A TECHNOLOGY GUIDE

For the Dialysis and Desalting of Macromolecules

VIVAproducts

Top considerations for sample dialysis and desalting

In 1861, chemist Thomas Graham at Anderson's University in Glasgow introduced dialysis, a method that used semipermeable membranes to selectively separate smaller molecules from larger ones in a solution (1). This concept laid the foundation for the first diffusion dialyzer available in the 1950s and became the preferred technique among scientists for removing salts and low molecular weight impurities from various solutions containing proteins, oligonucleotides, and polysaccharides (1). Efficient dialysis preserves the integrity and function of these key molecules, allowing scientists to generate reliable and reproducible data for many downstream applications, including mass spectrometry and enzyme activity assays (2). During dialysis a semipermeable membrane is flanked by a sample and a buffer solution. This membrane contains pores that retain or permit molecules to pass to the other side depending on their size. Buffer exchanges remove contaminants that have transferred from the sample to the buffer and allow more unwanted molecules to diffuse through the membrane.

When purifying macromolecules using dialysis, researchers should consider the following:

1. Shared buffer volume

Many conventional dialyzers require researchers to manually pipette dialysis buffer into individual wells on a 96-well plate, which takes considerable hands-on time. Using a shared buffer volume increases scalability and allows multiple samples to receive uniform buffer exposure, expediting dialysis. The XDB™ *Xpress* Dialysis Box from scienova™ can hold up to 750 ml of buffer and pairs with XDB Refill Kits that hold sample volumes between 100-1000 µl. This enables the simultaneous desalting or purification of up to 96 macromolecule samples in half the time compared to other systems (3,4). These samples include DNA, RNA, oligonucleotides, antibodies and other proteins.

2. Diffusion length

Dialysis time accelerates exponentially with decreasing distance between the sample and the dialysis membrane, known as diffusion length (5). One way to decrease diffusion length is to increase the ratio of membrane surface to sample volume. A larger membrane provides a bigger surface for the sample to contact, which increases dialysis efficiency. Dialysis devices with smaller ratios of membrane area to sample volume require much longer times to achieve the same results as dialyzers with larger membrane area to sample volume ratios (2). The membranes in the XDB Refill Kits minimize diffusion length and maximize the ratio of membrane area to sample volume, increasing dialysis speed (5).

3. Membrane composition

Different dialysis and desalting membranes can nonspecifically bind macromolecules depending on membrane composition, potentially causing sample loss. The XDB Refill Kit membranes contain regenerated cellulose, a low protein and low hormone binding material that helps achieve sample recoveries of up to 98 percent (6). Another aspect to consider is the membrane's pore size. A 10 kilodalton (kDa) membrane will retain most molecules larger than 10 kDa. Since the molecular weight cutoff is not a precise boundary, scientists should choose membranes that are one half to one third the molecular weight of their molecule of interest (7). The membranes used in the XDB Refill Kits have molecular weight cutoffs ranging from 2 to 140 kDa. They can retain proteins, nucleic acids, and macromolecular complexes, and can be used for equilibrium dialysis, antibody and protein purification, cell-free protein synthesis, protein renaturation and denaturation, and nanoparticle cleaning (6).

4. Continuous buffer circulation

Continuous buffer circulation prevents the buildup of small molecules on the outer side of the membrane, maintaining a consistent concentration differential for efficient diffusion (5). Increasing circulation through stirring the buffer prevents small molecules from re-entering the membrane and reintegrating into the sample. Researchers can achieve continuous buffer circulation by pairing the XDB *Xpress* Dialysis Box with an external peristaltic pump, which maintains consistent conditions across all samples in the shared buffer system. The external pump can also be used to exchange the buffer without interrupting dialysis.

Flexible, accelerated dialysis

Scientists can increase dialysis efficiency and reduce hands-on time through shared buffer volumes, large membrane surface area to sample volume ratios, and continuous buffer circulation. The XDB *Xpress* Dialysis Box and the XDB Refill Kits feature these characteristics and are also compatible with multichannel pipettes and automated liquid handling systems. Automating dialysis ensures that all samples undergo the same dialysis conditions, eliminating variability and enhancing reliability and reproducibility. Scientists can customize automated liquid handling systems to suit diverse dialysis needs. This flexibility is vital in drug discovery where different compounds or formulations might demand distinct dialysis parameters.

