HAM #HR2130

Crystal Screen HT™

HAMPTON RESEARCH
Solutions for Crystal Growth

User Guide

Crystal Screen HT™ is a high throughput reagent kit designed to provide a rapid screening method for the crystallization of biological macromolecules. The kit is straightforward, effective, and practical for the determination of preliminary crystallization conditions. The kit is also effective in determining the solubility of a macromolecule in a wide range of reagents and pH.

Crystal Screen HT is supplied in a sterile, polypropylene DeepWell block, each reservoir containing 1 ml of sterile filtered reagent. The block is heat sealed using a special polypropylene backed film.

Crystal Screen and Crystal Screen 2 offer a sparse matrix of trial crystallization reagent conditions based upon the original Jancarik and Kim screen (3). The primary screen variables are salt, pH, and precipitant (salts, polymers, volatile organics, and non-volatile organics).

Sample Preparation

The macromolecular sample should be homogenous, as pure as is practically possible (>95%) and free of amorphous and particulate material. Remove amorphous material by centrifugation or microfiltration prior to use (1, 2, 4).

The recommended sample concentration is 5 to 25 mg/ml in sterile filtered, deionized water or dilute (25 mM or less) buffer. For initial screens, the sample should be free of unnecessary additives in order to observe the effect of the Crystal Screen and Crystal Screen 2 variables. However, agents that promote and preserve sample stability and homogeneity can and should be included in the sample. For additional sample preparation recommendation see Crystal Growth 101 - Preliminary Sample Preparation bulletin from Hampton Research.

Preparing the DeepWell Block for Use

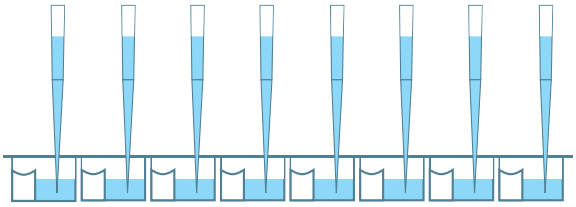
It is recommended the DeepWell block be centrifuged before removing the sealing film. Centrifugation at 500 rpm for five minutes will remove stray reagent from the sealing film. Removing the reagent from the film prevents stray reagent droplets from falling into neighboring wells during film removal. After centrifugation the film can be removed by grasping a corner of the film and gently peeling the film from the plate. Alternatively, the film can be left intact and the pierced for reagent access.

Performing The Screen

Manual Method - Sitting Drop Vapor Diffusion

1. Using a 96 well sitting drop vapor diffusion plate, pipet the recommended volume (typically 100 microliters) of crystallization reagent from the DeepWell block into the reservoirs of the crystallization plate. The DeepWell block is compatible with 8 and 12 channel pipets as well as many automated liquid handling systems. Use clean pipet tips for each reagent set transfer and change pipet tips when changing reagents. For an 8 channel pipet, transfer reagents A1-A8 to reservoirs A1-A8 of the crystallization plate. Repeat this procedure for reagent columns B through L. Change pipet tips when moving between reagent columns. For a 12 channel pipet, transfer reagents A1-L1 to reservoirs A1-L1 of the crystallization plate. Repeat this procedure for reagent rows 1 through 8. See figure 1. Time and pipet tips can be conserved by batch pipetting multiple plates with the same (row or column) of reagent before changing reagent and pipet tips.

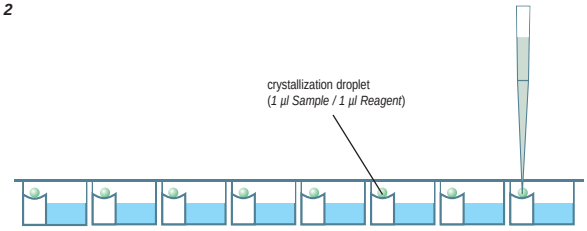
figure 1



2. Using clean pipet tips, pipet 0.05 to 2 microliters of crystallization reagent 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. See figure 2.

3. Using a clean pipet tip, pipet 0.05 to 2 microliters of sample to the reagent drop in the sitting drop well. One may choose to simply dispense the sample with no mixing or dispense with mixing by gently aspirating and dispensing the sample several times, keeping the tip in the drop during mixing to avoid foaming. Work carefully but quickly to minimize evaporation from the crystallization plate. See figure 2.

figure 2



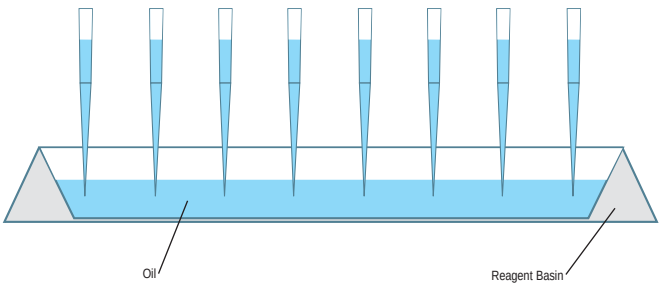
4. Seal the crystallization plate as per the manufacturer's recommendation. Most 96 well crystallization plates are sealed using a clear sealing tape or film. View and score the experiment as desired. See Hampton Research technical bulletin Crystal Growth 101 - Viewing Crystallization Experiments for additional information on viewing drops.

5. Seal the remaining reagent in the DeepWell block using either tape/film or a mat.

Manual Method – MicroBatch 96 well format

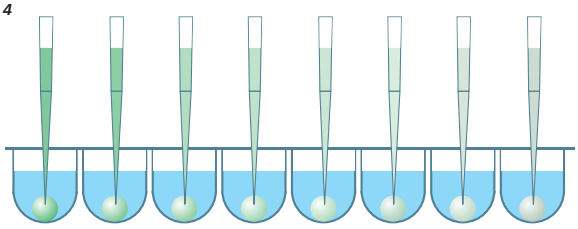
1. Using a 96 well clear polystyrene microplate (U-bottom recommended for best drop centering, flat-bottom recommended for best optics) pipet approximately 150 microliters of microbatch compatible oil into each of the 96 reservoirs. This can be accomplished using an 8 or 12 channel pipet and pipetting the oil from a reagent basin. See figure 3.

figure 3



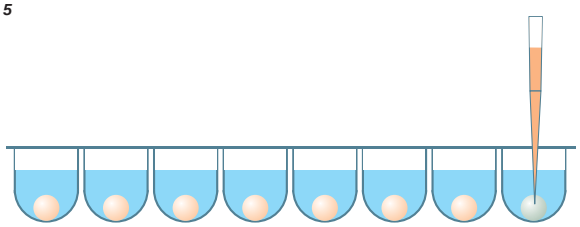
2. Once the plate is oiled, use an 8 or 12 channel pipet to aspirate reagent from the DeepWell block and dispense the reagent under the oil in the MicroBatch plate. Change tips when changing reagent to prevent cross reagent contamination. To save time and pipet tips, set multiple plates at one time. See figure 4.

figure 4



3. Using a single channel pipet, aspirate the sample and dispense the sample under oil in the MicroBatch plate. It is not necessary to dispense the sample drop into the reagent drop or mix the drops. See figure 5.

figure 5



4. After all reagent and sample drops have been dispensed to the MicroBatch plate, place the loose fitting clear cover on the MicroBatch plate and centrifuge the plate for 10 minutes at 500 rpm. Centrifugation will cause the drops to coalesce into a single drop. Note: If the drops appear flat or is fragmented into multiple drops, the centrifugation speed is too high and the centrifugation time it too long – adjust to obtain a spherical single drop in the center of the well.

5. Store the plates with the loose fitting clear polystyrene cover and observe for crystals. See Hampton Research technical bulletin Crystal Growth 101 - Viewing Crystallization Experiments for additional information on viewing drops.

Crystal Screen and Crystal Screen 2 DeepWell Block and Automated Liquid Handling Systems

The polypropylene DeepWell block is designed to be compatible with the SBS standard 96 microwell format and is therefore compatible with numerous automated liquid handling systems that accept 8x12 96 well assay blocks. Follow the manufacturer's recommendation for handling deep well microplates.

Examine The Drop

Carefully examine the drops under a stereo microscope (10 to 100x magnification) immediately after setting up the screen. 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 6 (on page 3) shows typical examples of what one might observe in a crystallization experiment.

Interpreting Crystal Screen HT

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 screen drops are clear 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 and repeat the screen condition. If more than 70 of the 96 screen drops contain precipitate and no crystals are present, consider diluting the sample concentration in half and repeating the entire screen. 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.

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 (pH, salt type, salt concentration, precipitant type, precipitant concentration, sample concentration, temperature, additives, and other crystallization variables) which produced the crystal in order to improve crystal size and quality.

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.

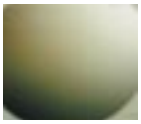
Crystal Screen HT Formulation

Crystallization reagents are formulated using the highest purity chemicals, ultrapure water (18.2 Megohm-cm, 5 ppb TOC) and are sterile filtered using 0.22 micron filters into sterile DeepWell blocks (no preservatives added).

Crystallization reagents are readily reproduced using Hampton Research Optimize™ and StockOptions™ stock solutions of salts, polymers and buffers. Optimize and StockOptions stock reagents make reproducing crystallization screen reagents accurate, precise, fast, convenient and easy. Dilutions can be performed directly into the crystallization plate using Optimize and StockOptions stock reagents.

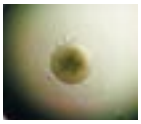
Crystallization reagents containing buffers are formulated by creating a 1.0 M stock buffer, titrated to the desired pH using hydrochloric acid or sodium hydroxide. The buffer is then diluted with the other reagent components and water. No further pH adjustment is required.

figure 6
Typical observations in a crystallization experiment.


Clear Drop


Skin/Precipitate


Precipitate


Precipitate/Phase

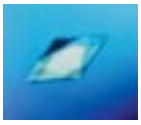

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

Crystallization reagents are stable at room temperature and are best used before the "Best If Used By". To enhance reagent stability it is strongly recommended that crystallization reagents be stored at 4°C or -20°C. Avoid ultraviolet light to preserve reagent stability.

If the sample contains phosphate, borate, or carbonate buffers it is possible to obtain inorganic crystals (false positives) when using crystallization reagents containing divalent cations such as magnesium, calcium, or zinc. To avoid false positives use phosphate, borate, or carbonate buffers at concentrations of 10 mM or less or exchange the phosphate, borate, or carbonate buffer with a more soluble buffer that does not complex with divalent cations.

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. Current approaches to macromolecular crystallization. McPherson, A. Eur. J. Biochem. 189, 1-23, 1990.
3. Sparse Matrix Sampling: a screening method for crystallization of proteins. Jancarik, J. and Kim, S.H. J. Appl. Cryst., 24,409-411, 1991.
4. Protein and Nucleic Acid Crystallization. Methods, A Companion to Methods in Enzymology, Academic Press, Volume 1, Number 1, August 1990.

Technical Support

Inquiries regarding Crystal Screen HT reagent formulation, interpretation of screen results, optimization strategies 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 5:00 p.m. USA Pacific Standard Time.

