Technical Data Sheet and Applications release_09-19-2013

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Technical Data Sheet

Xpress Micro Dialyzer

Specifications:

- max. sample volume 100 µl
- mass: 2.92 g (dry)
- dimensions: max. length = 87 mm, max. width = 35 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)
- membrane: low binding regenerated cellulose, contain 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 rebuffering
- Glycoprotein modification and engineering
- Protein in vitro translation
- Cell culture: studies of a cell line with a virus strain
- Enzyme activity assays
- Plasmid or primer purification

Chemical Resistance

Xpress Micro Dialyzer

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

Xpress Micro Dialyzer

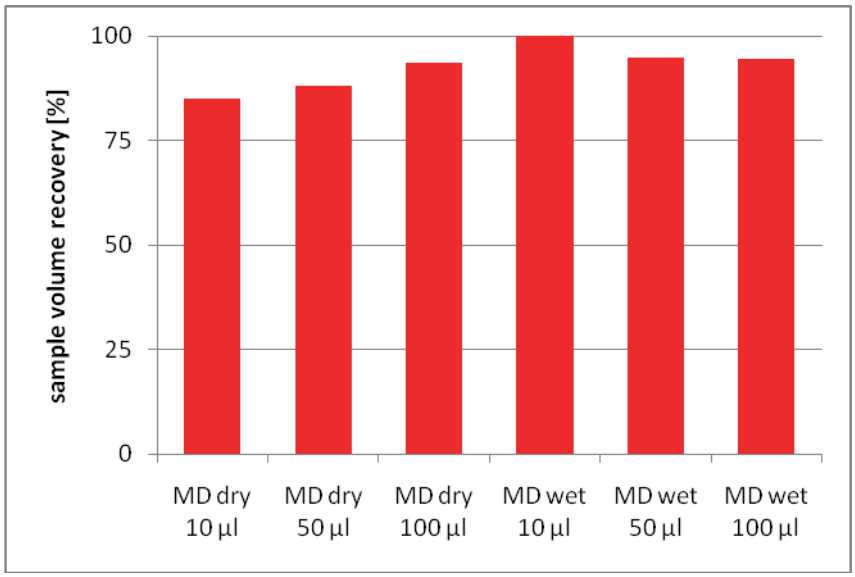
Materials and Methods

MWCO 6-8 kDa

Sample: 10 µl, 50 µl, or 100 µl respectively of Aq. dest.

Determination Method: Gravimetry

Sample	Recovery [%]
MD dry 10 µl	84.86
MD dry 50 µl	88.01
MD dry 100 µl	93.60
MD wet 10 µl	100.00
MD wet 50 µl	94.53
MD wet 100 µl	94.37
MD Micro Dialyzer	
Dry: dry membrane	
Wet: prewetted membrane	



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Application Note — Dialysis efficiency at different temperatures, dye

Xpress Micro Dialyzer

Materials and Methods

MWCO 6-8 kDa; MD 100

Sample: 100µl of 1mM p-Nitrophenol, Mr=129,11g/mol, in PBS

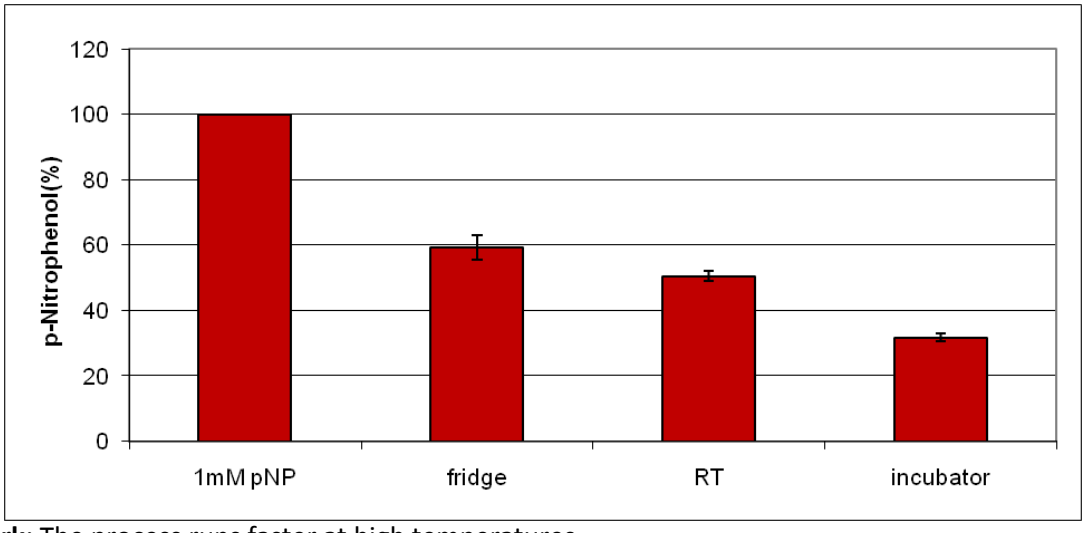
Dialysis Buffer: 1.8ml of PBS (Phosphate buffered saline, pH 7,4),