References

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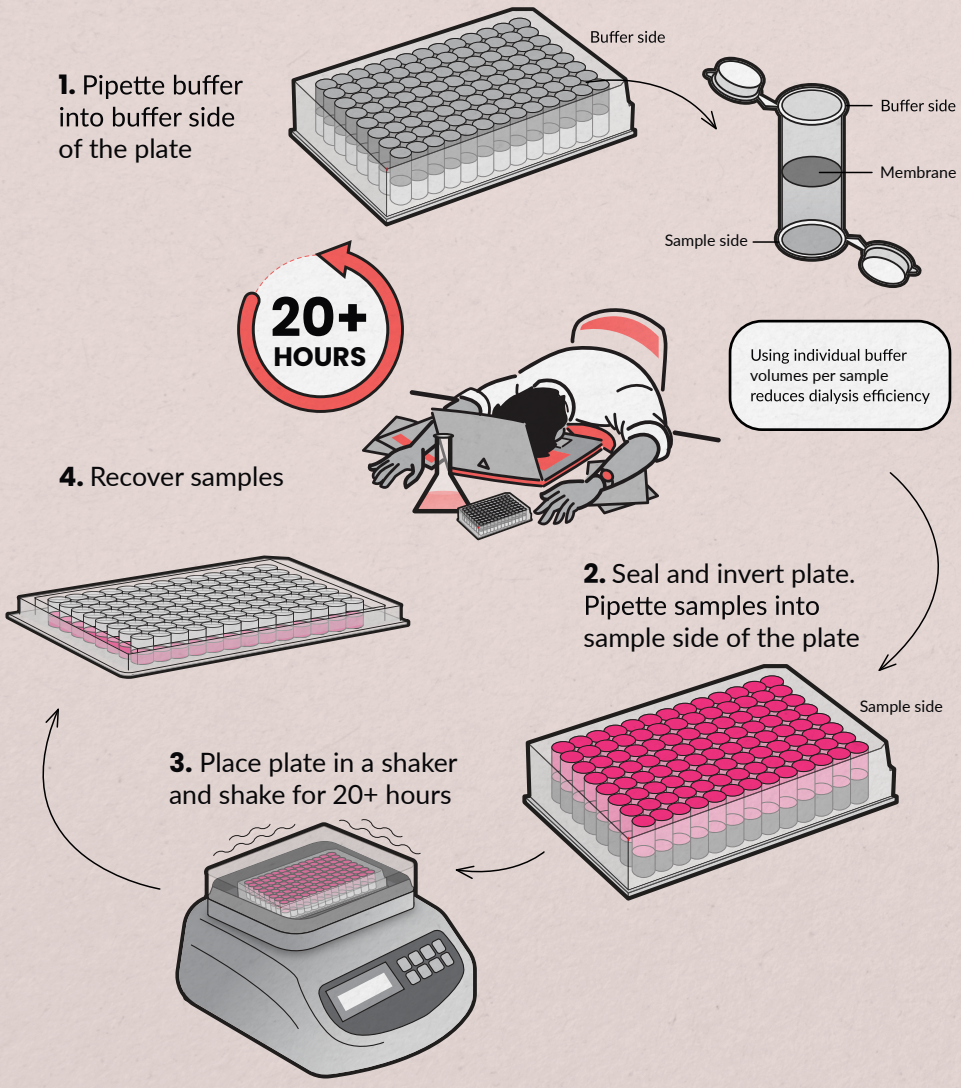
