

Crystallization Screening

Crystallization screening is the process of evaluating methods, reagents, and other chemical and physical variables with the objective of producing crystals and/or identifying the variables which are positively or negatively associated with crystallization of the sample.

At the time of this writing, up to 40% of samples screened for crystallization will produce some kind of crystalline result and 10% of samples will produce a crystal suitable for X-ray diffraction analysis. About 75% of proteins screened require optimization. Optimization is the systematic manipulation and evaluation of variables which influence the crystallization of the sample.

Primary Screen

Primary screens are front line screens used for initial screening. If one does not have the knowledge or desire for a specific bias or focus on a reagent class, one may choose a sparse matrix screen composed of salts, polymer, organics, buffers at various pH levels, and mixtures thereof. Or one may have knowledge that a specific reagent class or mixture is desired and choose a screen biased for salt, polymer, polymer and salt, or other formulations.

Secondary Screen

Secondary screens are follow up screens to primary screens. The score from a primary screen such as Index may indicate crystals or promising results in polymer and polymer – salt mixtures. In such an instance, one may choose a secondary screen such as PEGRx 1 and PEGRx 2, as well as PEG/Ion and PEG/Ion 2. Or, primary screens may show promising scores in salt based reagents, where secondary screens such as SaltRx 1 and SaltRx 2 would be appropriate for follow up screening.

Grid Screen

Grid Screens are simple, logical methods for systematically screening on a pH versus precipitant (reagent) grid.¹ For example, the pH range 4 to 9 might be screened in 1 pH increments across the 6 wells of the X-axis of a 24 well crystallization plate, while a reagent, such as Polyethylene glycol 6,000 might be screened in 4 concentrations (5, 10, 20, 30% w/v) across the 4 wells of the Y-axis of a 24 well crystallization plate. The method depends on the ability to identify preliminary crystallization conditions while coarsely or finely sampling two variables, typically pH and reagent concentration. Grid Screening can be used as a primary or secondary screen strategy and is most often employed in optimization of initial crystallization conditions (hits). Grid Screens can be designed to cover a broad range of pH and reagent concentration in big steps, casting a broad net to identify an initial promising pH and reagent concentration (hit). Subsequently, successively finer grids can be generated to identify the optimal pH and reagent concentration for crystallization. The Grid Screen strategy was an original approach to protein crystallization, prior to the development and popularization of sparse matrix screening.

Sparse Matrix Screen

Sparse Matrix Screens are composed of a sampling of reagent formulations that have previously crystallized a protein.² The formulations found in a Sparse Matrix Screen have emerged over time from the accumulated wisdom and experience of generations of many crystal growers. Initial ideas are assembled, formulated, and tested against previously crystallized and not yet crystallized proteins. Duds are dropped and winners move onto subsequent rounds of testing. Testing also employs formulations from the literature as well as databases, such as the Protein Data Bank (PDB)³, Biological Macromolecule Crystallization Database (BMCD)^{4,6}, in house data, or data shared through centers and collaborators. When data mining, one must carefully review the data, as screens have existed long enough now that they themselves are within the database, and one must avoid getting caught in some local minima; one should also avoid cherry picking formulations to create a screen that, while looking good on paper, produces redundant hits, rather than sample an appropriate and balanced chemical space of home run conditions as well as singles; one needs both to win the crystallization game. And though one should appreciate and respect data mining, one must also remind oneself to look outside the box, for new chemicals and formulations. The unprecedented success of polyethylene glycols, detergents, salt libraries (Tacsimate), small molecular libraries, and numerous other reagents would not have happened had it not been from looking outside the box, and a bit of dumb luck.⁷⁻¹¹

Sparse Matrix Screens can be broad, sampling many different salts, polymers, organics, buffers, pH, and mixtures thereof. They can also be biased towards a specific reagent class, such as Polyethylene glycols or Salts, or focused on mixtures. In developing a novel Sparse Matrix Screen, one first chooses the number of conditions in the screen (24, 48, 96, 192... 1,536). Second, one defines the intention of the screen, for example, a focus on polymers, polymer salt mixtures, and pH. Third, the appropriate concentration of the reagents must be determined and tested, paying particular attention to matching reagent pH with buffer pH, chemical compatibility, solubility, and stability. Finally, the Sparse Matrix Screen must be thoroughly tested with a portfolio of proteins, both previously and not previously crystallized, and the results compared to existing screens. Care should be taken to avoid redundancy of reagent formulation within the new screen and between existing screens, to prevent unnecessary oversampling of chemical space and wasting of the sample.

