Dialysis

Conventional dialysis separates small molecules from large molecules by allowing diffusion of only the small molecules through selectively permeable membranes. Dialysis is usually used to change the salt (small-molecule) composition of a macromolecule-containing solution. The solution to be dialyzed is placed in a sealed dialysis membrane and immersed in a selected buffer; small solute molecules then equilibrate between the sample and the dialysate. Concomitant with the movement of small solutes across the membrane, however, is the movement of solvent in the opposite direction. This can result in some sample dilution (usually <50%).

This section describes dialysis of large- and small-volume samples using cellulose membranes with pore sizes designed to exclude molecules above a selected molecular weight (Basic and Alternate Protocols). A Support Protocol describes preparation of membranes for dialysis.

LARGE-VOLUME DIALYSIS

BASIC PROTOCOL

This protocol describes the use of membranes, prepared using the support protocol, for dialysis of samples in large, easily handled volumes, typically 0.1 to 500 ml.

Materials

Dialysis membrane (see Support Protocol)
Clamps (Spectrapor Closures, Spectrum, or equivalent)
Macromolecule-containing sample to be dialyzed
Appropriate dialysis buffer

1. Remove dialysis membrane from ethanol storage solution and rinse with distilled water. Secure clamp to one end of the membrane or knot one end with double-knots.

   Always use gloves to handle the dialysis membrane because the membrane is susceptible to cellulolytic microorganisms.

   See support protocol for a discussion of commercially available membranes and preparation and storage procedures.

   Clamps are less likely to leak than knots, but either type of closure should be carefully tested as described in steps 2 and 3.

2. Fill membrane with water or buffer, hold the unclamped end closed, and squeeze membrane. A fine spray of liquid indicates a pinhole in the membrane; discard and try a new membrane.

   Pinholes are rare but cause traumatic sample loss. Hard squeezing will cause some liquid to seep from the bag; this is normal.

3. Replace the water or buffer in dialysis membrane with the macromolecule-containing sample and clamp the open end. Again, squeeze to check the integrity of the membrane and clamps.

   If dialyzing a concentrated or high-salt sample, leave some space in the clamped membrane; there will be a net flow of water into the sample, and if sufficient pressure builds up the membrane can burst.

4. Immerse dialysis membrane in a beaker or flask containing a large volume (relative to the sample) of the desired buffer. Dialyze at least 3 hr at the desired temperature with gentle stirring of the buffer.

   Dialysis rates are dependent on membrane pore size, sample viscosity, and ratio of membrane surface to sample volume. Temperature has little effect on dialysis rate, but low

Contributed by Louis Zumstein
Current Protocols in Protein Science (1995) A.3B.1-A.3B.4

temperatures (i.e., 4°C) are usually chosen to enhance macromolecule stability. Common salts will equilibrate across a 15,000-MWCO membrane (see Support Protocol) in ~3 hr with stirring.

Some compounds, especially detergents above their critical micelle concentration (CMC), dialyze very slowly.

5. Change dialysis buffer as necessary.

   Usually two to three dialysis buffer changes are sufficient. For example, when 100 mM Tris·Cl is removed from a protein for sequence analysis or other amino-reactive chemistry, two equilibriums against a 1000-fold volume excess of buffer will decrease the Tris concentration 10^6-fold, to 100 nM; three dialyses will decrease the concentration to 100 pM.

6. Remove dialysis membrane from the buffer. Hold the membrane vertically and remove excess buffer trapped in end of membrane outside upper clamp. Release upper clamp and remove the sample with a Pasteur pipet.

ALTERNATE PROTOCOL

SMALL-VOLUME DIALYSIS

For solution volumes less than ~100 µl, the use of dialysis membrane as described above can result in unacceptable sample loss. The method described below can easily dialyze volumes as small as 10 µl. The sample is held in a 0.5-ml microcentrifuge tube, with dialysis membrane covering the open end of the tube.

Additional Materials

0.5-ml microcentrifuge tube
Cork borer

1. Cut a hole in the cap of a 0.5-ml microcentrifuge tube using a cork borer. Be sure that there are no rough edges on the inside of the cap (which will be in contact with the dialysis membrane).

   The success of this protocol is dependent on a tight-fitting cap, so make sure the cap is well-seated. On the other hand, an excessively tight-fitting cap can rip the membrane when it is inserted. Sarstedt tubes work well.

