

Protein renaturation and equilibrium dialysis with fully automated liquid handling and SBS compatible microdialysis devices

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Introduction

The removal of denaturing agents and equilibrium dialysis cabe time-consuming and difficult process. The scienova GmbH offers simple, cost efficient and fast dialysis systems like GridKit48 and ED300 in popular 96-well microplate formate. Through their patented design and low-binding regenerated cellulose membranes those "Xpress Micro Dialyzers" (MD) are easy to handle and have excellent sample recoveries. They can be used for an extensive variety of applications like:

• Removal of denaturating agents from samples

Sample dialysis of proteins, oligonucleotides, DNA, or RNA

- Equilibrium dialysis
- Removal of dyes

• Buffer exchange, rebuffering

High throuput enzyme reactivation by dialysis with liquid handling device

Trypsin was reversible inactivated in the presence of 8 M urea. Through removal of urea by dialysis about 75 % of the activity was regained (Fig. 2 A and B). It has been shown that up to 90 % of protein could be restored after urea removal by dialysis (BSA recovery, fig. 2C).



Fig. 1 | Fully automated handling of scienova Xpress Micro Dialyzer with Analytic Jena's liquid handling device Cybio® FeliX.

- Desalting

Free serum cortisol determination by equilibrium dialysis with ED300

The quantification of bioactive, free serum cortisol has high significance in medical diagnostics. The aim of this study was to demonstrate the quantification of free serum cortisol by equilibrium dialysis with scienova's *Xpress* Equilibrium Dialyzer ED300 and Analytik Jena's liquid handling device Cybio[®] FeliX.





Time	Sample (Inside)		Sample (outside)	
	Cortisol (nmol/ml)	SD	Cortisol (nmol/ml)	SD
0	0.182	0.00082	0.008	0.008
15	0.153	1.28.10-5	0.021	0.00794
60	0.108	0.00055	0.056	0.01545
120	0.079	0.00068	0.076	0.03631



Fig. 2 | (A) Release of PNA as an indicator for regained trypsin activity. (B) The decrease of urea concentration during dialysis. (C) BSA recovery after dialysis with MD100 devices.

The results show that a regain of about 75 % tryptic activity could be achieved after one hour. In total 48 samples have a low standard derivation which indicated a good constancy and reproductivity. The combination of scienova Xpress Micro Dialyzer MD100 GridKit48 and Cybio[®] FeliX enables high sample throuput without losing quality.

Material & Methods

Dialysis device: scienova *Xpress* Micro Dialyzer MD100 GridKit48

Liquid handling device: CyBi-Felix multi-channel/single-channel pipettor with Head R96/250 µl



Enzyme reactivation through urea removal: 100 µl samples, 0.5 mg/ ml trypsin, 20 mM CaCl2, 8 M urea (reference w/o urea). Samples were dialysed against 4.4 ml dialysis buffer (35 mM Tris HCl pH 7.8, 20 mM CaCl2.

Trypsin activity: 200 µl (4.7 mM DL-Benzoyl-Arg p-nitroanilline (DL BAPNA) in 10 % DMSO + 0.05 mg/ml trypsin in 35 mM Tris HCl pH 7.8 + 20 mM CaCl2).

Measurement: Photometer BioTek ELx800, 405 nm (measurement), 620 nm (reference).

Urea concentration: Wescor VAPRO 5520 Osmometer Protein determination according to Bradford.

0.17053 0.078 180 0.080 0.006

Concentration of free cortisol (nmol/ Tab. 1 ml) in human plasma after dialysis with ED300 device and liquid handling device Cybio[®] FeliX. Fig. 3 | Course of cortisol concentration during equilibrium dialysis in ED300 and Cybio[®] FeliX. Samples were taking from human plasma.

Scienova's modern dialysis devices ED300 are suitable for the usage in liquid handling devices, like the Analytik Jena Cybio[®] FeliX. The equilibrium is built up in less than 3 hrs (Fig. 3, Tab. 1). In combination with Cybio[®] FeliX the ED300 opens the opportunity of a high throuput sample preparation.

Material & Methods

Dialysis device: scienova *Xpress* Equilibrium Dialyzer ED300 3.5 kDa Liquid handling device: CyBio[®] Felix multi-channel/single-channel pipettor with Head R96/250 µl **Cortisol extration and analysis** with Cortisol ELISA-Kit (Neogen #402710). Cortisol extraction from human plasma and mesurement according to Neogen #402710 protocoll. Measurement: Photometer Tecan Sunrise 650 nm.

Hier fehlt noch das Logo BMWi-Logo mit Förderzusatz



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Video demonstartion: