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Innovating for better science

Guide
Sample dialysis in the lab

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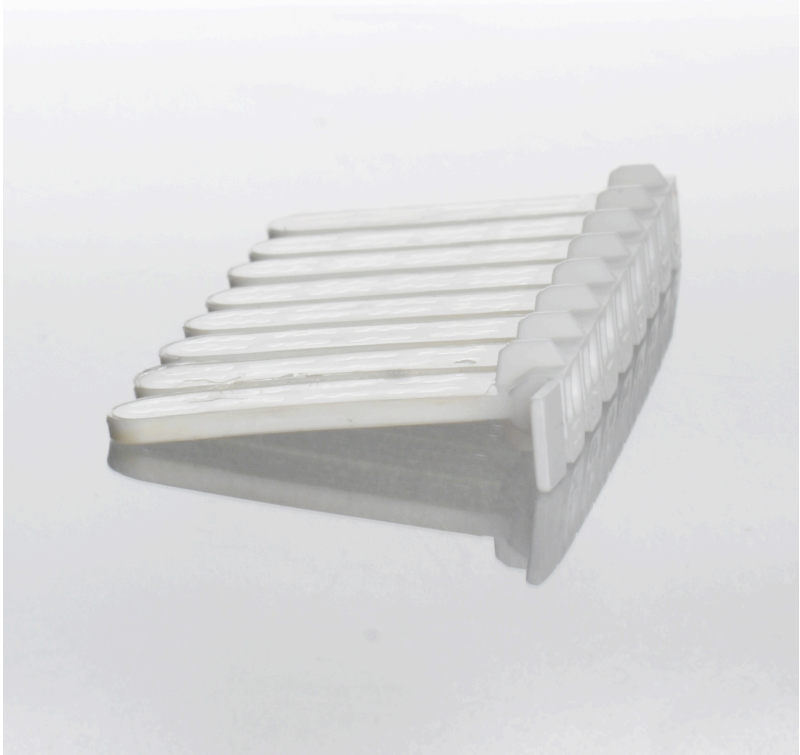


Introduction to Dialysis

Dialysis is the diffusion based size selective transportation of molecules or particles through a semipermeable membrane from higher concentration to lower concentration. Selectivity of dialysis is determined by pore size of semipermeable membranes. The end point of dialysis is the concentration equilibrium. Dialysis is an often used method to separate bigger molecules like proteins or DNA from accompanying substances such as salt or detergents. It is a very gentle method for sensitive substances.

Example: A protein is dissolved in a highly concentrated NaCl solution. NaCl disturbs following step such as ion exchange chromatography. The sample volume is pipetted into a dialysis device with a semipermeable membrane. The sample volume is in contact with higher volume of dialysis buffer through the semipermeable membrane. Only NaCl ions can pass through membrane pores. Protein molecules cannot pass through pores and will be retained. The NaCl concentration in the sample will be clearly reduced during dialysis process. The salt concentration can be adjusted to any desired level through multiple exchanges of dialysis buffer volume.