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Tube Number	**Salt**	**Buffer** †	**Precipitant**
A1.	0.02 M Calcium Chloride dihydrate	0.1 M Sodium Acetate trihydrate pH 4.6	30% v/v 2-Methyl-2,4-pentanediol
A2.	None	None	0.4 M Potassium Sodium Tartrate tetrahydrate
A3.	None	None	0.4 M mono-Ammonium dihydrogen Phosphate
A4.	None	0.1 M Tris Hydrochloride pH 8.5	2.0 M Ammonium Sulfate
A5.	0.2 M tri-Sodium Citrate dihydrate	0.1 M HEPES - Na pH 7.5	30% v/v 2-Methyl-2,4-pentanediol
A6.	0.2 M Magnesium Chloride hexahydrate	0.1 M Tris Hydrochloride pH 8.5	30% w/v Polyethylene Glycol 4000
A7.	None	0.1 M Sodium Cacodylate pH 6.5	1.4 M Sodium Acetate trihydrate
A8.	0.2 M tri-Sodium Citrate dihydrate	0.1 M Sodium Cacodylate pH 6.5	30% v/v iso-Propanol
A9.	0.2 M Ammonium Acetate	0.1 M tri-Sodium Citrate dihydrate pH 5.6	30% w/v Polyethylene Glycol 4000
A10.	0.2 M Ammonium Acetate	0.1 M Sodium Acetate trihydrate pH 4.6	30% w/v Polyethylene Glycol 4000
A11.	None	0.1 M tri-Sodium Citrate dihydrate pH 5.6	1.0 M mono-Ammonium dihydrogen Phosphate
A12.	0.2 M Magnesium Chloride hexahydrate	0.1 M HEPES - Na pH 7.5	30% v/v iso-Propanol
B1.	0.2 M tri-Sodium Citrate dihydrate	0.1 M Tris Hydrochloride pH 8.5	30% v/v Polyethylene Glycol 400
B2.	0.2 M Calcium Chloride dihydrate	0.1 M HEPES - Na pH 7.5	28% v/v Polyethylene Glycol 400
B3.	0.2 M Ammonium Sulfate	0.1 M Sodium Cacodylate pH 6.5	30% w/v Polyethylene Glycol 8000
B4.	None	0.1 M HEPES - Na pH 7.5	1.5 M Lithium Sulfate monohydrate
B5.	0.2 M Lithium Sulfate monohydrate	0.1 M Tris Hydrochloride pH 8.5	30% Polyethylene Glycol 4000
B6.	0.2 M Magnesium Acetate tetrahydrate	0.1 M Sodium Cacodylate pH 6.5	20% Polyethylene Glycol 8000
B7.	0.2 M Ammonium Acetate	0.1 M Tris Hydrochloride pH 8.5	30% v/v iso-Propanol
B8.	0.2 M Ammonium Sulfate	0.1 M Sodium Acetate trihydrate pH 4.6	25% w/v Polyethylene Glycol 4000
B9.	0.2 M Magnesium Acetate tetrahydrate	0.1 M Sodium Cacodylate pH 6.5	30% v/v 2-Methyl-2,4-pentanediol
B10.	0.2 M Sodium Acetate trihydrate	0.1 M Tris Hydrochloride pH 8.5	30% w/v Polyethylene Glycol 4000
B11.	0.2 M Magnesium chloride hexahydrate	0.1 M HEPES - Na pH 7.5	30% v/v Polyethylene Glycol 400