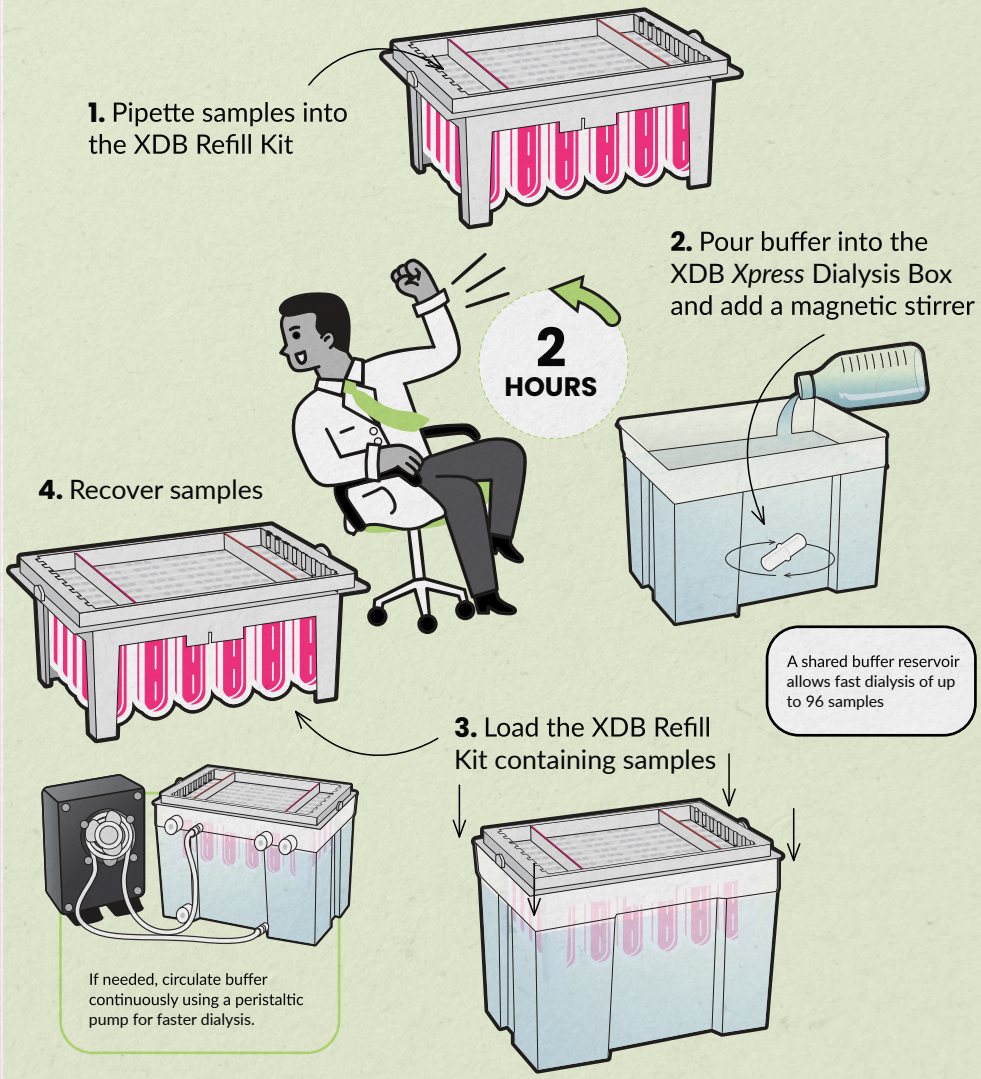
WITH OTHER DIALYZERS