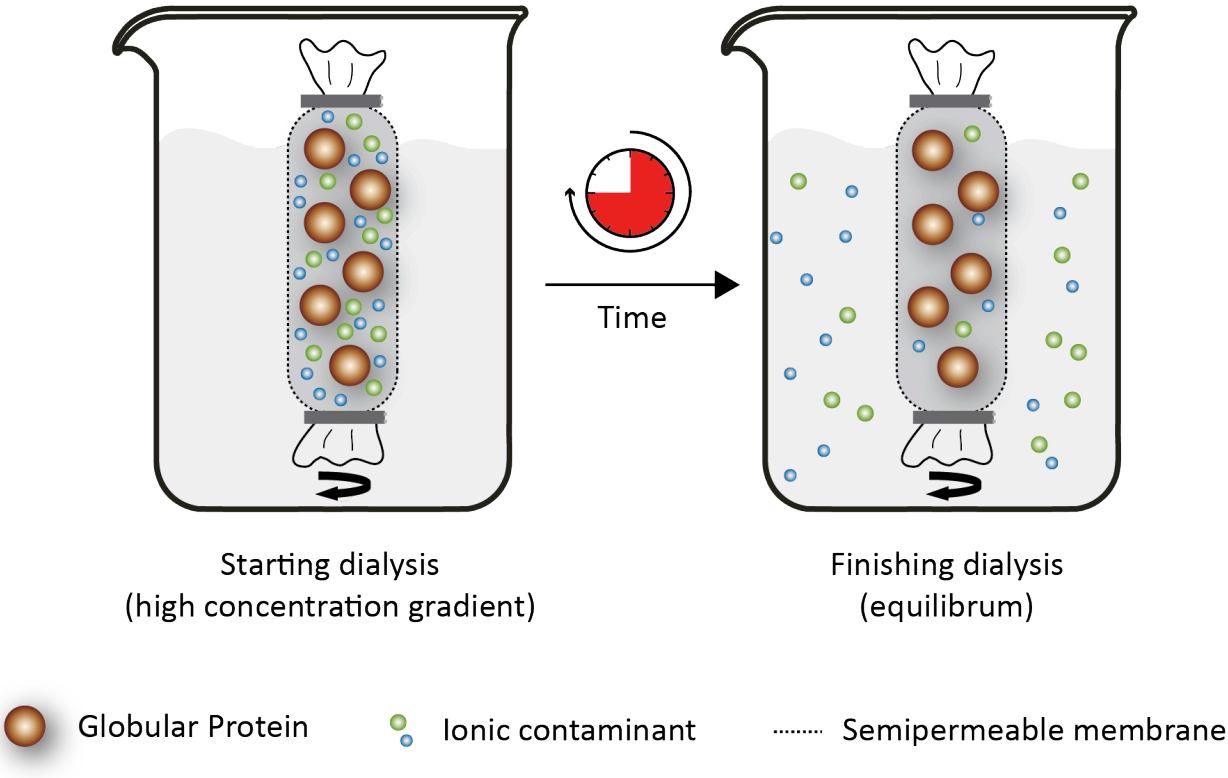


Fig. 1 | Principle of protein sample dialysis.

Advantages of dialysis:

- Very gentle method for sensitive substances like proteins (preservation of protein activity)
- Easy application
- Usage of standard lab equipment
- Different membranes available (pore size, membrane material)
- Dialysis devices are available as disposables
- Small volume change compared with ultrafiltration through dialysis
- Minimal membrane fouling compared with ultrafiltration

Applications:

- Desalting of proteins and other macromolecules
- Rebuffering of proteins and other macromolecules
- Renaturation / Denaturation of proteins
- Binding studies (plasma protein binding studies)
- Fractionation and characterization of nano particles
- Protein sample concentration
- Electroelution
- Removal of disturbing substances like urea from macromolecular substances

Disadvantages of Dialysis:

- Classical dialysis devices, like dialysis tubes require longer dialysis time. scienova´s™ *Xpress* MicroDialyzer reduces dialysis time drastically through its geometry compared with classical devices.
- Dialysis of substances is limited by concentration gradient
- Osmotic pressure effects can influence sample volume

Tips for Dialysis:

Concentration of substance

The dialysis speed depends on the concentration gradient, which is highest at the start of dialysis. Through the dialysis process the concentration gradient between the sample and dialysis buffer will be reduced. In order to keep high dialysis speed (high concentration gradient) it is recommended to (1) use a high sample volume to dialysis buffer volume ratio. For example, 5 ml sample volume could be dialysed in 5 l dialysis buffer, or (2) exchange the dialysis buffer volume periodically. For example, when desalting using scienova´s™ *Xpress* MicroDialyzer, it is recommended to exchange the buffer every 30 to 60 minutes.

Temperature

Dialysis speed is higher at a higher temperature of the solutions. Certainly, application of higher temperature is limited by sensitivity of samples like proteins (denaturation). Usually dialysis is performed at ambient temperature. Dialysis of more sensitive samples should be performed at about 4 °C. Limitations at this temperature are freezing sample and dialysis buffer as well as a lower dialysis rate. Figure 2 shows an example of salt removal with scienova´s™ *Xpress* MicroDialyzer at different temperatures.

Dialysis duration

Several aspects influence the dialysis duration: Substance, concentration, membrane, or length of diffusion pathway, as well as temperature. Some manufacturers, like scienova,™ depict protocols for frequently used substances in their technical data sheets. These can be used as a basis for optimization of dialysis conditions for similar substances. When operating under special conditions or with less common substances, it is often necessary to perform preliminary tests to find out the optimal parameters, especially required dialysis time, necessary to obtain the desired result.