preincubated in Deepwell plates

Determination Method: Tecan Sunrise Photometer; 420nm and 620nm

Experiment: Microdialyzers were filled with 100µl of p-Nitrophenol and placed in 1.8ml PBS. In each case four samples (n=4) were dialysed for 30min in a fridge(4°C), at room temperature(20°C) and in a incubator(37°C).

Time 30min	1mM pNP	fridge (4°C)	RT (20°C)	incubator (37°C)
Mean (420/620nm)	2,771	0,844	0,721	0,469
standard deviation	0,008	0,037	0,016	0,012
coefficient of variation	0,304	4,428	2,171	2,479
concentration (%)	100	59,34	50,55	31,87



Remark: The process runs faster at high temperatures.

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Application Note — Dialysis efficiency at different temperatures (salt)

Xpress Micro Dialyzer

Materials and Methods

MWCO 6-8 kDa; MD 100

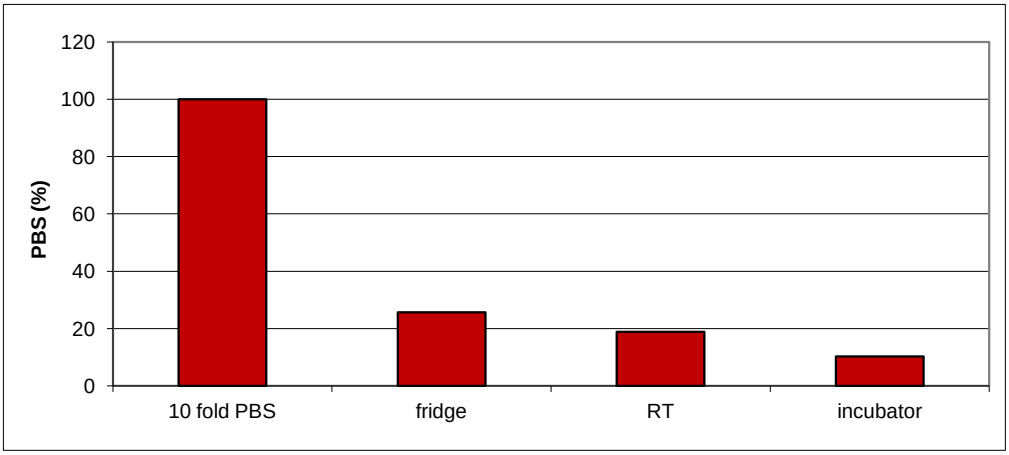
Sample: 100µl of PBS (10fold; NaCl=84,738g/l; $Na_2HPO_4*2H_2O$=53,6g/l; $NaH_2PO_4*2H_2O$=13,8g/500ml)

Dialysis Buffer: 1.8ml of aqua dest; preincubated in Deepwell plates

Determination Method: Osmometer; Fa. Kreienbaum

Experiment: Microdialyzers were filled with 100µl of PBS and placed in 1.8ml aqua dest. In each case four samples (n=4) were dialysed for 30min in a fridge(4°C), at room temperature(20°C) and in a incubator(37°C).

Time 30min	PBS 10-fold	fridge (4°C)	RT (20°C)	incubator (37°C)
Mean (mmol/kg)	3210	822,75	603,25	328,25
standard deviation	-	22,322	37,384	16,500
coefficient of variation	-	2,713	6,197	5,027
concentration (%)	100	25,63	18,79	10,23



Remark: The process runs faster at high temperatures.