B12.	0.2 M Calcium Chloride dihydrate	0.1 M Sodium Acetate trihydrate pH 4.6	20% v/v iso-Propanol
C1.	None	0.1 M Imidazole pH 6.5	1.0 M Sodium Acetate trihydrate
C2.	0.2 M Ammonium Acetate	0.1 M tri-Sodium Citrate dihydrate pH 5.6	30 % v/v 2-Methyl-2,4-pentanediol
C3.	0.2 M tri-Sodium Citrate dihydrate	0.1 M HEPES - Na pH 7.5	20% v/v iso-Propanol
C4.	0.2 M Sodium Acetate trihydrate	0.1 M Sodium Cacodylate pH 6.5	30% w/v Polyethylene Glycol 8000
C5.	None	0.1 M HEPES - Na pH 7.5	0.8 M Potassium Sodium Tartrate tetrahydrate
C6.	0.2 M Ammonium Sulfate	None	30% w/v Polyethylene Glycol 8000
C7.	0.2 M Ammonium Sulfate	None	30% w/v Polyethylene Glycol 4000
C8.	None	None	2.0 M Ammonium Sulfate
C9.	None	None	4.0 M Sodium Formate
C10.	None	0.1 M Sodium Acetate trihydrate pH 4.6	2.0 M Sodium Formate
C11.	None	0.1 M HEPES - Na pH 7.5	0.8 M mono-Sodium dihydrogen phosphate 0.8 M mono-Potassium dihydrogen phosphate
C12.	None	0.1 M Tris Hydrochloride pH 8.5	8% w/v Polyethylene Glycol 8000
D1.	None	0.1 M Sodium Acetate trihydrate pH 4.6	8% w/v Polyethylene Glycol 4000
D2.	None	0.1 M HEPES - Na pH 7.5	1.4 M tri-Sodium Citrate dihydrate
D3.	None	0.1 M HEPES - Na pH 7.5	2% v/v Polyethylene Glycol 400, 2.0 M Ammonium Sulfate
D4.	None	0.1 M tri-Sodium Citrate dihydrate pH 5.6	20% v/v iso-Propanol, 20% w/v Polyethylene Glycol 4000
D5.	None	0.1 M HEPES - Na pH 7.5	10% v/v iso-Propanol, 20% w/v Polyethylene Glycol 4000
D6.	0.05 M mono-Potassium dihydrogen Phosphate	None	20% w/v Polyethylene Glycol 8000
D7.	None	None	30% w/v Polyethylene Glycol 1500
D8.	None	None	0.2 M Magnesium Formate
D9.	0.2 M Zinc Acetate dihydrate	0.1 M Sodium Cacodylate pH 6.5	18% w/v Polyethylene Glycol 8000
D10.	0.2 M Calcium Acetate hydrate	0.1 M Sodium Cacodylate pH 6.5	18% w/v Polyethylene Glycol 8000
D11.	None	0.1 M Sodium Acetate trihydrate pH 4.6	2.0 M Ammonium Sulfate
D12.	None	0.1 M Tris Hydrochloride pH 8.5	2.0 M mono-Ammonium dihydrogen Phosphate

† *Buffer pH is that of a 1.0 M stock prior to dilution with other reagent components. pH with HCl or NaOH.*

Crystal Screen (DeepWell Block) contains forty-eight unique reagents beginning at position A1. To determine the formulation of each reagent, simply read across the page.

