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µ-PrepCell 2.0™

User Manual

204.0010A, Edition 5 2022

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Warning Symbols

The following symbols are used in this guide:

This sign warns about the risk of electric shock. It calls attention to a procedure or practice which, if not adhered to, could result personal injury or even loss of life by electrocution. Do not proceed beyond a danger sign until the indicated conditions are fully understood and met.

The warning sign denotes a hazard. It calls attention to a procedure or practice which, if not adhered to, could result in severe injury, loss of life or damage or destruction of parts or all of the equipment. Do not proceed beyond a warning sign until the indicated conditions are fully understood and met.

The caution sign denotes a hazard. It calls attention to a procedure or practice which, if not adhered to, could result in damage or destruction of parts or all of the equipment and/or erratic results. Do not proceed beyond a cautions sign until the indicated conditions are fully understood and met.

The biohazard sign draws attention to the fact that use of biological materials, viral samples may carry a significant health risk.

The toxic hazard sign draws attention to the fact that use of toxic solvents or samples may carry a significant health risk.

The attention sign signals relevant information. Read this information.

The note sign signals additional information. It provides advice or a suggestion that may support you in using the equipment.

Intended use

The µ-PrepCell 2.0 flow cell is used in combination with the ROXY EC(/LC) system or ROXY potentiostat for controlled REDOX reactions up-front Mass Spectrometric detection. It can be used in a wide range of application, for example:

- Fast synthesis of metabolites (µ-preparative)
- Rapid risk assessments of drug-protein binding
- Signal enhancement in MS
- Electrochemical cleavage of proteins/peptides
- Reduction of disulfide bonds in proteins/peptides
- Oxidative stress/damage of proteins, DNA, lipids, etc.

For research purposes only. **While clinical applications may be shown, this instrument is not tested by the manufacturer to comply with the In Vitro Diagnostics Directive.**

Operation of the µ-PrepCell 2.0 in combination with the ROXY EC(/LC) system or ROXY potentiostat can involve the use of hazardous materials including corrosive fluids and flammable liquids. The flow cell should only be operated by users with the following expertise:

- Completed degree as chemical laboratory technician or comparable vocational training.
- Fundamental knowledge of liquid chromatography & mass spectrometry
- Participation in an installation of the system performed by the manufacturer or a company authorized by the manufacturer and suitable training on the system, flow cell and control software.
- Knowledge and experience in the safe handling of toxic and corrosive chemicals and knowledge of the application of fire prevention measures prescribed for laboratories.

Information on safety practices is provided with your equipment operation manuals. Before using your equipment or accessories, you must thoroughly read these safety practices. This manual is written for laboratory technicians skilled in the art.

Unskilled, improper, or careless use of this equipment can create fire hazards, or other hazards which can cause death, serious injury to personnel, or severe damage to equipment and property. Observe all relevant safety practices at all times. Only use the device for applications that fall within the scope of the specified intended use. Else the protective and safety equipment of the device could fail

The µ-PrepCell 2.0 is ROHS compliant and in conformity with Directive 2011/65/EU Restricted use of Hazardous Substances in electrical and electronic Equipment (ROHS).

Antec Scientific is an ISO 9001 certified company.

Warranty, spare parts and service

The warranty period of this flow cell is 1 year on workmanship, wear and tear parts are excluded. Manufacturer provides operational spare parts of the instrument and current accessories for a period of five years after shipment of the final production run of the flow cell. Spare parts will be available after this five years period on an 'as available' basis.

Manufacturer provides a variety of services to support her customers after warranty expiration. Repair service can be provided on a time and material basis. Contact your local supplier for servicing. Technical support and training can be provided by qualified chemists on both contractual and as-needed basis.

Safety Instructions

Adhere to the following guidelines when using The µ-PrepCell2.0. The safety practices are intended to ensure safe operation of the flow cell.

Working environment & safety

The intended use of the µ-PrepCell 2.0 flow cell is to perform controlled REDOX reactions of target compounds (in a suitable liquid electrolyte medium) up-front

Mass Spectrometric detection. Operators using the system should have the appropriate education an extensive understanding of GLP rules and be skilled in the art. Use this system ONLY for the intended use. Use of the system for any other purpose might cause unsafe situations.

Operation

To assure optimal performance keep of the flow cell we recommend that the flow cell is checked regularly and maintenance procedures are carried out. Preventive maintenance contracts are available for that Purpose. Please contact your local dealer or the nearest sales office for more information.

Solvents

The solvents used may be flammable, toxic or corrosive. The room in which the system is installed should be well ventilated to prevent that solvent vapors cause poisoning or ignite and cause a fire. Use of open fire in the vicinity of this system must be strictly prohibited. Do not install the system in the same room with any other equipment that emits or could potentially emit sparks. Provide protective equipment near the instrument, when solvent gets into the eyes or on the skin, it must be flushed away immediately. Provide equipment, such eye wash stations and safety showers, as close to system as possible. Use proper eye and skin protection when working with solvents. Additional safety requirements or protection may be necessary depending on the chemicals used in combination with this equipment. Make sure that you understand the hazards associated with the chemicals used and take appropriate measures with regards to safety and protection. Sample containers (vials) should be sealed to minimize any risks related to solvent vapor.

Biological Hazard

When you analyze biological fluids you need possible precautions and treat all specimens as potentially infectious. Always wear protective and gloves when handling toxic or biologically infectious samples to prevent bio hazards or hazards while working with the flow cell. If necessary the flow cell must be decontaminated before decommissioning or shipment of the flow cell for repair to Antec or its representatives. When shipped to Antec every flow cell has to be accompanied with a decontamination form which should be completely filled in and signed by the customer. Without this decontamination form the flow cell will not be processed by Antec (either repaired or disposed).

Waste disposal

Perform periodic leak checks on LC tubing and connections. Do not close or block the drain in the oven compartment. Do not allow flammable and/or toxic solvents to accumulate. Follow a regulated, approved waste disposal program. Never dispose of flammable and/ toxic solvents through the municipal sewage system.

Using the flow cell in other ways than indicated in the manual might result in erratic or unsafe operation.

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C H A P T E R 1

Introduction

Congratulations on your purchase of the new µ-PrepCell 2.0™. With more than 20 years of experience in Electrochemistry (EC), Antec introduces a new successor of the well-established µ-PrepCell to perform controlled REDOX reactions upfront MS. The µ-PrepCell 2.0™ in combination with the ROXY™ Potentiostat generates metabolites of drugs or xenobiotics, similar to those generated during in vivo metabolic processes, in a significantly shorter time span (seconds vs. days or weeks) without any interfering components (no isolation steps required). Examples of other possible applications are:

- Fast synthesis of metabolites (µ-preparative)
- Rapid risk assessments of drug-protein binding
- Signal enhancement in MS
- Electrochemical cleavage of proteins/peptides
- Reduction of disulfide bonds in proteins/peptides
- Oxidative stress/damage of proteins, DNA, lipids, etc.
- Pharmaceutical Stability Testing and Degradant Synthesis

Figure 1. Front view of µ-PrepCell 2.0™

The µ-PrepCell 2.0 is a thin-layer electrochemical cell designed for the synthesis of metabolites high yield.

The µ-PrepCell 2.0 has an auxiliary electrode made from maintenance-free conductive PEEK, with a reference electrode positioned in a separate REF chamber.

The active surface of the working electrode is about 10 times the size of a standard ReactorCell™, and guarantees a significantly higher efficiency in formation of reactive metabolites. The cell can be used in the ROXY EC system for off-line collection of metabolites. Flow rate and working potential can be optimized using the Dialogue Elite software.

The µ-PrepCell 2.0™ is available in two dedicated kit specifically targeted for oxidative and reductive applications, respectively:

P/n 204.4310 µ-PrepCell 2.0 GC/MD
P/n 204.4312 µ-PrepCell 2.0 TiBlue

The flow cells include an accessory kit (p/n 204.0200) with parts for the installation and maintenance of the cell. The µ-PrepCell 2.0™ has exchangeable working electrode, which offers maximum flexibility for multiple applications requiring different working electrode materials. There are several working electrode types available for specific applications which can be purchased separately: TiBlue, Glassy carbon, Magic Diamond, Gold, Platinum and Titanium. A detailed description is given further on in this manual.

As a reference electrode the HyREF™ Pd/H_2 electrode is supplied. This reference electrode is maintenance free and can be used under harsh conditions. The µ-PrepCell is operating in a three-electrode configuration (more details about three-electrode configuration can be found in the next paragraph).

Flow cell description

An exploded view of the µ-PrepCell 2.0 is shown in Figure *2* to identify all parts of the cell.