   Alternatively, some researchers use a microcentrifuge tube without a cap. The sample is placed in the microcentrifuge tube, a dialysis membrane is placed over the top of the tube, and the membrane is secured with a rubber band. With this method, however, there is a risk that a small sample—e.g., 10 to 20 µl—may be lost around the edge of the tube.

2. Place sample in the microcentrifuge tube, cover tube opening with dialysis membrane, and snap on the tube cap in which a hole has been cut.

   This places a fair amount of stress on the dialysis membrane, so use a fairly thick membrane and make sure there are no rough edges on the cap.

3. To get the sample in contact with the dialysis membrane, *gently* centrifuge the microcentrifuge tube in an inverted position in a tabletop centrifuge.

4. Keep the tube inverted, immerse it in dialysis buffer, and anchor it so that it will remain inverted and immersed.

   This is easily done by inserting tubes in a styrofoam or foam sheet (e.g., Fisherbrand foam microcentrifuge tube rack) and placing the inverted tubes on the surface of the dialysis buffer.

5. Use a bent Pasteur pipet to blow out any air bubbles trapped under the cap to allow dialysis buffer to contact the membrane.

This step is usually not necessary if the membrane/rubber band method is used (see step 1 annotation).

6. Stir the dialysis buffer and dialyze at an appropriate temperature for at least 3 hr (see Basic Protocol, step 4).

7. To recover the sample, remove microcentrifuge tube from the buffer and centrifuge briefly right-side-up.

   Commercially available alternatives to this method include use of individual hollow-fiber filters from Spectrum (with sample capacities of 1 to 140 µl), and many different microdialysis machines, both single-sample and multisample (Spectrum, Cole-Parmer, Hoefer Pharmacia, and others).

SELECTION AND PREPARATION OF DIALYSIS MEMBRANE

SUPPORT PROTOCOL

Dialysis membranes are available in a number of thicknesses and pore sizes. Thicker membranes are tougher, but restrict solute flow and reach equilibrium more slowly. Pore size is defined by "molecular weight cutoff" (MWCO)—i.e., the size of the smallest particle that cannot penetrate the membrane. The MWCO designation should be used only as a very rough guide; if accurate MWCO information is required, it should be determined empirically (see Craig, 1967, for a discussion of parameters affecting MWCO). Knowledge of the precise MWCO is usually not required, however; it is necessary only to use a membrane with a pore size that is much smaller than the macromolecule of interest. For most plasmid and protein dialyses, a MWCO of 12,000 to 14,000 is appropriate, whereas a MWCO of 3500, 2000, or even 1000 is appropriate for peptides.

Most dialysis membranes are made of derivatives of cellulose. They come in a wide variety of MWCO values, ranging from 500 to 500,000, and also vary in cleanliness, sterility, and cost. Spectrum has the most impressive inventory. The least expensive membranes come dry on rolls; these contain glycerol to keep them flexible as well as residual sulfides and traces of heavy metals from their manufacture. If glycerol, sulfur compounds, or small amounts of heavy metals are problematic, cleaner membranes should be purchased or membranes should be prepared as described below. Protein dialysis should only be done with clean membranes.

Additional Materials

10 mM sodium bicarbonate
10 mM Na_2EDTA, pH 8.0
20% to 50% (v/v) ethanol

1. Remove membrane from the roll and cut into usable lengths (usually 8 to 12 in.).

   Always use gloves to handle dialysis membrane, as it is susceptible to a number of cellulolytic microorganisms. Membrane is available as sheets or preformed tubing.

2. Wet membrane and boil it for several minutes in a large excess of 10 mM sodium bicarbonate.

3. Boil several minutes in 10 mM Na_2EDTA. Repeat.

   Boiling speeds up the treatment process but is not necessary. A 30-min soak with some agitation can substitute for the boiling step.

4. Wash several times in distilled water.

5. Store at 4°C in 20% to 50% ethanol to prevent growth of cellulolytic microorganisms.

   Alternatively, bacteriostatic agents (e.g., sodium azide or sodium cacodylate) may be used for storage; however, ethanol is preferred for ease and convenience.

LITERATURE CITED

Craig, L.C. 1967. Techniques for the study of peptides and proteins by dialysis and diffusion. *Methods Enzymol.* 11:870-905.

McPhie, P. 1971. Dialysis. *Methods Enzymol.* 22:23-32.

Describe the theory and practice of dialysis and diffusion.

Contributed by Louis Zumstein
Introgen Therapeutics, Inc.
Houston, Texas