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コスモ・バイオ株式会社

Tube Number	**Salt**	**Buffer** †	**Precipitant**
E1.	2.0 M Sodium chloride	None	10% w/v PEG 6000
E2.	0.01 M Hexadecyltrimethylammonium Bromide	None	0.5 M Sodium Chloride, 0.01 M Magnesium Chloride hexahydrate
E3.	None	None	25% v/v Ethylene Glycol
E4.	None	None	35% v/v Dioxane
E5.	2.0 M Ammonium Sulfate	None	5% v/v iso-Propanol
E6.	None	None	1.0 M Imidazole pH 7.0
E7.	None	None	10% w/v Polyethylene Glycol 1000 10% w/v Polyethylene Glycol 8000
E8.	1.5 M Sodium Chloride	None	10% v/v Ethanol
E9.	None	0.1 M Sodium Acetate trihydrate pH 4.6	2.0 M Sodium Chloride
E10.	0.2 M Sodium Chloride	0.1 M Sodium Acetate trihydrate pH 4.6	30% v/v MPD
E11.	0.01 M Cobaltous Chloride hexahydrate	0.1 M Sodium Acetate trihydrate pH 4.6	1.0 M 1,6 Hexanediol
E12.	0.1 M Cadmium Chloride dihydrate	0.1 M Sodium Acetate trihydrate pH 4.6	30% v/v Polyethylene Glycol 400
F1.	0.2 M Ammonium Sulfate	0.1 M Sodium Acetate trihydrate pH 4.6	30% w/v Polyethylene Glycol Monomethyl Ether 2000
F2.	0.2 M Potassium Sodium Tartrate tetrahydrate	0.1 M tri-Sodium Citrate dihydrate pH 5.6	2.0 M Ammonium Sulfate
F3.	0.5 M Ammonium Sulfate	0.1 M tri-Sodium Citrate dihydrate pH 5.6	1.0 M Lithium Sulfate monohydrate
F4.	0.5 M Sodium Chloride	0.1 M tri-Sodium Citrate dihydrate pH 5.6	2% w/v Ethylene Imine Polymer
F5.	None	0.1 M tri-Sodium Citrate dihydrate pH 5.6	35% v/v tert-Butanol
F6.	0.01 M Ferric Chloride hexahydrate	0.1 M tri-Sodium Citrate dihydrate pH 5.6	10% v/v Jeffamine M-600®
F7.	None	0.1 M tri-Sodium Citrate dihydrate pH 5.6	2.5 M 1,6 Hexanediol
F8.	None	0.1 M MES pH 6.5	1.6 M Magnesium Sulfate heptahydrate
F9.	0.1 M Sodium dihydrogen phosphate mono 0.1 M mono-Potassium dihydrogen Phosphate	0.1 M MES pH 6.5	2.0 M Sodium Chloride
F10.	None	0.1 M MES pH 6.5	12% w/v Polyethylene Glycol 20,000
F11.	1.6 M Ammonium Sulfate	0.1 M MES pH 6.5	10% v/v Dioxane
F12.	0.05 M Cesium Chloride	0.1 M MES pH 6.5	30% v/v Jeffamine M-600®
G1.	0.01 M Cobaltous Chloride hexahydrate	0.1 M MES pH 6.5	1.8 M Ammonium Sulfate
G2.	0.2 M Ammonium Sulfate	0.1 M MES pH 6.5	30% w/v Polyethylene Glycol Monomethyl Ether 5000
G3.	0.01 M Zinc Sulfate heptahydrate	0.1 M MES pH 6.5	25% v/v Polyethylene Glycol Monomethyl Ether 550
G4.	None	None	1.6 M tri-Sodium Citrate dihydrate pH 6.5
G5.	0.5 M Ammonium Sulfate	0.1 M HEPES pH 7.5	30% v/v MPD
G6.	None	0.1 M HEPES pH 7.5	10% w/v Polyethylene Glycol 6000, 5% v/v MPD
G7.	None	0.1 M HEPES pH 7.5	20% v/v Jeffamine M-600®
G8.	0.1 M Sodium Chloride	0.1 M HEPES pH 7.5	1.6 M Ammonium Sulfate
G9.	None	0.1 M HEPES pH 7.5	2.0 M Ammonium Formate
G10.	0.05 M Cadmium Sulfate hydrate	0.1 M HEPES pH 7.5	1.0 M Sodium Acetate
G11.	None	0.1 M HEPES pH 7.5	70% v/v MPD
G12.	None	0.1 M HEPES pH 7.5	4.3 M Sodium Chloride
H1.	None	0.1 M HEPES pH 7.5	10% w/v Polyethylene Glycol 8000, 8% v/v Ethylene Glycol
H2.	None	0.1 M HEPES pH 7.5	20% w/v Polyethylene Glycol 10,000
H3.	0.2 M Magnesium Chloride hexahydrate	0.1 M TRIS pH 8.5	3.4 M 1,6 Hexanediol
H4.	None	0.1 M TRIS pH 8.5	25% v/v tert-Butanol
H5.	0.01 M Nickel(II) Chloride hexahydrate	0.1 M TRIS pH 8.5	1.0 M Lithium Sulfate monohydrate
H6.	1.5 M Ammonium Sulfate	0.1 M TRIS pH 8.5	12% v/v Glycerol anhydrous
H7.	0.2 M mono Ammonium dihydrogen Phosphate	0.1 M TRIS pH 8.5	50% v/v MPD
H8.	None	0.1 M TRIS pH 8.5	20% v/v Ethanol
H9.	0.01 M Nickel(II) Chloride hexahydrate	0.1 M TRIS pH 8.5	20% w/v Polyethylene Glycol Monomethyl Ether 2000
H10.	0.1 M Sodium Chloride	0.1 M Bicine pH 9.0	20% w/v Polyethylene Glycol Monomethyl Ether 550
H11.	None	0.1 M Bicine pH 9.0	2.0 M Magnesium Chloride hexahydrate
H12.	2% v/v Dioxane	0.1 M Bicine pH 9.0	10% w/v Polyethylene Glycol 20,000