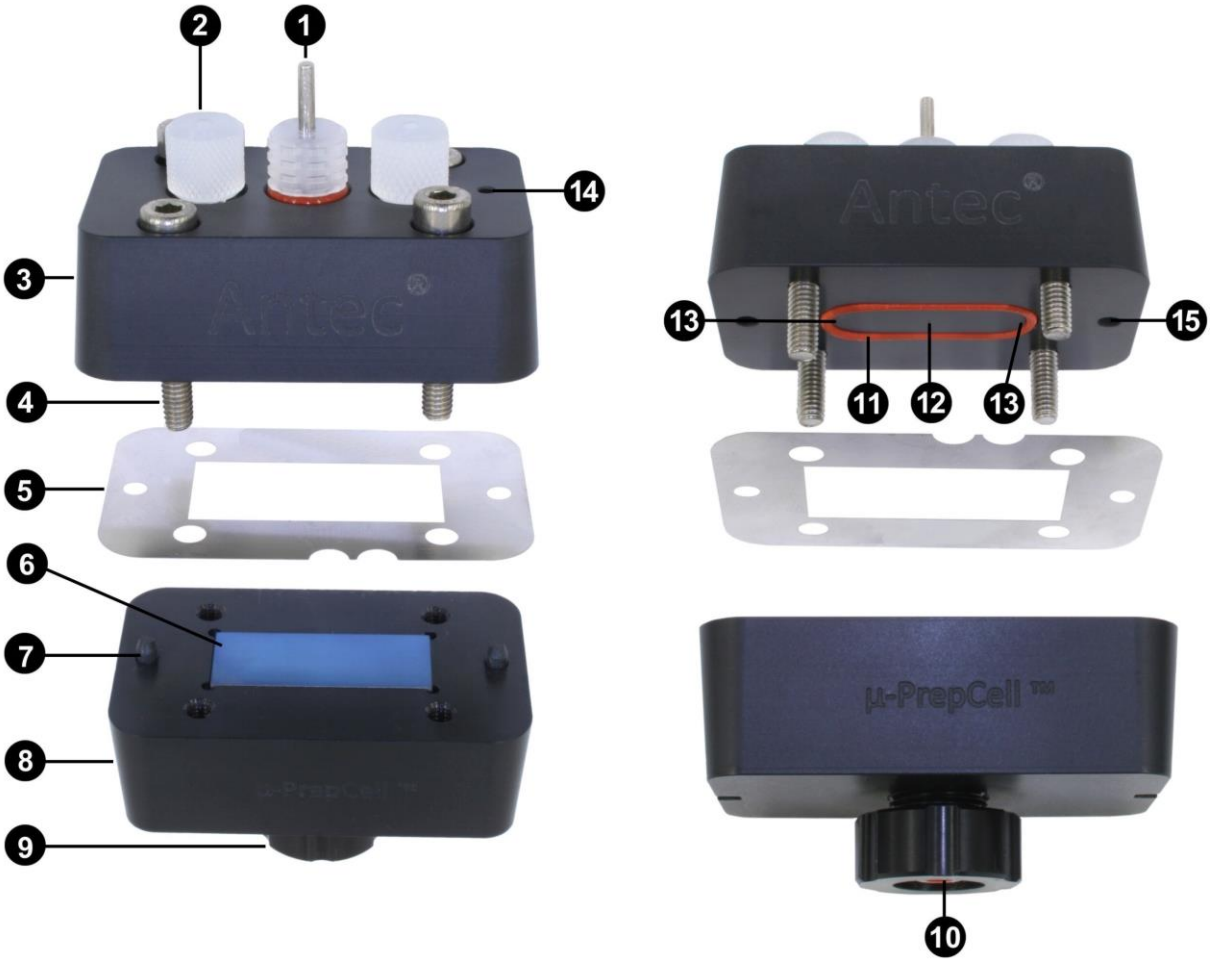


Figure 2. Exploded view of the µ-PrepCell 2.0™

1	HyREF™ Pd/H_2 electrode	9	Nut (POM)
2	Inlet/outlet fingertight (PTCFE)	10	Working electrode contact
3	Inlet block (conductive PEEK)	11	O-ring (Silicone)
4	Mounting screws (stainless steel)	12	Reference electrode port
5	Spacer 50 or 100 µm (stainless steel)	13	Inlet/out port
6	Working electrode (TiBlue)	14	Auxiliary electrode contact
7	Positioning pins for spacer (PEEK)	15	Positioning pin hole
8	Working electrode block (PEEK)		

In the next paragraph and chapters a more detailed description will be given of all parts and there function in the flow cell.

Warning: the spacers can be considered as SHARP METAL OBJECT. Take care handling this part during assembly of the cell, avoid contact with the sharp metal edges of the spacer.

The construction of the µ-PrepCell is symmetrically so the fluid connections can be used as either inlet or outlet.

Three-electrode configuration

In the µ-PrepCell 2.0 a three-electrode configuration is used (Figure 3). The working potential is set between the working electrode and the auxiliary electrode (AUX). The auxiliary electrode is kept at a precisely defined reference electrode potential by means of the so-called voltage clamp. This is an electronic feedback circuit that compensates for polarisation effects at the electrodes. At the working electrode, which is kept at virtual ground, the electrochemical reaction takes place, i.e. electrons are transferred at the working electrode. This results in an electrical current to the I/E converter, which is a special type of operational amplifier. The output voltage can be measured by an integrator or recorder.

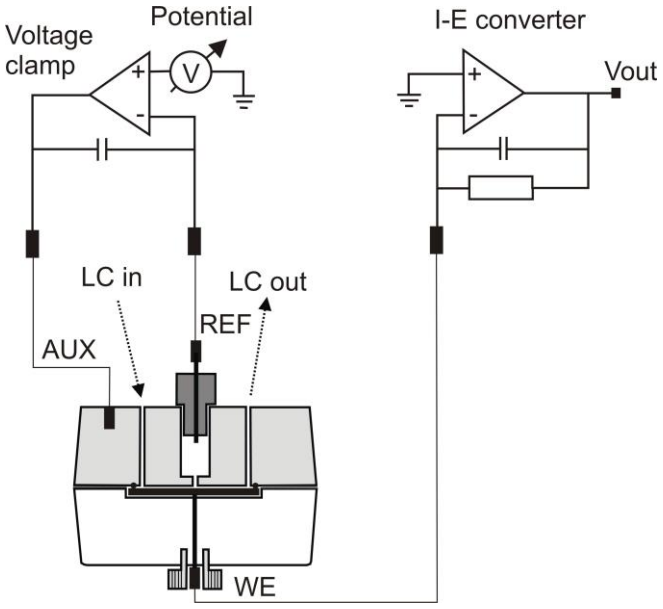


Figure 3. Schematic representation of the µ-PrepCell and electronics in a three-electrode configuration.

Essentially, for the oxidation or reduction reaction it would be sufficient to use only two electrodes. However, the three-electrode configuration has several advantages over a two-electrode configuration.

- If the working potential would be applied only over an auxiliary electrode versus the working electrode (without reference electrode), the working potential would continuously change due to polarisation effects at the electrodes, resulting in highly unstable working conditions.

- If the working potential would be applied only over the reference electrode versus the working electrode (without auxiliary electrode), the working potential would be very well defined. However, the potential of a reference electrode is only well defined if the current drawn is extremely low (pico-amperes) resulting in a very limited dynamic range.

A three-electrode configuration combines the best of both configurations. The reference electrode stabilizes the working potential and the auxiliary electrode can supply high currents. This results in the tremendous dynamic range of a three-electrode system.

HyREF™ reference electrode

The µ-PrepCell™ is equipped with a HyREF (Pd/H_2) reference electrode. The HyREF can be used under harsh conditions and is free of maintenance under normal use. An important characteristic of the HyREF is the pH dependence of the reference potential.

It is important to realise that if the pH of the mobile phase is changed, also the optimum conversion potential changes. In such case it is advisable to re-determine the optimum conversion potential by constructing a voltammogram.

HyREF versus Ag/AgCl Reference electrode

The reference potential of an Ag/AgCl and HyREF are different.

pH	E (V)
3.3	232
6.2	130
7.5	90
11.8	0

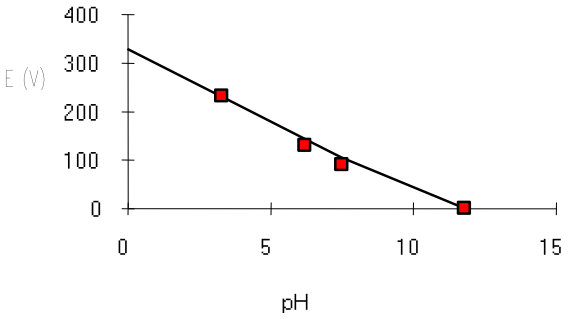


Figure 4. Potential difference between HyREF and Ag/AgCl REF versus pH.

So, if an Ag/AgCl REF is compared to a HyREF, the pH effect on HyREF, compared to Ag/AgCl must be taken into account. The pH-voltage relation is described by:

$E_{HyREF} = E_{Ag/AgCl} - 328 + 29.9\ pH$ (1)

Example

If an Ag/AgCl REF is exchanged for a HyREF a working potential of 800 mV (*vs*. Ag/AgCl) at pH 3, has to be changed to:

$E_{HyREF} = 800 - 328 + 29.9*3 = 560$ mV (*vs*. HyREF)

As a rule of thumb, the working potential should be chosen such that a similar background signal (I-cell) is measured with the HyREF as in using the Ag/AgCl REF.