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Application Note — Dialysis efficiency at different temperatures (Urea)

Xpress Micro Dialyzer

Materials and Methods

MWCO 6-8 kDa; MD 100

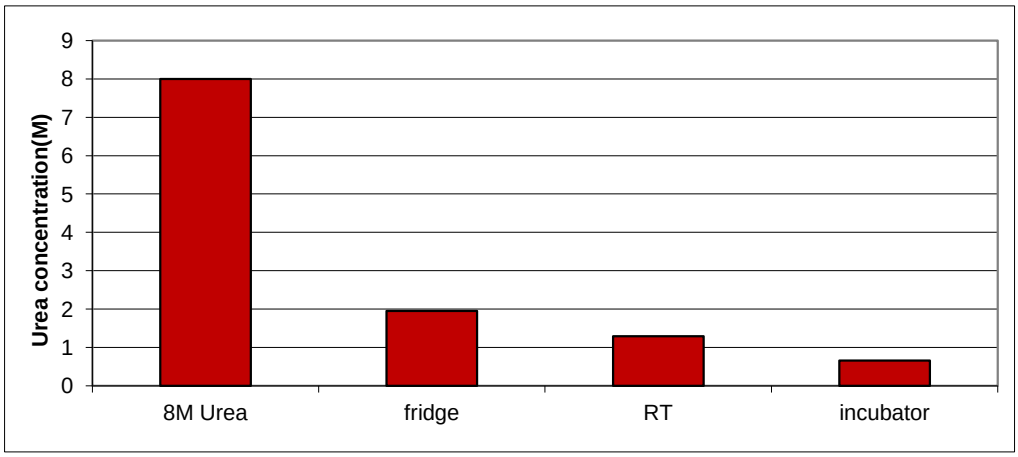
Sample: 100µl of 8M Urea

Dialysis Buffer: 1.8ml of aqua dest; preincubate in Deepwell plates

Determination Method: Osmometer, Fa. Kreienbaum

Experiment: Microdialyzers were filled with 100µl of Urea solution and placed in 1.8ml aqua dest. In each case four samples (n=4) were dialysed for 30min in a fridge(4°C), at room temperature(20°C) and in a incubator(37°C).

Time 30min	fridge (7°C)	RT (20°C)	incubator (37°C)
Mean (mmol/kg)	1758,75	1180	624,75
standard deviation	28,7170101	44,5570795	88,57153418
coefficient of variation	1,63280796	3,77602369	14,17711632
concentration (M)	1,947	1,29	0,66



Remark: The process runs faster at high temperatures.

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Application Note Protein Binding (BSA)

Xpress Micro Dialyzer

Materials and Methods

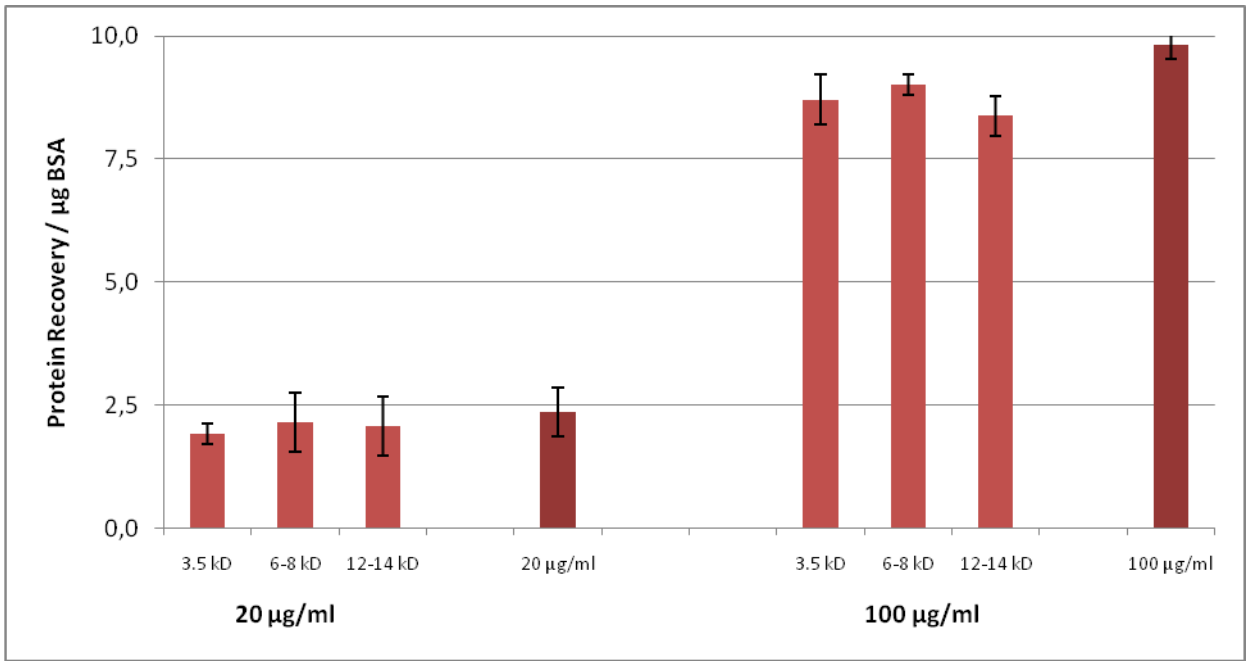
Sample: 100µl of BSA solution in PBS (20 and 100 µg/ml), n= 5 - 6

