Antec Scientific
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ROXY

Installation Guide

210.7011, Edition 5, 2018

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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 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 ROXY EC(/LC) system can involve the use of hazardous materials including corrosive fluids and flammable liquids. The system 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 and scientists 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 ROXY EC system is ROHS compliant and in conformity with Directive 2011/65/EU Restricted use of Hazardous Substances in electrical and electronic Equipment (ROHS).

Antec Leyden B.V. is an ISO 9001 certified company.

WEEE directive

All equipment of Antec Leyden which are subjected to the WEEE directive shipped after August 13, 2005 are compliant with the WEEE marking requirements. Such products are labelled with the “crossed out wheelie”, depicted on the left site.

The symbol on the product indicates that the product must not be disposed as unsorted municipality waste. Please take care of proper disposal and recycling.

If needed Antec offers the possibility for recycling. In that case please ship the instrument back to the manufacturer together with a decontamination form (Antec Leyden, the Netherlands). They will take care of the proper disposal and recycling of the instrument.

Manufacturer shipping address for the end-of-life products:

Antec Leyden B.V.
Industrieweg 12
2382NV Zoeterwoude
The Netherlands

In case of questions, or if further information is required about the collection & recycling procedure, please contact your local distributor.

Warranty, spare parts and service

The warranty period of this ROXY EC system 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 ROXY EC system. The safety practices are intended to ensure safe operation of the flow cell.

Working environment & safety

The intended use of the ROXY EC system 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 it is recommend that the ROXY EC system 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 to take the necessary precautions and treat all specimens as potentially infectious. Always wear protective and gloves when handling toxic or biologically infectious samples to prevent biohazards or hazards while working with the system. When the system or parts of it needs to be shipped to Antec for service/repair or disposal, it has to be accompanied with a decontamination form which should be completely filled in and signed by the customer. Without this decontamination form the system or parts of it 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 ROXY EC system in other ways than indicated in the manual might result in erratic or unsafe operation.

C H A P T E R 1

Introduction

To assure an efficient and successful onsite installation of the ROXY EC system, we have written this guide. This documents describes all the steps that should be taken. It contains references to relevant user manuals and qualification documents.
There are a few different configurations applied for electrochemistry coupled to MS (EC/MS). The EC reactor can be used for continuous flow experiment, or flow injection analysis both are not using chromatography. Sometimes a secondary solvent is added to study post EC reaction conjugation with glutathione for example. In combination with HPLC the reactor can be placed prior to the HPLC injector (EC/HPLC/MS) or post column (HPLC/EC/MS).
This document primarily outlines the ROXY EC/MS installation, for the other configurations most of the described procedure is the same. Except that for installation of a autosampler or pump the corresponding user manuals should be used.

Fig. 1. ROXY EC/MS with syringe pump for continuous flow experiments.

This document is intended as a general check list to guide you through the installation of a ROXY EC system and by no means a replacement of the installation sections in the individual user manuals supplied with the system.

C H A P T E R 2

Preparing for installation

Pre-installation preparations

Installation requirements document

To assure an efficient and successful onsite installation of the ROXY EC system by engineers of Antec or its distributors, we have a guideline with pre-installation requirements available (document 210.7050). This document contains hardware and site requirements as well as information about chemicals required for the installation and performance qualification of the ROXY EC system. The 'site preparation reply form' in the document confirming that all requirements are met, has to be signed and returned by the user prior to installation.

It is the customer's responsibility that the installation site requirements are met and all chemicals are available onsite at the date of installation. It is the installation engineer's responsibility to send this pre-installation requirement document (well in advance of the installation date) and get a signed copy in return.

IQ and OQ

A printed copy of an Installation Qualification (IQ) document is provided with each ROXY. The IQ/OQ document is also available for download from Antec's website. It is a document for installation and training/instruction of the user that should be signed-off. An Operational Qualification (OQ) of a ROXY is a dummy cell test and output measurement. This test is always part of the installation procedure.
A completed and signed IQ/OQ document will serve as an objective proof that the installation was completed successful and was carried out to the satisfaction of both parties. Familiarize yourself with the procedure, perform the installation according to the guidelines given herein, and fill in the IQ/OQ document during the installation.

PQ

A performance Qualification (PQ) for the ROXY demonstrates the performance of the system using a solution with a standard (substrate). In a continuous flow experiment, the substrate is converted in the electrochemical reactor and the reaction products are measured using an MS. For the

oxidative mode and reductive mode a separate performance test is available based on the conversion of Amodiaquine and Insulin, respectively. Which test is required is depending on the specific ROXY system installed and/or customers application (OX/RED).
A PQ should be performed as last step of the installation a to assure the proper operation of the full ROXY EC system (Syringe pump, ROXY potentiostat, flow cell) in combination with MS. See chapter 4 and 5 of this installation guide and familiarize yourself with the procedure.

For the PQ specific chemicals and solutions should be available at the customer site at the day of installation

Test solutions for oxidative mode

To perform a performance qualification in oxidative mode the following solution should be available:

1. Mobile phase: about 100mL of 20mM Ammonium formate (pH 7.4 adjusted with ammonium hydroxide) in 50% acetonitrile.
2. Sample: 20mL of 10µM Amodiaquine in the solution described in point 1.

