

GUIDELINES ON THE USE OF ReactorCell AND ACTIVATED TITANIUM ELECTRODES

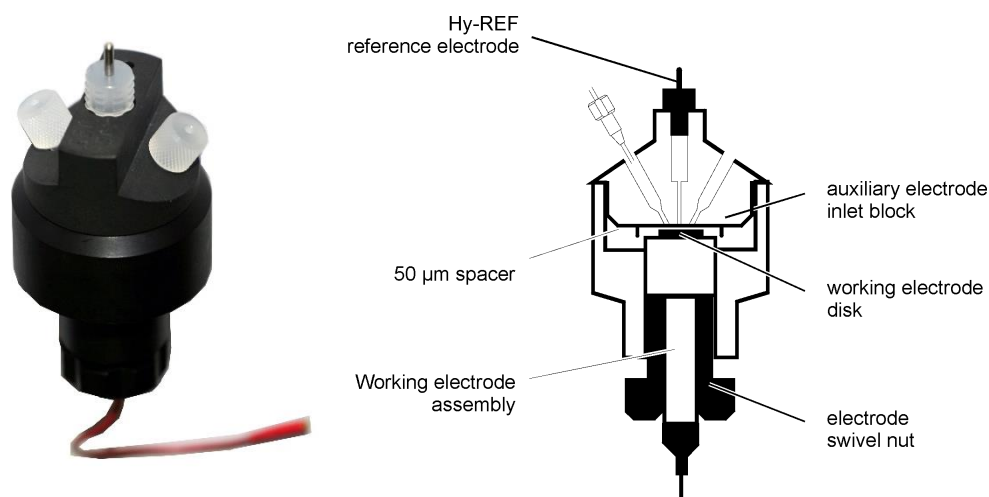


Fig. 1. ReactorCell.

General precautions

1. Always make sure that the surfaces of the spacer and working electrode are dry and free from particulate matter before assembling the cell. Clean fingerprints from spacer and electrode surfaces with acetone or methanol.
2. If the auxiliary electrode needs to be cleaned, wipe the surface careful with a soft tissue soaked in acetone or methanol. Do not apply force you may damage the electrode surface.
3. Use only our factory-supplied nuts to connect the tubing to the cell inlets, others may damage the internal thread of the cell inlets!
4. 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 (p/n 250.0035) must be used. If not used it may lead to damage of the ROXY potentiostat.

Never switch on the ReactorCell if:



- the 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.

Installation of the ReactorCell

The ReactorCell is shipped assembled!

The details, how to assemble, disassemble and install the cell, are described in the ReactorCell user manual (210.7014). In addition, the tutorial videos are available at:

<http://www.myantec.com/support/cell-and-reactor-videos/flexcell-and-reactorcell-instructions>

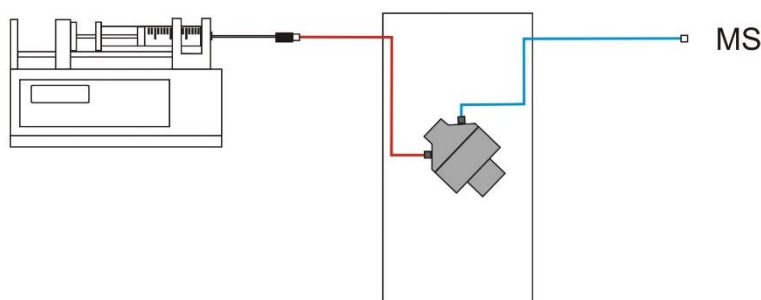


Fig. 2. Schematic drawing of the ReactorCell placed in a ROXY EC system.

Activated Titanium working electrode

- The surface of the electrode has a blue color.
- Place the electrode in the holder with blue side up (both sides are active).
- Do not touch the surface with fingers to prevent contamination.
- You may clean the surface by rinsing it with ethanol and gently drying with a soft tissue.



Do not polish Ti blue electrodes! The activated Titanium electrodes are disposable and should be replaced when its performance decreases.

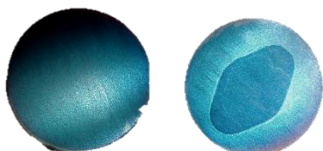


Fig. 3. Activated Titanium electrode: before (left) and after (right) use.