Development & History of Hampton Research Screens

PCT Pre-Crystallization Test

PCT is based on work done by Jarmila Jancarik and offers a quick, convenient, effective way to achieve the appropriate sample concentration for screening, using a minimal amount of sample.

GRAS Screens

GRAS Screens were developed by Hampton Research for the crystallization of proteins, including monoclonal antibodies. The screens utilize GRAS reagents that promote crystallization of biotherapeutics for bioprocess, bioformulation, and continuous flow manufacturing applications as well as crystallization of proteins for X-ray crystallography. Each of the chemicals in the GRAS Screens have been used under one or more of the following categories. As (1) a Generally Recognized As Safe (GRAS) substance, (2) a pharmaceutical excipient, (3) a normal physiological constituent, (4) a metabolic byproduct, and/or (5) a Everything Added to Food in the United States (EAFUS) substance. Formulation is based on a) data mining databases such as the Protein Data Bank (PDB), BMCD, and in house Hampton Research data, b) review and analysis of the patent and scientific literature, c) input from academic and pharma colleagues, and d) in house testing.

The primary crystallization reagents in GRAS Screen 1 and 2 are Polyethylene glycol 300, 400, MME 550, 600, 1,000, MME 2,000, 3,350 and 4,000 (high concentration) versus 24 unique secondary salts (low concentration), sampling pH 4 to 9 without an added buffer. The screens can be considered an extension to the PEG/Ion screens, albeit with a focus on GRAS reagents.

The primary crystallization reagent in GRAS Screen 3 and 4 are 24 unique salts (high concentration), versus Polyethylene glycol 300, 400, MME 550, 600, 1,000, MME 2,000, 3,350 and 4,000 as secondary reagents (low concentration), sampling pH 4 to 9 without an added buffer.

The primary crystallization reagents in GRAS Screen 5 and 6 are Polyethylene glycol 300, 400, MME 550, 600, 1,000, MME 2,000, 3,350 and 4,000, each sampled at 3 concentrations versus pH 4.5 to 10 sampling 8 unique buffers.

The primary crystallization reagents in GRAS Screen 7 and 8 are Ammonium - acetate, chloride, citrate, formate, phosphate, sulfate, tartrate, Potassium phosphate, Sodium acetate, Sodium - chloride, citrate, formate, phosphate, and tartrate, each sampled at 4 concentrations versus pH 4.5 to 10 sampling 8 unique buffers.

Index (Index HT)

Index is a primary, diverse reagent system crystallization screen for proteins, complexes, peptides, nucleic acids, and water soluble small molecules. The screen is a data-driven biased sparse matrix and grid screen. Index is based on a collaboration between Hampton Research and Allan D'Arcy. The objective of the collaboration was to develop a chemically balanced screen composed of 96 reagents. Both grid and sparse matrix based reagents were conceived, formulated and tested in an iterative process over a period of time utilizing a portfolio of pharma, academic, and standard biological macromolecular samples. The final formulation, a chimera of grid and sparse matrix, is one of the most frequent front line screens used at this time.

Index, as the name implies, efficiently samples a series of specially formulated reagent zones to identify which reagent class or classes and pH are effective in producing crystals or limiting sample solubility. Results from Index can be used to design optimization experiments and to identify follow up screens by reagent class. For example, positive results with salt based reagents in Index may be followed up with further screening using SaltRx and Grid Screen Salt HT. Success with polymer based reagents in Index may be followed up with further screening using PEGRx and PEG/Ion.

Index utilizes a broad, yet refined portfolio of crystallization reagent systems. These include the following: (1) traditional salts such as Ammonium sulfate and Sodium chloride versus pH; (2) neutralized organic acids such as Sodium malonate and Tacsimate; (3) High salt concentration mixed with low polymer concentration as well as high polymer concentration mixed with low salt concentration and; (4) Low ionic strength using polymers such as PEG, MPD, and Pentaerythritol versus pH. These reagent systems are formulated across a sparse matrix and incomplete factorial of concentration ranges, sampling a pH range of 3 to 9.¹²

Crystal Screen & Crystal Screen 2 (Crystal Screen HT)

Crystal Screen and Crystal Screen 2 are primary sparse matrix screens for proteins, soluble peptides, nucleic acids, and water soluble small molecules. Crystal Screen is based on the publication by Jarmila Jancarik and Sung Ho Kim², which was the first appearance of a sparse matrix crystallization screen in the literature.

