

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 can be a 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 format. 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 denaturing agents from samples
- Sample dialysis of proteins, oligonucleotides, DNA, or RNA
- Buffer exchange, rebuffing
- Equilibrium dialysis
- Removal of dyes
- Desalting

High throughput enzyme reactivation by dialysis with liquid handling device

Trypsin was reversibly 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).

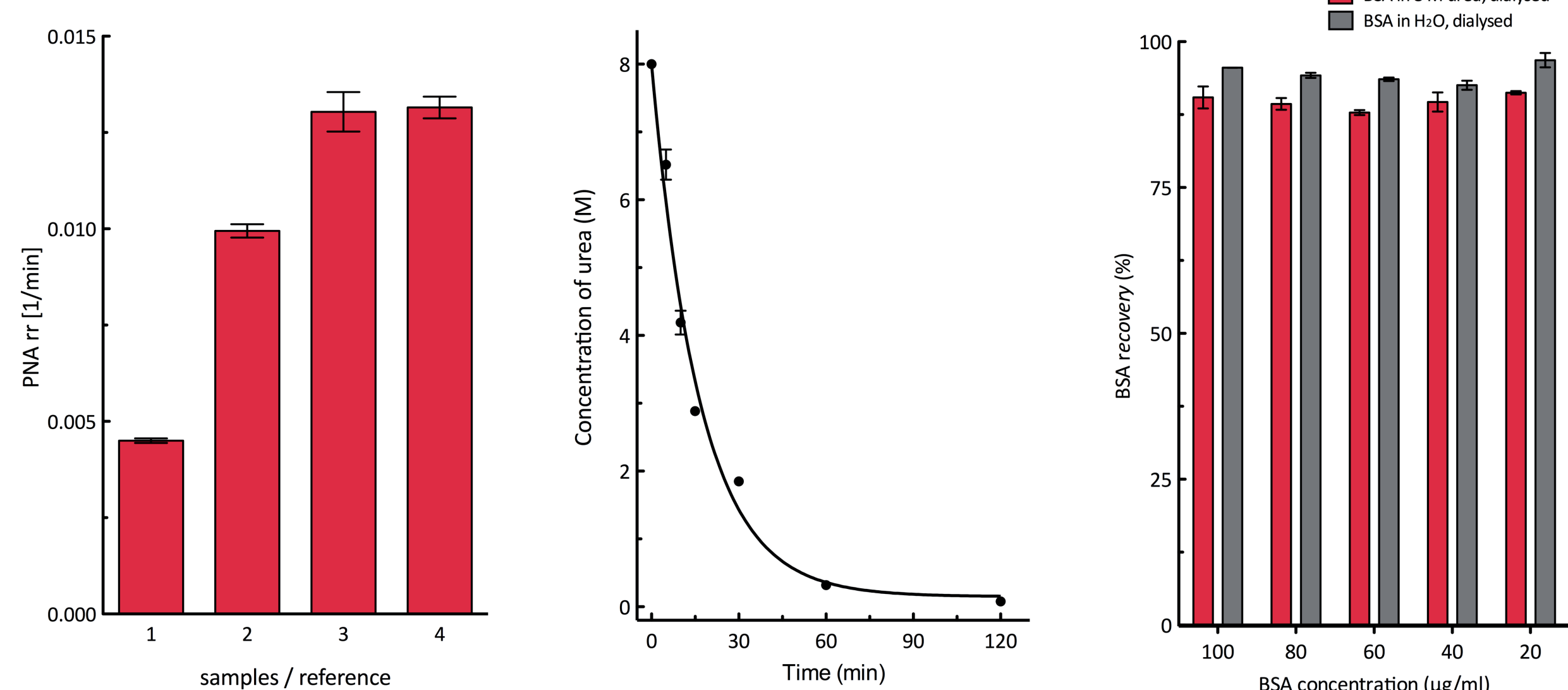


Fig. 2 | (A) Release of PNA as an indicator for regained trypsin activity. (B) The decrease of urea concentration during dialysis. (C) BSA recovery after dialysis with MD100 devices.

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 deviation which indicated a good constancy and reproducibility. The combination of scienova Xpress Micro Dialyzer MD100 GridKit48 and Cybio® FeliX enables high sample throughput without losing quality.

Material & Methods

Dialysis device: scienova Xpress Micro Dialyzer MD100 GridKit48
Liquid handling device: CyBio-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-nitroaniline (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



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

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
180	0.080	0.006	0.078	0.17053

Tab. 1 | Concentration of free cortisol (nmol/ml) in human plasma after dialysis with ED300 device and liquid handling device Cybio® FeliX.

