µ-PrepCell™ SS
Reduction of S-S Bonds

- **Fast and efficient reduction of S-S bonds**
- **Long term stability and reproducibility**
- **Reagent free, no interfering DTT, TCEP**
- **Superior peptide sequencing and S-S bond assignment**

The new µ-PrepCell SS consists of a dual electrode set-up, i.e., working electrode and counter electrode, unlike its predecessor, which contained an additional reference electrode (3 electrode set-up). This new 2 electrode cell configuration results in distinguished advantages such as: superior stability and longevity, no undesired peptide/protein oxidation during the reduction and overall much easier in use. Another advantage of the new design is the much higher pressure stability of up to 350 bar, allowing for its use in HDX-MS and pre-column LC settings (EC-LC-MS).

Cell description

The inlet block of the cell (upper part) is made out of titanium and serves as working electrode, meanwhile the black lower part contains the rectangular counter electrode made of platinum, see Figure 1.

Figure 1: Ti inlet block used as active (working) electrode with groove for sealing O-ring (left), rectangular Pt counter electrode in recess of black electrode holder (right)

Pulse mode for highest stability and reduction efficiency

For highest stability and reduction efficiency of the S-S bonds the µ-PrepCell SS should be operated in pulse mode. In Figure 2, a typical pulse setting is shown. For most peptides/proteins including mAbs a pulse (E1) of 1 to 1.5 V is sufficient for the reduction of inter- and intra-molecular disulfide bonds.

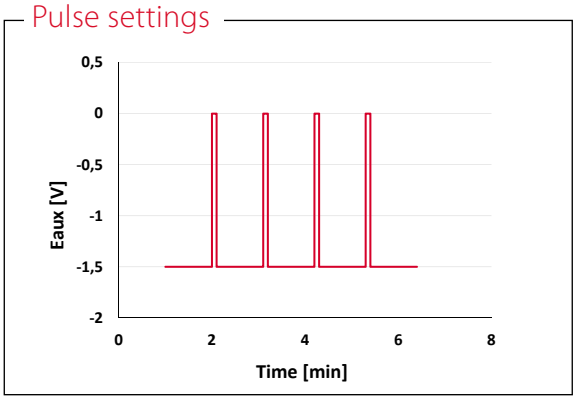

Figure 2: Applied pulse settings for reduction of S-S bonds in peptides/proteins. E1 = 1.5V, t1 = 1s, E2 = 0V, t2 = 0.1s, ts = 40 ms. Note: E1=1.5 is effectively -1.5V vs. the active surface of the Ti electrode

In Figure 3 the reduction of insulin is used to illustrate the stability and robustness of the new cell. The reduction efficiency remained unaffected and > 90% for over 100 injections without the need of

any electrode maintenance. The pictures shown in Figure 1 were actually taken from such a long-term experiment. The clean, shiny surfaces of both the Pt counter electrode as well as the Ti working electrode demonstrate the cleanliness of the reduction condition, resulting in overall stable and robust S-S bond cleavage.

Long term stability and reproducibility

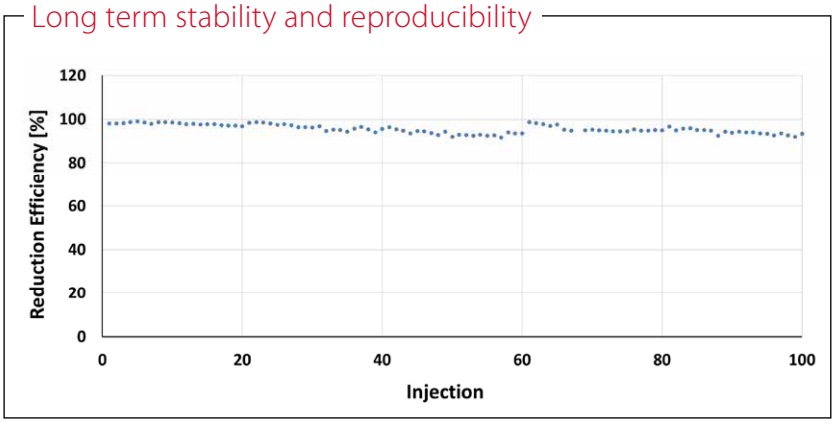

Figure 3: Long term stability and reproducibility of S-S bond cleavage measured by the reduction of insulin (> 90% reduction yield).

Reduction of Avastin

In Figure 4 the spectra of the intact and reduced Avastin (bevacizumab, Roche) Fab fragment after HPLC separation on a C4 column are shown. In Figure 4A the intact Fab fragment is shown with the post-column electrochemical cell off and in Figure 4B the reduced Fab fragment is depicted with the cell on (1 V, pulse mode).

From the deconvoluted MS spectrum in Figure 4B and the two fragments with mass 23435.25 Da for the light chain and mass 24612.86 Da for the heavy chain, the reduction of both the inter- and intramolecular disulfide bonds is unambiguously confirmed.

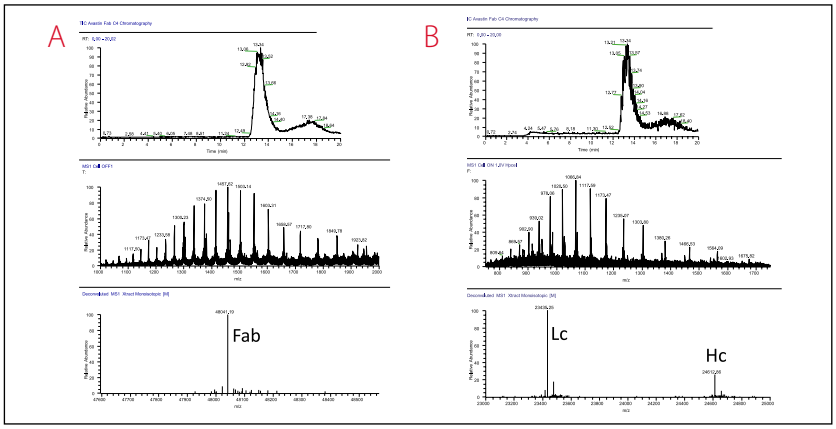

Figure 4: On-line LC-EC-MS of intact Fab fragment (A) and Fab fragment with electrochemically reduced S-S bonds (B). From top to bottom for (A) and (B): TIC, MS spectrum and deconvoluted MS spectrum with monoisotopic mass, measured on Orbitrap Fusion Lumos (Thermo). **Courtesy: Dr. Theo M. Luider, Yesim Ikiz and Dr. Martijn van Duijn, Erasmus Medical Centre, Rotterdam, The Netherlands**

Conclusion

With the new dual electrode µ-PrepCell SS, efficient and robust reduction of S-S bonds in top-down and bottom-up proteomics including HDX-MS becomes possible in routine.

Ordering information

204.4304	µ-PrepCell for S-S bond reduction, consisting of: µ-PrepCell with mounting bracket, cell cable, spacers, Ti working electrode (inlet block) and Pt counter electrode.

Replacement Electrode

204.5022	Platinum (Pt) counter electrode

Antec Scientific (USA)
info@AntecScientific.com
www.AntecScientific.com
T 888 572 0012

Antec Scientific (worldwide)
info@AntecScientific.com
www.AntecScientific.com
T +31 71 5813333

Antec® Scientific