The formulation was an iterative process, with input from crystallographers in pharma and academia, including planning, testing, and advice from Marcos Hatada. Crystal Screen was the world's first crystallization kit offered for purchase by the research community and was first offered by Hampton Research in 1991. The Crystal Screen formulation has had a tremendous impact on the structural biology community, that continues to this day, with more than 2,000 citations to the original publication, and remains one of the most popular crystallization screens today.

Crystal Screen 2 was developed by Hampton Research as an extension to the original 50 conditions in Crystal Screen.¹⁹ The formulation is of a reduced reagent relative supersaturation, to balance the higher relative supersaturation in Crystal Screen, along with exploring then novel reagents such as Jeffamine, Polyethylene glycol monomethyl ether, and mixed component precipitant systems such as [High Salt]/[Low Salt], [High Salt]/[Low PEG], [High PEG]/[Low PEG], and [High PEG]/[Low Salt]. Formulation is based on a) data mining of the BMCD, in house data at Hampton Research, and data supplied by a pharma partner, and b) testing of novel reagents with a portfolio of proteins.

Crystal Screen Cryo & Crystal Screen 2 Cryo (Crystal Screen Cryo HT)

Crystal Screen Cryo and Crystal Screen 2 Cryo are primary biased sparse matrix screens with cryo for proteins, soluble peptides, nucleic acids, and water soluble small molecules. Crystal Screen Cryo and Crystal Screen 2 Cryo are based on the original Crystal Screen and Crystal Screen 2 formulation, with added glycerol for the growth of cryo ready crystals. The optimal glycerol concentrations were described by Elspeth Garman¹³ and Eddie Snell¹⁴.

PEGRx 1 & PEGRx 2 (PEGRx HT)

PEGRx 1 and PEGRx 2 are primary and secondary, polymer and pH based crystallization screens for biological macromolecules. Both screens were developed at Hampton Research. The screens are designed to evaluate polymer based crystallization reagents and pH in low (PEGRx 1) to medium (PEGRx 2) ionic strength. Both screens are designed for use as primary screens, or as secondary screens to follow the Hampton Research Index screen and other screens when low to medium ionic strength polymer based reagents produce hits and interesting solubility leads. Chemical selection, buffer, pH, and formulation were based on the literature, public databases (PDB, BMCD), and in house data as well as the creation and sampling of novel polymers and formulations. Formulations were tested against previously crystallized and not yet crystallized samples, and an iterative process of removing, replacing, and retesting carried out until screen performance criteria were met.

PEG/pH & PEG/pH 2 (PEG/pH HT)

PEG/pH and PEG/pH 2 are primary or secondary screen using Polyethylene glycol 4,000 as the primary reagent in addition to sampling a total of 20 unique buffers at 96 unique pH levels. The buffer concentration of 0.2 M results in a PEG/Ion like screen but using the buffer to drive the ionic strength, instead of salt as in the PEG/Ion screens. Using PEG/pH, PEG/pH 2, or PEG/pH HT one isolates pH, buffer type and relative supersaturation from other chemical and physical variables to screen the effect that pH and buffer type have on the solubility, stability, homogeneity, monodispersity and crystallization of the sample. Varying the pH can alter the protonation state and charge of amino acid residues in the protein, generating different species of the protein for solubility and crystallization screening. The change in pH can have a dramatic effect on inter and intramolecular contacts in the protein and can manipulate how the protein interacts with itself, the surrounding solvent and chemicals in the drop. By screening buffer type and pH in a low ionic strength environment of Polyethylene glycol, PEG/pH simultaneously delivers as a solubility and a crystallization screen for proteins.

PEG/Ion & PEG/Ion 2 (PEG/Ion HT)

PEG/Ion and PEG/Ion 2 are primary or secondary, polymer, salt and pH matrix crystallization screens for biological macromolecules. Both screens were developed at Hampton Research. PEG/Ion is a sparse matrix profile of anions and cations in the presence of monodisperse Polyethylene glycol

(PEG) 3,350 over pH 4.5 - 9.2. The screen is designed to evaluate monodisperse, high purity PEG 3,350 and 48 unique salts representing a very complete range of anions and cations frequently used in the crystallization of biological macromolecules. The primary screening variables are PEG, ion type, ionic strength, and pH. More than 60% of the published crystallizations utilize PEG as a primary crystallization reagent and in approximately 50% of those reports, the PEG was combined with an ion as a secondary crystallization reagent. PEG/Ion reagents are formulated without a buffer and are not pH titrated, yet the formulation encompasses a broad pH range of 4.5 to 9.2 due to the diverse inclusion of 0.2 M salt in the presence of PEG.

