organic can be used, but optimal content should be evaluated in practice.

- *Non-aqueous:* For organic compounds that are insoluble in aqueous mobile phases, non-aqueous mobile phase can be used, e.g., 0.1 M TBAP (tetrabutylammonium perchlorate) dissolved in ACN or ACN/H_2O 99/1 (v/v) (Note that the samples containing TBAP require a dilution prior MS analysis).

Step 3 & 4 – Connecting the cell in the system: To assure optimal operation the Cell should be installed and properly primed (air bubble-free) with degassed mobile phase. (1) Install the inlet and outlet tubing on the two ports of the cell. (2) Remove the reference electrode, and (3) switch on the flow. Assure that there is no air bubbles trapped in the REF opening. Fix the reference electrode and remove excess of mobile phase with a tissue (wear protective gloves!).

Step 5 – Optimal sample concentration: It is recommended to use a sample concentration between 10 – 100µM in mobile phase, however higher concentration can also be used but stronger adsorption can occur and loss in performance observed. In case of high sample concentration, more frequent cleaning of the electrode may be required (See the cleaning procedures in User manual) to recover full performance of the cell.

Step 6 – Optimal flow rate: A flow rate of 10 – 100 µL/min can be used for metabolite synthesis with the µ-PrepCell. The recommended flow rate is between 20 – 50 µL/min. For ReactorCell the recommended flow rate is between 1-10 µL/min.

Step 7 – Optimization of the potential: The cell potential is the driving force of an electrochemical reaction! Always record a MS Voltammogram! A MS Voltammogram will give you a good indication of the optimal potential required for the formation of specific metabolites. Verify that the potential determined with a MS Voltammogram is indeed the optimal potential by executing a direct measurement at that specific potential before starting your metabolite synthesis.

Step 8 – Selecting the operation mode: In the ROXY potentiostat™ the DC and Scan mode are available for efficient metabolite synthesis. The DC mode is based on applying a static (single) potential during the whole conversion process. Note that the synthesis of different metabolites of one compound may require operation at different potential settings. In the Scan mode stabile oxidation conditions are obtained by continuous scanning between two preset potentials values (E1 and E2) with a certain scan rate (unit: mV/s). More details about using the DC and Scan mode can be found in the User manual.

Step 9 – Start the measurements: Apply the optimized potential using the DC mode or range of potentials in the Scan mode. You can use the event table to generate user defined programs.