ZelluTrans/Roth Mini Dialyzer (MD300)

Vol. 50-300 µl

MWCO	1 x MD300 in microtube	12 x MD300 in microtube	1 strip (= 8 MD300)
3500 (3.5 kDa)	NH90.1	NH91.1	NH92.1
6000-8000 (6-8 kDa)	NH93.1	NH94.1	NH95.1
12000-14000 (12-14 kDa)	NH96.1	NH97.1	NH98.1
Schwimmhilfe/Float			4768.1
Abdeckfolie/Sealing Film			4781.1

Technical Data Sheet and Applications

Content

Technical Data Sheet

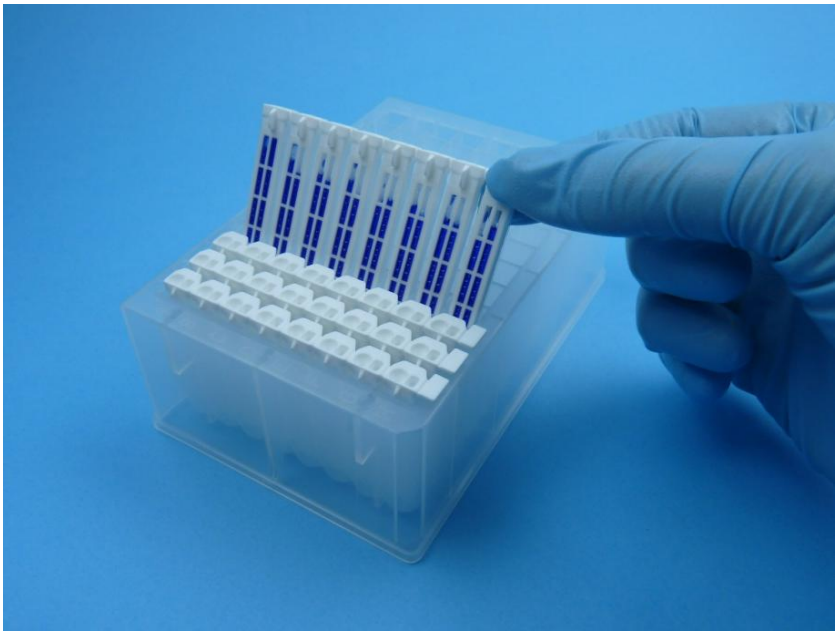
ZelluTrans/Roth Mini Dialyzer (MD300)

Specifications:

- max. sample volume 300 µl, min. sample volume 50µl
- mass: 4.9 g (dry)
- dimensions: max. length = 85 mm, max. width = 41 mm, max. thickness = 8 mm
- temperature 1-60 °C
- for aqueous solution
- incubation time up to 24 h
- pH 3-10
- cut off 3.5, 6-8, 12-14 kDa
- storage 4-22°C
- cartridges with 8 samples, scalable from 1 to 96 samples
- conform to Microplate Standard (SBS)
- usable with 2.2 ml Deep Well Plate as dialysis buffer container
- membrane: low binding regenerated cellulose, contains glycerole to prevent embrittlement and traces of elements like sulphides and heavy metals

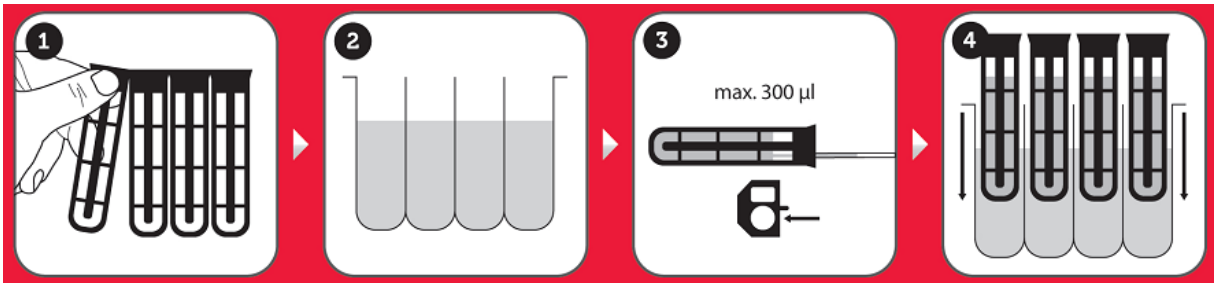
Applications:

- Protein and peptide sample purification e.g. desalting before mass spectrometry
- Optimization of protein renaturation with different renaturation buffers and steps
- Removal of dyes after protein labeling
- Protein sample desalting and rebuffering
- Glycoprotein modification and engineering
- Protein in vitro translation
- Enzyme activity assays
- Plasmid or primer purification



Manual – English Version

ZelluTrans/Roth Mini Dialyzer (MD300)



Preparation

For smaller number of samples, break carefully the desired number of segments from the MD300. Please don´t touch the membrane!