WITH THE XDB *XPRESS* DIALYSIS BOX AND XDB REFILL KITS



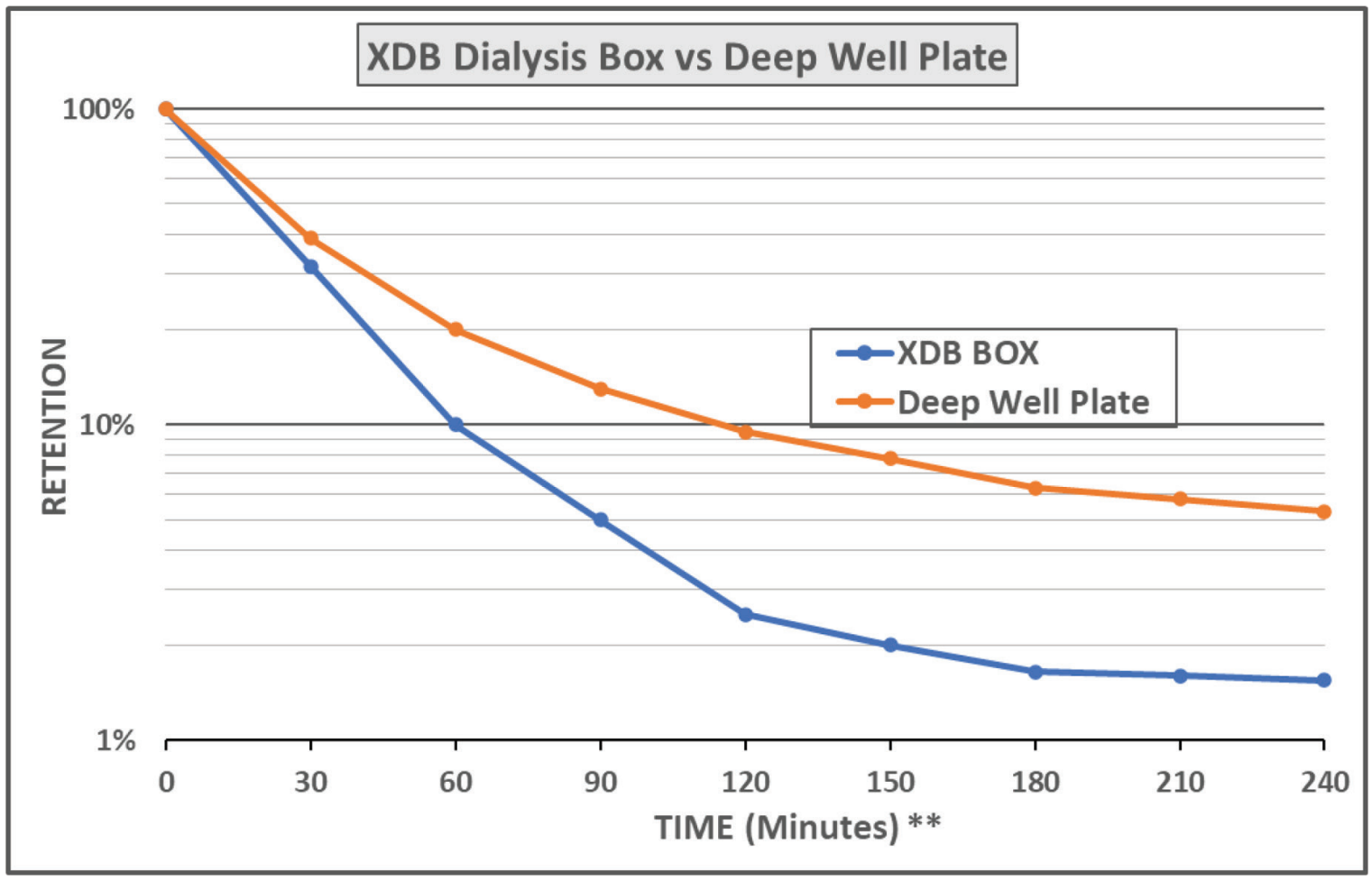
The XDB *Xpress* Dialysis Box and XDB Refill Kits allow the simultaneous dialysis of multiple small volume samples. The shared buffer reservoir of the XDB *Xpress* Dialysis Box speeds up dialysis times and the unique membrane geometry in the XDB Refill Kits maximizes efficiency and sample recovery.

Essential Materials

Material	Description
XDB *Xpress* Dialysis Box	The XDB *Xpress* Dialysis box can dialyze up to 96 samples in a shared buffer reservoir. The box can be assembled with refill kits containing membranes that can dialyze a wide range of macromolecules. It comes with a lid and small fixing rubbers to reduce sample and buffer evaporation and contamination.
XDB Refill Kits	The XDB Refill Kits contain dialyzer cartridges that researchers can fill using single pipettes, multichannel pipettes, or automated liquid handlers. Researchers can easily insert the XDB Refill Kits into the XDB box containing the buffer. The kits contain low binding regenerated cellulose membranes with molecular weight cutoffs between 2 and 140 kDa and can handle sample volumes ranging from 10 µl to 1000 µl.
Magnetic stirrer	A magnetic stirrer is recommended for buffer circulation. Researchers should place the magnetic stir bar in the XDB *Xpress* Dialysis Box before inserting the refill kit.
Tube connectors for a peristaltic pump (optional)	Researchers can connect the XDB *Xpress* Dialysis Box to a peristaltic pump for automated buffer exchanges.

