Microdialysis Crystallization

HAMPTON RESEARCH
Solutions for Crystal Growth

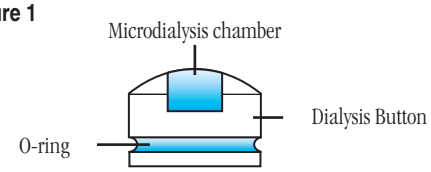
Method

In the microdialysis crystallization method the sample is separated from the crystallization reagent by a semi-permeable membrane. The semi-permeable membrane allows small molecules, such as salts, additives, and other crystallization reagents to pass, but prevents biological macromolecules from crossing the membrane. Crystallization of the sample takes place due to the diffusion of crystallization reagent out of, or into the sample, at constant sample concentration.

Microdialysis Buttons

The Dialysis Buttons are injected molded polystyrene. The button has a chamber which varies from 5 to 350 microliters depending upon which size button one chooses to use. The sample is placed in this chamber so as to create a slight dome of liquid at the top edge of the button. A Dialysis Membrane (having the appropriate molecular weight cut-off) is placed over the top of the button/sample and is held in place with an O-ring. The O-ring is held in place by a groove in the dialysis button. See Figure 1.

Figure 1



A typical dialysis experiment is used to take the sample from the presence of a high ionic strength solution to a lower ionic strength solution (however, the technique can just as easily be used to proceed from low ionic strength to a higher ionic strength). This is accomplished by placing the sample in high ionic strength in the Dialysis Button, sealing the button with a dialysis membrane and placing the sealed button in a solution of ionic strength lower than that inside the button. Salts, ligands, and compounds smaller than the pore size of the dialysis membrane will leave the button as long as their concentration is lower on the opposite side of the membrane. Once the concentration of the diffusible species is the same on both sides of the membrane, the system is in equilibrium.

Using the Dialysis Buttons

The following two practical's offer examples of how to set up a dialysis experiment using the Dialysis Buttons.

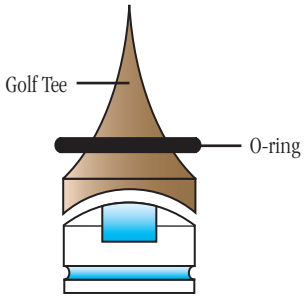
Practical 1 - Carboxypeptidase A

1. Using Carboxypeptidase A, make an 8 to 20 mg/ml solution of the Carboxypeptidase A in 20 mM TRIS HCl pH 7.5, 1.5 M LiCl.

2. Place 100 µl of 10 mg/ml Carboxypeptidase A in 20 mM TRIS HCl pH 7.5, 1.5 M LiCl in a 100 µl Dialysis Button. The droplet should have a slight dome shape following the hemispheric edge of the top of the Dialysis Button.

3. Seal the Dialysis Button with the Dialysis Membrane. Using a one inch (2.5 cm) square of Dialysis Membrane which has equilibrated in water, place the membrane over the top of the button. Place an inverted golf tee on top of the membrane and button. Roll the O-ring down the golf tee until the O-ring rolls of the golf tee onto the edge of the button. Roll the O-ring into the machined groove on the edge of the button. Remove the golf tee. There should be no bubbles between the membrane and the sample inside the button. Bubbles will prevent dialysis.

Figure 2

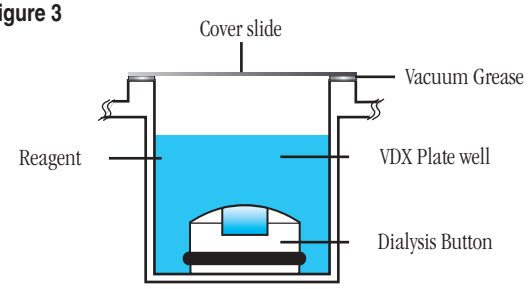


4. Place 0.9 ml of 20 mM TRIS HCl pH 7.5 in the reservoir of a VDX™ plate.

5. Place the Dialysis Button in the well, membrane side up. Be sure the reservoir solution covers the top of the membrane/button. Seal the VDX plate using vacuum grease and a cover slide. See Figure 3 below.

6. Observe under a microscope. Crystals will appear within 2 to 3 days. Final concentration of LiCl will be 0.15 M.

Figure 3



Practical 2 - Lysozyme

1. Prepare 20 mg/ml Lysozyme in 50 mM Sodium acetate trihydrate pH 4.6. Filter the solution using a 0.2 micron filter.

2. Fill and seal a 100 microliter Dialysis Button with 100 microliters of the Lysozyme solution as described for the Carboxypeptidase A practical.