Dialysis Buffer: 1,8 ml of Aq. dest., exchange after 1 h, Incubation time: 4h at RT

Determination Method: Protein determination according to Bradford,

Tecan Sunrise photometer

Protein recovery	µg Protein	SD
3.5 kD (20 µg/ml)	1.92	0.2
6-8 kD (20 µg/ml)	2.14	0.6
12-14 kD (20 µg/ml)	2.08	0.6
Initial value (20 µg/ml)	2.35	0.5
3.5 kD (100 µg/ml)	8.71	0.5
6-8 kD (100 µg/ml)	9.02	0.2
12-14 kD (100 µg/ml)	8.38	0.4
Initial value (100 µg/ml)	9.83	0.3



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Application Note Urea Removal

Xpress Micro Dialyzer

Materials and Methods

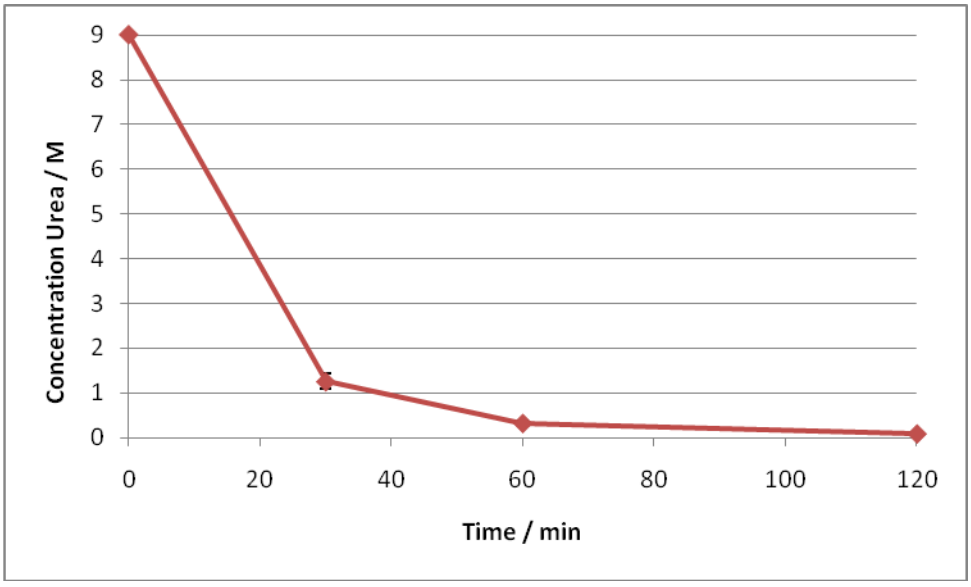
MWCO 3,5 kDa

Sample: 100 µl of 1 mg/ml BSA in 9 M Urea

Dialysis Buffer: 1.8 ml 10 mM Tris, pH 8.3, Buffer exchange interval 30 min, RT

Determination Method: Wescor VAPRO 5520 Osmometer

t in min	Residual Urea in M	SD
0	9	0,00
30	1,25	0,17
60	0,32	0,06
120	0,09	0,02



Remark: Due to 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.

