Sitting Drop Vapor Diffusion

For Small Molecules & Peptides

HAMPTON RESEARCH

Solutions for Crystal Growth

Crystal Growth 101

Principle

A drop composed of a mixture of sample and reagent is placed in vapor equilibration with a liquid reservoir of reagent. Over time, a reagent concentration equilibrium is reached between the drop and the reservoir (figure 1).

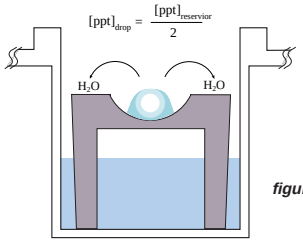


figure 1

If the sample is to be crystallized using salts, polymers, non-volatile organics, and pH, the drop will typically contain a lower reagent concentration than the reservoir. To achieve equilibrium, water vapor leaves the drop and eventually ends up in the reservoir. As water leaves the drop, the sample undergoes an increase in relative supersaturation. Both the sample and reagent in the drop increase in concentration as water leaves the drop for the reservoir. Equilibration is reached when the reagent concentration in the drop is approximately the same as that in the reservoir.

If the sample is to be crystallized using volatile organics, the drop can contain either a lower or higher initial reagent (volatile organic) concentration than the reservoir. In other words, one can choose to vapor diffuse volatile organics from the reservoir to the drop by having a higher concentration of volatile organic in the reservoir than the drop. Or one could have a lower concentration of volatile organic in the reservoir compared to the drop and the volatile organic would diffuse from the drop to the reservoir.

If the sample is more soluble in a higher concentration of volatile organic (typical), then one would begin the vapor diffusion experiment with the drop containing a higher concentration of volatile organic than the reservoir. As the volatile organic leaves the drop for the reservoir, the relative supersaturation of the sample in the drop would increase.

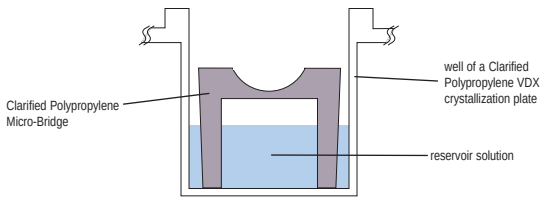
If the sample were less soluble in a higher concentration of volatile organic (atypical), then one would begin the vapor diffusion experiment with the drop containing a lower concentration of volatile organic than the reservoir. As the volatile organic leaves the reservoir for the drop, the relative supersaturation of the sample in the drop would increase.

Other scenarios are possible, especially if one screens reagent systems which are mixed with volatile organics and salts and/or polymers. Here, one must consider both the equilibration path of the volatile organic and the non-volatile salt and/or polymer.

Clarified Polypropylene VDX Plate for Sitting Drop Vapor Diffusion Crystallization

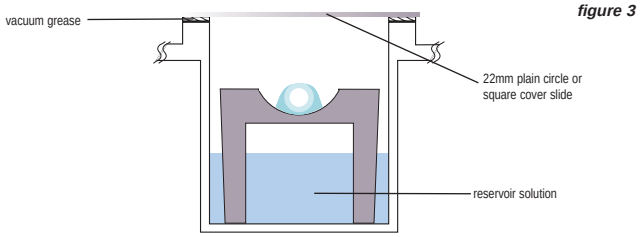
1. Using a single Clarified Polypropylene VDX Plate, place a single Clarified Polypropylene Micro-Bridge in each reservoir. See figure 2 below.

figure 2



2. Apply a bead of high vacuum grease to the rim of each of the 24 reservoirs. Apply the bead in a smooth circular motion, leaving a small gap, creating an incomplete circle in the bead of grease. This gap will allow trapped air to escape from the reservoir when the cover slide is applied. When sealing the cover slide to the reservoir, simply place the cover slide on the bead of grease, press gently, then twist approximately 30 degrees or more to seal the gap. See figure 3.

figure 3



3. Pipet 1.0 milliliter of the crystallization reagent into reservoir A1 of the Polypropylene VDX Plate.

4. Pipet the sample, solubilized in the volatile organic, into the depression of the Polypropylene Micro-Bridge. The Polypropylene Micro-Bridge can hold up to 40 microliters of liquid.

5. Seal the reservoir with a 22 mm diameter plain glass cover slide (22 mm circles or squares may be used to seal the reservoir).

6. Repeat steps 1 through 5 for the remaining 23 reservoirs. In the remaining reservoir one may evaluate the effects of various additives, varying concentrations of the volatile organic, pH, or other chemical crystallization variables.

Temperature as a Crystallization Variable

If it is suspected that the sample might have temperature dependent solubility, the crystallization screen or reagent conditions may be repeated at one or more additional temperatures below or above room temperature.

Making Changes to the Reservoir and Drop

Additions, deletions, or modifications to the drop and reservoir can be performed by simply removing the glass cover slip, performing the modification and then resealing the cover slide to the reservoir.