Buffer Preparation

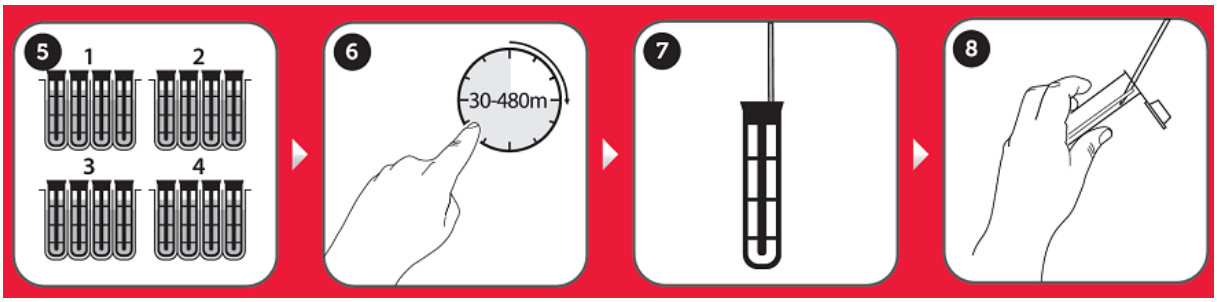
Pipette dialysis buffer either in
a) a 96-deep-well plate (2.2 ml/well), V< 1.3 ml, or
b) a 48-deep-well plate (5 ml/well), V< 3.5 ml.

Loading the sample

Insert the pipette tip with the sample into the round opening. We recommend to hold the MD300 horizontally for safer and easier loading of the sample. Max. volume is 300 µl.

Introduction

Insert the MD300 cartridge (or single segments of it) into the prepared deep-well plate.



Dialysis

One step dialysis can be done in one well of the deep-well plate. If more than one dialysis step is required, simply change the position of the MD300 in the deep-well plate to wells with fresh buffer. It is also possible to use a float for dialysis.

Dialysis Time

The dialysis time is depending on the used compound and the cut-off of the semipermeable membrane. For the MD300, typical dialysis times range from 1 h to 8 h, with repeated replacement of the dialysis buffer. Replacement of the buffer should occur after about 30 min to 1 h depending on the compounds.

Sample retrieval

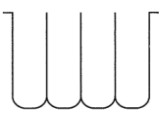
Set the pipette volume to 450 µL and press the pipette button to the first stop, hold it and bring the pipette upright into the round opening with a little bit of pressure. Aspirate the sample.

Further analytics

Finally, pipette the sample into a microcentrifuge tube or a microtest plate for further analytics.

Materials Required

Single dialyzer or dialyzer strip (consisting of 8 connected single dialyzers)



Deep well plate 2.2 ml or 5 ml volume (96 or 48 samples, respectively)

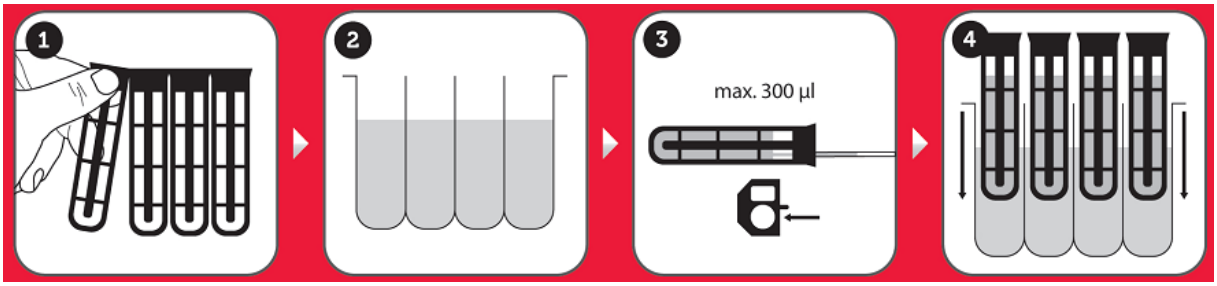
Pipette for 100-1000 µl and 1-5 ml, each with suitable pipette tips

Microcentrifuge tubes or micro-test plates for sample storage

Suitable protection gloves and goggles

Manual – German Version

ZelluTrans/Roth Mini Dialyzer (MD300)



Vorbereitung

Falls die Verwendung von einzelnen oder mehreren Segmenten des MD300 gewünscht ist, werden diese vom MD300 vorsichtig abgebrochen. Bitte die Membran nicht berühren!