† *Buffer pH is that of a 1.0 M stock (0.5 M for MES) prior to dilution with other reagent components. pH with HCl or NaOH.*

Crystal Screen 2 (DeepWell Block) contains forty-eight unique reagents beginning at position E1. To determine the formulation of each reagent, simply read across the page.

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Sample: _____ ***Sample Concentration:*** _____

Sample Buffer: _____ ***Date:*** _____

Reservior Volume: _____ ***Temperature:*** _____

Drop Volume: Total _____ ***µl Sample*** _____ ***µl Reservior*** _____ ***µl Additive*** _____ ***µl***

1 Clear Drop
2 Phase Separation
3 Regular Granular Precipitate
4 Birefringent Precipitate or Microcrystals
5 Posettes or Spherulites
6 Needles (1D Growth)
7 Plates (2D Growth)
8 Single Crystals (3D Growth < 0.2mm)
9 Single Crystals (3D Growth > 0.2mm)

Crystal Screen HT - Scoring Sheet	Date:	Date:	Date:	Date:	Date:
A1. 30% MPD, 0.1 M Na Acetate pH 4.6, 0.02 M Calcium Chloride					
A2. 0.4 M K, Na Tartrate					
A3. 0.4 M Ammonium Phosphate					
A4. 2.0 M Ammonium Sulfate, 0.1 M Tris HCl pH 8.5					
A5. 30% MPD, 0.1 M Na Hepes pH 7.5, 0.2 M Sodium Citrate					
A6. 30% PEG 4000, 0.1 M Tris HCl pH 8.5, 0.2 M Magnesium Chloride					
A7. 1.4 M Sodium Acetate, 0.1 M Na Cacodylate pH 6.5					
A8. 30% iso-Propanol, 0.1 M Na Cacodylate pH 6.5, 0.2 M Sodium Citrate					
A9. 30% PEG 4000, 0.1 M Na Citrate pH 5.6, 0.2 M Ammonium Acetate					
A10. 30% PEG 4000, 0.1 M Na Acetate pH 4.6, 0.2 M Ammonium Acetate					
A11. 1.0 M Ammonium Phosphate, 0.1 M Na Citrate pH 5.6					
A12. 30% iso-Propanol, 0.1 M Na Hepes pH 7.5, 0.2 M Magnesium Chloride					
B1. 30% PEG 400, 0.1 M Tris HCl pH 8.5, 0.2 M Sodium Citrate					
B2. 28% PEG 400, 0.1 M Na Hepes pH 7.5, 0.2 M Calcium Chloride					
B3. 30% PEG 8000, 0.1 M Na Cacodylate pH 6.5, 0.2 M Ammonium Sulfate					
B4. 1.5 M Lithium Sulfate, 0.1 M Na Hepes pH 7.5					
B5. 30% PEG 4000, 0.1 M Tris HCl pH 8.5, 0.2 M Lithium Sulfate					
B6. 20% PEG 8000, 0.1 M Na Cacodylate pH 6.5, 0.2 M Magnesium Acetate					
B7. 30% iso-Propanol, 0.1 M Tris HCl pH 8.5, 0.2 M Ammonium Acetate					
B8. 25% PEG 4000, 0.1 M Na Acetate pH 4.6, 0.2 M Ammonium Sulfate					
B9. 30% MPD, 0.1 M Na Cacodylate pH 6.5, 0.2 M Magnesium Acetate					
B10. 30% PEG 4000, 0.1 M Tris HCl pH 8.5, 0.2 M Sodium Acetate					
B11. 30% PEG 400, 0.1 M Na Hepes pH 7.5, 0.2 M Magnesium Chloride					
B12. 20% iso-Propanol, 0.1 M Na Acetate pH 4.6, 0.2 M Calcium Chloride					
C1. 1.0 M Sodium Acetate, 0.1 M Imidazole pH 6.5					
C2. 30% MPD, 0.1 M Na Citrate pH 5.6, 0.2 M Ammonium Acetate					
C3. 20% iso-Propanol, 0.1 M Na Hepes pH 7.5, 0.2 M Sodium Citrate					
C4. 30% PEG 8000, 0.1 M Na Cacodylate pH 6.5, 0.2 M Sodium Acetate					
C5. 0.8 M K, Na Tartrate, 0.1 M Na Hepes pH 7.5					
C6. 30% PEG 8000, 0.2 M Ammonium Sulfate					
C7. 30% PEG 4000, 0.2 M Ammonium Sulfate					
C8. 2.0 M Ammonium Sulfate					
C9. 4.0 M Sodium Formate					
C10. 2.0 M Sodium Formate, 0.1 M Na Acetate pH 4.6					
C11. 1.6 M Na, K Phosphate, 0.1 M Na Hepes pH 7.5					
C12. 8% PEG 8000, 0.1 M Tris HCl pH 8.5					
D1. 8% PEG 4000, 0.1 M Na Acetate pH 4.6					
D2. 1.4 M Sodium Citrate, 0.1 M Na Hepes pH 7.5					
D3. 2% PEG 400, 0.1 M Na Hepes pH 7.5, 2.0 M Ammonium Sulfate					
D4. 20% iso-Propanol, 0.1 M Na Citrate pH 5.6, 20% PEG 4000					
D5. 10% iso-Propanol, 0.1 M Na Hepes pH 7.5, 20% PEG 4000					
D6. 20% PEG 8000, 0.05 M Potassium Phosphate					
D7. 30% PEG 1500					
D8. 0.2 M Magnesium Formate					
D9. 18% PEG 8000, 0.1 M Na Cacodylate pH 6.5, 0.2 M Zinc Acetate					
D10. 18% PEG 8000, 0.1 M Na Cacodylate pH 6.5, 0.2 M Calcium Acetate					
D11. 2.0 M Ammonium Sulfate, 0.1 M Sodium Acetate pH 4.6					
D12. 2.0 M Ammonium Phosphate, 0.1 M Tris HCl pH 8.5					

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Sample: _____ *Sample Concentration:* _____

Sample Buffer: _____ *Date:* _____

Reservior Volume: _____ *Temperature:* _____

Drop Volume: Total _____ *µl Sample* _____ *µl Reservior* _____ *µl Additive* _____ *µl*

1 Clear Drop
2 Phase Separation
3 Regular Granular Precipitate
4 Birefringent Precipitate or Microcrystals
5 Rosettes or Spherulites
6 Needles (1D Growth)
7 Plates (2D Growth)
8 Single Crystals (3D Growth < 0.2mm)
9 Single Crystals (3D Growth > 0.2mm)