Working electrodes

There is a wide range of working electrode materials available for the µ-PrepCell 2.0 for various fields of application. In Table 1 the available WE's are listed and in Figure 5 a photo of the electrodes is shown for reference. The structure of the electrode is dependent on the type of active material. 'Bulk' means an homogeneous electrode consisting completely out of the active material.' Thin layer' means an electrode consisting of thin layer (in the µm range) of the active material deposited on top of a conductive substrate. 'Laminated layer' means an electrode with a layer (typically 200 – 300 µm thick) of the active material laminated on top of a conductive substrate.

Table 1 Working electrodes available for the µ-PrepCell.

Part	Description	Active material	Structure of electrode
204.5007	WE GC for µ-PC	Glassy Carbon	Bulk
204.5010	WE Ti for µ-PC	Titanium*	Bulk
204.5010A	WE TiBlue for µ-PC2.0	Titanium oxide*	Thin layer
204.5022	WE Pt for µ-PC	Platinum	Laminated layer
204.5027	WE Au for µ-PC	Gold	Laminated layer
204.5050	WE MD for µ-PrepCell	Boron-doped Diamond	Thin layer

*) WE's which can be used on both sides (top and bottom), the other WE's can be used one side only.

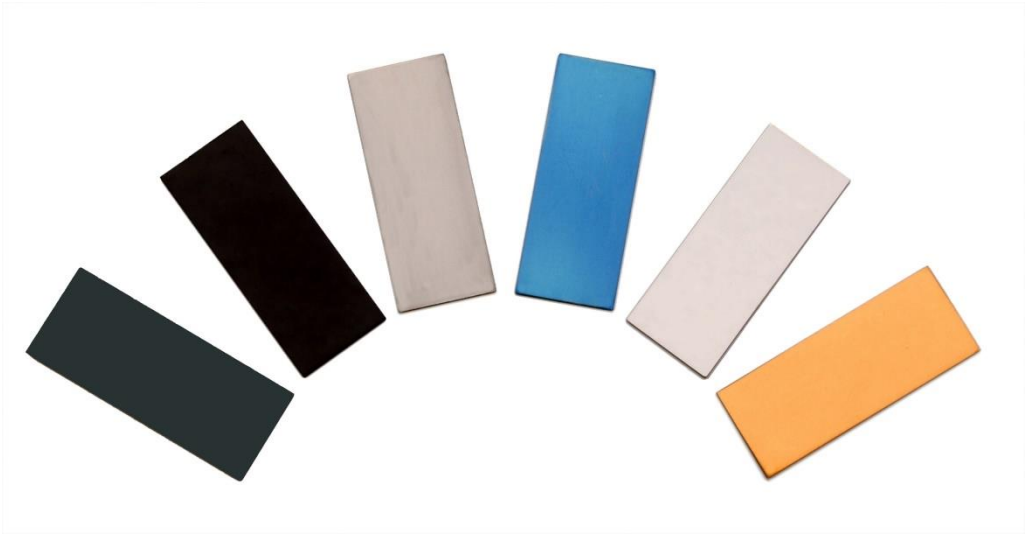


Figure 5. Working electrode materials available for the µ-PrepCell 2.0. From left to right: Boron-doped Diamond (MD), Glassy Carbon (GC), Titanium (Ti), TiBlue, Platinum (Pt) and Gold (Au).

Oxidative mode

Glassy Carbon (GC) and Boron-Doped Diamond (BDD, trade name Magic Diamond™) are the recommended electrodes for use in electrochemical reactions in oxidative Mode. In Table 2 the approximate working potential limits are listed for GC and Boron-Doped Diamond in aqueous mobile phases for reference. The values presented in the table are only an estimation and will depend on mobile phase composition (pH, supporting electrolyte, modifier) and the analyte itself.

At high positive working potentials the water in the mobile phase electrolyses and results in decrease in metabolites formation. In case of electrolysis of the water/mobile phase the cell current (Icell) readout will display the message "overload" and auxiliary potential (Eaux) will have the extreme value (-9.9V) (With the default range value of 20 mA of the ROXY potentiostat). Furthermore, the electrospray signal will become very unstable because of gas formation in the cell and loss of the signal can be observed. When such a phenomenon is observed it is recommended to adjust the potential to a lower value.

Table 2 Working potential limits for GC and Boron-doped diamond WE.

WE material	potential limits *vs*. HyREF (V)	
	Oxidation	reduction
Glassy carbon	+2.5V	-1.5V
Magic diamond	+3.5V	-2.5V

A too high potential with aqueous mobile phases may lead to unstable operation of the µ-PrepCell due to gas formation (hydrolysis). It is important to recognize such situation and adjust the oxidation potential of the potentiostat to a lower value.

A Magic Diamond™ (MD) electrode consisting of an ultra-thin film of boron-doped diamond material deposited on a Si wafer. The special properties of doped diamond electrodes, such as a wide potential window, their inertness and excellent response stability, makes them well suited for electrochemical conversion of a wide variety electro-analytical application. The MD electrode is perfect choice if the oxidation of the analyte requires high potential in aqueous electrolytes.

Reductive mode

For cleavage of disulfide bonds in proteins/peptides electrochemical reactions in reductive mode the new TiBlue electrodes are available. With the µ-PrepCell 2.0 in combination with the new TiBlue™ electrode stable and reproducible reduction can be achieved in DC mode. The TiBlue electrodes are made by a proprietary

surface treatment of a Titanium alloy, resulting in a blue-coloured crystalline TiO_2 layer. The electrodes can be used both sided and are disposable.

Identification of WE materials

Working electrodes can be identified by their part number, see Table 1. The part number can be found on the packaging bag of the electrode. The different type of WE's can also be identified quite easily by their appearance. See Figure 6.

The surface of the Titanium electrode has a grey matt appearance, which is identical on both sides. The Titanium electrode can therefore in principle be used on both sides. The TiBlue electrode has a clear blue colour, this blue titanium oxide layer is applied on both sides and can be used double sided.

The Magic Diamond electrode (Boron-doped diamond) has a dark blue/grey metallic appearance on the top side (boron-doped diamond). The back side of the electrode is a film of gold (backside Au- metallized) to assure good electrical contact with the substrate. This electrode can therefore only be used on one side (top).

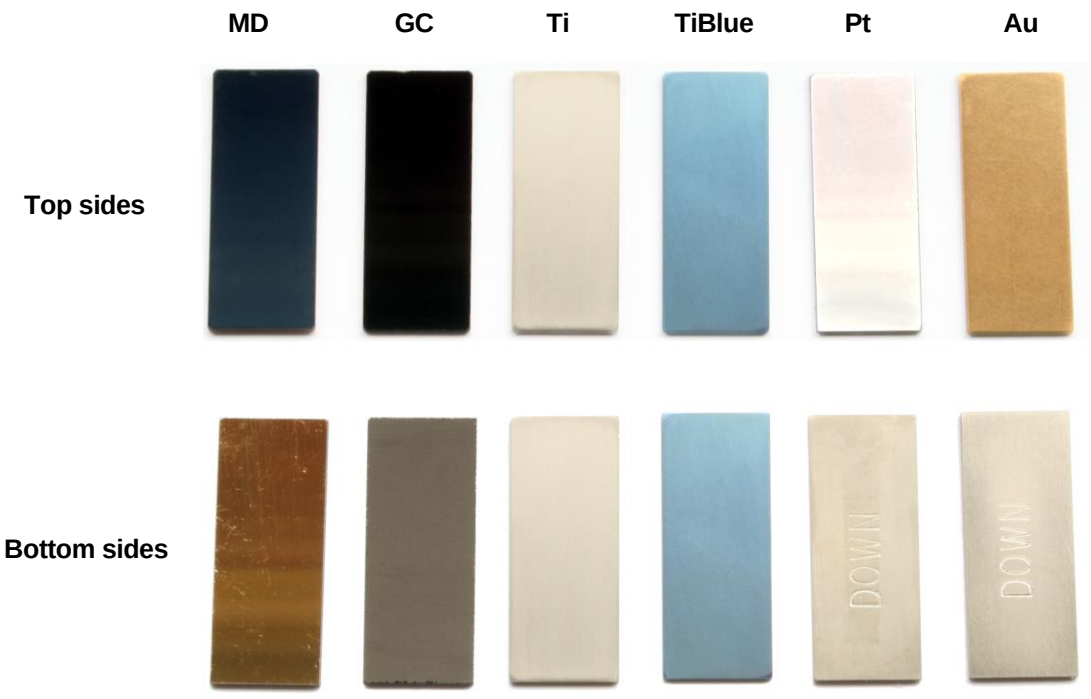


Figure 6. µ-PrepCell working electrode identification. Photo of top and bottom side for all available electrodes.

The Glassy Carbon electrode has a black color, the polished top side has a shiny mirror like appearance, the unpolished back-side has a matt black color. This electrode should only be used on the polished top side.