Vorlegen

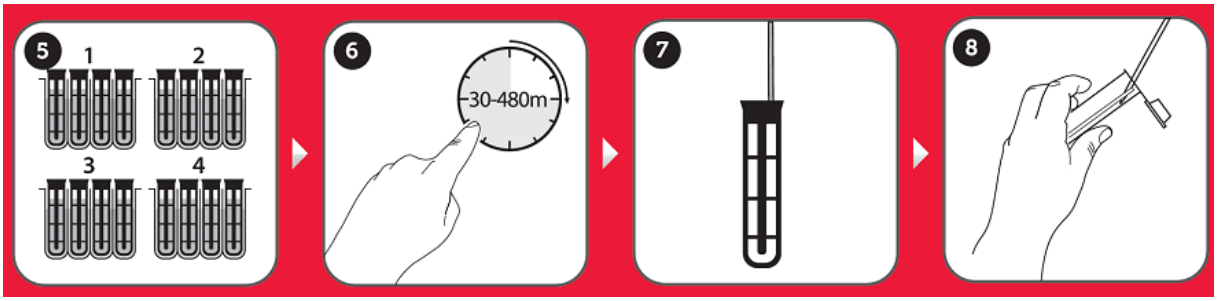
Vorlegen des Dialyse-puffers entweder in a) eine 96-deep-well Platte (2,2 ml/well), V< 1,3 ml oder in b) einer 48-deep-well Platte (5 ml/well), V< 3,5 ml, bzw. schwimmende Dialyse mit einem Floß.

Probe einfüllen

Zum Füllen der Segmente, die Pipettenspitze mit Probe in die runde Öffnung einführen, leicht andrücken und die Probe einfüllen. Für ein einfacheres Befüllen empfehlen wir, den MD300 waagrecht zu halten.

Einsetzen

Setzen Sie den MD300 in die mit Dialysepuffer vorbereitete Deep-well Platte.



Mehrfach-Dialyse

Man kann die Dialyse entweder in einem Schritt durchführen oder durch Wechsel des Dialysepuffers in mehreren Schritten dialysieren. Der Wechsel kann durch einfaches Umsetzen des MD300 innerhalb der Deep-well Platte erfolgen.

Dialyse-Zeit

Die Dialysezeit hängt von der zu dialysierenden Substanz ab und der Ausschlussgröße der Membran. Bei dem MD300 ist die typische Dialysezeit zwischen 1 und 8 Stunden mit wiederholtem Wechsel des Dialysepuffers (je nach Substanz etwa alle 30 bis 60 min).

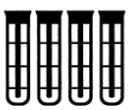
Entnahme der Proben

Für die Rückgewinnung der Proben sollte die Pipette auf ein Volumen von 450 µl eingestellt werden. Die Pipettenspitze wird in die runde Öffnung eingeführt und leicht angedrückt und die Probe herauspipettiert.

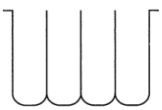
Weitere Verwendung

Für weitere Analysen wird die Probe in ein vorbereitetes Gefäß pipettiert.

Materialien



Microdialyzer mit bis zu 8 Segmenten (MD300) oder Einzelsegmente



Wahlweise Deep-well Platte mit 2,2 ml oder 5 ml Volumen (96 oder 48 Proben)

Pipette 100-1000 µl sowie Pipette 1-5 ml mit jeweils passenden Spitzen

Mikrozentrifugen-röhrchen oder Mikrotiterplatten als Probengefäß

Geeignete Schutzhandschuhe und Schutzbrille

Chemical resistance

ZelluTrans/Roth Mini Dialyzer (MD300)

Materials and Methods

MWCO 3,5, 6-8, 12-14 kDa

Test Sample: 100 µl of Congo Red color solution in water dd

Dialysis solution: 1.4 ml of tested chemical

Incubation: 18 h

Determination Method: Optical integrity and leak-tightness to air pressure

Acetonitrile	Good
Acetone	Good
Chloroform	Good
Dimethyl sulfoxide	Good
Ethanol 70%	Good
Ethanol 98%	Good
Ethylacetate	Good
Ethylene glycol	Good
Glycerol	Good
n-Hexane	Good
iso-Propanol	Good
Methanol 98%	Good
Methylene chloride	Good
1-Propanol	Good
Tetrahydrofuran	Good
Toluene	Good

Hydrogen peroxide 30%	Good

Acetic acid 25%	Good
Acetic acid 96%	Good
Formic acid 25%	Good
Formic acid 100%	No
Hydrochloric acid 10%	Limited
Hydrochloric acid 25%	No
Hydrochloric acid 37%	No
Hydrofluoric acid 50%	No
Nitric acid 25%	No
Nitric acid 65%	No
Phosphoric acid 25%	Limited
Phosphoric acid 85%	No
Sulfuric acid 98%	No