Settings for reduction of disulfide bonds in the test sample

1. Test sample: 5 μ g/mL insulin dissolved in solution described in the point 2.
2. A solution used for reduction of disulfide bonds should contain 1% formic acid in 50% acetonitrile.
3. Flow rate is 2 μ L/min
4. Potential DC = -2.0V

Solution composition can be optimized, if needed. Minimum content of formic acid is 0.5%. Increasing flow rate will result in less efficient reduction. The lower flow rate the efficiency of electrochemical reduction is increasing.

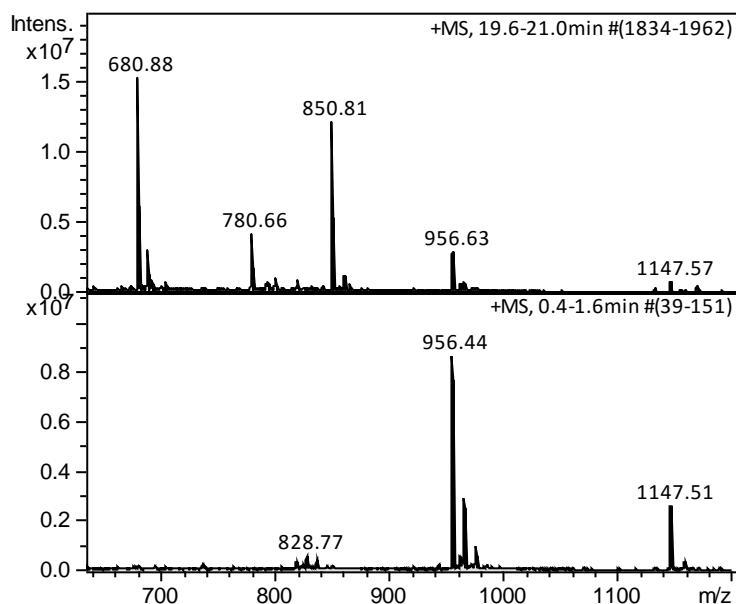
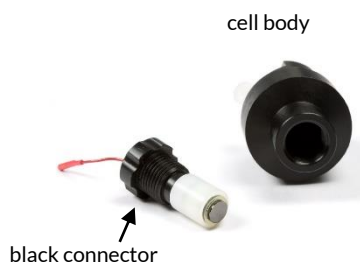


Fig. 4. Mass spectra recorded with cell off (bottom) and cell on ($E_{DC} = -2.0V$; top). We used HCT Ion trap mass spectrometer (Bruker Daltonik, Germany). The mass spectra may differ depending on applied settings and a type of a mass spectrometer.

Troubleshooting

The following actions can be taken if reduction efficiency decreased:

1. Switch the cell off. 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 the cell on.
2. Refill REF electrode chamber with electrolyte.
3. Disassemble and check for leakages. Clean the cell. Replace the spacer. There are spacers about 130 and 50 μ m thickness available. 50 μ m spacer may leak when working in high organic conditions. Ensure you are fixing the black connector **very tight** to the cell body to prevent leakage!



4. Replace the working electrode. Please note that activated titanium working electrode may require 20 min of initialization with potential ON.

APPENDIX

A list of chemicals is shown below as a guideline for the purchase of chemicals at the customer site. The listed brands/purities are not necessarily the best chemicals, but the applications were evaluated/developed at the Antec R&D laboratory using these specific brands/purities. If for any reason alternative chemicals need to be purchased use the following guidelines:

- The chemicals should have at least the same purity or better than the chemicals listed in the table below
- Do not purchase ultra-dry grade or anhydrous chemicals

Table 2. Brands and purities of chemicals used for application development at Antec.

Component	Purity	Brand	Order no:	Mw	kg/L
1. Insulin from bovine pancreas	Meets USP testing specifications	Sigma Aldrich	I8405- 100mg		
Formic acid	98% for mass spectroscopy	Fluka	94318- 250mL-F	46.03	
Acetonitrile	HPLC grade, 99.9%	Acros	268260025	41.05	D:0.781
Water	TOC <10ppb and deionized, resistivity >18 MOhm-cm (Barnstead Easypure II)				

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