SPECIAL APPLICATION NOTE
CyBi®-FeliX

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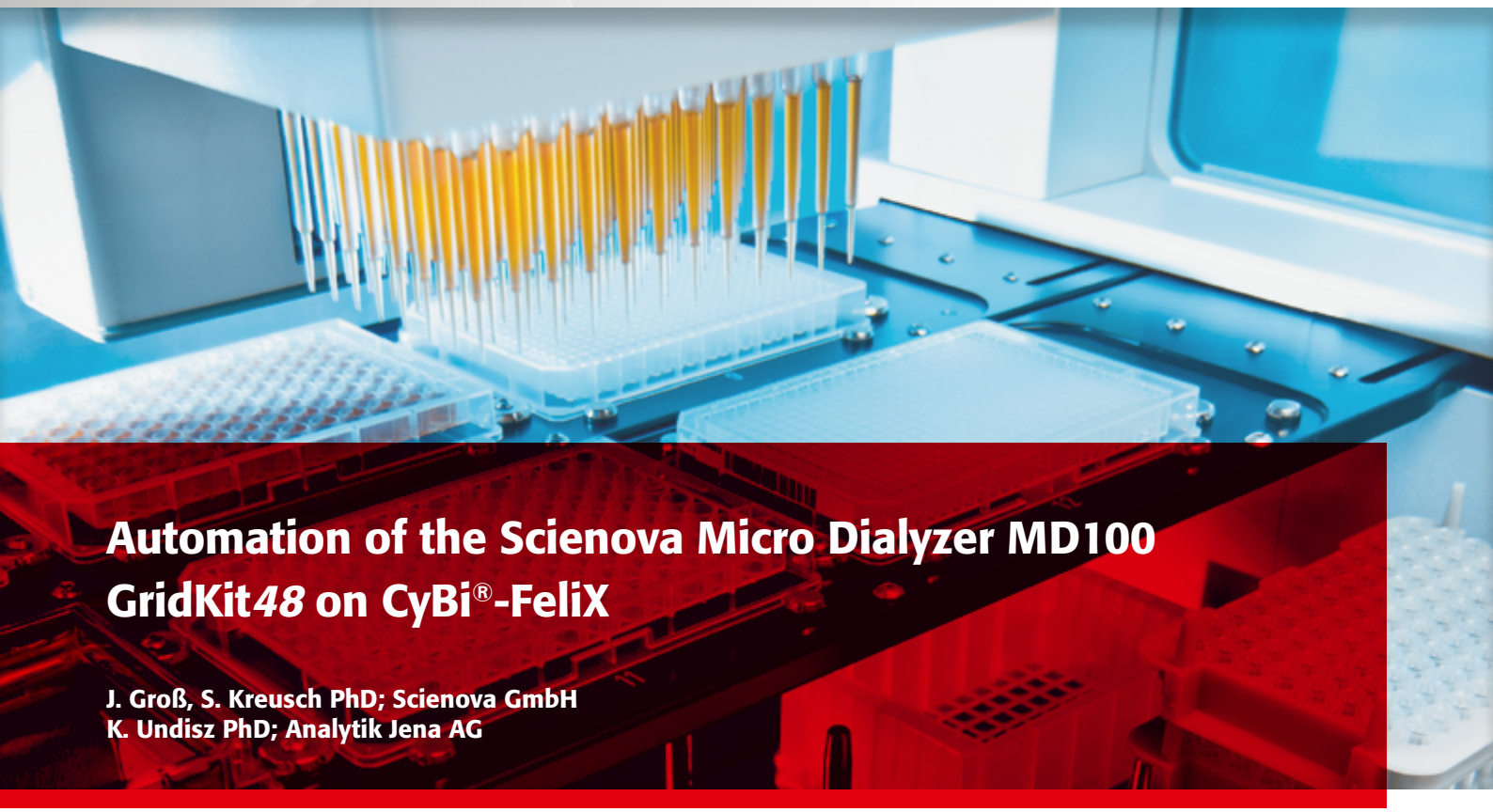

Automation of the Scienova Micro Dialyzer MD100 GridKit*48* on CyBi®-FeliX

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ABSTRACT

The CyBi®FeliX automated liquid handling system with Head R 96/250 µl was used in conjunction with the Scienova MD100 GridKit48 for complete dialysis process of enzyme reactivation through urea removal and protein recovery. The procedure was shown to significantly increase sample throughput and processing accuracy.

