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Laboratory Dialysis: FAQ's

Q1: How do you prepare dialysis membrane tubing?

Preparation instructions will vary based on the membrane type as follows. Note that boiling membrane is not recommended as it can damage the membrane and alter the pore rating.

A) Biotech RC, CE, and PVDF membranes should be rinsed in DI water for 15 to 30 minutes to remove sodium azide preservative.

B) Spectra/Por® 7 Standard RC has been pretreated to remove the trace levels of heavy metals and sulfides and only requires a 15 to 30 minute soak in DI water to remove the sodium azide preservative.

C) Spectra/Por® 1 through 6 Standard RC membranes may require some extra preparation. While rinsing Spectra/Por® 1 through 6 in water is typically sufficient to remove glycerin or preservative, Spectrum offers two membrane pre-treatment solution kits for the removal of the trace levels of heavy metals and sulfides introduced during manufacturing. Heavy Metal Cleaning Solution and Sulfide Removal Solution Kits are recommended for ultra-sensitive dialysis applications like binding studies or when low level presence of these contaminants may interfere with downstream analysis of the dialysis sample. Refer to the Membrane Dialysis Accessories webpage for more product information.

Q2: How does dialysis work and how long does it take to complete?

Dialysis is the diffusion of dissolved solutes across a selectively permeable membrane against a concentration gradient in an effort to achieve equilibrium. While small solutes pass through the membrane larger solutes are trapped on one side.

By exchanging the dialysate buffer on the outside side of the membrane, you can continually pull away the smaller solutes to purify the trapped larger molecules. In general, dialysis will be most effective when the buffer is replaced a few times over the course of a day and then left overnight at room temperature on a stir plate. A standard protocol for dialysis is 16 to 24 hours. Many factors affect the rate dialysis, including: diffusion coefficients, pH, temperature, time, concentration of species, sample volume, dialysate (buffer) volume, number of dialysate changes, membrane surface area, membrane thickness, molecular charges and dialysate agitation (stirring).

Q3: How much volume of dialysate is needed to dialyze a sample and how often does the dialysate need to be changed?

The larger the dialysate volume, the greater the driving force for diffusion of small molecules. We generally recommend a 100:1 buffer to sample volume ratio. By replacing the buffer just as the rate of diffusion slows down and the solutions are approaching equilibrium, you can maintain the driving force and the rate of dialysis. We generally recommend two or three buffer changes over the period of 12 - 24 hrs as follows:

First buffer change:	After 2-3 hours
Second buffer change:	After 4-5 hours
Last buffer change:	Prior to leaving overnight.

Q4: How do I select the correct Molecular Weight Cut Off (MWCO)?

The MWCO should be chosen as high as possible in order to maximize the dialysis rate. However, in order to achieve a higher sample recovery you can select the MWCO that is about half of the molecular weight of the macromolecules that need to be retained. For Applications in which separation of molecules is required, there must be at least a 5x difference between the molecular weight of both species for membrane dialysis to be effective. Otherwise, you may require other separation techniques such as chromatography or TFF filtration.

Q5: Which membranes minimize protein binding the best?

Each type of membrane displays a different affinity for various molecules. For globular proteins, the relative binding affinity is CE < RC < PVDF.

Q6: How do you select the right closure and closure size for membrane tubing?

Standard RC membrane (Spectra/Por 1-7) and Biotech RC is constructed of flexible regenerated cellulose polymers and can be sealed using any of the dialysis tubing closures. Biotech CE and PVDF are constructed of a more rigid polymer requiring gentler Universal closures. Since these work well for all dialysis tubing; when in doubt, use Universal Closures. Standard Closures should ONLY be used with Standard RC tubing.

It is recommended to use a closure with a sealing width of 4-10 mm longer than the flat width of the dialysis tubing. The smallest Universal Closure has a sealing width of 50 mm. This will seal all flat widths of our Biotech Grade tubing.

Q7: What is the shelf life for dialysis membranes?

The dry packaged dialysis membranes have a shelf-life of 5 years. The wet packaged (0.1% sodium azide solution) membranes have a shelf-life of 3 years. The irradiated membranes have a shelf life of 1.5 years.

Q8: Can I still use the membrane if it dries out or freezes?

If wetted membrane dries out, the pore size is adversely affected and the membrane becomes brittle and will likely leak. The membrane should be discarded.

If the membrane freezes, the ice crystals may rupture the membrane and also cause leaking. It is recommended not to use the membrane. However, you can try slowly increasing the temperature until the storage solution completely melts. The possibility remains that the membrane is not longer integral.

Q9: Are dialysis membranes endotoxin free? (I don't think this is true for Biotech membranes)

No. Spectrum manufactures dialysis membranes intended for laboratory use.

Q10: Should I be concerned about the creases, roller marks, or fold lines on membranes?

The roller marks or fold lines along the membranes will not affect the diffusion properties as long as the membrane is integral.

Q11: Why is the CE membrane more rigid and opaque than RC?

While the polymers in the cellulose ester (CE) membrane cross-link to form a more rigid molecular lattice, the regenerated cellulose (RC) polymers form a more flexible lattice structure. The opaqueness comes from the pores in a more rigid frame. The larger the pore size, the more opaque the membrane.

Q12: Can dialysis membranes be chemically or heat sealed?