Test solution for reductive mode

To perform performance qualification in reductive mode the following solution should be available:

1. Mobile phase: about 100mL of 1% formic acid in 50% acetonitrile.
2. Sample: 20mL of 5µg/mL insulin in the mobile phase described in point 1.

The customer should prepare the solution(s) preferable a day before the installation day. The sample solution of both Amodiaquine and Insulin should be stored at 4°C in the dark (light sensitive).

Relevant documents for installation

The detailed description of ROXY EC system installation is part of the following user manuals:

210.7050 - ROXY Installation Requirements (prior to installation!)
210.7011 - ROXY EC Installation guide (this document)
210.7010 - ROXY Potentiostat user manual
210.7014 - ReactorCell user manual
204.0010A-µ-PrepCell2.0 user manual
206.0010 - SynthesisCell user manual
175.0015 - Dialogue Elite user manual

For the latest versions of the Antec's manuals and application notes visit www.antecscientific.com.

When preparing for an installation, we advise to print out any document/manual that needs to be signed off.

Software download

To assure that the system will be installed with the most recent software version available we recommend to download the most recent copy of the Dialogue Elite software and drivers (i.e. USB/COM port driver) before the installation.

http://www.antecscientific.com/support/documents-and-downloads/software

Contents of ROXY

Although there are a few exceptions, most ROXY EC systems are delivered as the following parts:

Part no.	Description	Qty
210.0010A	ROXY Potentiostat, High Current	1
210.0200D	ROXY Potentiostat accessory kit	1
188.0035	Dual syringe infusion pump*	1
188.0300	Syringe 1mL, TLL	2
188.0302	Syringe 100µL, TLL	1
250.0123C	Dual port USB-RS232 converter	1
180.0161A	ROXY LC conn. kit, EC*	1
250.0139L	ROXY cell cable incl 0.5uF, 2m	1
250.0035	ROXY grounding kit	1
171.9015	Dialogue Elite OQ, PQ, ROXY (standard)	1
204.4310[1]	µ-PrepCell 2.0 GC/BDD	1
204.4312[2]	µ-PrepCell 2.0 TiBlue	1
204.4302[3]	µ-PrepCell High Pressure Ti	1
210.0040[4]	ReactorCell kit (incl. BDD, GC, Au Pt)	1

[1] ROXY EC system with µ-PrepCell 2.0 (210.0074A)
[2] ROXY EC system for S-S reduction (210.0072A)
[3] ROXY EC system for HDX (210.0073A)
[4] ROXY EC system with ReactorCell (210.0070A)
* A ROXY for HDX has no syringe pump.

The details are in the packing list that comes with the delivery.

C H A P T E R 3

Installation

Take the following steps to unpack and install the devices:

1. Check all carton boxes for damage. We advise to store the transport boxes in case of further transport.
2. To unpack the detector, lift it from its box by both hands. **Never lift the detector at its front door**, but at its sides.
3. Unpack the syringe pump.
4. Align the system components as indicated in the photo below
5. Check the part and serial numbers of the individual system parts and write it down in the IQ document.
6. Check the contents of accessory kits using the packing list, to be sure it is complete and undamaged.
7. Follow the installation instructions in the next section of this document.

Fig. 2. ROXY EC with syringe pump.

A tutorial movie how to install a ROXY with an µ-PrepCell is available on the Antec website:
https://www.antecscientific.com/support/tutorials/roxy-installation

Electrical connections

1. Connect the power cable, trigger cable and serial communication cable to the ROXY Potentiostat as it is shown in the Fig. 3. Use cables provided by Antec or ensure that the used cables meet all relevant safety and EMC and local requirements.

The manufacturer is not liable if power cords are used which do not meet the relevant safety and EMC requirements (CE, CSA and UL).