Dialyze Samples in Half the Time

The new XDB™ dialyzer kits can purify DNA, RNA, oligonucleotide, antibody or other protein samples up to 98% in only two hours.



Need to quickly remove salts or other small molecules from large quantities of samples? The new XDB *Xpress* Dialysis Box kits can purify samples in a convenient well plate design compatible with automated liquid handling systems. The XDB Dialysis Box uses an easy to fill, shared buffer volume to achieve up to 90% removal of small molecules in an hour and 98% in two hours. This is about twice as fast as traditional deep well plate dialyzers.

The innovative design of the XBD Refill Kits allows all your samples to be loaded securely into the Dialysis Box in one step. The Refill Kits are available in three formats to accommodate samples ranging from 10-1000 µl. Regenerated cellulose membranes provide maximum sample recovery and are available in a wide assortment of molecular weight cutoffs.

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** Dialysis performed at ambient temperature using 1 mM 4-nitrophenol samples (100 µL each) and PBS 1-fold, pH 7.4 buffer. Dialysis membranes were 6-8 kDa MWCO. Buffer in the XDB Dialysis Box was mixed with a magnetic stirrer. Absorption of 4-nitrophenol measured at 405 nm (625 nm reference) with micro plate spectrometer.

Adapting Dialysis to Large-Scale Synthetic RNA Purification

Conventional dialysis paired with automated liquid handling systems allows overnight desalting of large RNA sample volumes.

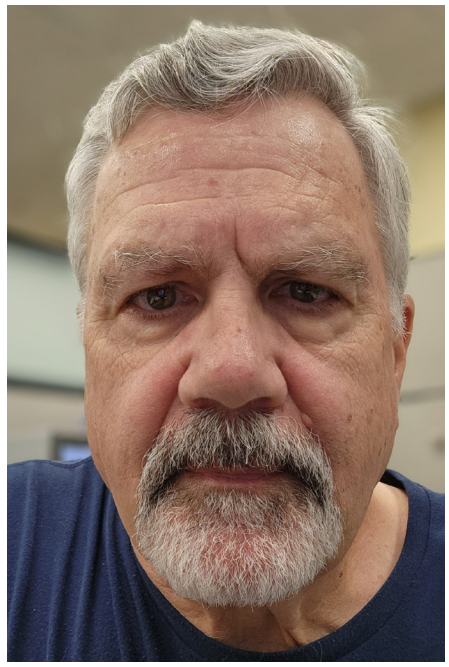
Residual salts and impurities compromise the functionality and accuracy of biomolecules like proteins and nucleic acids. Desalting ensures the integrity of synthetic molecules for various downstream applications, from molecular biology research and diagnostic assays to RNA-based therapeutics. Robert Kaiser, a research and development (R&D) scientist at Agilent Technologies, routinely synthesized DNA and RNA oligonucleotides with specific properties. One day, he ran into trouble when desalting large quantities of synthetic RNA and adapted the XDB *Xpress* Dialysis Box and XDB Refill Kits to quickly desalt large sample volumes with minimal manual work. Through his innovation, Kaiser achieved overnight desalination of sizable RNA volumes, a development that quickly transitioned into manufacturing to meet customer demands.

Can you describe how you improved your workflow for desalting synthetic RNAs?

We were making synthetic RNAs on a commercial oligo synthesizer in a 96-well format. We had an eight by 12 column arrangement and we needed to find a way to dialyze it to get pure synthetic RNA without residual salt or small molecules. We were handling RNA quantities in the 200 microgram to one milligram range. None of the commercial 96-well dialysis plates could process that much material at one time, and those that did took too long or required too much manual intervention. I found the XDB *Xpress* Dialysis Box and XDB Refill Kits from Vivaproducts, which had the same dimensions and footprint as a standard 96-well plate. We just needed a liquid handling robot to do all the manipulations. Our engineering team built a liquid handling system that could do dialysis overnight in an unattended fashion. This was the answer to a problem that had been difficult to solve, and we were able to take it from an R&D environment into manufacturing.