Ammonium hydroxide 1N	Limited
Ammonium hydroxide 25%	Limited
Potassium hydroxide 1N	Limited
Potassium hydroxide 32%	No
Sodium hydroxide 1N	Limited
Sodium hydroxide 32%	No

Good chemical resistance	Good
Limited chemical resistance, e.g. pore size cannot be guaranteed	Limited
No chemical resistance, use not recommended	No

Sample Volume Recovery

For successful experimental work, it is not only necessary to dialyze the samples fast, but also to retrieve as much as possible from the sample volume.
With ZelluTrans/Roth Mini Dialyzer (MD300), the sample recovery rate is more than 90 % in average. To achieve best results, the pipette has to be adjusted to a bigger volume, e.g. 450 µL.

Experimental conditions:

ZelluTrans/Roth Mini Dialyzer (MD300)
MWCO 6-8 kDa

Sample: 50 µl, 150 µl or 300 µl respectively of Aq. dest.

Recovery: Pipette adjusted to 450 µL. Water was recovered at once after filling in order to prevent negative influence due to evaporation caused by the big surface area.

Determination Method: Gravimetry

Number of samples: n=7.

MWCO: molecular weight cut off

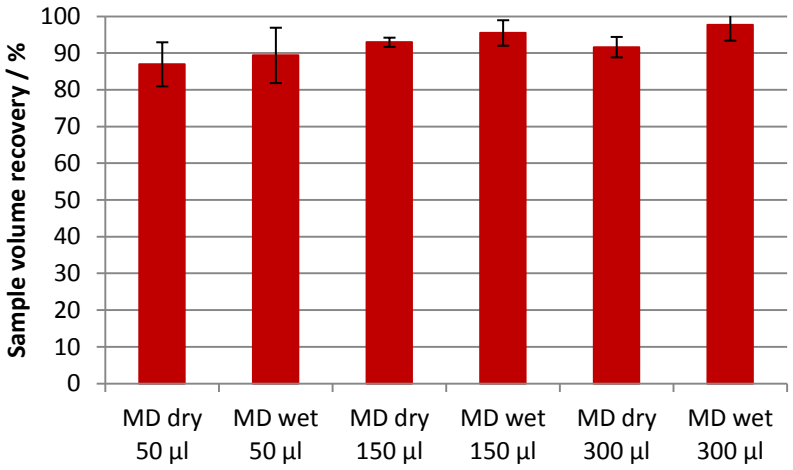


Figure 1: Comparison of sample volume recovery with dry and prewetted ZelluTrans/Roth Mini Dialyzer (MD300), using samples of 50 µl, 150 µl or 300 µl Aqua dest respectively, n=7, error bars indicate standard deviation.

Table 1: Data

Sample	Recovery %	SD %
MD dry 50 µl	86.94	6.00
MD wet 50 µl	89.37	7.54
MD dry 150 µl	92.95	1.25
MD wet 150 µl	95.48	3.47
MD dry 300 µl	91.62	2.78
MD wet 300 µl	97.72	4.33

Comparison of dialysis speed of ZelluTrans/Roth Mini Dialyzer (MD300), with different MWCO

When comparing the different available molecular weight cut-offs of the ZelluTrans/Roth Mini Dialyzer (MD300), it becomes evident that the MWCO of 3.5 kDa is in tendency slower than the others. The reason for this can be found in the smaller size of the pores in membrane. But for everyday use, this difference is negligible small and all MWCOs can be used in the same way.

Experimental conditions:

ZelluTrans/Roth Mini Dialyzer (MD300)
MWCO 3.5 kDa, 6-8 kDa and 12-14 kDa

Sample: 300µl of 1mM *p*-Nitrophenol (MW 139.11 g mol^{-1}, CAS 100-02-7) in PBS (Phosphate buffered Saline, 145 mM NaCl (58,44 g·mol^{-1}, CAS 7647-14-5), 10 mM Na_2HPO_4 (141,96 g·mol^{-1} CAS 7558-79-4 (wasserfrei), pH 7.4).

Dialysis buffer: 3.5 ml of PBS, pH7.4.

5 mL Deep-well plates (for 48 samples).

Buffer exchange interval 60 min. Samples were taken as indicated.

Determination Method for *p*-Nitrophenol: Tecan Sunrise Photometer, 420 nm (vs. 620 nm as reference wavelength).

Number of samples: n=3.