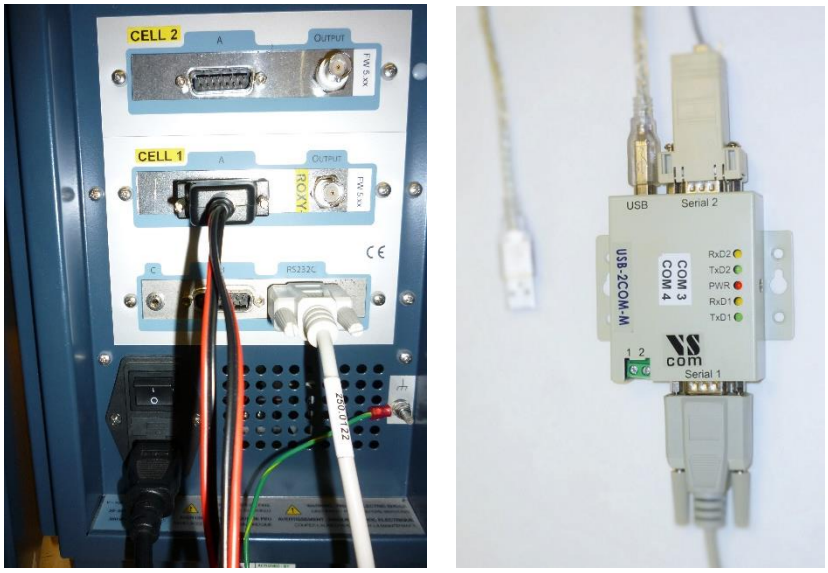
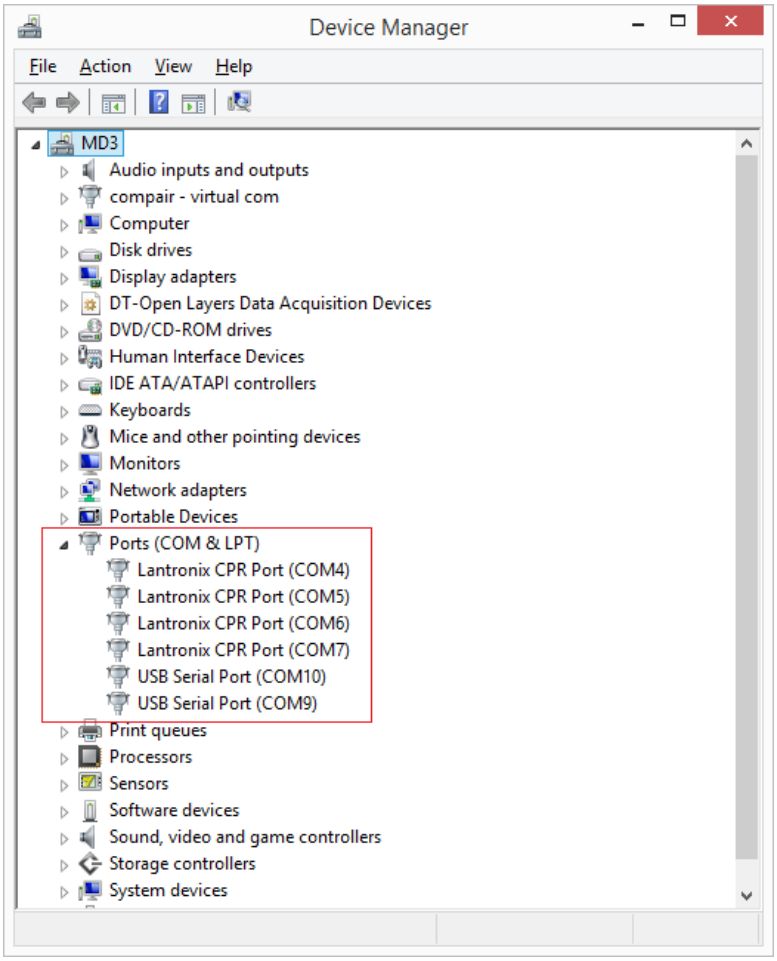


Fig. 3. Back panel of a ROXY Potentiostat (left) and VScom RS232-USB converter. Converter is connected to computer using an USB port.

2. Install the driver of the VScom USB-to-RS232 converter.
3. Connect the VScom port “Serial 1” to the ROXY with the serial cable.
4. Connect the VScom port “Serial 2” to the syringe pump “RS232 IN” port (Harvard 11 pump), or connect the PC directly to the pump using the USB cable supplied with the pump (Legato 101 pump).
5. Connect the cell cable to the Cell 1 connector inside the potentiostat.
6. Connect the USB cable(s) to computer and wait for Windows to finish installation, in case needed. Right bottom of the Windows screen shows the progress, in case a Windows driver is loaded.

7. Check in the Windows Device manager the numbers assigned to USB serial ports. Look for “USB Serial Port”. The highest number is “Serial 2” the other is “Serial 1”. Also the Legato port is recognizable as such.

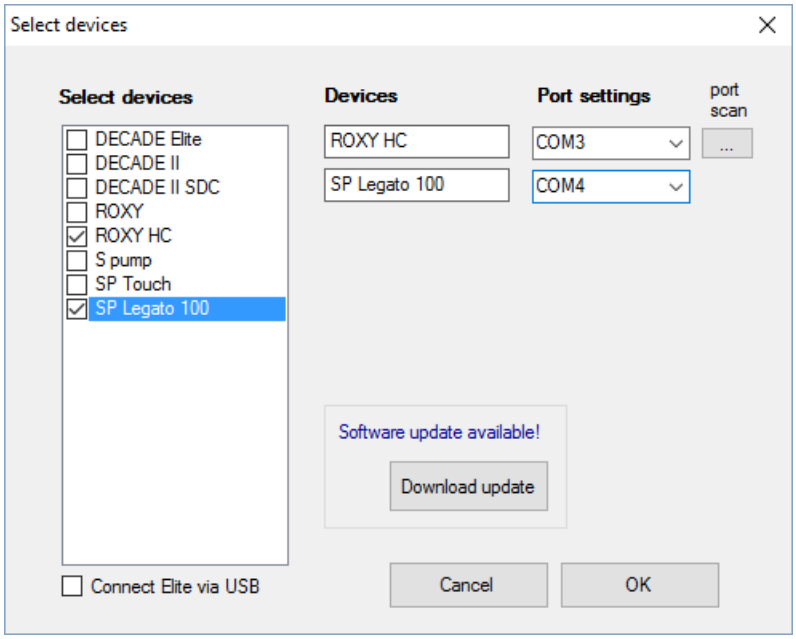
In case of doubt unplug the cable and identify the port that disappears in the device manager.