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Application Note Salt Removal

Xpress Micro Dialyzer

Materials and Methods

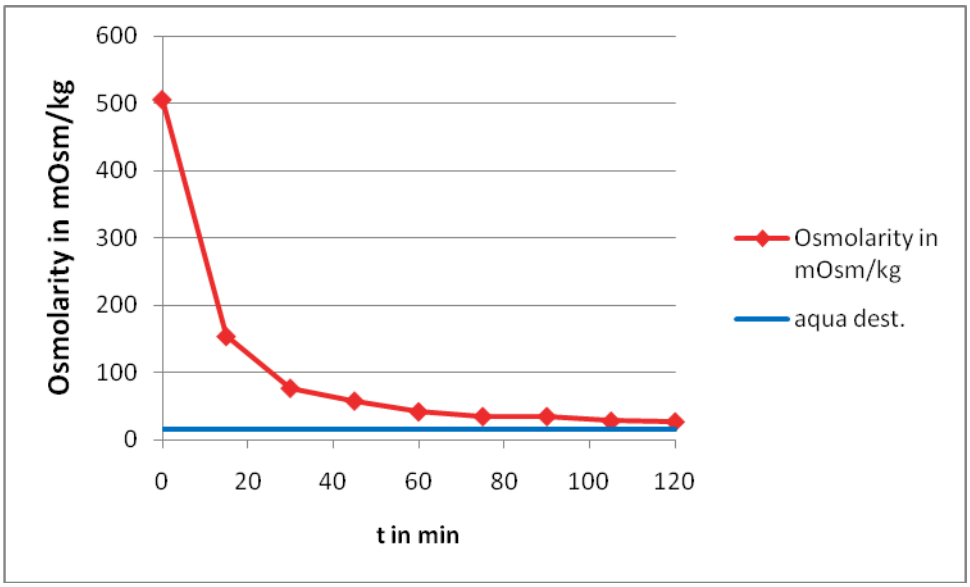
MWCO 6-8 kDa

Sample: 100µl of 50mM H_2NaPO_4/ HNa_2PO_4, 150mM NaCl, pH 7.4

Dialysis Buffer: 1.8 ml Aq. dest., Buffer exchange interval 15 min, 20 °C

Determination Method: Wescor Osmometer Vapor 5520

t in min	Osmolarity in mOsm/kg
0	506
15	154
30	77
45	58
60	42
75	35
90	35
105	28
120	27
aqua	15



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Application Note Dialysis Speed of different MWCO