MWCO: molecular weight cut off

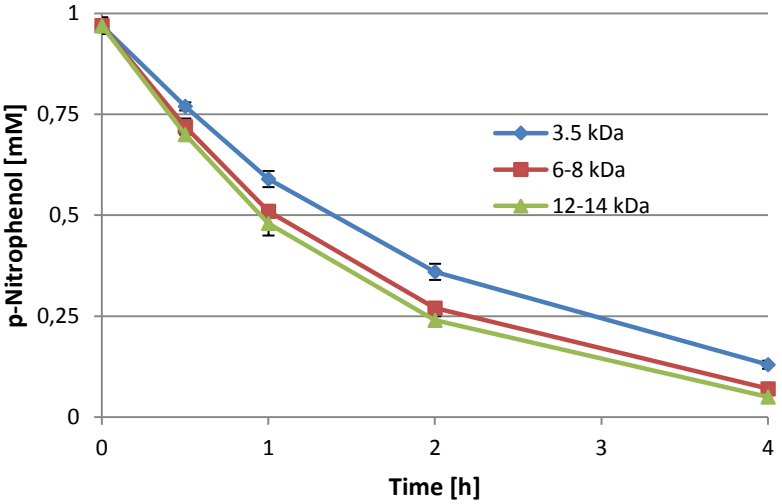


Figure 1: Comparison of dialysis speed of ZelluTrans/Roth Mini Dialyzer (MD300) with 3.5 kDa, 6-8 kDa and 12-14 kDa MWCO. Time course of removal of *p*-Nitrophenol by dialysis. n=3, error bars indicate standard deviation.

Table 1: Data. Photometric determination of *p*-Nitrophenol concentration.

Time / h	*p*-Nitrophenol / mM					
	3.5 kDa	SD	6-8 kDa	SD	12-14 kDa	SD
0	0.97	0.02	0.97	0.02	0.97	0.02
0.5	0.77	0.01	0.72	0.02	0.7	0.01
1	0.59	0.02	0.51	0.01	0.48	0.03
2	0.36	0.02	0.27	0.01	0.24	0.01
4	0.13	0.01	0.07	0.00	0.05	0.01

Comparison of dialysis speed of ZelluTrans/Roth Mini Dialyzer (MD300) using 2 ml and 5 ml deep-well plates

It is common knowledge that the dialysis efficiency can be increased by increasing the volume of the dialysis buffer. Another way to speed up the dialysis is to use smaller volumes and to change the buffer on a regular basis. The comparison of deep-well plates with 5 ml and 2 ml volume using ZelluTrans/Roth Mini Dialyzer (MD300) shows, that regular change of the dialysis buffer is decisive for dialysis speed and that the volume then is of minor importance.

Experimental conditions:

ZelluTrans/Roth Mini Dialyzer (MD300)
MWCO 6-8kDa

Sample: 300µl of 1mM *p*-Nitrophenol (MW 139.11 g mol^{-1}, CAS 100-02-7) in PBS (Phosphate buffered Saline, 145 mM NaCl (58,44 g·mol^{-1}, CAS 7647-14-5), 10 mM Na_2HPO_4 (141,96 g·mol^{-1} CAS 7558-79-4 (wasserfrei)), pH 7.4.

Dialysis buffer: 1.3 ml or 3.5 ml respectively of PBS, pH7.4.

2 mL (96 samples) and 5 mL (48 samples) deep-well plates.

Buffer exchange interval 60 min. Samples were taken as indicated.

Determination Method for *p*-Nitrophenol: Tecan Sunrise Photometer, 420 nm (vs. 620 nm as reference wavelenght).

Number of samples: n=3.

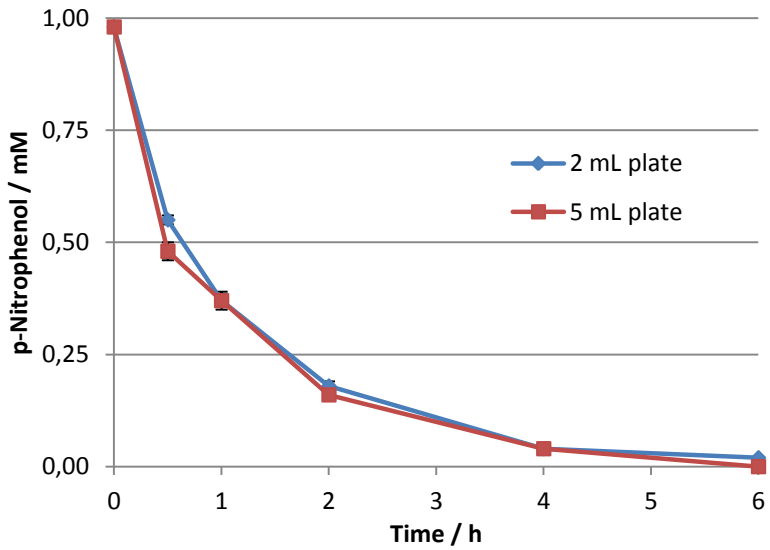


Figure 1: Comparison of dialysis speed of ZelluTrans/Roth Mini Dialyzer (MD300) in 2 mL and 5 mL deep-wall plates. Time course of removal of *p*-Nitrophenol by dialysis. n=3, error bars indicate standard deviation.