Software installation

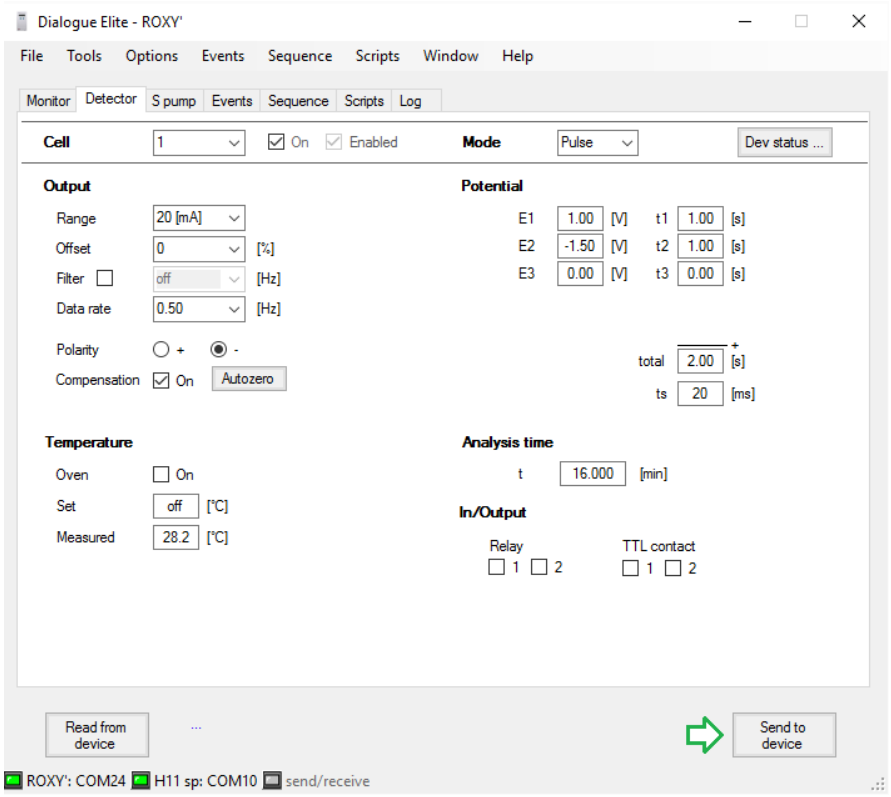
1. Install Dialogue Elite and connect the license key (dongle). Without a license, a message appears and the program starts with limited functionality (demo mode).

2. After installing Dialogue software, switch on potentiostat and syringe pump.
3. Run the Dialogue software from Windows start menu under 'Antec software'. The program starts with the 'device selector', where active devices and ports can be selected. Choose the correct port numbers to connect Potentiostat and syringe pump, respectively.



4. In case an update is available a message appears. It is advisable to always use the latest updates.

5. The green LEDs in the left bottom corner of the Dialogue window show that the devices are successfully connected.
6. Use the 'Send to device' button to apply changes in settings as the green arrow (right bottom) indicates. 'Read from device' refreshes the current settings.
7. The monitor tab shows the actual status of most relevant parameters. The S pump tab is for control of the syringe pump.



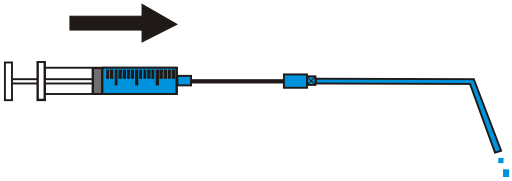
8. For more details about the Dialogue Elite software, see the user manual. Our website contains a number of screen recordings of most common actions.

Installation of a flow cell

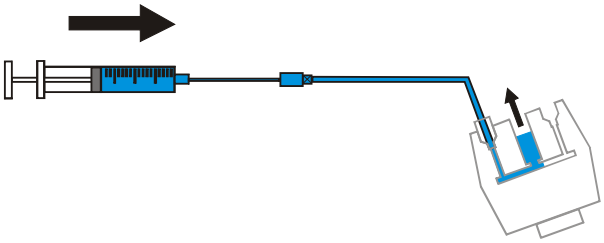
1. Follow the specific procedure to assemble the flow cell as described in the flow cell user manual.
2. The schematic drawing how to connect the inlet and outlet tubing with the cell and to place the grounded union is delivered with the ROXY EC system (see Fig. 4).

For successful installation it is of utmost importance to install the reactor cell bubble free.