The CE and RC dialysis membranes can only be mechanically sealed. However, the PVDF dialysis membranes can be mechanically sealed or heat sealed and is often used in this manner for the purposes of sample "encapsulation".

Q13: Can Spectra/Por® dialysis membranes be re-used for the same protein samples?

We do not recommend re-using dialysis membranes since they can be contaminated through handling and dialysis conditions (pH, temperature, chemical exposure, etc.) can alter the membrane integrity and/or cause leaking, especially when removing and reapplying closures. Dialysis membranes are designed for single use.

Q14: How accurate are the membrane pore sizes and what is MWCO?

Since dialysis membrane consists of a spongy matrix, it is more appropriate and practical to measure the "pore size" indirectly by rating its retention performance characterized by its "Molecular Weight Cut Off" (MWCO). The MWCO is defined by the molecular weight solute that is 90% retained by the membrane during a 17 hour period. For this reason, you should select a MWCO that is just smaller than the size of the solutes you want to retain.

Q15: What is the difference between Spectra/Por® 2 and 4 dialysis membranes?

Both Spectra/Por® 2 and 4 have a MWCO of 12–14 kD. While Spectra/Por® is more suited for general dialysis, Spectra/Por® 2 offers special higher and lower FW's and/or higher permeability; i.e. water permeability of Spectra/Por® 2 is superior to that of Spectra/Por® 4.

Q16: Is there a "rule of thumb" regarding membrane surface area to sample volume?

The surface area to volume ratio is a function of the tubing flat width. If you have a two equal length pieces of tubing with two different flat widths, the smaller flat width piece possesses a higher surface area to volume ratio and dialyzes quicker while the larger flat width piece possesses a lower surface area to volume ratio and dialyzes slower. The smaller flat width has a shorter distance for diffusion and less solute "competition" through the membrane pores. Larger flat widths have a longer distance to the membrane and more solute competition through the pores. In general, the greater the surface-area-to-volume ratio, the quicker the dialysis.

Q17: How good is the mass transfer across the membrane if the osmolarity is equal on both sides but concentration gradients still exist?

Most dialysis is done with no osmotic pressure across the membrane. The dialysis process is driven by the concentration gradient from the inside and outside of the dialysis tubing. If there is a large difference in osmotic pressure, water will move across the membrane. If too much water migrates across the membrane, the dialysis tubing can potentially burst or collapse, depending upon the direction of water movement.

Q18: How much pressure can a dialysis membrane withstand if used for ultrafiltration?

Dialysis membranes are not designed for pressure filtration. The maximum recommended pressure is 1.5 psi without affecting the MWCO.

Q19: Which dialysates (buffers) are commonly used in dialysis?

Biomolecules must be maintained under strict pH control to stabilize their molecular properties. The typical pH range for dialysis buffers is 6 – 8. The following are some of the common solutions/buffers found in biochemical solutions:

- Water
- PBS: Phosphate buffer saline
- TBS: Tris buffered saline
- HEPES
- Amino Acid Buffers

Q20: Why use microns vs. Daltons (MW) units and how do you convert between them?

While the size of dissolved molecules is defined by molecular weight (MW) units in Daltons, particle and cell size is defined by metric diameter because the MW units become impractical and do not account for shape in the microscopic range. Since microns are a measure of a 2-dimensional distance and Daltons are a measure of 3-dimensional size based on atomic weight units, there is no direct conversion from one to the other. For this reason, many common biological materials were characterized for dialysis, ultrafiltration and microfiltration purposes and plotted on a conversion chart to correlate the approximate scales as a reference for estimating conversions. Refer to Spectrums' Pore Size Chart on our website for converting between Daltons and metric units.

Q21: What is the best way to dialyze a sample with a high salt concentration?

We recommend performing dialysis against a low salt concentration buffer if possible. It is also not advisable to dialyze against pure DI water as this will cause the osmotic pressure to draw water into the tubing, "balloon" and potentially rupture the membrane. A serial dialysis using buffers with decreasing concentration of solutes (salt) will prevent the osmotic pressure from swelling the membrane. Try to reduce the order of magnitude of solute concentration by a factor of 10 to 1 at each buffer exchange; i.e. dialyze a sample with 5 M NaCl against a buffer with 500 mM NaCl.

Q22: Can I tie knots in the dialysis tubing instead of using closures?

We strongly recommend against tying knots in the tubing. Tied knots do not provide an effective seal against leakage. Only dialysis closures will provide the adequate seal necessary for safe dialysis of your sample.

Q23: How long should I cut the dialysis tubing?

Along with each tubing flat width, Spectrum lists the correlating volume/length ratio that can be used to calculate how much length is required to contain your sample volume. For example if the FW is 16 mm, the volume/length ratio is 0.79 ml/cm. To contain a sample of 5 ml, you will need a length of approximately 6.5 cm. However, you also need to add about 10 – 20% (but at least 1 cm) more length to account for head space (air) to keep your sample buoyant. Lastly you need to add enough about 2 cm at each end to allow for applying two closures. The total tubing length would be at least 11.5 cm.

The simple equation to calculate total required tubing length is as follows:

Total length = (sample volume) / (vol/length) + (additional 10-20%) + 4 cm

Visit our website and use our convenient Dialysis Tubing Calculator to calculate the required tubing length based on your sample volume and tubing flat width.