Compatibility with Volatile and Aggressive Organic Solvents

The clarified polypropylene used in the Polypropylene VDX and Polypropylene Micro-Bridges are compatible with a wide number of volatile and aggressive organic solvents. However, to be certain of compatibility, it is recommended that the organic solvent (or reagent) to be used for crystallization be tested with the Clarified Polypropylene VDX Plate and Clarified Polypropylene Micro-Bridge prior to setting the experiment to confirm compatibility. Pipet 1 milliliter of the reagent into the reservoir. Seal with a glass slide and vacuum grease. Observe for compatibility.

Vapor Diffusing Volatile Organics Out of the Drop Into the Reservoir

If the sample is more soluble in high rather than low concentrations of volatile organic, then one might consider vapor diffusing the volatile organic out of the crystallization drop. In such an experiment, the reservoir should initially contain a con-

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centration of volatile organic that is lower than the volatile organic concentration in the drop. For example, if the sample is solubilized in 100% volatile organic, one might fill the reservoir with 80% volatile organic in order to equilibrate the sample drop to 80% volatile organic. If the sample remains soluble, the glass slide sealing the reservoir can be removed and the reservoir solution exchanged (or diluted) with an even lower concentration of the volatile organic. This procedure can be repeated until the appropriate concentration of volatile organic for crystallization is determined. To accelerate the screening of reagent concentration, one might pipet different concentrations of the reagent into different reservoirs. For example, if the sample were solubilized in 100% volatile organic, one might pipet twelve different organic concentrations (95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 45%, 40%, and 35%) into twelve different reservoir in the first two rows of the Polypropylene VDX Plate to screen ten different concentrations simultaneously.

Vapor Diffusing Volatile Organics Into the Drop from the Reservoir

If the sample is less soluble in high rather than low concentrations of volatile organic, then one might consider vapor diffusing the volatile organic from the reservoir into the drop. In such an experiment, the reservoir should initially contain a concentration of volatile organic that is higher than the volatile organic concentration in the drop.

Crystallization Screening

Using the Clarified Polypropylene VDX Plate together with the Clarified Polypropylene Micro-Bridge, it is possible to rapidly and conveniently screen any variety of different organic solvents, concentrations of organic solvents, sample concentrations, temperature, pH, ionic strength, additives, as well as other chemical and physical crystallization variables. Each Clarified Polypropylene VDX Plate has 24 reservoirs and can screen 24 unique conditions in each plate. Crystallization screens based on organic solvents and other chemicals can be custom formulated for a particular small molecule and rapidly testing using the 24 well Clarified Polypropylene VDX Plate.

Optimization of Crystallization Conditions

Once preliminary crystallization conditions have been determined using crystallization screens, optimization of reagent conditions leading to sample crystals can be formulated and tested using the same methods used for screening. During optimization, varying the concentration of the sample, the concentration of the organic solvent (and/or other reagents used in the reagent) can be systematically varied to determine optimal conditions for growing the best crystals.

Using Non-Traditional Reagents for Small Molecule Crystallization

Volatile organics are traditionally the sample solubilization and crystallization reagent of choice for small molecule and peptide crystal growers. With small molecules and peptides that can be solubilized in water or water/solvent mixtures, it is possible to screen 'water based' crystallization reagents (typically used for protein crystallization) such as salts (ammonium sulfate) and polymers (polyethylene glycol) as well as pH and temperature using the vapor diffusion method. A rapid, convenient, and cost effective way to screen a variety of reagents, reagent concentrations, and pH is to use a sparse matrix crystallization such as Crystal Screen™ (HR2-110). Numerous other sparse matrix and grid based crystallization screens are available from Hampton Research and can be applied to the crystallization of small molecules, including peptides. Note: If the sample can be solubilized in water based solvent systems it is not necessary to use the Clarified Polypropylene VDX Plate. Other plates and crystallization methods are available for water based crystallization methods. Contact Hampton Research for additional information.

Solubilizing Peptides and Small Molecules in Water

If the peptide is basic, it will probably dissolve in water. If it does not, try 10% and higher solutions of acetic acid. If the peptide still does not dissolve, add trifluoroacetic acid (TFA) (<50 µL) to solubilize the peptide and dilute with water.

Acidic peptides may also dissolve in water or acetic acid. If the peptide does not dissolve, add ammonium hydroxide (< 50 µL) and dilute with deionized water.

Peptides that are neutral may require the addition of organic solvents, such as methanol, iso-propanol, or acetonitrile. The addition of denaturants such as urea or guanidinium-HCl may also be required.

Small molecules and peptides that cannot be solubilized in water can sometimes be solubilized in mixtures of water and organic solvent, or water which has been titrated to an acidic or alkaline pH as described above. Other potent solubilizing agents include dilute formic acid, hexafluoro-2-propanol (HFIP), hexafluoroacetone, trifluoroacetic acid (TFA), and triethylamine formate. In some cases as little as 10 to 100 mM will help to solubilize stubborn proteins, peptides, and small molecules within the solvent system of choice.

Hampton Research
27632 El Lazo Road, Suite 100
Laguna Niguel, CA 92677-3913 U.S.A.
Tel: (949) 425-1321 • Fax: (949) 425-1611
Technical Support e-mail: tech@hrmail.com
Internet: www.hamptonresearch.com