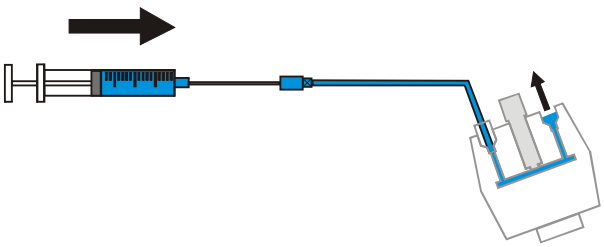
1. Place the Luer Lock connector on the syringe. Fill the syringe with solvent containing the substrate and connect it with the inlet tubing. Ensure that the tubing is filled with liquid and does not contain any bubbles.



2. Connect the tubing with the cell. The outlet tubing and REF electrode should not be connected yet. Hold the cell tilted (45° angle) and fill it until the REF chamber of cell is filled.



3. Mount the REF electrode and remove excess of the liquid around it. Push the solution firmly through the outlet port. You may see some air escaping from the outlet.



4. Place the syringe in the infusion pump and start the flow.

5. Connect the outlet tubing to the cell and, if the tubing is filled, to MS
6. Ensure that all connections are tight and leak-free.

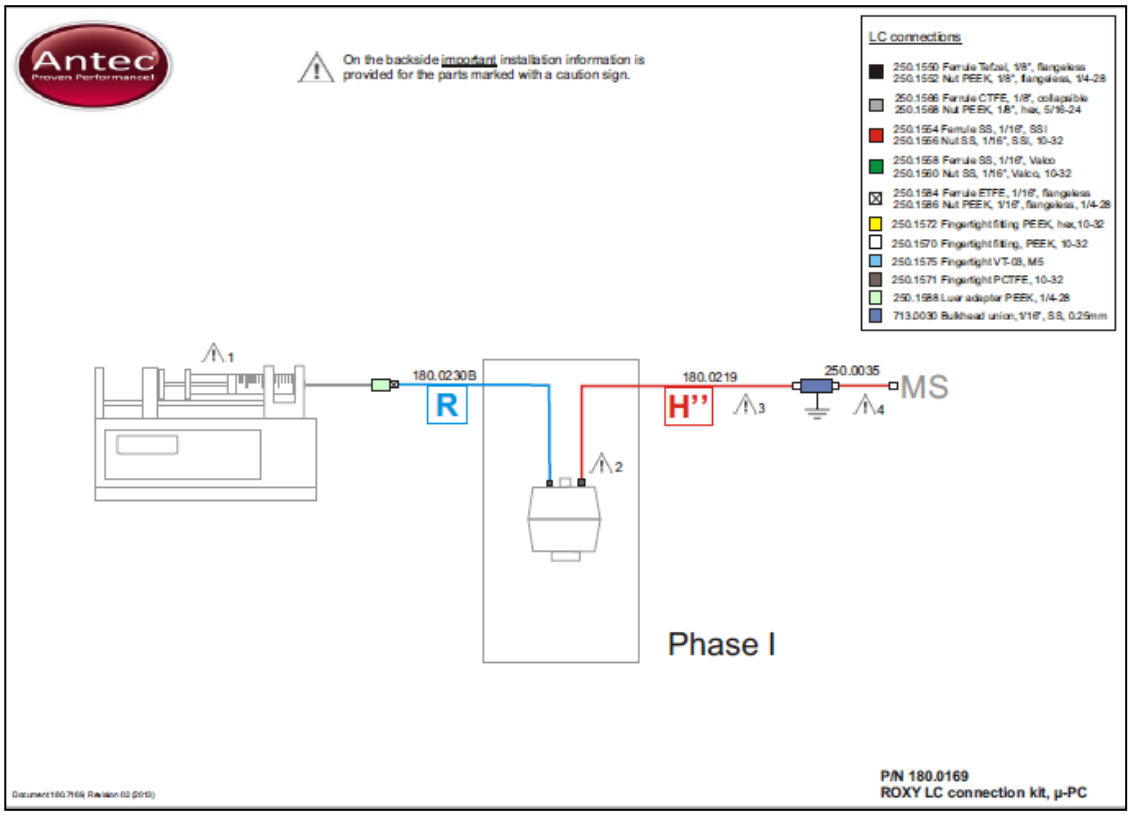


Fig. 4. The schematic of installation of LC connections (180.7169).

The details on cell installation, maintenance and troubleshooting are described in the following user manuals:

210.7014 - ReactorCell user manual
204.0010A - µ-PrepCell 2.0 user manual

Installation of grounding kit

For safety, to protect a user from the high voltage of the electrospray, a grounding kit is applied. A grounding point can be placed inside the unit, or outside, close to the electrospray source. The grounding point is a metal union that is connected to a ground wire, and connected to the ROXY.
Of course, the ROXY should be grounded as well, by using a grounded power socket.

Fig. 5. The grounding kit inside the ROXY (left) or for external use (right).

Performing an experiment

The general instruction how to perform an experiment using ROXY EC system are summarized in the steps below.

Step 1 – Choice of electrode

Electrochemical behavior of a substrate is electrode-dependent, among other things. For new/unknown substrates it is recommended to test samples with both the Diamond and GC electrode. The boron doped diamond (BDD) electrode is more inert, has less absorption and has a wider working potential range in aqueous solution. For reduction of disulfide bonds the TiBlue working electrode is used. Select the electrode and mount the cell as described in the corresponding manuals.