Make sure that the working electrode is properly positioned in the PrepCell with the correct active surface facing upwards in the working electrode block. If not it may lead to irreproducible and erratic measurements.

The Au and Pt electrodes with their characteristic yellow and grey metallic appearance are laminated electrodes. The back layer of these laminated electrodes is Titanium. The Ti bottom side is clearly marked with the text 'down'.

C H A P T E R 2

Installation

Unpacking

Inspect the transport box for possible damage as it arrives. Immediately inform the transport company in case of damage, otherwise she may not accept any responsibility. Keep the transport box as it is designed for optimum protection during transport and it may be needed again. Carefully unpack the system and inspect it for completeness and for possible damage. Contact your supplier in case of damage or if not all marked items on the checklist are included. Prior to shipment, your detector has been thoroughly inspected and tested to meet the highest possible demands. The results of all tests are included.

See check list below for reference:

(1)	Delivery is in accordance with order	O
(2)	Delivery is undamaged	O
(3)	All items on checklist(s) are included	O
(4)	Certificate of performance is included	O
(5)	User manual is included on USB stick	O

Environmental

Your flow cell is intended for indoor use only in an industrial or commercial environment. The flow cell can be controlled and operated with the ROXY potentiostat or ROXY EC system (not included with the Flow cell, has to be ordered separately). For the installation instructions of the ROXY potentiostat, see document 210.7010 ROXY potentiostat user manual. The environmental conditions for use of the ROXY potentiostat are:

Table III. Environmental specifications

Parameter	Requirement
Operating temperature	10 – 35 ºC (50 – 95 °F)
Maximum Altitude	2000 meter (7500 ft)
Operating humidity	20 – 80%, non-condensing

The flow cell itself can be used in the temperature range between 10 – 50 ºC

Chemicals

Mobile phase and flush/storage solutions must be of sufficient purity as it is in direct contact with the working electrode in EC reactions and might introduce interferences in MS detection. High purity chemicals including water is a prerequisite. So all chemicals should be electrochemically clean, HPLC/MS grade or better. For water used for the preparation of mobile phases a water purification apparatus is advised which is able to supply high purity deionized water with resistivity of >18 MOhm.cm and low TOC level (<10 ppb).

Installation of the µ-PrepCell holder

The µ-PrepCell is delivered with a special holder to mount it inside the oven compartment of the ROXY potentiostat. The µ-PrepCell holder is fixed to the back-panel of the oven compartment using the M4 screw (red arrow) supplied with part 204.0102 the µ-PrepCell holder kit.

Figure 7. µ-PrepCell holder mounted on the centre position in the ROXY potentiostat oven compartment.

The µ-PrepCell holder has a protrusion/notch on both sides (blue-dotted circle) which clamp into the grooves on the sides of the µ-PrepCell. Insert the cell from the top into the clamp as indicated by the arrow.

Figure 8. Inserting the µ-PrepCell 2.0 into the cell holder. The groove in the sides of the cell should slide through the plane with the notches. The cell will snap into place (locked by the notches).

The cell should be firmly pushed inside the clamp to snap/click into place (notches of the clamp will lock the cell).

General precautions

Before starting to use the µ-PrepCell please take into account the following precautions when handling the cell.

1. Always make sure that the surfaces of the spacer, working electrode, inlet block and WE block are dry and free from particulate matter before assembling the cell.
2. Check the inlet block O-ring for any damage/wear and replace by new O-ring if needed to assure leak tightness.
3. Assure that the spring-loaded WE electrode contact holder (Figure 16) is never exposed to (corrosive) liquids because it may lead to corrosion of the contact and malfunction of the cell.

4. Make sure that the working electrode surface is uniform, clean and undamaged. Check it before every installation/new experiment. Follow the maintenance procedure for the specific working electrode as specified in chapter 3 Maintenance.

Bulk electrodes and laminated electrodes like Glassy Carbon, Ti , Au and Pt may be polished mechanically. Thin layer electrodes like Magic Diamond (Boron-Doped Diamond) and TiBlue cannot be polished. Polishing the MD and TiBlue electrode surface will damage the electrode and lead to loss of performance. Follow the specific maintenance instructions in chapter 3.

Make sure that the working electrode is properly positioned in the PrepCell with the correct active surface facing upwards in the working electrode block. If not it may lead to irreproducible and erratic measurements.

5. If the cell is not in use we recommend to switch of the cell and removed it out out of the EC system. Disassemble the cell and clean all surfaces) and store them dry in a dust-free environment (for example the plastic Raaco box in which the cell and its spares was delivered).

Assembling the µ-PrepCell

See also Figure 2 (exploded view of the µ-PrepCell2.0) for reference how to mount the different parts of the flow cell. Take the following precautions before assembling the cell.

Never switch on the flow cell if:

- **the (black, red and blue) cell cable is not correctly connected,**
- **the cell is only partly (or not at all) filled with mobile phase containing the supporting electrolyte (e.g., ammonium formate, ammonium acetate, formic acid), because damage to the working electrode or the electronics may occur.**

Use proper eye and skin protection when working with solvents.

<u>Warning:</u> the spacers can be considered as <u>SHARP METAL OBJECT</u>. Take care handling this part during assembly of the cell, avoid contact with the sharp metal edges of the spacer.

Execute the following steps to assemble the µ-PrepCell:

1. Check if all parts of the µ-PrepCell are clean and dry.

2. Install a silicone O-ring into the O-ring groove of the conductive PEEK inlet block as shown in the photo below.

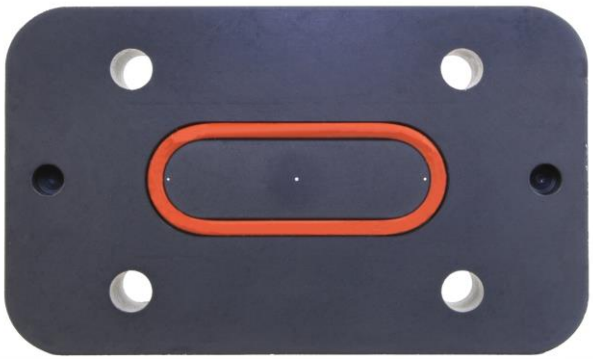


Figure 9. Bottom side of inlet block with Silicone O-ring installed.

3. Place the working electrode in the WE recess in the PEEK WE block.

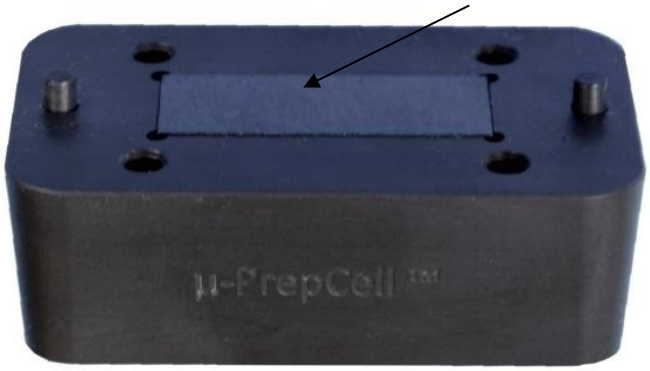


Figure 10. The PEEK WE block with MD working electrode inserted (arrow).

The WE contact (see Figure 16) should not be installed in the WE block yet. Make sure that the active side of the WE electrode is facing upwards, see previous chapter for reference. For example the MD electrode in *Figure 10* is positioned with the blue/grey crystalline diamond side upwards (and the gold back layer downwards). Furthermore, make sure that the electrode is laying levelled and centered in the recess and that it is free of any (hard) particulate matter below and on top of the electrode. It may lead to damage to the electrode and cell blocks when tightening the cell.

4. Place a stack of (minimum) 2x 100 µm stainless steel spacers for BBD, GC and laminated WE electrodes (Au, Pt). For TiBlue WE electrodes place a stack of 1x 50 µm and 1x 100 µm stainless steel spacers on top of the WE block, as shown in the *Figure 11*.

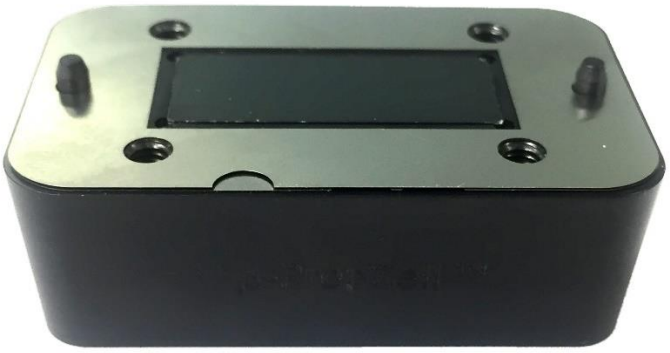


Figure 11. PEEK WE block with MD working electrode and stainless steel spacers.

The µ-PrepCell is standard assembled at the factory with 2x 100 µm (MD, WE WE) or 1x 100µm + 1x 50µm spacers (TiBlue WE), this corresponds to a total thickness of 200 or 150µm, respectively. More spacers can be added to adjust the working volume of the cell, but spacer thickness larger than 300µm might result in leakage.