INTRODUCTION

The removal of denaturing agents can be time-consuming and difficult. Therefore the Scienova GmbH offers a simple, cost-efficient and fast dialysis system in the 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 denaturating agents from samples
- Sample dialyses of proteins, oligonucleotides, RNA or DNA
- Buffer exchange, rebuffering
- Removal of dyes
- Desalting
- Sample concentration

Figure 1: One cartridge of micro dialyzers MD100 with 8 micro dialyzer units

To simplify and improve the dialysis process, Xpress Micro Dialyzers is combined in a kit which enables the simultaneous handling of up to 48 samples without human intervention.

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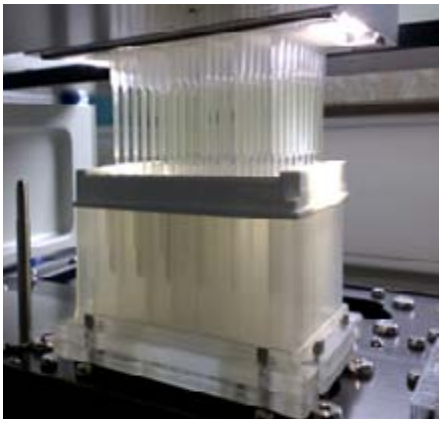
AIM

Trypsin is reversibly inhibited in the presence of high urea concentrations. To regain tryptic activity, by paranitroaniline (PNA) release, urea removal is needed, which is achieved through dialysis.

DL – benzoyl– Arg p – nitroaniline $\xrightarrow{\text{Trypsin}}$ p – nitroaniline + benzoyl – Arg

To show that there is no sample loss in consequence of protein binding with the membrane, different bovine serum albumin (BSA) solutions were dialyzed for two hours and the recollected samples were analyzed.

MATERIALS AND METHODS



CyBi®-FeliX

The CyBi®-FeliX is a multi-channel/single-channel pipettor for automated liquid handling. Depending on the specific application needs different pipetting heads and liquid handling adapters are available. The CyBi®-FeliX with the Head R 96/250 µl enables a simultaneous handling of up to 96 samples on 12 deck positions.

Figure 2: CyBi®-FeliX with Head R 96/250 µl in combination with Scienova MD 100 GridKit*48*

Scienova MD100 GridKit*48*

The Scienova GridKit*48* consists of a 48-deep well plate, a grid and six cartridges à eight Micro Dialyzers (48 samples) with the chosen cutoff (3.5, 6-8, 12-14 kDa).
By the help of the grid, all Micro Dialyzers have a well-defined position and a good grip. Additionally the free positions in the grid can be used for buffer handling while dialysis. For the following experiments MD 100 cartridges with a cutoff from 6-8 kDa and a maximal sample volume of 100 µl were used.

Figure 3:
Scienova MD100 GritKit*48*

Experiment 1: Enzyme reactivation through urea removal	
Dialysis samples	100 µl trypsin samples: ***1.*** 0.5 mg/ml trypsin in 8 M urea, 20 mM CaCl2 ***2.*** 0.5 mg/ml trypsin in 20 mM CaCl2 as reference
Dialysis buffer	4.4 ml of dialysis buffer (35 mM Tris pH 7.8, 20 mM CaCl2)
Measurement solution	200 µl (4.7 mM DL-Benzoyl-Arg p-nitroaniline (DL BAPNA) in 10 % DMSO + trypsin 0.05 mg/ml in 35 mM Tris pH 7.8, 20 mM CaCl2)
Determination method	Photometer BioTek ELx800, 405 nm (measurement wavelength) and 620 nm (reference wavelength) PNA-release rate (rr) indicating tryptic activity is evaluated as: $rr = \frac{\text{absorbance}_{405\ nm} - \text{absorbance}_{620\ nm}}{\Delta t \text{ in min}''}$
Urea determination	Wescor VAPRO 5520 Osmometer