Step 2 – Assembling the cell

Follow the steps as described in the user manual of the cell. Make sure to use the correct PTCFE finger tights supplied in the flow cell kit. The thread of a PEEK inlet block (the black ones) are rather fragile. Put the spacer(s) in place, and mount the cell according instructions.
µ-PrepCell 2.0: Do not forget to insert the metal spacers and O-ring (if applicable). For the BDD, GC and laminated electrodes at least a thickness of 200 µm metal spacers should be inserted., For the TiBlue electrodes us a thickness or 150 µm. The WE electrode contact pin should be screwed in completely (be aware of the locking mechanism) to assure proper contact.

Do not overtighten the nut holding the WE contact. Screw it gently into the threaded hole until you feel resistance and stop.It is recommended to check if none of the electrodes are short-circuited using a voltmeter.

Step 3 - Mobile phase

The EC reactor cells require an electrolyte of 10 mM or higher (e.g. ammonium formate, ammonium acetate) in the mobile phase. Also, formic acid at concentration 0.1 – 1 % can be used as supporting electrolyte. The supporting electrolyte will assure the stable working conditions and conversion.

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 BDD 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 substrates that are insoluble in aqueous mobile phase, 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 SPE prior MS analysis).

Step 4 – Connecting the cell in the system

To assure optimal operation the cell should be installed and properly primed (bubble free). The solvents should be thoroughly degassed prior to use.

Step 5 – Optimal sample concentration

It is recommended to use a substrate concentration between 10 – 100µM in solvent. A higher concentration can be used but secondary reactions or adsorption can occur resulting in loss of performance (lower percentage yield). 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. It therefore recommended for new or unknown substrates to obtain a MS voltammogram. A MS voltammogram will give 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 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 one constant potential. Note that the synthesis of different metabolites of one compound may require operation at different potential settings. Scan mode applies continuous scanning between two preset potentials values (E1 and E2) with a certain scan rate (unit: mV/s). The Pulse mode applies a step potential between 2 or 3 preset values. More details about using the DC, Pulse 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.

C H A P T E R 4

PQ Oxidative mode (metabolite synthesis)

PQ procedure

For a successful performance test it is important that the ROXY EC has been installed as described and that the MS has been optimized for the analysis of the substrate. Set up the system according to the conditions as indicated in table below. More details are in the application note 210_004 “Metabolism of Amodiaquine” which is on Antec’s website.

Preparations

1. Prepare a test solution of 10 µmol/L Amodiaquine (AQ). Fill the syringe & tubing with the substrate solution.
2. Fill the cell as described, avoid the inclusion of bubbles. Connect the outlet tubing to the grounding point and MS. Place the syringe in the syringe pump.
3. Load and start the event table in Dialogue which runs the following steps in the procedure automatically:
4. Start the syringe pump manually. Start measuring the signal with the cell off for about 10 min or until a good spectrum is obtained.
5. Switch on the cell, increment the potential from 0, to 0.4 and 1.2 Volt. Measure the substrate and metabolites at each potential setting for about 5-10 min until a good spectrum is obtained.
6. The test is passed when the substrate signal (m/z 356) is below 30% at 1.2 Volt, and a number of metabolite peaks appear at m/z 354, 326 and 299.

Settings:

Configura-tion	ROXY EC/MS for oxidative metabolites
Cell	ReactorCell or µPrepCell
Electrode	GC, or BDD
Solvent	AQ in 20mM ammonium formate (pH 7.4)/50% acetoni-trile
Flow rate	10 or 50 µL/min
Event table	AQ oxidation GC.evt or AQ oxidation BDD.evt

Results

Below mass spectra are from AQ and its oxidative metabolites, measured intensities vary from MS to MS. At 400mV: a dehydrogenation reaction results in m/z 354. At 1200mV several reaction products are found: N-dealkylation (m/z 326), aldehyde formation (m/z 299), oxidation of the aldehyde to carboxylic acid (m/z 315) and an N-oxidation of AQ (m/z 370).

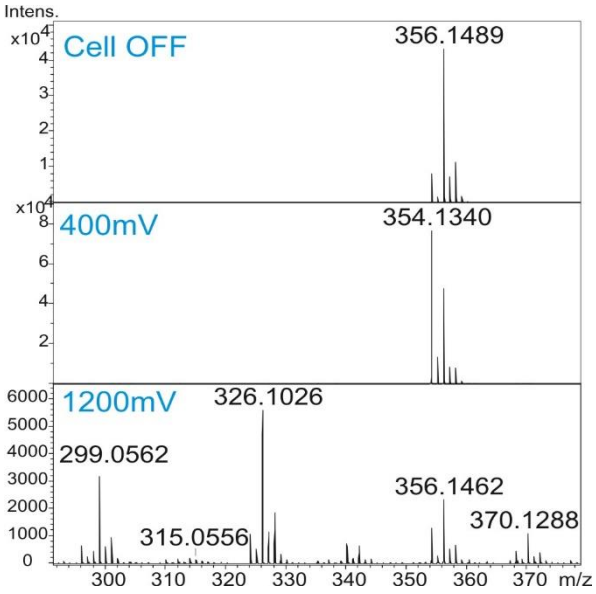


Fig. 6. Results of Amodiaquine (AQ) oxidation. Cell off shows the 100% level of AQ.

	Specified	**Measured**	**Passed/Failed**
Substrate signal cell on vs off (m/z 356.1)	< 30%	……..	……..
Three m/z 326, 299, and 354 found	Yes	……..	……..

Final result (passed / failed) _____

C H A P T E R 5

PQ Reductive mode (disulfide bond reduction)

PQ procedure TiBlue electrode (DC mode)

For a successful performance test it is important that the ROXY EC has been installed as described and that the MS has been optimized for the analysis of the substrate. In case a Ti working electrode is used, please follow the instructions in the next chapter. Set up the system according to the conditions as indicated in table below.

Preparations

1. Prepare a test solution of 5 µg/mL Insulin. Fill the syringe & tubing with the substrate solution.
2. Fill the cell as described, avoid the inclusion of bubbles. Connect the outlet tubing to the grounding point and MS. Place the syringe in the syringe pump.
3. Load and start the event table 'Insulin reduction DC mode.evt' in Dialogue which runs the following steps in the procedure automatically.
4. In the case the test is performed manually: Start the syringe pump manually. Start measuring the signal with the cell off for about 10 min or until a good spectrum is obtained.
5. Switch on the cell in DC mode, increment the potential from -1.5 to -2.5 Volt in DC mode. Measure the substrate and metabolites at each potential setting for about 5-10 min until a good spectrum is obtained.
6. The test is passed when the substrate signal (m/z 1147.5) is below 30% at -2.5 Volt, and metabolite peaks appear at m/z 680.9 and 850.6.

Settings & conditions for TiBlue WE:

Configuration	ROXY EC/MS for disulfide bond reduction
Cell	ReactorCell or µPrepCell
Electrode	TiBlue WE
Solvent	5 µg/mL Insulin in 1% formic acid in a 50% acetonitrile and 49% water solution
Flow rate	10 or 50 µL/min (for ReactorCell or µPrepCell resp)
Mode	DC mode, Ec between -1.5 and -2.5V
Event table	Insulin reduction DC mode.evt

PQ procedure Ti electrode (Pulse mode)

For a successful performance test it is important that the ROXY EC has been installed as described and that the MS has been optimized for the analysis of the substrate. In case a TiBlue working electrode is used, please follow the instructions in the previous chapter. Set up the system according to the conditions as indicated in table below.

Preparations

7. Prepare a test solution of 5 µg/mL Insulin. Fill the syringe & tubing with the substrate solution.
8. Fill the cell as described, avoid the inclusion of bubbles. Connect the outlet tubing to the grounding point and MS. Place the syringe in the syringe pump.
9. Load and start the event table 'Insulin reduction Pulse mode.evt' in Dialogue which runs the following steps in the procedure automatically.
10. In the case the test is performed manually: Start the syringe pump manually. Start measuring the signal with the cell off for about 10 min or until a good spectrum is obtained.
11. Switch on the cell in Pulse mode with the pulse settings as mentioned in the table below, increment the E1 from -1.5 to -2.0 Volt. Measure the substrate and metabolites at each potential setting for about 5-10 min until a good spectrum is obtained.
12. The test is passed when the substrate signal (m/z 1147.5) is below 30% at -2.0 Volt, and metabolite peaks appear at m/z 680.9 and 850.6.

Settings & conditions for Ti WE:

Configuration	ROXY EC/MS for disulfide bond reduction
Cell	ReactorCell or µPrepCell
Electrode	Ti WE
Solvent	5 µg/mL Insulin in 1% formic acid in a 50% acetonitrile and 49% water solution
Flow rate	10 or 50 µL/min (for ReactorCell or µPrepCell resp)
Mode	Pulse mode, E1 between -1.5V and -2.0V, E2=0.5V, E3= 0.0V, t1=1.5s, t2=0.5s, t3=0s and ts=40 ms
Event table	Insulin reduction Pulse mode.evt

Results

Below mass spectra are from Insulin and its reductive metabolites, measured intensities vary from MS to MS. The molecule has a light chain and heavy chain that fall apart when the disulfide bridges are reduced.