Xpress Micro Dialyzer

Materials and Methods

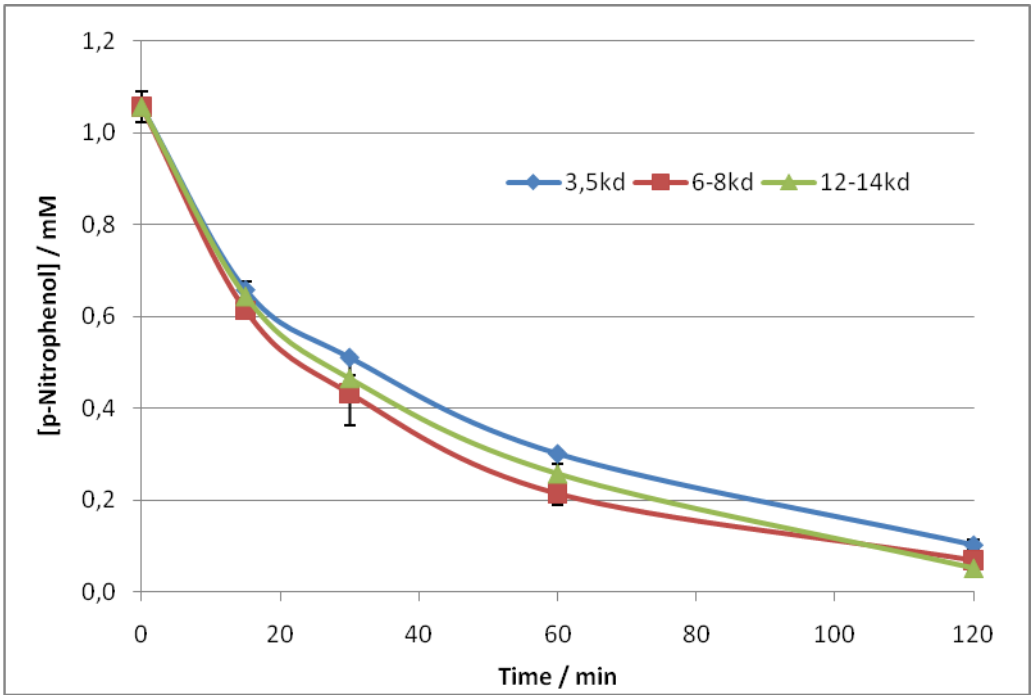
MWCO 3.5 kD, 6-8 kD, 12-14 kD

Sample: 100µl of 1mM *p*-Nitrophenol in PBS, pH 7.4, n=3

Dialysis buffer 1.8 ml of PBS, pH7.4, exchange interval 30 min.

Determination Method: Tecan Sunrise Photometer, 420 nm

mM *p*-NP			
Time / min	**3.5 kD**	**6-8 kD**	**12-14 kD**
0	1.06	1.06	1.06
15	0.66	0.61	0.64
30	0.51	0.43	0.47
60	0.30	0.21	0.26
120	0.10	0.07	0.05



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Application Note Dialysis of Sucrose

Xpress Micro Dialyzer

Materials and Methods

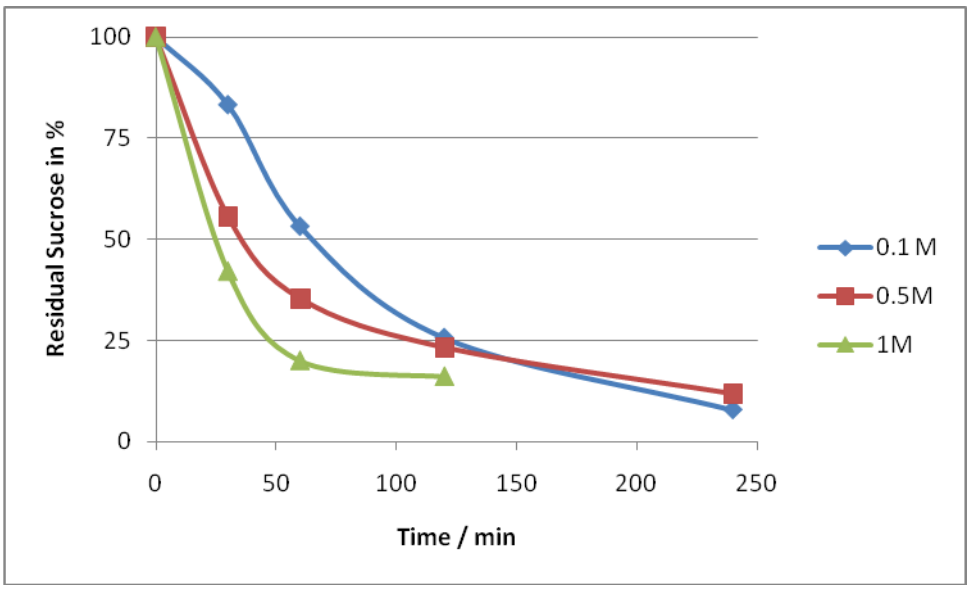
Xpress Micro Dialyzer MWCO 6-8 kDa

Sample: 100µl of 1M, 0,5 M or 0,1 M Sucrose, respectively, in Aq. dest

Dialysis Buffer: 1.8 ml Aq. dest., Buffer exchange interval 30 min, 20 °C

Determination Method: Wescor Osmometer Vapor 5520

Time	Residual Sucrose in % of original concentration		
min	0.1 M	0.5M	1M
0	100.0	100.0	100.0
30	83.2	55.5	42.1
60	53.1	35.3	20.0
120	25.6	23.2	16.1
240	7.8	11.8	



Remark: With higher molarities of sucrose (e.g. 0,5 M and 1 M), an increasing osmotic effect appears resulting in dilution of the sample, which is simulating a higher speed of dialysis. To prevent this, it is best to keep the concentration difference around 100 mM. If this is not possible or desired, reduce the sample volume to 50 µl and close the Micro Dialyzer with scienova sealing for MD. For salt, this osmotic effect was not as pronounced as for sugars.