Micro Dialyzers were placed in 4.4 ml dialysis buffer into the grid and filled with 100 µl trypsin sample by CyBi®-FeliX. Forty samples (n=40) with urea and eight samples (n=8) without urea as reference were dialyzed 60 min at room temperature (22 °C). After incubation time the samples were transferred to a 96-well microplate for measurement.

Experiment 2: BSA recovery	
Dialysis samples	***1.*** 100 µl of BSA solution in 8 M Urea (n = 6) ***2.*** 100 µl of BSA solution in A. deion. (n = 2) as reference for each concentration: 10, 20, 40, 60, 80 and 100 µg/ml
Dialysis buffer	4.4 ml of A. deion.
Determination methods	Protein determination according to Bradford, Tecan Sunrise photometer: λ (590/450 nm) Urea determination: Wescor VAPRO 5520 Osmometer

Micro Dialyzers were placed in 4.4 ml A. deion. into the grid and filled with 100 µl BSA sample by CyBi®-FeliX. For each concentration six samples (n=6) and two references (n=2) were dialyzed for 120 min at room temperature (22 °C). After Incubation time the samples were transferred to a 96-well microplate for measurement.

RESULTS ENZYME REACTIVATION

The following table shows the PNA-release rate (rr) depending on the urea concentration.

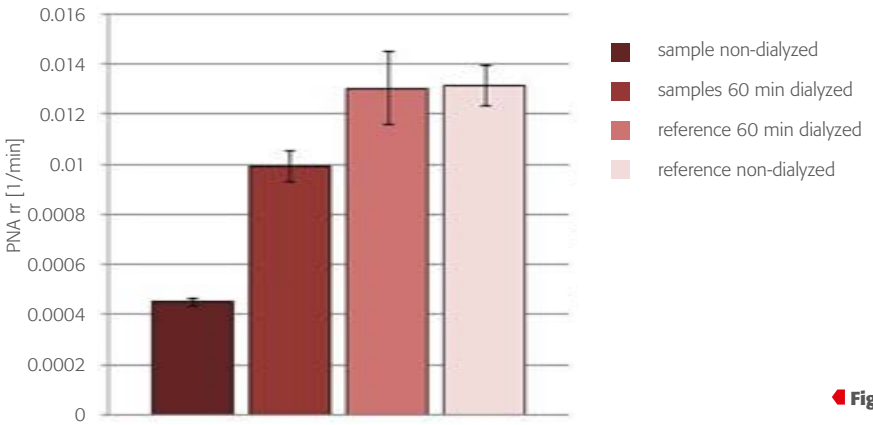
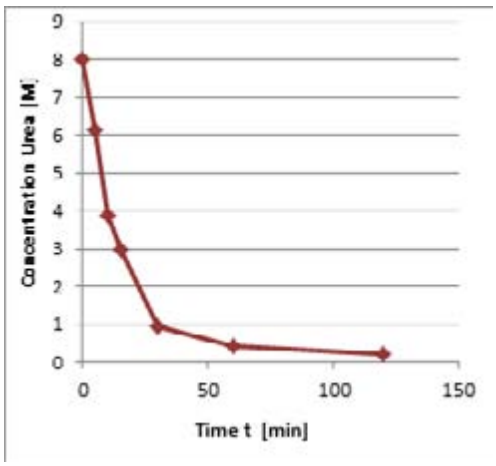


Figure 4: Regain of tryptic activity

	sample non-dialyzed	sample 60 min dialyzed	reference dialyzed	reference non-dialyzed
Mean PNA release rate (1/min)	0.0045	0.0099	0.0130	0.0131
Standard deviation	0.0001	0.0006	0.0014	0.0008
Urea concentration (M)	8	0.30	0	0

After one hour of dialysis the urea concentration dropped from 8 M to 0.30 M.