The laminated Au and Pt electrodes should be used with a minimum spacer thickness of 200 µm in the µ-PrepCell. This thickness can be created by installation of the following stack of spacers: 2x pn 204.2218 spacer 100 µm

Using a smaller spacer thickness might lead to blockage of the cell, subsequent pressure build-up and may lead to damage.

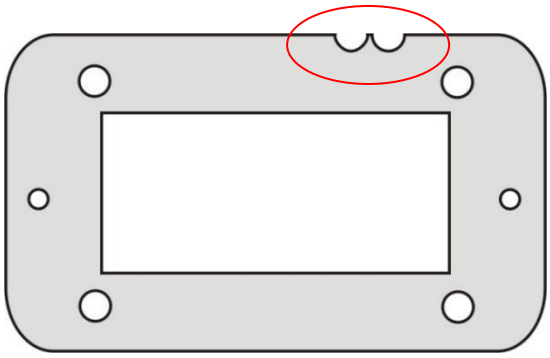


Figure 12. Schematic drawing of 100 µm µ-PrepCell spacer. Red circle: marking of the spacer thickness. One notch: 50 µm, two notches: 100 µm.

Effective spacer thickness

The effective spacer thickness and thus the volume of the cell is determined by the total thickness of the metal spacers minus the height which the WE electrode is protruding above the top plane of the PEEK WE block.

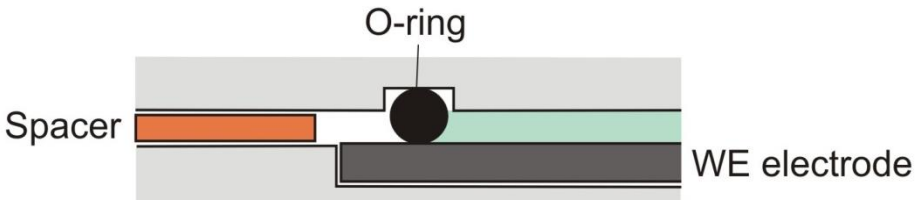


Figure 13. Schematic drawing of the sealing construction in an µ-PrepCell.

The WE electrode is protruding approximately100 µm above the WE block resulting in an *effective spacer thickness of 50 µm* in the case 150 µm of metal spacers are used.

5. Close the Cell by placing the auxiliary (inlet) block on the top of WE block. Place the four M4 stainless steel allen screws in the screw holes and tighten them gently in a cross wise manner using the hex key delivered in the accessory kit (max 0.13 Nm). Don't over-tighten the screws.

Figure 14. µ-PrepCell inlet and WE block fixed with 4x M4 stainless steel allen screws.

6. Close the Cell by placing the auxiliary (inlet) block on the top of WE block. Place the four hex screws in the screw holes and tighten them gently in a cross wise manner using the hex key delivered in the accessory kit (max 0.13 Nm). Don't over-tighten the screws.

Before closing make sure that both blocks and the holes in the AUX block for the positioning pins are dry. In case the holes contain liquid it could result in wet spacers or liquid accumulating in the area between the Viton O-ring and the spacer. This could negatively affect the performance of the cell.

7. The spring-loaded contact pin from the WE contact holder will assure proper electrical contact with the working electrode (Fig. 12).

Figure 15. µ-PrepCell WE contact.

First insert the WE contact in the centre hole of the PEEK WE block. Fix the holder by means of the black nut. Make sure that the WE contact is inserted deep enough and 'locked' within the hole, note that the round holder has two straight sides which uniquely fit within the bottom part of the centre hole (see Figure 16). This prevents rotation of the WE contact holder when fixing the black nut.

Do not overtighten the black nut. Screw it gently into the threaded hole until you feel resistance and stop. Overtightening might deform the WE recess area of the WE block (protrusion) and block the flow through the cell.

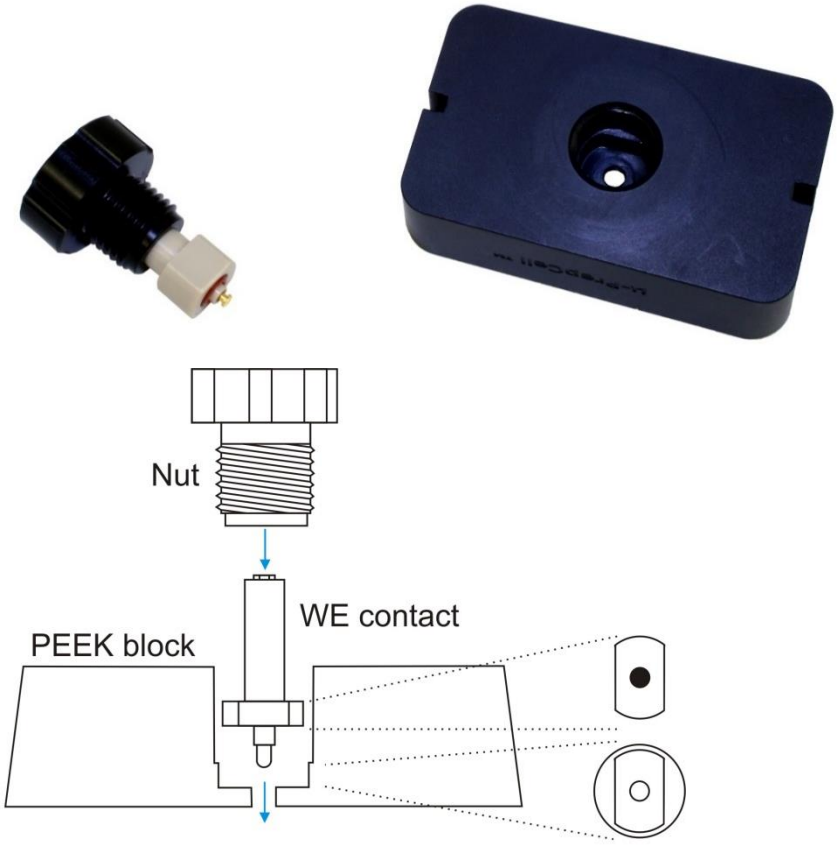


Figure 16. µ-PrepCell – WE contact, nut and WE block. Below: schematic intersection.

Assure that the spring-loaded WE electrode contact holder is never exposed to (corrosive) liquids because it may lead to corrosion of the spring inside of the contact and malfunction of the cell.

8. Before using the µ-PrepCell, it is recommended to check if none of the electrodes are short-circuited. This can be done by measuring the Ohmic resistance with a voltmeter between the WE–AUX; WE–REF and AUX–REF contacts. Note that the cell should be completely dry for a valid measurement.

For installation and priming of the µ-PrepCell see the installation section on the next page. Bubbles in the µ -PrepCell are the number 1 source of poor reproducibility. The next chapter contains important information how to prime/fill the µ-PrepCell without the introduction of air-bubbles. This procedure should be followed strictly to assure optimal performance of the µ-PrepCell.

9. The µ-PrepCell 2.0 is equipped with a Pd/H_2 reference electrode (HyREF, see Figure 17), make sure that the orange silicone O-ring is mounted onto the PTCFE body as depicted by the arrow in Figure 17. The Silicone O-ring assures that no leakage can occur during operation.

Figure 17. µ-PrepCell 2.0 HyREF (Pd/H_2) reference electrode with Silicone O-ring (black arrow).

Before mounting the HyREF reference electrode onto the µ-PrepCell 2.0 install and prime the cell as described in the next sections.

.

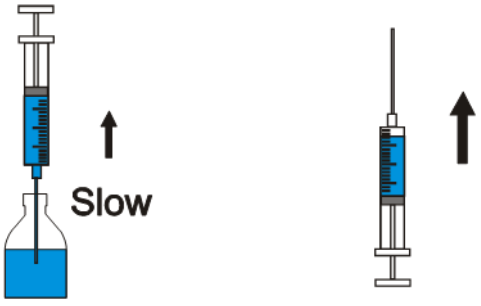
Priming the µ-PrepCell 2.0

Air bubbles in the u-PrepCell are the number 1 source of poor reproducibility. Bubbles can be generated at the electrode (electrolysis) or by connecting the syringe Luer lock. This document contains important information how to prime/fill the µ-PrepCell without the introduction of air-bubbles. This procedure should be followed strictly during the installation and use of the cell for electrochemical oxidation/reduction to assure optimal performance of the µ-PrepCell. An instruction video and additional information can be found on the Antec support web site (video base).

Use proper eye and skin protection when working with solvents. Additional safety requirements or protection may be necessary depending on the chemicals used in combination with this equipment. Make sure that you understand the hazards associated with the chemicals used and take appropriate measures with regards to safety and protection.