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Application Note CHAPS Removal

Xpress Micro Dialyzer

Materials and Methods

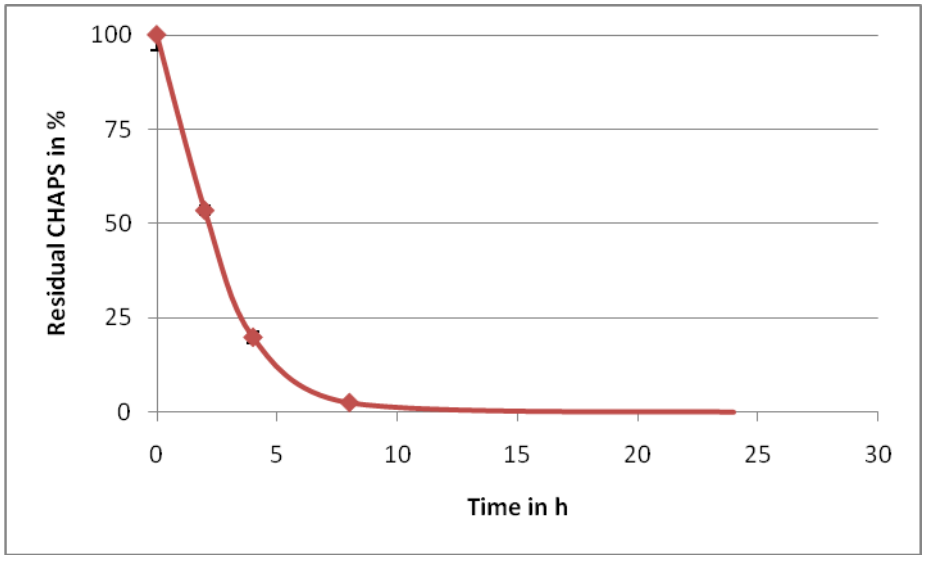
MWCO 6-8 kDa

Sample: 100µl of 1 mg/ml BSA in Aq. dest., 1% (w/v) CHAPS

Dialysis Buffer: 1.8 ml Aq. dest., Buffer exchange interval 2 h for 8 h, 20 °C

Determination Method: Agilent HP1100/MSD

t in h	Residual CHAPS in %
0	100
2	53,4
4	19,8
8	2,5
24	0,0



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Application Note SDS Removal

Xpress Micro Dialyzer

Materials and Methods

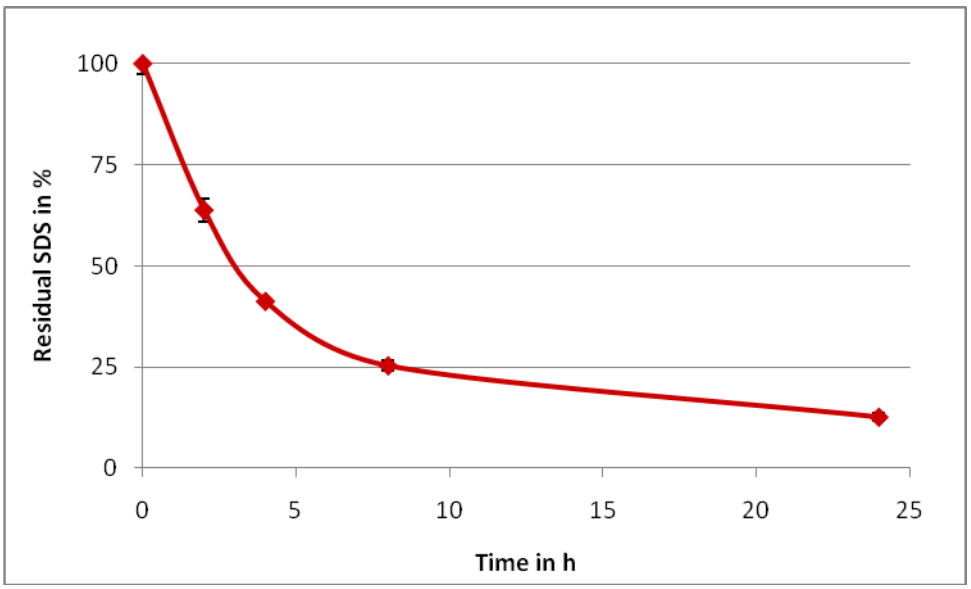
MWCO 6-8 kDa

Sample: 100µl of 1 mg/ml BSA and 0.1 % (w/v) SDS in PBS, pH 7.4

Dialysis Buffer: 1.8 ml Aq. dest., Buffer exchange interval 2 h for 8 h, 20 °C

Determination Method: Agilent HP1100/MSD, ESI negative mode

t in h	Residual SDS in %
0	100
2	63,8
4	41,2
8	25,3
24	12,6



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Application Note Volume reduction by evaporation

