Dialysis Buttons™

HAMPTON RESEARCH
Solutions for Crystal Growth

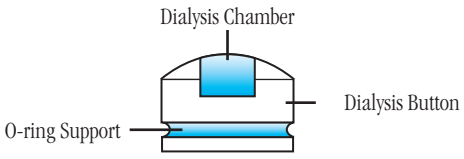
Method

Crystallization by dialysis is an easy variation to the typical vapor diffusion method used to grow crystals. In the dialysis method the sample in question is separated from the "precipitant" by a semi-permeable membrane which allows small molecules such as ions, additives, buffers, and, salts to pass but prevent biological macromolecules from crossing the membrane. Equilibration kinetics depend upon the molecular weight cut-off of the Dialysis Membrane, the precipitant, the ratio of the volume, the concentration of the components inside and outside of the dialysis cell, and the geometry of the cell.

Description

The Dialysis Buttons offered by Hampton Research are machined from transparent Perspex®. The button has a chamber which varies from 5 to 350 microliters depending upon which size button one chooses to use. See Figure 1. 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. Dialysis buttons are notoriously tricky to set up since beginners often trap air bubbles between the sample solution and the membrane which impedes dialysis. With a little practice using the "Applicator for Dialysis Buttons" one can master the technique. Dialysis Buttons from Hampton Research are supplied with O-rings and a Golf Tee Applicator. The package of Dialysis Buttons does not include Dialysis Membranes. See the Related Product section on page 3 for more information.

Figure 1



Using the Dialysis Button

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.

Cleaning

The Dialysis Buttons can be cleaned with soap and deionized water. Do not clean the buttons with organic solvents as this may turn the Perspex® opaque.

Practical Example

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

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, 1.5 M LiCl, pH 7.5 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 Applicator on top of the membrane and button. Roll the O-ring down the Applicator until the O-ring rolls off the Applicator and onto the edge of the button. See Figure 2b. Continue to roll the O-ring into the machined groove on the edge of the button. Remove the Applicator. There should be no bubbles between the membrane and the sample inside the button. Bubbles will prevent dialysis.

Figure 2a

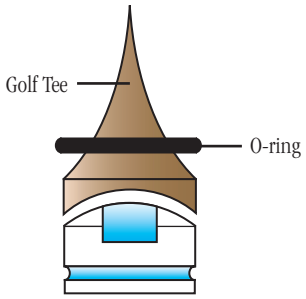


Figure 2b

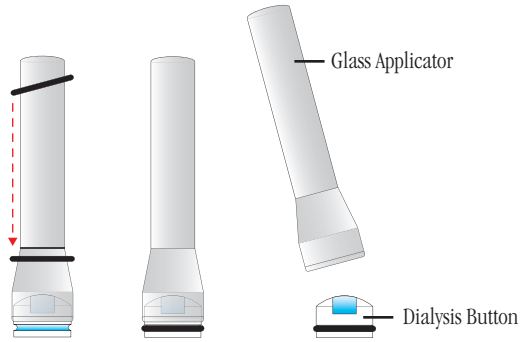


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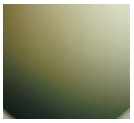
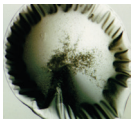
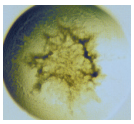
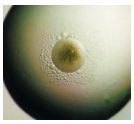
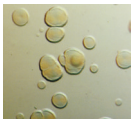
Figure 4
Typical observations in a crystallization experiment

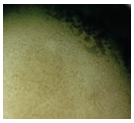

Clear Drop


Skin / Precipitate

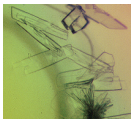

Precipitate


Precipitate / Phase

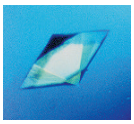

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster

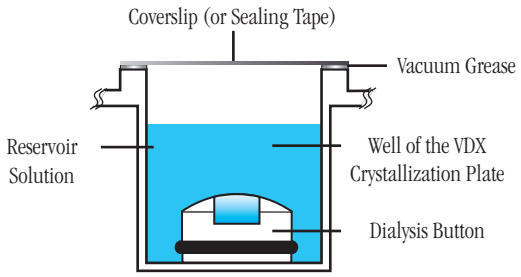

Single Crystal

4. Place 0.9 ml of 20 mM TRIS HCl, pH 7.5 in the reservoir of a VDX™ plate (or Linbro® plate, or small chamber which can be sealed).

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 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



Reference for the above protocol: Dr. Jim Pflugrath and Dr. Gary Gilliland, Cold Spring Harbor Laboratory Protein Crystallography Workshop.