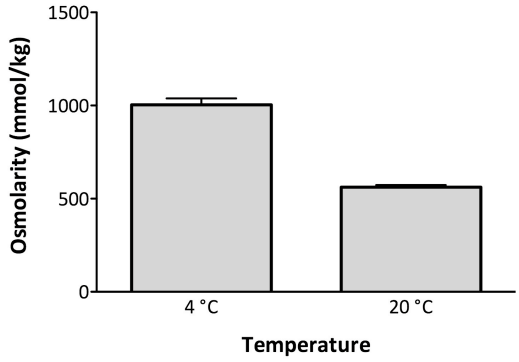


Fig. 2 | Dialysis of PBS under different temperatures
scienova *Xpress* MicroDialyzer MD100, MWCO 6-8 kDa, sample: 100 µl PBS; dialysis buffer: a. dest., dialysis duration: 30 mins.; measuring of osmolarity (Vapro Osmometer 5520, Kreienbaum)

Membrane selection

The flow through the membrane depends on the active membrane area, the ratio of active membrane area to sample volume, pore size, and porosity. A larger area to sample volume ratio, higher porosity, and broader pores enhances dialysis speed. Scienova´s™*Xpress* MicroDialyzer has a special geometry that maximizes the active membrane area and helps achieve the best ratio of membrane area to sample volume, ensuring high dialysis efficiency. The unit for pore size is most often a Dalton (D). The selected pore size of the membrane should be as wide as possible while still retaining the desired sample substance. The pore size or cutoff (MWCO) in D should be about double the molecular weight in D of the desired sample substance. The characteristic of the membrane is indicated in the technical data of the manufacturer. Cutoff means that at least 90% of globular substance with the same molecular weight of cutoff should be retained. For example, non globular molecules or chain like molecules of similar molecular cutoff could pass pores easier. Therefore, preliminary tests should be performed for molecules with different cutoffs. To remove a low molecular substance, the cutoff should be selected at lower than half the molecular weight of the substance.

Membranes can bind substances in an unspecific manner, potentially causing loss of the sample. Especially proteins have very different binding affinities. Cellulose and cellulose ester are well-proven low binding materials for a lot of substances, especially proteins. The test of membrane binding for samples is recommended if loss of sample substance was detected.

Length of diffusion pathway

Time consumption of the dialysis grows exponentially with the length of the diffusion pathway (Example Fig. 3). Therefore it is very important to select a dialysis device with a short diffusion length. The scienova™ *Xpress* MicroDialyzer has a special geometry to minimize length of diffusion pathway.

Stirring or shaking the dialysis buffer and sample is recommended if possible. It will increase the transportation of substance through diffusion.

Dialysis buffer

The dialysis buffer is in contact with the dialysis sample. In the simplest case the dialysis buffer can be water.

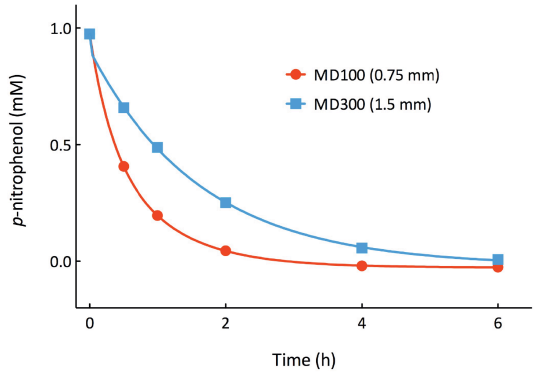


Fig. 3 | Comparsion of dialysis speed at different diffusion length

scienova *Xpress* MicroDialyzer MD100 (0.75 mm diffusion length) and MD300 (1.5 mm diffusion length), sample: 1 mM *p*-nitrophenol; dialysis buffer: 1x PBS, dialysis duration: 30 mins.; absorption at 420 nm (Tecan Sunrise)

Small compounds of dialysis buffer can pass through the semipermeable membrane. The chosen dialysis buffers have to be compatible with the required steps of sample preparation. It can be used for stabilization of pH or redox potential to protect sensitive substances. If the sample has a high concentration of a low molecular weight substance like saccharose, then the concentration gradient will cause an osmotic pressure resulting in significant transportation of water into the sample. In this case it is recommended to first use a step with a dialysis buffer that contains the same substance in a lower concentration to reduce the concentration gradient and the osmotic pressure.

Frequently used buffers for protein samples are phosphate buffer, TRIS, MOPS, and HEPES. Additional substances for protein dialysis are amino acids, EDTA, inhibitors, activators, cofactors, or DTT.

Substance

The diffusion speed depends on molecular size (in solution hydrodynamic diameter). A smaller molecule diffuses faster. Therefore, the required dialysis time for most salts is shorter than for dyes with typical molecular weights of 200-500 Da.

