PIERCE
3747 N. Meridian Road
P.O. Box 117
Rockford, IL 61105

Dialysis: an overview

TR0020.0

Introduction

Pierce offers a variety of unique products to facilitate the buffer exchange and removal of small contaminants by dialysis. SnakeSkin® Dialysis Tubing, Slide-A-Lyzer® Dialysis Cassettes and Slide-A-Lyzer® MINI Dialysis Units (see Related Pierce Products) all offer unprecedented convenience and performance for dialysis of samples ranging from 10 µl to 100 ml. Although these high-performance dialysis products are much easier to use than traditional dialysis tubing, which requires boiling and soaking before use, they make use of the same principles of dialysis.

Dialysis is Diffusion Through a Semi-Permeable Membrane

Dialysis is the movement of molecules by diffusion from high concentration to low concentration through a semi-permeable membrane. Only those molecules that are small enough to fit through the membrane pores are able move through the membrane and reach equilibrium with the entire volume of solution in the system. Once equilibrium is reached, there is no further net movement of the substance because molecules will be moving through the pores into and out of the dialysis unit at the same rate. By contrast, large molecules that cannot pass through the membrane pores will remain on the same side of the membrane as they were when dialysis was initiated.

To remove additional unwanted substance, it is necessary to replace the dialysis buffer so that a new concentration gradient can be established. Once the buffer is changed, movement of particles from high (inside the membrane) to low (outside the membrane) concentration will resume until equilibrium is once again reached. With each change of dialysis buffer, substances inside the membrane are further purified by a factor equal to the volume difference of the two compartments. For example, if one is dialyzing 1 ml of sample against 200 ml of dialysis buffer, the concentration of the dialyzable substance at equilibrium will be diluted 200 less than at the start. Each new exchange against 200 ml of new dialysis buffer will dilute the sample 200 times more. For example, for three exchanges of 200 ml, the sample will be diluted 200 x 200 x 200 or 8,000,000 times, assuming complete equilibrium was reached each time before the dialysis buffer was changed.

Factors Affecting Dialysis Rate

Factors that affect the completeness of dialysis include (1) dialysis buffer volume, (2) buffer composition, (3) the number of buffer changes, (4) time, (5) temperature and (6) particle size vs. pore size. Substances that are very much smaller than the pore size will reach equilibrium faster than substances that are only slightly smaller than the pores. SnakeSkin® and Slide-A-Lyzer® Dialysis Products use membranes that contain convoluted pores, not the tube-like pores often found in traditional dialysis tubing. Convoluted pores allow small molecules to pass through the twists and turns of the pores with greater ease than do larger molecules. The greater the difference in molecular weight of the unwanted molecule vs. the molecular weight cut-off (MWCO) of the pore size, the greater the rate of dialysis.

SnakeSkin® and Slide-A-Lyzer® Dialysis Products are available in 3.5K, 7K and 10K MWCO membranes types. The MWCO describes the molecular weight at which a compound will be 90% retained following overnight (17-hour) dialysis. The MWCO is determined by testing many different proteins of known molecular weight. In general, these MWCO apply to globular molecules, such as most proteins. More linear proteins may be able to pass through the pores, even though their molecular weight exceeds the stated MWCO. To compensate for this, choose a dialysis device with a smaller MWCO. For DNA or RNA, a MWCO no greater than one-third the MW should be used in order to prevent excessive sample loss.

The aforementioned factors highlight the difficulty in predicting dialysis time required for a particular sample. For thorough exchange of buffering salts, most researchers use a volume of dialysis buffer 200-500 times that of the sample and dialyze at room temperature for a total of 6-8 hours with 3 changes of the dialysis buffer. After the last change, many researchers continue to dialyze overnight at 4°C. This general guideline is also appropriate for removal of most excess labeling reagents and reaction byproducts from biotinylation or cross-linking experiments. However, some dyes form large aggregates and so do not dialyze efficiently despite having low monomer molecular weights.

Other Factors in Dialysis

Because dialysis involves molecules passing through the semi-permeable membrane in both directions and each substance reaches its own equilibrium independent of other substances, sample dilution can occur. If a substance's concentration is higher on the outside of the membrane and is small enough to pass through the pores, it will have a net movement from the dialysis buffer into the sample.

Water is such a small molecule that it is capable of passing through the pores of virtually all dialysis membranes. When dialyzing a high solute concentration against a dilute dialysis buffer, there will be a net movement of water (and possibly salts) into the dialysis unit through the membrane. Glycerol and some sugars are especially hygroscopic, and as rapidly as they diffuse across the membrane to reach equilibrium, they also significantly affect the osmosis of water across the membrane and so may cause a change in volume of the sample. Take care when dialyzing with large differences in glycerol or sugar concentration between sample and dialysis membrane.

Prevent this movement of water and consequent change in sample volume by dialyzing in a "stepwise" fashion, minimizing the difference in water concentration between sample and dialysis buffer at each stage in the dialysis process. For example, when processing a sample with very high solute concentration against a buffer with very low solute concentration, dialyze first against a fairly concentrated dialysis buffer. With each subsequent replacement of dialysis buffer, use a less concentrated buffer until the desired final buffer concentration is reached.

Finally, some molecules may stick to the dialysis membrane, which is made of regenerated cellulose. This nonspecific binding can result in sample loss. The percent of total protein lost is partially dependent on the protein concentration. Protein loss caused by nonspecific binding to the membrane is negligible for concentrated samples (>0.5 mg/ml) but may be significant with dilute protein samples (<0.1 mg/ml). Adding a “carrier” protein such as BSA to dilute protein sample before dialysis will prevent this loss.

Related Pierce Products

Slide-A-Lyzer® Dialysis Cassettes:

MWCO	Capacity	Product No. 10 Pack*	Product No. Kit*
3,500	0.1-0.5 ml	66333	66335
3,500	0.5-3 ml	66330	66332
3,500	3-12 ml	66110*	66107*
7,000	0.1-0.5 ml	66373	66375
7,000	0.5-3 ml	66370	66372
7,000	3-12 ml	66710*	66707*
10,000	0.1-0.5 ml	66383	66385
10,000	0.5-3 ml	66380	66382
10,000	3-12 ml	66810*	66807*

*Packs and kits of 3-12 ml cassettes have 8 units per package.

68035 **SnakeSkin® Dialysis Tubing,** 3.5K MWCO, 35 linear feet for multiple 10-100 ml samples

68700 **SnakeSkin® Dialysis Tubing,** 7K MWCO, 35 linear feet for multiple 10-100 ml samples

68100 **SnakeSkin® Dialysis Tubing,** 10K MWCO, 35 linear feet for multiple 10-100 ml samples

69550 **Slide-A-Lyzer® MINI Dialysis Units,** 3.5K MWCO, 50 units, each for 10-100 µl samples

69560 **Slide-A-Lyzer® MINI Dialysis Units,** 7K MWCO, 50 units, each for 10-100 µl samples

69570 **Slide-A-Lyzer® MINI Dialysis Units,** 10K MWCO, 50 units, each for 10-100 µl samples

References

McPhie, P. (1971). Dialysis. *Meth. Enzymol.* **22**, 23-33.