*Desalting, Rebuffering, Renaturation – Dialysis for Optimized Sample Preparation (and II)**

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Rule dialysis buffer: buffer should be compatible with sample molecules and the following analysis or preparation steps, if necessary check the osmotic pressure in a pretest. Selection of buffer volume depends on the required end concentration of the substance which should be removed. Mostly an exchange of dialysis buffer is recommended in order to save dialysis buffer and time.

Which sample volume is needed?
Selection according to manufacturer's data.
Suitable cut off?
Half of the molecular weight of the retained molecule, double of molecular weight of the removable compounds.
Costs per sample?
Price for dialysis device + costs for operational hours + costs for dialysis buffer.
Requirement of additional equipment?
Selection according to manufacturer's instructions and application protocol for additional equipment.
For higher sample numbers: Ready for automated handling?
Selection according to manufacturer's instructions and application protocol for compatibility with microplate format and automation.

3.8. Selection of the best dialysis device

Main point for optimal dialysis process is the selection of a suitable dialysis device. There are different products of several suppliers on the market (Table 1, see part I page 590).

Figure 5. 96 deep well plate Riplate® (Ritter Medical) with 12 cartridges ED300 (scienova GmbH) dialysis cartridges for 96-fold dialysis.

Every user has to ask some simple questions to find the optimal device for his task. Diffusion is the force for the transportation process of the dialysis. According to Fick's first law the flux depends in inverse proportion of the diffusion length. Therefore the geometry of the dialysis device is essential for dialysis speed. The diffusion length is the distance between the membrane at one side to the membrane at the other

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side. It should be as short as possible. The dialysis buffer should be stirred or shaken. The dialysis buffer movement avoids a concentration gradient of the buffer and raise the dialysis speed.

A new development is a Deep Well Plate in combination with dialysis cartridges (Figure 5).

Deep Well Plates are common for storage of samples with higher volumes then 300 µl in microplate format but also as reaction vessel and bioreactor for cell culture. In case of the new dialysis application, the Deep Well Plate is used as dialysis buffer container and the cartridge is placed in the wells of the plate. The system has the following advantages:

1. *Geometry: short diffusion ways and vertical membranes*
2. *High volume recovery*
3. *Easy handling with standard lab pipettes*
4. *Ready for automation*
5. *Easy and downscaling of sample number.*

An example for an application with this new system is given below.

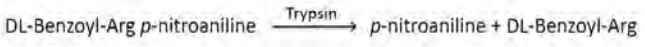
Rule dialysis device: Select according to manufacturer's data the sample volume and cutoff. The diffusion length should be as short as possible. Easy handling with the available lab equipment.

4. Dialysis application

Dialysis is suitable and gentle method for the removal of denaturing agents. But it can be time-consuming and difficult and was limited in handling high sample quantities.

In the following application example of enzyme reactivation by urea removal, we demonstrate the possibility to handle 48 separate dialysis samples with an automated liquid handling device in one single process.

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



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.

4.1. Methods & material

Dialysis samples: *100 µl trypsin samples (0.5 mg/ml trypsin in 8 M urea, 20 mM $CaCl_2$), reference sample (0.5 mg/ml trypsin in 20 mM $CaCl_2$), dialysis buffer: 4.4 ml of dialysis buffer (35 mM Tris·HCl pH 7.8, 20 mM $CaCl_2$).*

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·HCl pH 7.8. 20 mM $CaCl_2$).*

Determination method: *Photometer BioTek ELx800. 405 nm (measurement wavelength) and 620 nm (reference wavelength), PNA-release rate (rr) indicating tryptic activity is evaluated (see below):*

$$rr = \frac{\text{aborbance}_{405\ nm} - \text{absorbance}_{620\ nm}}{\Delta t\ (min)}$$

Urea determination: *Wescor VAPRO 5520 Osmometer.*

Dialysis device: *scienova Xpress Micro Dialyzer MD100 GridKit48 (48 dialysis samples in 6 cartridges with 8 sample segments each, delivered in a Riplate® 48-deep well plate, Ritter Medical).*

Liquid handling device: *CyBi®-FeliX, multi-channel / single-channel pipettor for automated liquid handling, head R96/250 µl, Analytik Jena.*

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 minutes at room

temperature (22 °C). After incubation time the samples were transferred to a 96-well microplate for measurement.

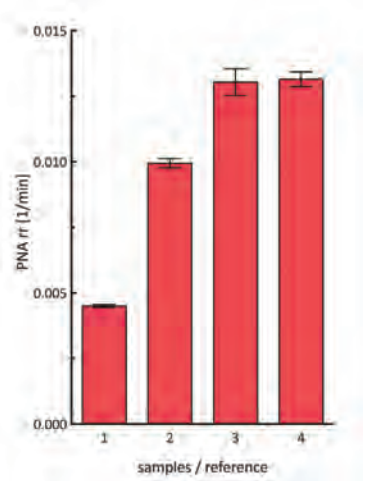


Figure 6. Regain of tryptic activity after dialysis (urea removal): 1: sample non-dialyzed, 2: samples 60 minutes dialyzed, 3: reference 60 minutes dialyzed, 4: reference non-dialyzed. Trypsin activity is illustrated in PNArr.

4.2. Results

After 1 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 modern dialysis device and liquid handling automate enables a high sample throughput without losing quality, useable for all dialysis applications (Figures 6 and 7).

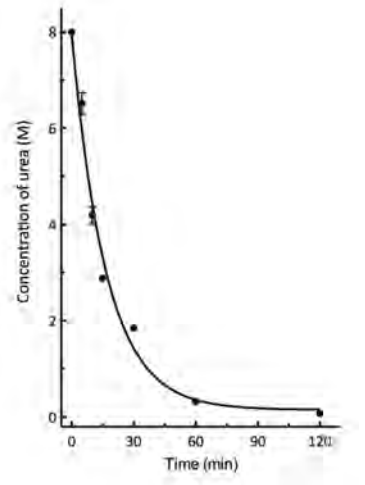


Figure 7. Urea removal during dialysis: The concentration of urea is shown in dialyzed samples.
A scienova Xpress Micro Dialyzer MD100 GridKit48 in 48 deep well Riplate® (Ritter Medical) and Analytik Jena CyBi®-FeliX (head R96/25 µl) were used. Performed at room temperature.