Definition and used terms

Diffusion and flux in solvents

Diffusion is a result of a temperature dependent random walk of molecules. Through random movement concentration gradients of molecules will be equilibrated.

Flux of molecules and particles are described by Fick's first law

$$J = -D\frac{\partial c}{\partial x}$$

J: Diffusion flux
D: Diffusion coefficient
c: Concentration
x: Diffusion length

Semipermeable membrane

Semipermeable membranes have a selective passage for molecules. Semipermeable membranes for dialysis have pores and can retain molecules according to their sizes. Selectivity depends on a tight size distribution of pore widths.

Osmotic pressure

Pure water tends to pass through semipermeable membrane toward higher concentration of molecules like salts or glucose.
Osmotic pressure is the pressure needed to compensate the inward flow of pure solvent across a semipermeable membrane.
Osmotic pressure can cause significant solvent flux during dialysis.

Scienova´s™ *Xpress Micro Dialyzer*

Fig. 4 | MD100 MicroDialyzer

Scienova´s™ *Xpress* MicroDialyzer has an excellent membrane area to sample volume ratio. Short length of diffusion pathways save dialysis time. It is easy and safe to handle small sample volumes with scienova´s™ *Xpress* MicroDialyzer. Different types are available. (table below)

	MD100	MD300
sample volume	10 - 100 µl	50 - 300 µl
MWCO	3,5; 6-8; 12-14 kDa	
Cartridge/ Single Segment	x / x	x / x

Characteristics

- Membrane: Regenerated cellulose
- High recovery (>85 %)
- Cartridges with 8 single segments
- Compatible with microplate standard (SBS)

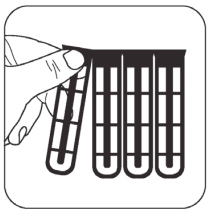
Membranes: Regenerated cellulose

- Cutoffs (MWCO): 3.5, 6-8, 12-14 kDa
- Temperature: 1 - 40 °C
- pH: 4 - 8
- Storage at: 4 - 22 °C
- Stable for different compounds (table see attachment)

Applications of the scienova™ *XPRESS* MicroDialyzer

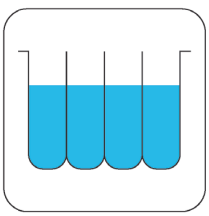
- Sample dialysis of proteins, oligonucleotides, RNA or DNA
- Buffer exchange or desalting
- Sample concentration
- Preparation of protein samples for MALDI-MS
- Dialysis of low-molecular compounds
- Purification of cell culture extracts
- Removal of salts, solvents, or detergents
- Formation studies (protein-protein, protein-DNA, protein-RNA)
- Enzyme activity assays
- Glycoproteins: Modification and formation
- Dye removal from protein samples

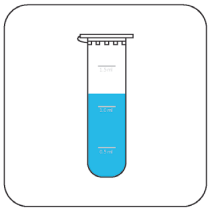
Handling
Xpress MicroDialyzer



Preparation

If only one segment is desired, break it carefully from the eight segmented MD cartridge. Be careful not to touch the membrane.



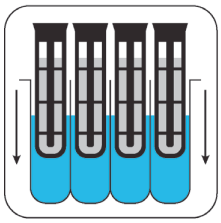


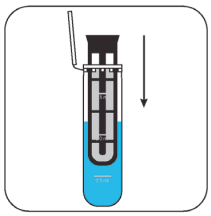
Buffer preparation

Pipette dialysis buffer either in a.) a deep-well plate V≤ 1800 µl or in b.) a microcentrifuge tube V ≤ 1400 µl.

Loading the sample

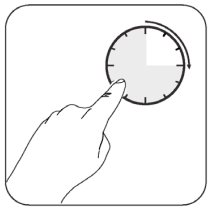
Bring the pipette with sample volume upright into the round opening. The sample volume should be between 10-100 µl (MD100) or 50-300 µl (MD300).





Introduction

Put the MD or the single segment into a.) a deep-well plate or b.) in the microcentrifuge tube used for buffer preparation.



Sample dialysis

One-step dialysis can be done in the same microcentrifuge tube or deep-well plate. If more than one dialysis step is required, then change the position of the MD into the deep-well plate channels or use a new microcentrifuge tube.
The dialysis time is dependent on the compound used and the cutoff of the semipermeable membrane. Normally it takes 15 to 60 minutes. For dialysis of salts, normal dialysis time is 30 minutes per step.