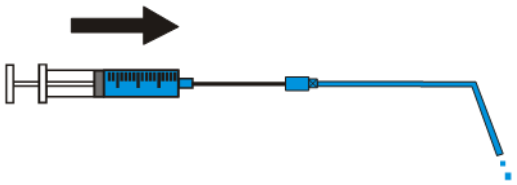
1. Fill the syringe by slowly aspirating degassed sample solution. Warning: too fast aspiration can lead to under pressure in the syringe and subsequent air bubble formation in the solution.

2. Hold the syringe upwards and remove any aspirated air from the syringe. If bubbles are present in the sample solution itself, tick against the syringe to move the bubbles upwards.

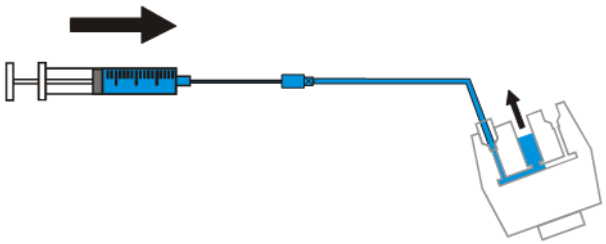


1. 2.

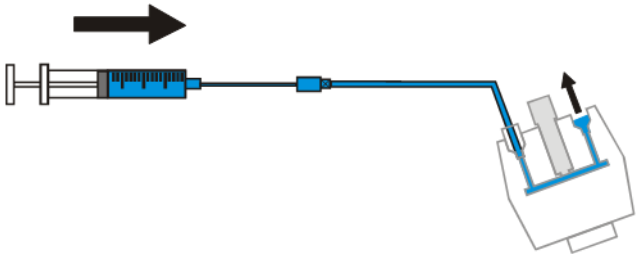
3. Connect a blue-striped PEEK (250 µm ID, 1/16” OD, typically 45 cm in length) inlet tubing to the syringe using a Luer lock connector. Fill the inlet tubing with sample solution.



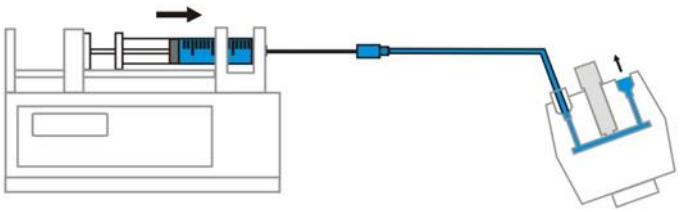
4. Connect the outlet of the tubing to the inlet of the µ-PrepCell using a 10-24 fingertight fitting. Make sure that the outlet tubing and REF electrode are removed from the cell. Fill the cell tilted (45° angle) until the sample solution is siphoning from the REF chamber of cell. Push the solution firmly through the cell in order to remove any air-bubble present.



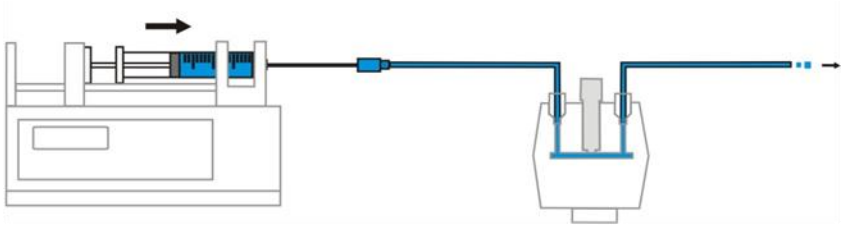
5. Check visually if there are no air bubbles present in the sample solution in the REF chamber. If any air bubbles are present try to remove them. Mount the REF electrode and make sure not to introduce any air bubble during the mounting process. Subsequently, continue filling the cell until the solution is siphoning (air bubble free) from the outlet port.



6. Place the syringe in the infusion pump and start the infusion pump to dispense at the designated flow rate.



7. Subsequently connect a red-striped PEEK (127 µm ID, 1/16" OD, typically 1 meter in length) outlet tubing to the outlet port of the cell using a 10-32 fingertight fitting. Fill the outlet tubing with sample solution.



8. The cell is now primed and ready for use.

µ-PrepCell 2.0 installation in a ROXY EC system

With a ROXY EC system a dedicated LC connection kit is supplied, part 180.0161A ROXY LC conn. kit, EC. For a detailed description please refer to the installation documentation supplied with the kit. In this section a brief description is given how to install an µ-PrepCell in your ROXY EC system. See Figure 18 on the next page.

For air bubble-free installation and use of the µ-PrepCell 2.0 follow the precautions described in the previous section.

To install the µ-PrepCell in your ROXY EC system follow the steps below:

1. Have some tissues ready and wear protective gloves as you probably will spill some mobile phase during the mounting procedure.

2. Connect the syringe filled with mobile phase to the inlet of the µPrepCell using tubing assembly Q'' (p/n 180.0230A) and tighten it carefully. Make sure that the syringe and tubing is air-bubble free when connecting it to the cell.

CAUTION

The mobile phase should contain the supporting electrolyte (e.g., ammonium formate, ammonium acetate, formic acid) to provide proper working conditions and to prevent the damage of the working electrode or the electronics.

3. Connect tubing assembly H'' (p/n 180.0219) to the outlet of the cell but not yet to the MS. Flush µ-PrepCell with the mobile phase and ensure that all air bubbles are removed. Keep it under an angle of 45 degree to clear out the air in the cell. During clearing tick against the cell to promote removal of air bubbles.

4. Place the cell levelled in the cell holder and unscrew the HyREF electrode from the AUX inlet block at low flow rate of mobile phase. Visually inspect if there are no air bubbles present anymore in the hole of the reference electrode in the AUX block (one can clearly see the working electrode when looking inside). In case of air bubbles tick against the cell to promote removal. When all air bubbles are removed mount the HyREF electrode back in the AUX block and remove the excess mobile phase which is pushed out the chamber during fixing of the HyREF electrode.

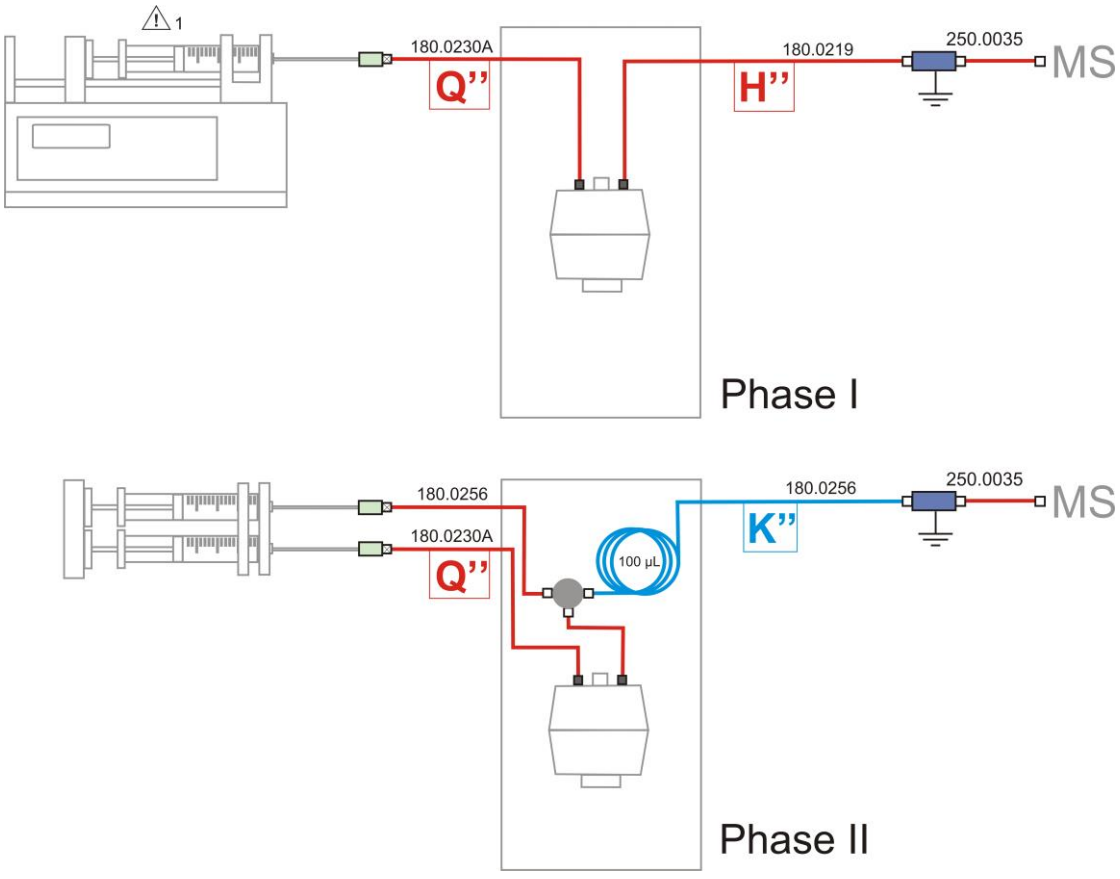


Figure 18. Schematic drawing of the µ-PrepCell in a ROXY EC system. Top part: Phase I configuration, bottom part: Phase II configuration.