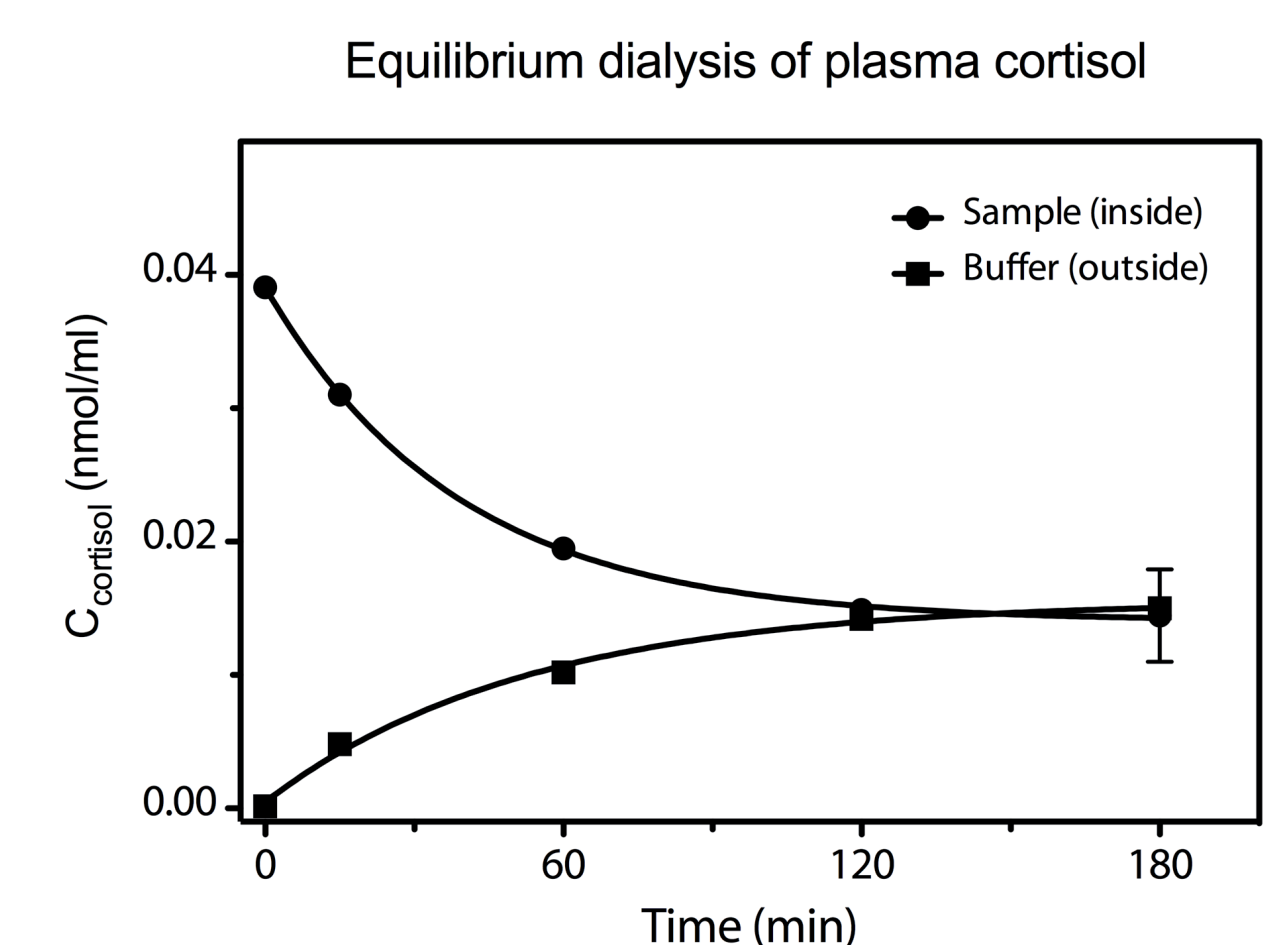


Fig. 3 | Course of cortisol concentration during equilibrium dialysis in ED300 and Cybio® FeliX. Samples were taken from human plasma.

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 throughput 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 extraction and analysis with Cortisol ELISA-Kit (Neogen #402710). Cortisol extraction from human plasma and measurement according to Neogen #402710 protocol.
Dialysis conditions: Sample vol. 150 µl, buffer vol. 650 µl, room temperature,
Dialysis buffer: 10 mM PBS (phosphate-buffered saline)
Measurement: Photometer Tecan Sunrise 650 nm.



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