PEG/Ion 2 is designed as an extension to PEG/Ion in order to generate a 96 reagent screen. PEG/Ion 2 screens a complete profile of titrated organic acids at varying pH levels (3.7 - 8.8), metals and Tryptone in the presence of monodisperse PEG 3,350 at varying concentrations.

Both screens are designed for use as primary screens or as secondary screens to follow the Hampton Research Index and other screens when polymer – salt based reagents in PEG 3,350 and similar M_r PEGs (3,000 to 8,000) produce hits and interesting solubility leads.

PEG/Ion 400

PEG/Ion 400 is a crystallization reagent kit designed to provide a rapid screening method for the crystallization of membrane and soluble biological macromolecules. Most G protein-coupled receptors (GPCRs) have been crystallized in Polyethylene glycol (PEG) 400 based reagents. PEG/Ion 400 is designed as a 96 reagent crystallization screen that is compatible with the Lipidic Cubic Phase (LCP). The screen combines a single concentration (30% v/v) of high purity Polyethylene glycol 400 and 48 different high purity salts, comprising both anions (malate, malonate, nitrate, phosphate, succinate, sulfate, tartrate, and thiocyanate) and cations (ammonium, cadmium, calcium, ethylammonium, lithium, magnesium, nickel, potassium, sodium, tetraethylammonium, and zinc) in two concentrations (0.1 and 0.4 M) which due to their unique pH characteristics also affords a reasonable pH screen (approximate pH range of 4 to 9.5). The primary screen variables are PEG, ion type, ionic strength, salt concentration, and pH.

PEG/Ionic Liquid 1 & PEG/Ionic Liquid 2 (PEG/Ionic Liquid HT)

PEG/Ionic Liquid 1 and PEG/Ionic Liquid 2 are primary crystallization screens where Polyethylene glycols are the primary, and ionic liquid the secondary reagent, sampling a broad range of pH without an added buffer. Ionic liquids have been found effective as additives in protein crystallization, with different ionic liquids used to increase crystallization rates and crystal size. The inclusion of ionic liquids in crystallization experiments has been reported to lead to less crystal polymorphism as well as less precipitation at higher precipitant concentrations. Ionic liquids have been used as additives

to produce crystals in reagents that had previously not resulted in crystallization and results suggest ionic liquids may be applicable for the solubilization and crystallization of membrane proteins.

SaltRx 1 and SaltRx 2 (SaltRx HT)

SaltRx and SaltRx 2 are primary or secondary, salt and pH matrix crystallization screens for biological macromolecules. Both screens were developed at Hampton Research. Salt is the only primary crystallization reagent (precipitant) utilized. Based on a design of 96 conditions, the screen evaluates a broad portfolio of crystallization salts of varying concentration and pH. The selection, concentration, and pH of the salts were determined by data mining the BMCD and PDB, crystallization reports in the literature, as well as in house data at Hampton Research. Based on this analysis, up to 35% of protein crystallizations involve salt as the primary crystallization reagent.

SaltRx 1 and SaltRx 2 can be used as a primary crystallization screen when salt, ionic strength and pH are desired or suspected as appropriate crystallization variables. The screens are also used as secondary screens when salt based reagents from Index, Crystal Screen, and Grid Screen produce crystals and when further screening for additional salt conditions or optimization is desired.

MembFac & Crystal Screen Lite (MembFac HT)

MembFac and Crystal Screen Lite are primary sparse matrix crystallization screens for membrane proteins and hydrophobic samples with limited solubility. The MembFac formulation is based on the research of Michael Stowell. Crystal Screen Lite is based upon Crystal Screen, with the primary precipitant concentration halved, while the secondary salts, ions, and buffers remain at the original concentration. Testing of the Crystal Screen Lite formulation was performed in collaboration between Jarmila Jancarik and Hampton Research utilizing a portfolio of membrane proteins, proteins of limited solubility, and soluble proteins.