Practical 2 - Lysozyme

1. Prepare 10 mg/ml Lysozyme in 50 mM Sodium acetate trihydrate pH 4.5. Filter the solution using a 0.2 micron filter.

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

3. Pipet 1 milliliter of 50 mM Sodium acetate trihydrate buffer into a small (5 ml) beaker.

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

5. Pipet a small amount of concentrated Sodium chloride into the beaker such that the final concentration of Sodium chloride in the beaker is 0.2 M. Seal the beaker with parafilm and store at room temperature.

6. Increase the concentration of Sodium chloride each day by 0.2 M. Repeat until crystals are observed in the button.

Reference for the above protocol: Crystallization of nucleic acids and proteins, a practical approach. Edited by A. Ducruix and R. Giege, Oxford University Press, 1992. Pages 95-96.

Considerations

Just as in a vapor diffusion experiment, the path is often as important as the endpoint in a Dialysis experiment. The path is the equilibration course which the solution inside and outside the button take towards achieving equilibrium. This course can be changed by manipulating the following:

- Ratio of Button volume/reservoir volume
- Button and Reservoir Components & Concentration
- Molecular Weight Cut Off of Dialysis Membrane
- Viscosity of Solutions
- Plus the usual assortment of crystallization variables including pH, sample concentration, temperature, etc...

Variations of Dialysis

Macrodialysis

The sample is loaded into dialysis tubing of the appropriate molecular weight cut-off and is dialysed against the appropriate reservoir solution. This method typically requires at least 100 microliters of sample and can be performed with liters of sample in large dialysis tubing.

Zeppenzauer Cells

Capillary tubes are closed with dialysis tubing or gel plugs. See Zeppenzauer, M. 1971, Methods In Enzymology, 22, 253.

Microcap Dialysis

The sample is placed in a glass capillary with one end sealed with wax, the other with Dialysis Membrane. The tube is placed in a microcap/small centrifuge tube filled with the appropriate reservoir. See Crystallization of nucleic acids and proteins, a practical approach, Edited by A. Ducruix and R. Giege, Oxford University Press, 1992.

Double Dialysis

This method reduces the rate of equilibration and can provide enhanced control over the crystallization of the sample. Simply put, a Dialysis Button is prepared and placed inside a reservoir sealed with a Dialysis Membrane, which is in turn placed inside another reservoir. Confused? See Thomas, D.H., et al, 1989, Protein Engineering, 2, 489.

Examine The Drop

Carefully examine the drops under a stereo microscope (10 to 100x magnification) immediately after setting up the screen.

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User Guide (pg 3)

Record all observations and be particularly careful to scan the focal plane for small crystals. Observe the drops once each day for the first week, then once a week there after. Records should indicate whether the drop is clear, contains precipitate, and or crystals. It is helpful to describe the drop contents using descriptive terms. Adding magnitude is also helpful. Example: 4+ yellow/brown fine precipitate, 2+ small bipyramid crystals, clear drop, 3+ needle shaped crystals in 1+ white precipitate. One may also employ a standard numerical scoring scheme (Clear = 0, Precipitate = 1, Crystal = 10, etc). Figure 4 (on page 2) shows typical examples of what one might observe in a crystallization experiment.

Interpreting The Results

Clear drops indicate that either the relative supersaturation of the sample and reagent is too low or the drop has not yet completed equilibration. If the drop remains clear after 3 to 4 weeks consider repeating the screen condition and doubling the sample concentration.

Drops containing precipitate indicate that either the relative supersaturation of the sample and reagent is too high, the sample has denatured, or the sample is heterogeneous. To reduce the relative supersaturation, dilute the sample twofold and repeat the screen condition. If sample denaturation is suspect, take measures to stabilize the sample (add reducing agent, ligands, glycerol, salt, or other stabilizing agents). If the sample is impure, aggregated, or heterogeneous take measures to pursue homogeneity. It is possible to obtain crystals from precipitate so do not discard nor ignore a drop containing precipitate. If possible, examine drops containing precipitate under polarizing optics to differentiate precipitate from microcrystalline material.

Compare the observations between the 4°C and room temperature incubation to determine the effect of temperature on sample solubility. Different results in the same drops at different temperatures indicate that sample solubility is temperature dependent and that one should include temperature as a variable in subsequent screens and optimization experiments.

Retain and observe plates until the drops are dried out. Crystal growth can occur within 15 minutes or one year.

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

Technical Support

Inquiries regarding Dialysis and general inquiries regarding crystallization are welcome. Please e-mail, fax, or telephone your request to Hampton Research. Fax and e-mail Technical Support are available 24 hours a day. Telephone technical support is available 8:00 a.m. to 4:30 p.m. USA Pacific Standard Time.

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