Table 1: Data. Photometric determination of *p*-Nitrophenol concentration.

	2 mL deep-well plate		5 mL deep-well plate	
Time / h	**p-NP / mM**	**SD**	**p-NP / mM**	**SD**
0	0.98	0.01	0.98	0.01
0.5	0.55	0.01	0.48	0.02
1	0.37	0.01	0.37	0.02
2	0.18	0.01	0.16	0.01
4	0.04	0.01	0.04	0.00
6	0.02	0.00	0.00	0.01

Comparison of dialysis speed of ZelluTrans/Roth Mini Dialyzer (MD) and ZelluTrans/Roth Mini Dialyzer (MD300)

p-Nitrophenol is our laboratory standard for the determination of dialysis speed. Due to its molecular weight, it offers a differentiated picture of dialysis speed, but still in a short period of time.
The comparison of the ZelluTrans/Roth Mini Dialyzer MD and Mini Dialyzer (MD300) shows, that the smaller Mini Dialyzer MD is quicker, but also the larger MD300 provides a more than sufficient speed for everyday use.

Experimental conditions:

ZelluTrans/Roth Mini Dialyzer MD and Mini Dialyzer (MD300)
MWCO 6-8 kDa

Sample: 100 µl or 300 µl of 1mM *p*-Nitrophenol (MW 139.11 g mol^{-1}, CAS 100-02-7) in PBS (Phosphate buffered Saline, 145 mM NaCl (58,44 g·mol^{-1}, CAS 7647-14-5), 10 mM Na_2HPO_4 (141,96 g·mol^{-1} CAS 7558-79-4 (wasserfrei), pH 7.4).

Dialysis buffer: 1.8 ml or 3.5 ml of PBS, pH7.4.

2 mL (for MD100) and 5 mL Deep-well plates (for MD300).

Buffer exchange interval 60 min. Samples were taken as indicated.

Determination Method for *p*-Nitrophenol: Tecan Sunrise Photometer, 420 nm (vs. 620 nm as reference wavelength).

Number of samples: n=3.

MWCO: molecular weight cut off

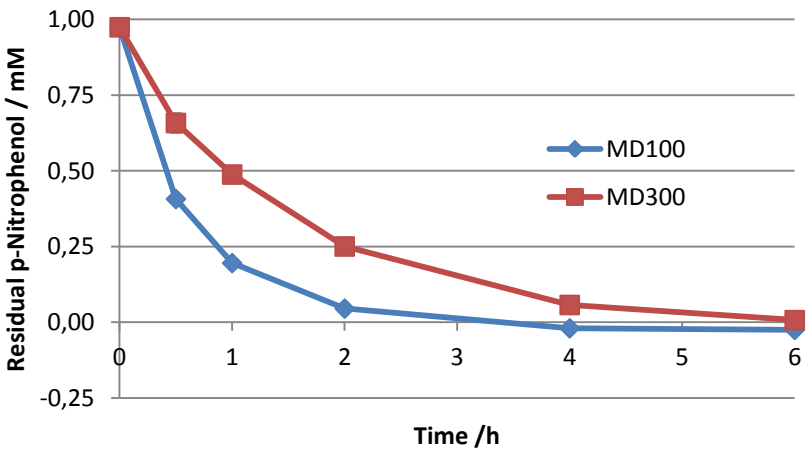


Figure 1: Comparison of dialysis speed of ZelluTrans/Roth Mini Dialyzer MD and Mini Dialyzer (MD300) with 6-8 kDa MWCO. Time course of removal of *p*-Nitrophenol by dialysis. n=3, error bars indicate standard deviation.

Table 1: Data. Photometric determination of *p*-Nitrophenol concentration.

Time / h	MD100		MD300	
	p-NP / mM	SD	*p*-NP / mM	SD
0	0.975	0.027	0.975	0.027
0.5	0.407	0.010	0.659	0.031
1	0.196	0.006	0.488	0.018
2	0.045	0.003	0.251	0.012
4	-0.020	0.000	0.057	0.013
6	-0.025	0.001	0.007	0.020

Comparison of dialysis speed of ZelluTrans/Roth Mini Dialyzer (MD300) with and without shaking

In classical dialysis, the dialysis tubes or devices needed to be placed in a stirred or shaken dialysis buffer.