Xpress Micro Dialyzer

Materials and Methods

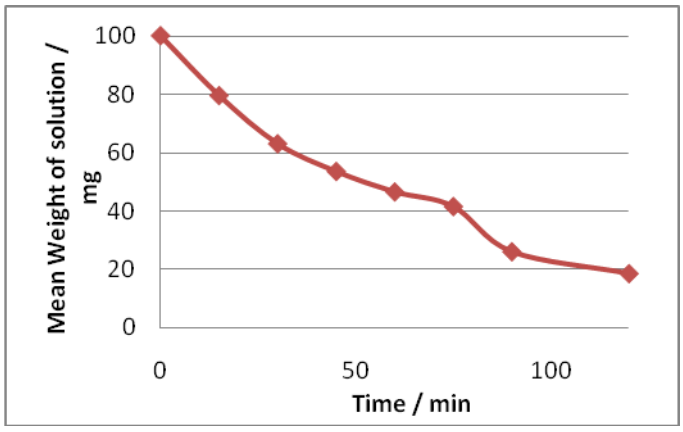
MWCO 3,5 kDa

Sample: 100 µl of PBS (Phosphate buffered saline: 10mM H_2NaPO_4/ HNa_2PO_4, 150mM NaCl, pH 7.4)

Determination Method: Gravimetric analysis of the weight of the residual solution in the Micro Dialyzer (MD).

Experimental: Microdialyzers were filled with 100 µl PBS and placed in an "Egg Crate" (from Corning) which was attached to a stand to allow air to pass the MD. Ambient temperature, n=2.

Time / min	Weight of residual solution in Micro Dialyzer/ mg		
	Sample 1	Sample 2	**Mean**
0	100	100	**100**
15	81	78	**79.5**
30	64	62	**63**
45	55	52	**53.5**
60	46	47	**46.5**
75	38	45	**41.5**
90	30	22	**26**
120	26	11	**18.5**



Remark: Results may vary with temperature and air flow

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Application Note — Enzyme reactivation through Rebuffering

Xpress Micro Dialyzer

Aim

Solved trypsin is best storable at acidic pH and low temperature. To regain tryptic activity, here demonstrated by para-nitroaniline (PNA) release, rebuffering towards a neutral pH is needed, which is achieved through dialysis.

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

Materials and Methods

Xpress Micro Dialyzer MWCO 6-8 kDa; MD 100
Dialysis-sample: 100 µl trypsin-sample (0.5 mg/ml in glycine-HCl buffer pH 2; 0.5 mg/ml in Aq. dest.)
Dialysis-buffer: 1.8 ml of dialysis-buffer (35 mM Tris pH 7.8, 7 mM $CaCl_2$, final volume adjusted with Aq. dest.)
Measurement-solution: 4.7 mM DL-Benzoyl-Arg p-nitroaniline (DL BAPNA) 10% DMSO in 35 mM Tris pH 7.8, 7 mM $CaCl_2$ + 20 µl Trypsin samples 0,05 mg/ml , final volume adjusted with Aq. dest.
Determination Method: Tecan Sunrise Photometer, 405 nm (measurement wavelength) and 620 nm (reference wavelength), PNA- release rate (rr) indicating tryptic activity is evaluated.

$$rr = \frac{\text{absorbance}_{405\,nm} - \text{absorbance}_{620\,nm}}{\Delta t \text{ in min}}$$

Protocoll: Micro dialyzers were filled with 100 µl trypsin-sample and placed in 1.8 ml dialysis-buffer. In each case four samples (n=4) were dialyzed for 5 min, 10 min, 15 min and 30 min at room temperature (20°C).

	Non-dialyzed control	5 min dialyzed	10 min dialyzed	15 min dialyzed	30 min dialyzed
Trypsin in pH 2 glycine-HCl buffer	0,0005	0,0074	0,0123	0,0135	0,0144
Trypsin in Aqu.dest. dialyzed		0,0121	0,0134	0,0122	0,0139
Trypsin untreated	0,0127