Sample retrieval

Set the pipette volume to 140 µl, press the pipette button to first stop, hold it, and bring the pipette tip upright into round opening with a low pressure. Aspirate the sample.

Finally, pipette out the sample into a microcentrifuge tube or micro plate.

A video demonstration of scienova´s™ MicroDialyzer can be viewed at:
http://www.youtube.com/user/scienovaDialyzer

Products
Ordering Information

Product number	Size	Cutoff	Sample number	Price (net)
40072	MD100	3.5 kDa	96 (12 x 8 Cartridge). in Deep well plate	330.94 €
40071	10 - 100 µl sampel volume		8 (1 Cartridge)	29.36 €
40782			12 (12 Single segments)	38.76 €
40071-X56			56 (56 Single segments), with 96 well rack for 1.5 and 2 ml tubes and 1 forceps	164.93 €
40071-X280			280 (280 Single segments), with 96 well rack for 1.5 and 2 ml tubes and 1 forceps	551.57 €
40076	MD100	6-8 kDa	96 (12 x 8 Cartridge), in Deep well plate	330.94 €
40075	10 - 100 µl sample volume		8 (1 Cartridge)	29.36 €
40783			12 (12 Single segments)	38.76 €
40075-X56			56 (56 Single segments), with 96 well rack for 1.5 and 2 ml tubes and 1 forceps	164.93 €
40075-X280			280 (280 Single segments), with 96 well rack for 1.5 and 2 ml tubes and 1 forceps	551.57 €
40078	MD100	12-14 kDa	96 (12 x 8 Cartridge), in Deep well plate	330.94 €
40077	10 - 100 µl sample volume		8 (1 Cartridge)	29.36 €
40784			12 (12 Single segments)	38.76 €
40077-X56			56 (56 Single segments), with 96 well rack for 1.5 and 2 ml tubes and 1 forceps	164.93 €
40077-X280			280 (280 Single segments), with 96 well rack for 1.5 and 2 ml tubes and 1 forceps	551.57 €
40787	MD300	3.5 kDa	96 (12 x 8 Cartridge), in Deep well plate	364.03 €
40786	50 - 100 µl sample volume		8 (1 Cartridge)	32.30 €
40786-X12			12 (12 Single segments)	43.50 €
40789	MD300	6-8 kDa	96 (12 x 8 Cartridge), in Deep well plate	364.03 €
40788	50 - 100 µl sample volume		8 (1 Cartridge)	32.30 €
40788-X12			12 (12 Single segments)	43.50 €

Product number	Size	Cutoff	Sample number	Price (net)
40791	MD300	12-14 kDa	96 (12 x 8 Cartridge), in Deep well plate	364.03 €
40788	50 - 100 µl sample volume		8 (1 Cartridge)	32.30 €
40788-X12			12 (12 Single segments)	43.50 €

scienova MicroDialyzer – Sample box
Economic box to test different types of MicroDialyzers

Product number	Selectable parts	Price (net)
SBMD1	☑ 1 Cartridge (Size and Cutoff selectable) ☐ MD100 or MD300 ☐ 3.5; 6-8 or 12-14 kDa	24.66 €
SBMD2	☑ 1 Cartridge and single segment (Size and Cutoff selectable) ☐ MD100 or MD300 ☐ 3.5; 6-8 or 12-14 kDa	29.04 €
SBMD3	☑ 3 Single segments 3 different Cutoffs (Size selectable) ☐ 3.5; 6-8; 12-14 kDa ☐ MD100 or MD300	13.14 €
SBMD4	☑ 2 Cartridge and 2 Single segments (Size and Cutoff selectable) ☐ 3.5; 6-8 or 12-14 kDa ☐ MD100 or MD300	58.09 €

Accessories

Product number	Selectable parts	Price (net)
40785	96-deep-well plate (MD100/MD300) Vol. 2.2 ml	3.50 €
40750	Floater for *Xpress* MicroDialyzer (MD100/MD300)	3.68 €
40751	Sealing Sheet for 12 *Xpress* MicroDialyzer (MD100/MD300)	3,15 €

Attachment

Chemical resistance

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

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%	
Phosphoric acid 85%	
Sulfuric acid 98%	
Ammonium hydroxide 1N	
Ammonium hydroxide 25%	
Potassium hydroxide 1N	
Potassium hydroxide 32%	
Sodium hydroxide 1N	
Sodium hydroxide 32%	

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