With the ZelluTrans/Roth Mini Dialyzer MD and Mini Dialyzer (MD300), this has changed. Due to its unique design with an ideal volume-to-membrane-surface-ratio it provides shortest diffusion distances. Used in standard 2 mL deep well plates with repeated change of the dialysis buffer, the difference in dialysis speed between shaken and not-shaken dialyzers has become negligible for everyday use.

Experimental conditions:

ZelluTrans/Roth Mini Dialyzer (MD300)
MWCO 6-8kDa

Sample: 300µl of 1mM *p*-Nitrophenol (MW 139.11 g mol^{-1}, CAS 100-02-7) in PBS (Phosphate buffered Saline, 145 mM NaCl (58,44 g·mol^{-1}, CAS 7647-14-5), 10 mM Na_2HPO_4 (141,96 g·mol^{-1} CAS 7558-79-4 (wasserfrei), pH 7.4.

Dialysis buffer: 1.3 ml of PBS, pH7.4.

2 mL Deep-well plates.

Buffer exchange interval 60 min. Samples were taken as indicated.

Turbo Shaker 3500 at 600 rpm where indicated.

Determination Method for *p*-Nitrophenol: Tecan Sunrise Photometer, 420 nm (vs. 620 nm as reference wavelenght).

Number of samples: n=3.

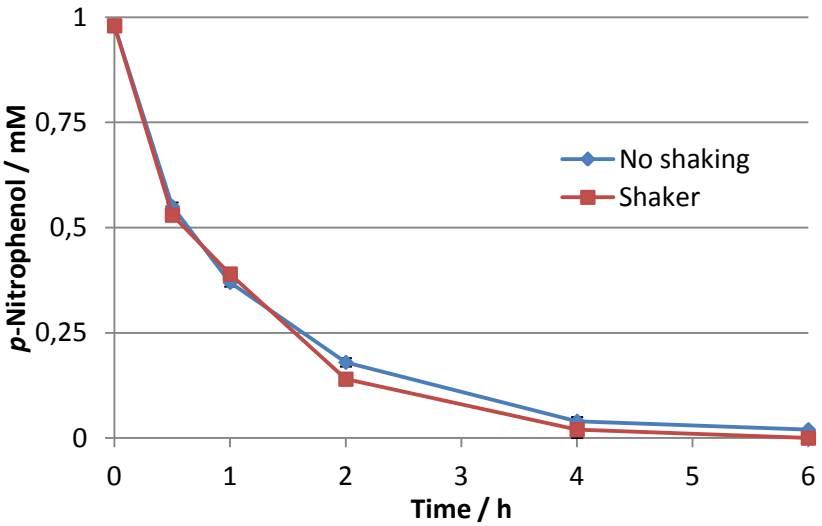


Figure 1: Comparison of dialysis speed of ZelluTrans/Roth Mini Dialyzer (MD) 3.5 kD MWCO with and without shaking. Time course of removal of *p*-Nitrophenol by dialysis. n=3, error bars indicate standard deviation.

Table 1: Data. Photometric determination of *p*-Nitrophenol concentration.

mM *p*-Nitrophenol				
Time / h	**No Shaking**	**SD**	**Shaking 600 rpm**	**SD**
0	0.98	0.01	0.98	0.01
0.5	0.55	0.01	0.53	0.01
1	0.37	0.01	0.39	0.01
2	0.18	0.01	0.14	0.01
4	0.04	0.01	0.02	0.02
6	0.02	0.00	0.00	0.01

Salt Removal

The most well known application of laboratory dialysis is the change of buffer conditions for protein solutions. With the nearly ideal relation between membrane surface area and the sample volume, ZelluTrans/Roth Mini Dialyzer (MD300) allow to remove sodium chloride, e.g. in form of phosphate buffered saline PBS, within 3 to 4 hours in a sufficient way for most applications.

Experimental conditions:

ZelluTrans/Roth Mini Dialyzer (MD300)
MWCO 6-8 kDa

Sample: 300µl of PBS (Phosphate buffered Saline, 145 mM NaCl (58,44 g·mol^{-1}, CAS 7647-14-5), 10 mM Na_2HPO_4 (141,96 g·mol^{-1} CAS 7558-79-4 (wasserfrei), pH 7.4)

Dialysis buffer: 3.5 mL of Aq. dest.

5 mL Deep-well plates (for 48 samples).

Buffer exchange interval 60 min. Samples were taken as indicated.