Natrix & Natrix 2 (Natrix HT)

Natrix and Natrix 2 are primary biased sparse matrix crystallization screens for nucleic acids & protein/nucleic acid complexes. Natrix is based on published formulations, including the sparse matrix formulation first described by William Scott in 1995.¹⁵ Natrix 2 is based upon published reagent formulations for the crystallization of nucleic acids and protein-nucleic acid complexes. A variety of hammerhead ribozymes and other ribozymes, RNAs, DNAs, RNA-drug complexes, and RNA-protein complexes have been crystallized using the Natrix and Natrix 2 formulations.

Natrix screens are unique in that rather than relying solely or heavily on the traditional nucleic acid precipitant (+/-)-2-Methyl-2,4-pentanediol (MPD), Natrix screens also utilize Polyethylene glycols (PEGs) in a variety

of molecular weights (200, 400, 4,000, 8,000) as well as 2-Propanol, Polyethylene glycol monomethyl ether (PEG MME), and 1,6-Hexanediol. Many of the polymeric and low molecular weight organic precipitants are combined with various monovalent salts as precipitating agents. This combination of salts and low molecular weight organics and polyalcohols, as well as the utilization of varying chain length PEGs, has proven to be a successful combination for producing nucleic acid and protein-nucleic acid complex crystals.

Grid Screens

The Grid Screens are premised on the simple, logical methods for systematically screening on a pH versus precipitant (reagent) grid.¹ The method depends on the ability to identify preliminary crystallization conditions while coarsely or finely sampling two variables, typically pH and reagent concentration. The Grid Screening strategy can be used as a primary crystallization screen when Ammonium sulfate or Sodium chloride, or Sodium malonate, or Sodium potassium phosphate, or middleweight PEG, or MPD and pH are desired or suspected as appropriate crystallization variables. It is also useful as a secondary screen when these same reagents from screens such as Index, Crystal Screen or similar screen produce crystals and further screening for additional conditions or optimization is desired.

The Grid Screens, save for Grid Screen Salt HT (96 reagents), are 24 reagent screens varying precipitant concentration versus pH. Chemical selection, buffer, pH, and formulation were based on the literature, public databases (PDB, BMCD), and in house data. Formulations were tested against previously crystallized and not yet crystallized samples, and an iterative process of removing, replacing, and retesting carried out until screen performance criteria were met.

Nucleic Acid Mini Screen

Nucleic Acid Mini Screen is a crystallization screen for nucleic acid fragments. The formulation is based upon the publication, "A Highly Efficient 24 Condition Matrix for the Crystallization of Nucleic Acid Fragments" where the preliminary crystallization conditions of 35 nucleic acids were determined.¹⁶ The unique formulation consists of separated reagents for the sample drop and for the reagent well (common dehydrant). Samples include DNA, DNA-Drug complexes, C-Tetrad and G-Quartet Motifs, RNA oligomers, and other nucleic acids.

Low Ionic Strength Screen

The Low Ionic Strength Screen is a crystallization screen for intact monoclonal antibodies, monoclonal antibody fragments, & proteins less soluble at low ionic strength. The formulation is based upon the publication, "Crystallization of intact monoclonal antibodies".¹⁷ The format of the screen is unique from other screens offered by Hampton Research in that the reagent Polyethylene glycol 3,350 and buffers are supplied as separate solutions al-

lowing one to customize the number of conditions, concentration, and pH covered to their liking. The unique formulation also consists of separated reagents for the sample drop and for the reagent well (common dehydrant).

Selecting the Screen(s)

If the protein, a similar protein, or a family member of a related protein has been previously crystallized, one may initially look to pursuing similar methods, reagents, and screens for the crystallization of the new sample.