The results show that a regain of about 75 % tryptic activity could be achieved after one hour. In total all 48 samples have a low standard deviation which indicates a good constancy and reproducibility. The combination of Scienova MD100 GridKit48 and CyBi®-FeliX enables a high sample throughput without losing quality, useable for all applications of Scienova Micro Dialyzers.

Figure 5: Urea Removal

RESULTS BSA RECOVERY

The following figures contain all recovery values compared with the references.

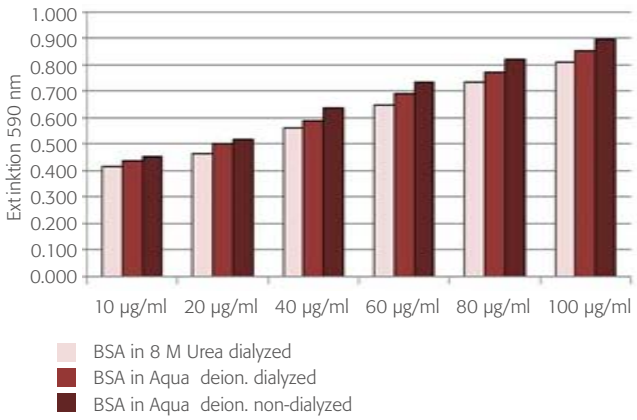


Figure 6: Extinction of different BSA samples at 590 nm

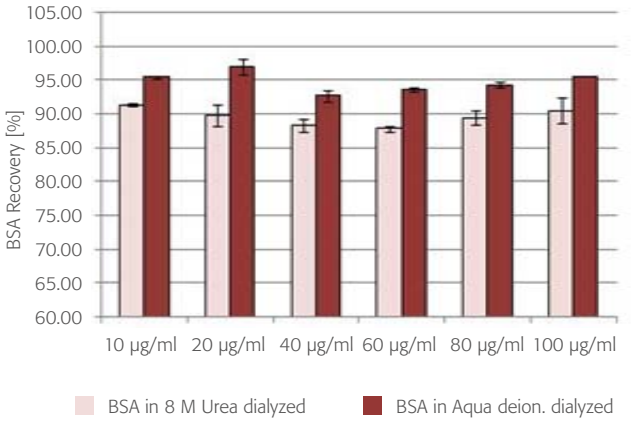


Figure 7: BSA recovery in %

	sample non-dialyzed	sample 60 min dialyzed	reference dialyzed	reference non-dialyzed
Mean PNA release rate (1/min)	0.0045	0.0099	0.0130	0.0131
Standard deviation	0.0001	0.0006	0.0014	0.0008
Urea concentration (M)	8	0.30	0	0

After two hours of dialysis the urea concentration dropped from 8 M to 0.23 M. The recovery values display a range of 87 to 90 % independent from the protein concentration.

DISCUSSION

As a result of repeated implementation the BSA recovery rates were significantly smaller (up to 5 %). There are no significant differences between automated and manual process. One reason for a difference is the high mobility of urea in solutions and the short diffusion distances in Scienova Xpress Micro Dialyzer. There is only a minor osmotic effect, resulting in a slightly higher volume recovery of about 110 µl. An advantage of automation is the small range of variation which also exists for volumes down to 25 µl. The manual filling and emptying of 48 micro dialyzers might take up to 20 min, whereas the CyBi®-FeliX manages it in less than one minute. Also manual pipetting errors will be eliminated. This BSA assay is an example to show the stability of proteins inside the Micro Dialyzer. It is recommended to make a pretest, because other proteins maybe behave differently. The enzyme reactivation shows, that an immediately automated removal by dialysis of denaturing agents is possible. Based on the results, the Scienova MD100 GridKit48 is one of the first disposables for a complete automation of dialysis process. The accurate height adjustment in tenth of millimeter and the simultaneous handling by CyBi®-FeliX of up to 48 samples enables a high throughput of samples to dialyze, efficiency can be further increased by automated mixing or changing the buffer while dialysis is running.

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