5. Replace the mobile phase in the syringe with the desired sample solution and place the syringe in the syringe pump. Make sure that the syringe is air bubble free. If necessary the sample can be degassed or sparged with argon (deaerated conditions) before introducing the sample to the cell.

In case of high sample concentration, more frequent cleaning of the electrode may be required (See the cleaning and activation procedures in the maintenance section of this user manual) to recover the full performance of the cell.

6. Carefully connect the syringe to the inlet tubing assembly Q'' (180.0230A), avoid the introduction of air bubbles in the system.

7. Start the syringe pump with a sufficiently low flow rate for efficient electrochemical conversion (e.g. 10µL/min - 50 µL/min).

8. Connect the outlet tubing with the MS source, make sure that the MS inlet is proper grounded using the ROXY grounding cable 250.0035 provided with the ROXY potentiostat (available for units purchased after 1 September 2010).

CAUTION

An ESI interface of an MS is usually operating at high voltages of typically 3 – 5 kV. In cases where the inlet of the ESI-MS is not grounded, the grounding kit (pn 250.0035) must be used. If not used it may lead to irreproducible/erratic results or damage of the ROXY potentiostat or flow cell. To prevent electric shock when connecting this grounding kit assure that the high voltage on the ESI source is switched off. Refer to the user manual of your specific MS for detailed instructions.

Figure 19. ROXY grounding kit (part 250.0035).

9. Connect the cell cable to the µ-PrepCell. The red connector should be connected to the Working electrode, the black one to the HyREF reference electrode and the blue connector to the Auxiliary electrode.

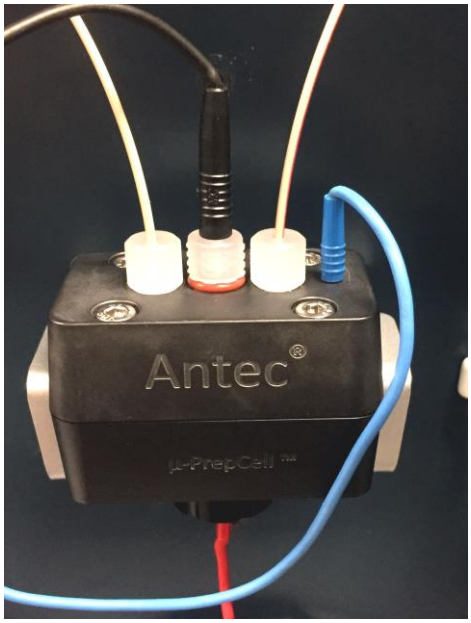


Figure 20. µ-PrepCell 2.0 with connected ROXY cell cable.

Your µ-PrepCell 2.0 is now ready for use.

The µ-PrepCell should be flushed with demi water after use, ensure that the cell is switched off during flushing with water! If the cell is not used for longer periods, disassemble the cell , dry all parts and store it in a dust-free environment.

It is recommended to optimize the conditions for metabolite synthesis with on-line MS detection. At first instance recording of MS Voltammogram helps with estimation of the potential. Once the conditions are established the synthesis of metabolites can be performed with off-line sample collection. How to record MS Voltammogram and the detailed background information about the supplied events files and relevant Dialogue settings are provided in the Dialogue for ROXY EC system User guide (210.7017).

C H A P T E R 3

Maintenance

Working electrode maintenance

Activation or polishing of the working electrode is necessary if the electrode surface has been electrochemically changed. This may be due to fouling by oxidation (reduction) reaction products. Excessively high currents also may change the electrode surface. This is noticed by a strongly decreased performance after prolonged use.

Decreased cell performance

Several actions can be taken at decreased flow cell performance. Avoid unnecessary polishing; take the next step only if the previous was not successful.

1. Wiping the electrode surface with a tissue wetted with methanol or acetone.
2. Polishing the electrode surface. Only apply polishing on Metal (Ti, Au, Pt) and GC electrode only. **The MD and TiBlue electrodes must not be polished mechanically.**
3. Electrochemical activation of the GC and MD electrodes using pulse mode (see separate section of this manual).
4. The (re)activation procedure for Diamond electrodes using scan mode (see separate section of this manual).

Disassembly of the µ-PrepCell

If the working electrode needs maintenance, the cell has to be disassembled.

Before disassembling the flow cell read the General precautions.

1. Switch off the cell and syringe pump and disconnect both fingertight fittings.
2. Unscrew the four screws from the PEEK inlet block and open the cell.
3. Remove the Working electrode
4. Remove the HyREF reference electrode
5. Clean and dry the spacers.
6. Clean/polish the WE as described in the next section.
7. Note that the GC WE has only 1 side with a mirror-like surface. This side is facing the sample and should be polished. The laminated Au and Pt electrodes

also have one active side (mirror-like surface) which should be polished, the backside is of Titanium and can be clearly recognized (word ‘down’ engraved). See chapter 1.

Use proper eye and skin protection when working with solvents.

Polishing of the GC electrode

The polishing procedure is for GC and metal working electrodes (Ti, Au, Pt) only. Do <u>not</u> polish the thin-film Magic Diamond and TiBlue electrodes, it will lead to damage of the electrode surface and a loss of performance. For Magic Diamond electrodes follow the activation procedure described in a separate section of this manual. For the TiBlue electrodes use the other side, if unused or replace it for a new one.

1. Shake diamond slurry thoroughly before use!
2. Rinse the polishing disc with demi water before applying the diamond slurry!
3. Apply a small amount of slurry on the wetted polishing disc, usually **one drop** is sufficient.
4. Put the working electrode with the face down on the disc and polish the electrode with a ‘figure 8’ motion for about one minute. Apply only gentle pressure with your fingers. The GC electrode is rather brittle and can break when applying too much force.
5. Clean the electrode with an ethanol-wetted tissue and check the surface visually; repeat the procedure if necessary.
6. Reassemble the cell.
7. Clean the polishing disc with demi water.
8. Store the polishing disc dust free in its plastic bag.

Inlet block & O-ring

The new Conductive PEEK inlet block is virtually maintenance-free under normal use and only require some cleaning after use with organic solvent (Ethanol, Acetone). In the event that for some reason the active area is damaged (by scratching, dropping or applying too extreme measurement conditions) resulting in leakage or erratic operation, contact Antec or your local distributor for service. Antec offers a refurbish service for the µ-PrepCell blocks.

Figure 21. Active area (AUX) of the µ-PrepCell 2.0 inlet block with Silicone O-ring.

For leak-free operation of the µ-PrepCell 2.0 the silicon O-ring in the inlet block should be in pristine conditions and not show signs of wear and tear. Regularly inspect (every time you assemble the cell) if the O-ring still has a round shape, that it is not damaged, swollen or dirty. Replace the O-ring immediately in case of damage. The re-ordering part number is 204.0501 µ-PrepCell O-ring inlet block, Silicone,5pcs. Remove the O-ring as shown in Figure 22. Gently remove the O-ring using the supplied stainless steel needle supplied in the µ-PrepCell accessory kit. Carefully lift the Silicone O-ring out of its chamber. Make sure not to cut the O-ring with the needle point or the PEEK surface.

Figure 22. Removal of Silicone O-ring. Gently lift the O-ring out of its chamber from the outside using the needle provided in the µ-PrepCell accessory kit.

<u>Warning:</u> the needle has a <u>SHARP TIP</u>. Take care not to cut/pierce yourself! Gently lift the O-ring, <u>do not</u> scratch the surface of the conductive PEEK inlet block.

Maintenance Magic Diamond™ electrode

The MD (Boron-Doped Diamond) working electrode consists of an ultra-thin crystalline Diamond layer deposited on top of a Si substrate. Therefore, such electrode cannot be polished to restore the electrode surface in case of loss of sensitivity due to fouling. The backside of the MD electrode is backside metalized with Gold for better contact. Note that the electrode edges on the gold side may show some blackish marking. This is caused by the activation procedure executed at the manufacturer. An effective method to restore the detection performance is by electrochemically reactivation of the electrode surface under acidic conditions.