How did automated overnight dialysis facilitate your work?

It was simple to use once we identified the right setup of the liquid handling robot. Our engineers designed and built a holder for Vivaproducts' 96-well dialysis unit. We needed something to hold down the dialysis cartridges, so they didn't accidentally pop out when the pipette tips went in them. We built a unit that held up



CREDIT: ROBERT KAISER

Robert Kaiser, a R&D scientist at Agilent Technologies, adapted the XDB Xpress Dialysis Box and XDB Refill Kits for automated, overnight desalting of large amounts of synthetic RNA.

to 96 samples in a metal clamshell of sorts. It was a rack and a top that came down to hold everything rigidly in place. That meant we could use a reproducible set of settings on the liquid handling robot to fill the cartridges, pull the liquid out, and move it to another 96-well plate.

What features of the Vivaproducts dialyzer made it more efficient than others?

There weren't any other dialyzers that worked with a 96-well plate format easily and that had the same sample volume ranges. This was the only one that allowed processing of up to 96 samples at once. The individual MD300 XDB Refill Kits from Vivaproducts were perfect, because they could dialyze 300 µl samples and that was the volume output from our purification process. Our engineers developed a small custom system for adding water to the dialysis chamber, mixing it with a pump, and then automatically removing it every two hours and refilling it. The robots we used to move liquid had tips that fit nicely with the ports on the dialysis devices so that we had at least 80 percent sample recovery. This was the only thing that worked easily and reliably. And we were able to determine the amount of time and the number of water changes necessary to get to a salt concentration that was below the limit of detection. It was a lot easier than doing ultrafiltration, individual gel filtration, or using desalting columns. It allowed us to process 96 samples at a time hands off. It was time and labor efficient, which removed a lot of the cost from the end product.

What should researchers consider to achieve successful dialysis?

Researchers should make sure the device holds a little more volume than they need. We used both the 300 µl and the 1000 µl (MD1000) version of Vivaproducts' dialyzers and we found that in both cases it was best to not fill them to the maximum volume, so no liquid came out the top of the device. We filled them to about 80 percent and that worked great. There is some inherent sample loss. The MD300 XDB Refill Kit has a U-shaped flow path. There was always around 50 µl left behind and we had to do multiple removal steps. The MD1000 cartridges have a slightly different design and work a lot better because they draw liquid from the bottom of the device, and you can get almost everything out in one go. The tricky part is ensuring that everything is rigidly held in the same position from run to run so the robotics and the pipette tips routinely find the right openings to get fluid instead of air. That was an automation problem that was straightforward to handle even though I'm not an automation engineer. The dialysis membranes are fragile and can damage easily, but if you're careful, they work fine. We were working with as much as one milligram of RNA. With much less sample, you might have to use additives to keep your material from adhering to the inside of the cartridges due to nonspecific binding.