3. Pipet 1 milliliter of 1.0 M Sodium chloride, 50 mM Sodium acetate pH 4.6 into reservoir of a VDX plate.

4. Place the filled button, membrane side up in the beaker.

5. Observe under a microscope. Crystals will appear within 2 to 3 days.

Microdialysis Crystallization

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Diaplate for Microdialysis Crystallization

The disposable Diaplate is a 96 well microdialysis plate used for the desalting or crystallization of proteins from very small volumes of up to a maximum of 3.2 µl. The sample solution is pipetted into each of the 96 microdialysis wells against up to 350 µl dialysis solution used for exchange. Each of the 96 wells has a separate regenerated cellulose membrane inside meaning no cross contamination or leakage between wells. The size of the compounds that pass through the membrane are determined by its Molecular Weight Cut Off (MWCO) of 10,000 Daltons.

Using the Diaplate for Microdialysis Crystallization

1. Peel back and remove the red adhesive cover tape from the 200 Micro Pressure Adhesive Spacer (Image 1).

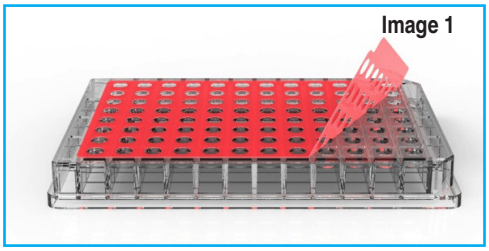
Image 1

2. Add up to 3.2 µl of protein into each of the 96 drop wells.

3. With the protective film facing up, position the 200 Micro UV Cover Film onto the Diaplate.

4. Use Paddle to press down and across the UV Cover Film to activate the pressure sensitive adhesive and seal the Film to the Diaplate.

5. Invert the plate. With the cut corners positioned to the left, load up to 350 µl of dialysis buffer or crystallization reagent into each of the 96 square reagent wells (Image 2).

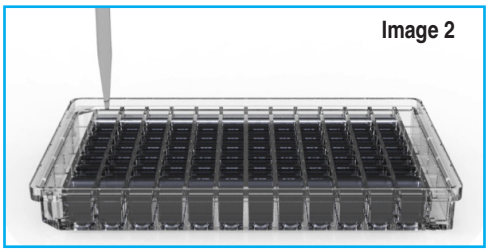
Image 2

6. With the well identification correctly positioned, place the engraved UV Screen Solution Cover over the reagents for protection during dialysis.

7. If the dialysis is to take place for a week or longer, place the Diaplate in a clear plastic sealable enclosure (bag) or humidification chamber. Evaporation rate can be <0.1% per week at 25° Celsius when the plate is stored in a sealed enclosure.

8. There are two options for viewing the Diaplate. 1) Pipette or pour off the reagents, remove the protective film from the 200 Micro UV Cover Film, invert the Diaplate and inspect for crystals. 2) Leaving the plate inverted and the reagents in place, view the plate through the reagents.

9. To remove crystals, pipette or pour off the reagents, right the plate, cut the film for access to crystals.

10. Dialysis buffers or crystallization reagents may be diluted, added to, or replaced during dialysis for a dynamic experiment. As well, the experimental results can be scored, the reagents removed and replaced with sample buffer, this sample buffer removed, and a different dialysis buffer or crystallization screen pipetted into the Diaplate for further experimentation.

References and Readings

1. Crystallization of nucleic acids and proteins, Edited by A. Ducruix and R. Giege, The Practical Approach Series, Oxford Univ. Press, 1992.

2. Preparation and analysis of protein crystals. McPherson, A. Eur. J. Biochem. 189, 1-23, 1990.

3. Zeppenzauer, M. et al, Crystal. of horse liver alcohol dehydrogenase complexes from alcohol solutions. Acta Chem Scan, 21, 1099, 1967.

Related Products

HR3-336	Dialysis Button Sampler - 5 of each size listed below
HR3-314	5 µl Dialysis Button - 50 pack
HR3-316	10 µl Dialysis Button - 50 pack
HR3-318	15 µl Dialysis Button - 50 pack
HR3-320	20 µl Dialysis Button - 50 pack
HR3-326	50 µl Dialysis Button - 50 pack
HR3-328	100 µl Dialysis Button - 50 pack
HR3-330	200 µl Dialysis Button - 50 pack
HR3-332	350 µl Dialysis Button - 50 pack
HR3-338	Dialysis Membrane Discs, cutoff 3,500 - 50 pack
HR3-344	Dialysis Membrane Discs, cutoff 6,000 to 8,000 - 50 pack
HR3-346	Dialysis Membrane Discs, cutoff 12,000 to 14,000 - 50 pack
HR3-253	Diaplate 10 kDa MWCO 3/pack

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