Room temperature.

Determination Method for NaCl: Wescor VAPRO 5520 Osmometer.

Number of samples: n=3.

MWCO: molecular weight cut off

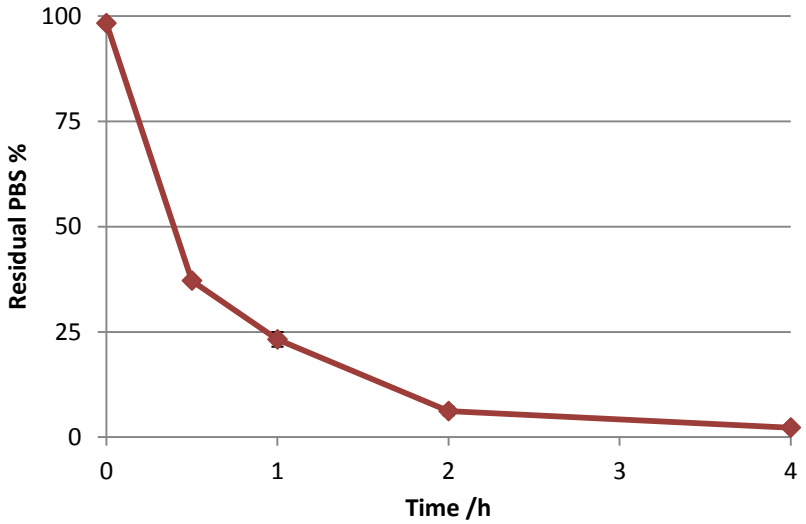


Figure 1: Dialysis of PBS with ZelluTrans/Roth Mini Dialyzer (MD300), n=3, error bars indicate standard deviation.

Table 1: Data. Measurement of concentration by osmometry.

Time / h	**Residual PBS / %**	**SD / %**
0	98.3	0.74
0.5	37.2	0.35
1	23.2	1.75
2	6.2	1.02
4	2.3	0.41

Urea Removal

In numerous applications in protein biochemistry urea is used as a denaturing agent. Just as important is the gentle removal of urea afterwards e.g. for renaturing the proteins.
Dialysis is a classical method for a gentle exchange of buffer conditions. With the ZelluTrans/Roth Mini Dialyzer (MD300) and its unique design it is possible to remove for for example 9 M urea within only 4 hours. Due to the advantageous membrane surface area in relation to the sample volume and because of the small molecular weight of urea, there is no noteworthy volume increase caused by osmosis.

Experimental conditions:

ZelluTrans/Roth Mini Dialyzer (MD300)
MWCO 6-8 kDa

Sample: 300 µl of 9 M Urea (MW 60.06 g mol^{-1}, CAS 57-13-6)

Dialysis buffer: 3.5 mL of Aq. dest.

5 mL Deep-well plates (for 48 samples).

Buffer exchange interval 60 min. Samples were taken as indicated.

Room temperature.

Determination Method for Urea: Wescor VAPRO 5520 Osmometer

Number of samples: n=3.

MWCO: molecular weight cut off

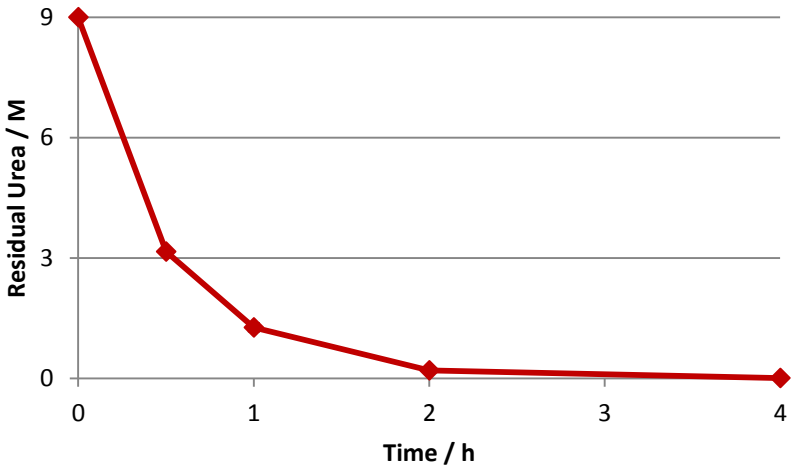


Figure 1: Dialysis of 9 M Urea with ZelluTrans/Roth Mini Dialyzer (MD300), n=3, error bars indicate standard deviation.

Table 1: Data. Measurement of concentration by osmometry.

Time / h	Residual Urea / M	SD
0	9.00	0.00
0.5	3.16	0.06
1	1.27	0.10
2	0.20	0.05
4	0.01	0.03