Activation of the electrodes using pulse mode

This activation procedure is suitable for both MD and GC electrodes. Dialogue Elite software can be used to control the ROXY potentiostat and to apply the cleaning pulse. Follow the steps below:

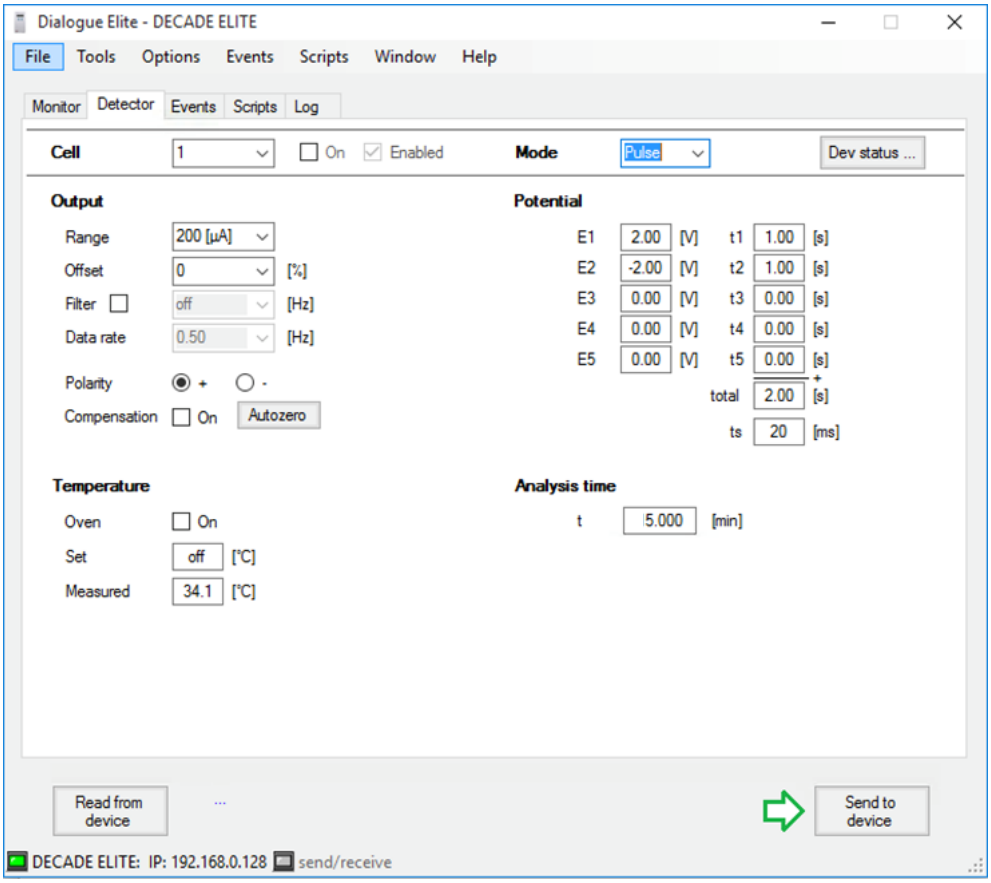


Figure 23. Dialogue Elite Detector tab with pulse settings for the electro-chemical activation of MD and GC WE electrodes.

1. Refill the syringe with the mobile phase containing the supporting electrolyte, e.g., the same mobile phase used for oxidation of the sample and start the flow rate (20-50µL/min).

2. Open Dialogue Elite, go to the detector tab and set the detection mode to pulse with the settings for the electrochemical activation, see Figure 23. The settings for the activation pulse are listed in Table 4.

Table 4 Activation pulse settings.

Parameter	*Value*
E1	+ 2 V
E2	- 2 V
E3	0 V
t1	1000 ms
t2	1000 ms
t3	0 ms
ts	20 ms

(It is recommended to set the Output Range to 200 µA.)

3. Set Run time value to 5 min and turn on the cell (check the Cell On box).

4. Click the 'Send to device' button to transfer all settings to the instrument. Note that pulse setting is instantly applied to the cell.

5. Push the F5 key (or goto the 'Options' menu and click 'Start analysis') to start a run of 5 minutes using the activation pulse settings.

6. After the activation procedure turn OFF the cell and leave the flow for additional 1-2min.

In case that no significant improvement of the cell performance is seen:

A. For MD electrode use the additional procedure described in the next chapter (Maintenance Magic Diamond electrode).
B. For GC electrode you can polish the electrode manually as described in the previous chapter (Polishing of the GC electrode).

Activation of the electrodes using scan mode in acidic conditions

1. Disassemble the µ-PrepCell as described on page 12. Keep the MD electrode disc fixed inside the working electrode assembly.
2. Wipe the electrode surface with a tissue wetted with methanol or acetone and subsequently with demi water to clean the electrode surface. **Under all circumstances try to avoid direct contact of the electrode surface with fingers.** The skin contains fatty substances which will foul the electrode.

3. Assemble the µ-PrepCell with MD electrode as described in the previous section.

4. Prepare a mobile phase of 0.5 M Nitric acid (HNO_3), install the µ-PrepCell and prime it with mobile phase to remove any air bubbles.

5. Set the flow rate of 50 µL/min (You will need at least a 5mL syringe).

Make sure that all parts that are not acid-resistant such as: nylon inlet filters, column are not connected in the system during this step.

6. Set the ROXY potentiostat detector in SCAN mode with the following settings: E1 = -3.00 Volt, E2 = +3 Volt, Scan rate: 50 mV/s, scan cycle: continuous and range 200 µA/V.

7. Start scanning under acidic conditions for 1 hours.

8. After scanning switch off the cell and the flow rate, replace the mobile phase by HPLC grade water and flush the cell to remove the acidic solution.

9. You can start your measurements again.

In the case there is no significant sensitivity improvement observed, repeat step 1 to 10 and increase the total scan time (step 7). One can also try to perform the activation procedure under the mobile phase conditions of the application. Furthermore, in literature anodic treatments are reported (for example the application of a static potential of + 3 Volt vs. Ag/AgCl for 5 – 10 minutes) to restore the electrode response. Such procedure could be executed as an alternative, if the above-mentioned procedure does not lead to satisfactory results.

Electrochemical reactivation procedure for MD electrode is part of the ROXY potentiostat firmware and available via display panel (Diag→Activate). The scan settings and the duration are as described in points 6 and 7, and only “push button” action is needed to execute it (See part 210.7010 ROXY Potentiostat user manual). Replace the Magic Diamond electrode when no improvement is seen after repeated reactivation/conditioning attempts.

Chemical compatibility: The MD electrode exhibits an excellent inertness and can be used with a large variety of mobile phase and chemicals. However it has been observed that the MD electrode operational lifetime is strongly reduced when exposed to fluorinated acids, such as tri-fluoroacetic acid. Even at relatively low concentrations (2% in aqueous solution) significant damage of the diamond electrode was seen within days of operation (delamination/’blister’ formation of the MD layer).

Storage

If the flow cell is not in use, switch off the cell and flush with water. Disconnect from the LC system, we recommend that you disassemble the cell and clean and dry all surfaces.

Before removing the cell from the detector, turn off the cell first !

C H A P T E R 4

Specifications µ-PrepCell

Type	Thin-layer electrochemical cell (micro-preparative work)
Spacers	50 or 100 µm, stainless steel, stackable (max. stack thickness 250 µm)
WE dimensions	12 x 30 mm, thickness 1 mm
WE area (wetted)	1.9 cm^2
Cell volume	10 µl (effective spacer thickness 50 µm)
Working electrode	Glassy Carbon (GC), Boron-Doped Diamond (BDD), Titanium (Ti), Titanium oxide (TiBlue), Platinum (Pt) and Gold (Au)
Reference electrode	HyREF™ (Pd/H_2 electrode)
Auxiliary electrode	Conductive PEEK (PEEK, 30% carbon fiber-reinforced)
Wetted materials	PEEK, Carbon, Methyl-vinyl silicone rubber (Silicone VMQ-70), PCTFE and WE material (see working electrode section)
Fluidic connections	1/16” OD PEEK or PEEKsil tubing, ID 250 µm or less, with 10-32 PCTFE fingertight fitting
Electrical connections	ROXY cell cable incl 0.5uF, 3m (part 250.0139L)
Flow rate	Typically 20 – 100 µL/min
Working temp range	10 - 50 °C
Max. pressure	25 bar (GC electrode) , 50 bar (with MD electrode)

C H A P T E R 5

Accessories µ-PrepCell 2.0

The Antec µ-PrepCell2.0 MD/GC (p/n 204.4310) and µ-PrepCell2.0 TiBlue (p/n 204.4312) are shipped together with a number of parts:

Table 4. µ-PrepCell parts and accessories.

Part number	Component
250.1025	Polishing disc for WE
250.1030	10 mL diamond slurry 1 µm
250.0062	Hex key for flow cell assembly
250.1571	Fingertight fitting PCTFE 10-32
204.2217	µ-PrepCell spacer 50 µm
204.2218	µ-PrepCell spacer 100 µm
204.0102	µ-PrepCell holder kit
204.0510	µ-PrepCell O-ring tool (inlet block)
204.0501	µ-PrepCell O-ring inlet block, Silicone,5pcs
204.0504	µ-PrepCell 2.0 O-ring HyREF electrode, Silicone
204.0503A	µ-PrepCell O-ring WE contact, Silicone

All available WE materials for the µ-PrepCell are listed below:

Table 5. µ-PrepCell WE's.

Part number	Component
204.5007*	WE GC for µ-PC
204.5010	WE Ti for µ-PC
204.5010A#	WE TiBlue for µ-PC2.0
204.5022	WE Pt for µ-PC
204.5027	WE Au for µ-PC
204.5050*	WE MD for µ-PrepCell

*#) shipped with µ-PrepCell2.0 TiBlue (p/n 204.4312). *) µ-PrepCell2.0 MD/GC (p/n 204.4310)*

For these and other Antec µ-PrepCell parts contact your local supplier.