Screens for Soluble Proteins

- Index
- Crystal Screen, Crystal Screen 2
- PEGRx 1, PEGRx 2
- PEG/Ion 1, PEG/Ion 2
- PEG/pH 1, PEG/pH 2
- PEG/Ion 400
- PEG/Ionic Liquid 1, PEG/Ionic Liquid 2
- SaltRx 1, SaltRx 2
- Grid Screens
- GRAS Screens

Screens for Membrane Proteins

- MembFac
- Crystal Screen Lite
- PEG/Ion 400
- GRAS 1
- GRAS 2
- GRAS 5
- GRAS 6

Screens for Protein Complexes

- PEGRx 1
- PEGRx 2
- PEG/Ion 1
- PEG/Ion 2
- GRAS 1
- GRAS 2

Screens for Nucleic Acids & Protein Nucleic Acid Complexes, Ribozymes, RNAs, DNAs, RNA-Drug Complexes, and RNA-Protein Complexes

- Natrix 1
- Natrix 2

Screen for Nucleic Acid Fragments, DNA, DNA-Drug Complexes, C-Tetrad and G-Quartet Motifs, and RNA Oligomers

- Nucleic Acid Mini Screen (NAM)
- Grid Screen MPD

Screens for Biological Therapeutics, including Monoclonal Antibodies

- GRAS Screens

Interpreting the Screen

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. If more than 70 of the 96 drops are clear, then consider doubling the sample concentration and

repeating the entire screen.

Drops containing precipitate indicate 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 with sample buffer and repeat the screen condition. If more than 70 of the 96 drops contain precipitate and no crystals are present, then consider diluting the sample concentration in half by adding an equal volume of sample buffer to the sample and repeating the entire screen. If sample denaturation is suspected, take measures to stabilize the sample (add reducing agent, ligands, additives, salt, or other stabilizing agents). If the sample is impure, aggregated, or heterogeneous take measures to pursue increased purity and 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 or UV optics to differentiate precipitate from microcrystals. If the drop contains a macromolecular crystal the relative supersaturation of the sample and reagent is appropriate for crystal nucleation and growth.

The next step is to optimize the preliminary conditions by varying the reagent concentration, screen pH, vary temperature between 4 and 30°C, screen additives, and evaluate other crystallization variables including sample construct, purity, stability, and homogeneity in order to achieve the desired crystal size and quality. See CG101 Optimization for further information.

When sample quantity permits, set screens in duplicate (4°C and 25°C) or triplicate (10°C and 20°C and 30°C) to evaluate the effect of temperature on crystallization. Compare the observations between the different temperatures 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.

When sample quantity permits, set screens using multiple drops and drop ratios, such as 1:2, 1:1, and 2:1. See Hampton Research Crystal Growth 101: Drop Ratio for details.

Getting Down to Business - Performing the Screen

Today, most screening is performed using either the sitting or hanging drop vapor diffusion method. Other methods such as microbatch, dialysis, and free interface diffusion are also used for screening.

Screening by sitting or hanging drop vapor diffusion may be accomplished using manual and automated sample handling and pipetting. Manual methods can be employed using an adjustable 0.1 to 2 µl or a 1 to 10 µl for

the drops, and an adjustable 1,000 μl pipette for the reagent well (reservoir). A 1 to 2 μl drop composed of sample and reagent is set in vapor equilibration with a reagent volume of 500 to 1,000 μl . Automated methods can be used to set a 50 to 400 nl drop composed of sample and reagent in vapor equilibration with a reagent volume of 50 to 100 μl . See CG101 Sitting Drop Vapor Diffusion Crystallization and CG101 Hanging Drop Vapor Diffusion Crystallization for details on these methods.

The Sample

Once the sample of interest is isolated in a pure, homogenous, stable, active, and concentrated form, it is time to commence screening methods and reagents towards producing crystals of the sample. For screening, the sample should be in the concentration range of 1 to 25 mg/ml in dilute, 5 to 25 mM buffer, and the salt concentration below 200 mM .

Other additives essential for sample homogeneity, stability, and activity should be present in the lowest effective concentration.

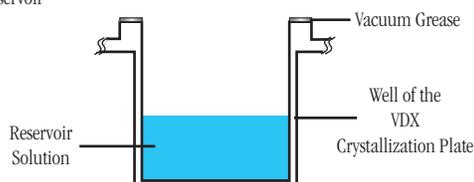
Manual Method – Tube Based Screen Kits- Hanging Drop Vapor Diffusion

The following procedure describes the use of a tube based screen with the Hanging Drop Vapor Diffusion method. Screens can also be performed using the Sitting Drop, Sandwich Drop, Microbatch, and Microdialysis methods. A complete description of the Hanging Drop, Sitting Drop, Microbatch, Dialysis and other crystallization methods are available from the Hampton Research Crystal Growth 101 Library. Note: Unscrew, open, pipette reagent, and close tubes one at a time to minimize evaporation and the risk of contamination.