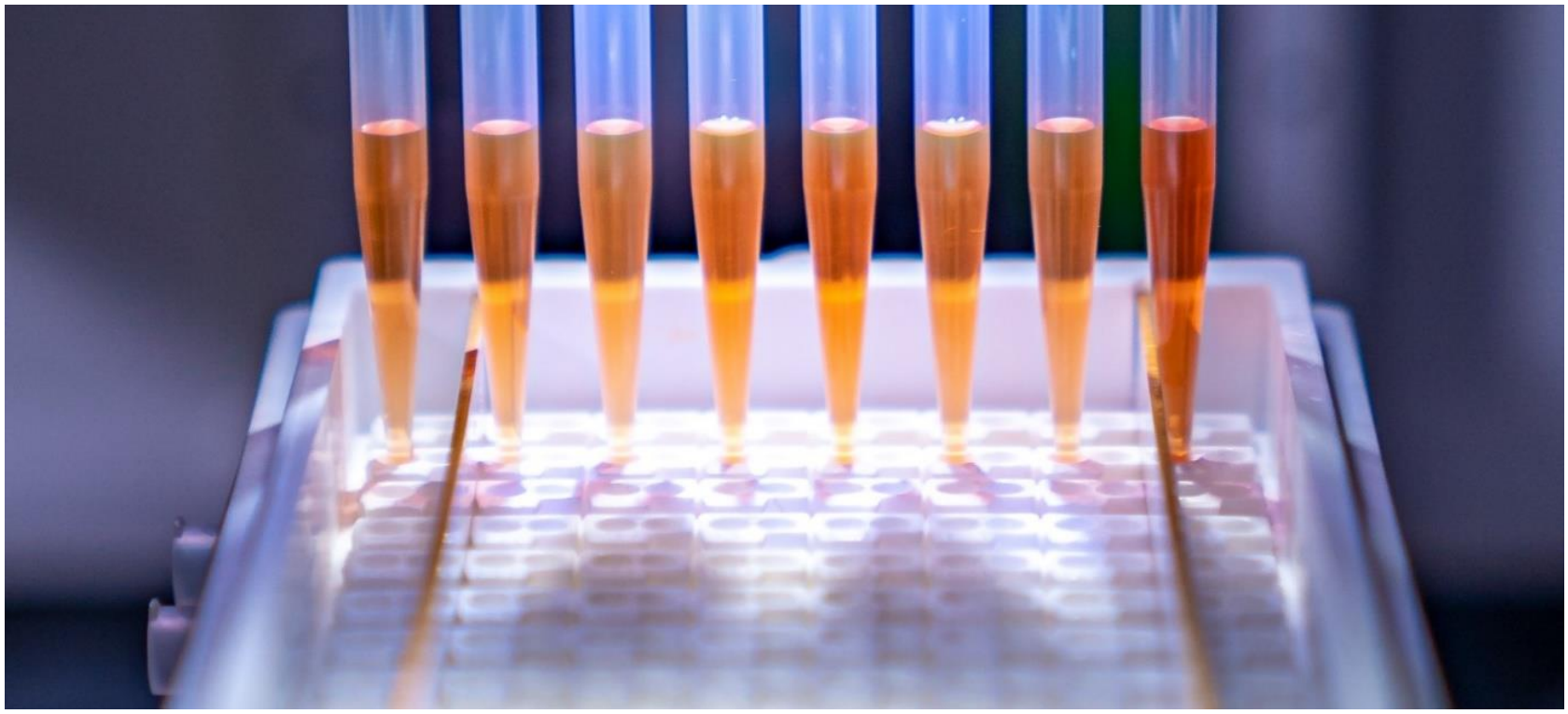
"There weren't any other dialyzers that worked with a 96-well plate format easily. This was the only one that allowed processing of up to 96 samples at once."

For what types of samples and applications would the Vivaproducts dialyzer be the most useful?

It was designed for proteins, but it worked great for nucleic acids. It works for any sufficiently large macromolecule considering the membrane's molecular weight cutoff. It works great for dialyzing large numbers of small volumes, for desalting samples, and for removing small molecules.

A MD1000 XDB Refill Kit can process large quantities of protein, RNA, and DNA from up to 48 1000 µl samples at a time.

CREDIT: VIVAPRODUCTS



Fast Automated Desalting & Dialysis

The new XDB™ micro-dialyzer kits are easy to automate and can purify your DNA, RNA, oligonucleotide, antibody or other protein samples up to 98% in two hours.

Need to quickly remove salts or other small molecules from large quantities of samples? The new XDB *Xpress* Dialysis Box kits can purify samples in a convenient well plate designed for simple use with automated liquid handling systems. The XDB Dialysis Box uses an easy to fill, shared buffer volume to achieve up to 90% removal of small molecules in an hour and 98% in two hours. This is about twice as fast as traditional deep well plate dialyzers.

The design of the XBD Refill Kits allows your samples to be loaded securely into the Dialysis Box in one step. The Refill Kits are available in three formats to accommodate samples ranging from 10-1000 µl in volume. Regenerated cellulose membranes provide maximum sample recovery and are available in a wide assortment of molecular weight cutoffs. Dialysis time can be reduced by using a magnetic stirrer or connecting the XDB Dialysis Box to an external pump using the built-in Luer tubing fittings.

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