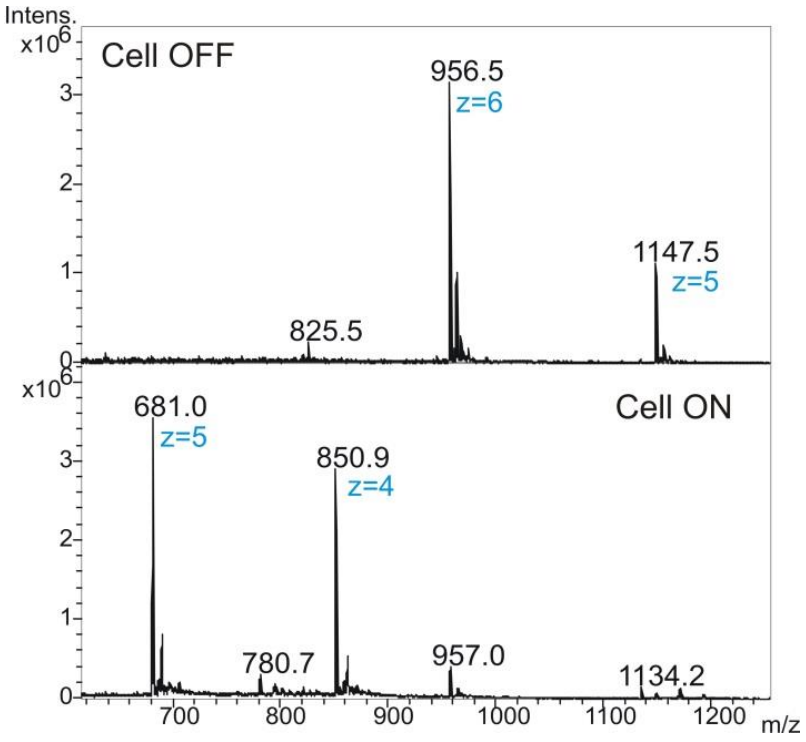


Fig. 7. Results of Insulin reduction (b). Cell off (a) shows the 100% level of the most abundant peak of Insulin (m/z 956.5).

	Specified	**Measured**	**Passed/Failed**
Substrate signal cell on vs off (m/z 956.5)	< 30%	……..	……..
The m/z 681.0 and 850.9 are found	Yes	……..	……..

Final result (passed / failed) _____

C H A P T E R 6

Trouble shooting

In Antec's knowledge base most common causes of problems can be found, with an explanation what to do. When starting up the ROXY it may happen that products are not immediately found in the MS spectra. Give the ROXY and MS some time to stabilize, and wait for the product to reach the MS.

If this does not help, there might be something wrong. The most common cause of problems are gas bubbles in the cell. Those can come from the solvent, or electrochemically generated by the EC reaction (electrolysis). In any way, bubbles fill the cell and disturb the EC reaction. The cell must really be bubble free.

To get rid of bubbles, switch off the cell. Disconnect outlet tubing and push the sample through the cell by using the syringe. You should observe the gas bubbles leaving the cell. Reconnect tubing and place the syringe in the pump before switching on the cell.

Another common cause is a problem with the solvents or standard solution. Prepare a fresh solution in case of any doubt.

See for more details and advise, for example the following Knowledge Base paper:

https://www.antecscientific.com/support/troubleshooting/knowledge-base?k=i-do-not-get-much-reaction-products

C H A P T E R 7

Dialogue Elite

Hands-on training

This hands-on training is intended for end-users who want to get familiar with the Dialogue software that is used for control and data-acquisition with the ROXY EC system.

Exercise 1. Setting DC potential and starting data acquisition.

1. Set EC Mode to DC
2. Set DC potential (E = 1V)
3. Switch the cell ON
4. Set the *Run time* to 3 minutes
5. Start data acquisition by: Options/Start analysis

After 3 minutes the acquisition will stop and excel file will open.

Exercise 2. Setting Pulse mode.

1. Choose EC Mode: Pulse
2. Set the Pulse settings to:
   E1 = 1V, E2 = 0.75V E3 = - 0.15V
   t1 = 400ms, t2=200ms, t3=400ms and ts =100ms
3. The Pulse will start immediately after checking *Cell ON* box. (If the data acquisition is required see the exercise 1 (Options→ Start analysis)!)

Exercise 3. Setting Scan mode.

1. Choose EC Mode: Scan (Changing EC Mode invokes “Cell OFF”)
2. Set Scan settings to: E1 = 0V, E2 = 3.5V, Scan: continuous,
   Rate: 50mV/s, number of scans: 3.
3. Check the *Cell ON* box (This command applies E1 potential only).
4. To start the scan, go to *Options → Start analysis* and start the acquisition (remember to set desired *Run Time*).

Exercise 4. Creating an events table

1. Choose Detection mode to *DC* at 0min.
2. Set syringe pump flow rate (*Syr Flow Rate*) to 5µL/min at 0.05min
3. Set the command Syr pump/Start infuse
4. Set the start signal to run MS (*Output A / Relay 1*) at 0.1min.
5. Don't forget to set it *"inactive"* at 0.2min.
6. Set the required *Ecell DC* potential (1V) at 0.3min.
7. Start the acquisition (*Acquisition ON/OFF*)
8. Choose the parameter to switch the cell on (*Cell on/off*) at 0.5min.
9. Choose the parameter to switch the cell off (*Cell on/off*) at 3.5min (the potential will be applied for 3 minutes).
10. Save the data by *Save chromatogram* command (ddd_exercise 4_nnn)
11. End the program by *End events* command at 5 min.

Check the User manuals & guides for maintenance procedure and operational details:

ReactorCell user manual	**p/n 210.7014**
µ-PrepCell 2.0 user manual	**p/n 204.0010A**
ROXY Potentiostat user manual	**p/n 210.7010**

For tutorials see screen recordings on Antec's website:

https://www.antecscientific.com/support/software-recordings/dialogue-elite-software-tutorials