1. Prepare a VDX Plate (HR3-141) for Hanging Drop Vapor Diffusion by applying a thin bead of cover slide sealant to the upper edge of each of the 24 reservoirs. One may also use a Greased VDX Plate (HR3-171). See Figure 1.

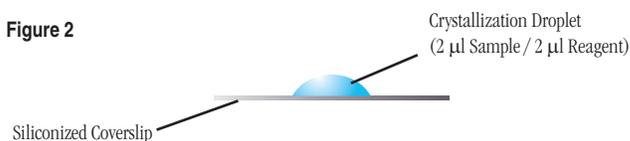
Figure 1

Cross section of a reservoir in the VDX plate.



2. Using a clean pipet tip, pipet 1 ml of screen reagent 1 into reservoir A1. Discard the pipet tip, add a new pipet tip and pipet 1 ml of screen reagent 2 into reservoir A2. Repeat the procedure for the remaining screen reagents using a clean pipet tip for each reagent so as to avoid reagent contamination and carry over.
3. Pipet 2 μl of the sample to the center of a clean, siliconized 22 mm diameter circle or square cover slide. See Figure 2.

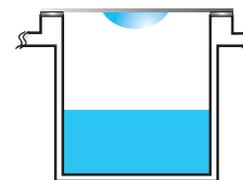
Figure 2



4. Pipet 2 μl of screen reagent 1 from reservoir A1 into the sample droplet and either a) dispense or b) dispense and mix by aspirating and dispensing the droplet several times, keeping the tip in the drop during mixing to avoid foaming. See Figure 2.
5. Working quickly to minimize evaporation, invert the cover slide and droplet over reservoir A1 and seal the cover slide onto the edge of the reservoir. See Figure 3.

Figure 3

Inverted siliconized coverslip placed over the reservoir.



6. Repeat operations 3 through 5 for the remaining screen reagents.

Manual Method – Screen in a 96 Deep Well Block Kits - Sitting Drop Vapor Diffusion

1. Using a 96 well sitting drop vapor diffusion plate, pipet the recommended volume (typically 50 to 100 microliters) of crystallization reagent from the Deep Well block into the reagent reservoirs of the crystallization plate. The Deep Well block is compatible with 8, 12, and 96 channel automated and manual pipettes. Use clean pipet tips for each reagent set, transfer and change pipet tips when changing reagents. For an 8 channel pipet, transfer reagents A1-H1 to reservoirs A1-H1 of the crystallization plate. Repeat this procedure for reagent columns 2 through 12. Change pipet tips when moving between reagent columns. For a 12 channel pipet, transfer reagents A1-A12 to reservoirs A1-A12 of the crystallization plate. Repeat this procedure for reagent rows B through H.
2. Using clean pipet tips, pipet the desired volume of crystallization reagent (typically 0.05 to 2 microliters) from the crystallization plate reservoir to the sitting drop well. Some 96 well crystallization plates allow this procedure to be performed using a multichannel pipet where other plates require the use of a single channel pipet. Change the pipet tip between reagents.
3. Using a clean pipet tip, pipet the same volume (typically 0.05 to 2 microliters) of sample to the reagent drop in the sitting drop well. Work carefully but quickly to minimize evaporation from the crystallization plate.
4. Seal the crystallization plate using an optically clear sealing film or tape. Seal the remaining reagent in the Deep Well block using AlumaSeal II sealing film.

Automated Method – Screen in a 96 Deep Well Block - Sitting Drop Vapor Diffusion

The Deep Well block is compatible with the SBS standard 96 well microplate format and is compatible with numerous automated liquid handling systems that accept 8 x 12, 96 well assay blocks. Follow the automation manufacturer's recommendation for handling Deep Well blocks.

1. Using a 96 well sitting drop vapor diffusion plate, dispense the recommended volume (typically 50 to 100 microliters) of crystallization reagent from the Deep Well block into the reagent reservoirs of the crystallization plate.
2. Dispense the desired volume of crystallization reagent (typically 50 to 200 nanoliters) from the crystallization plate reservoir to the sitting drop well.
3. Transfer the equivalent volume of sample to the reagent drop in the sitting drop well.
4. Seal the crystallization plate using a clear sealing tape or film. View and score the experiment. See Hampton Research Crystal Growth 101 - Viewing Crystallization Experiments for more information.
5. Seal the remaining reagent in the Deep Well block using AlumaSeal II Sealing Film.

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