Crystal Screen 2 HT - Scoring Sheet	Date:	Date:	Date:	Date:	Date:
E1. 10% PEG 6000, 2.0 M Sodium Chloride					
E2. 0.5 M Sodium Chloride, 0.01 M CTAB, 0.01 M Magnesium Chloride					
E3. 25% Ethylene Glycol					
E4. 35% Dioxane					
E5. 5% iso-Propanol, 2.0 M Ammonium Sulfate					
E6. 1.0 M Imidazole pH 7.0					
E7. 10% PEG 1000, 10% PEG 8000					
E8. 10% Ethanol, 1.5 M Sodium Chloride					
E9. 2.0 M Sodium Chloride, 0.1 M Na Acetate pH 4.6					
E10. 30% MPD, 0.1 M Na Acetate pH 4.6, 0.2 M Sodium Chloride					
E11. 1.0 M 1,6 Hexanediol, 0.1 M Na Acetate pH 4.6, 0.01 M Cobalt Chloride					
E12. 30% PEG 400, 0.1 M Na Acetate pH 4.6, 0.1 M Cadmium Chloride					
F1. 30% PEG MME 2000, 0.1 M Na Acetate pH 4.6, 0.2 M Ammonium Sulfate					
F2. 2.0 M Ammonium Sulfate, 0.1 M Na Citrate pH 5.6, 0.2 M K/Na Tartrate					
F3. 1.0 M Lithium Sulfate, 0.1 M Na Citrate pH 5.6, 0.5 M Ammonium Sulfate					
F4. 2% Polyethyleneimine, 0.1 M Na Citrate pH 5.6, 0.5 M Sodium Chloride					
F5. 35% tert-Butanol, 0.1 M Na Citrate pH 5.6					
F6. 10% Jeffamine M-600, 0.1 M Na Citrate pH 5.6, 0.01 M Ferric Chloride					
F7. 2.5 M 1,6 Hexanediol, 0.1 M Na Citrate pH 5.6					
F8. 1.6 M Magnesium Sulfate, 0.1 M MES pH 6.5					
F9. 2.0 M Sodium Chloride, 0.1 M MES pH 6.5, 0.2 M Na/K Phosphate					
F10. 12% PEG 20,000, 0.1 M MES pH 6.5					
F11. 10% Dioxane, 0.1 M MES pH 6.5, 1.6 M Ammonium Sulfate					
F12. 30% Jeffamine M-600, 0.1 M MES pH 6.5, 0.05 M Cesium Chloride					
G1. 1.8 M Ammonium Sulfate, 0.1 M MES pH 6.5, 0.01 M Cobalt Chloride					
G2. 30% PEG MME 5000, 0.1 M MES pH 6.5, 0.2 M Ammonium Sulfate					
G3. 25% PEG MME 550, 0.1 M MES pH 6.5, 0.01 M Zinc Sulfate					
G4. 1.6 M Sodium Citrate pH 6.5					
G5. 30% MPD, 0.1 M Hepes pH 7.5, 0.5 M Ammonium Sulfate					
G6. 10% PEG 6000, 0.1 M Hepes pH 7.5, 5% MPD					
G7. 20% Jeffamine M-600, 0.1 M Hepes pH 7.5					
G8. 1.6 M Ammonium Sulfate, 0.1 M Hepes pH 7.5, 0.1 M Sodium Chloride					
G9. 2.0 M Ammonium Formate, 0.1 M Hepes pH 7.5					
G10. 1.0 M Sodium Acetate, 0.1 M Hepes pH 7.5, 0.05 M Cadmium Sulfate					
G11. 70% MPD, 0.1 M Hepes pH 7.5					
G12. 4.3 M Sodium Chloride, 0.1 M Hepes pH 7.5					
H1. 10% PEG 8000, 0.1 M Hepes pH 7.5, 8% Ethylene Glycol					
H2. 20% PEG 10,000, 0.1 M Hepes pH 7.5					
H3. 3.4 M 1,6 Hexanediol, 0.1 M Tris pH 8.5, 0.2 M Magnesium Chloride					
H4. 25% tert-Butanol, 0.1 M Tris pH 8.5					
H5. 1.0 M Lithium Sulfate, 0.1 M Tris pH 8.5, 0.01 M Nickel (II) Chloride					
H6. 12% Glycerol, 0.1 M Tris pH 8.5, 1.5 M Ammonium Sulfate					
H7. 50% MPD, 0.1 M Tris pH 8.5, 0.2 M Ammonium Phosphate					
H8. 20% Ethanol, 0.1 M Tris pH 8.5					
H9. 20% PEG MME 2000, 0.1 M Tris pH 8.5, 0.01 M Nickel (II) Chloride					
H10. 20% PEG MME 550, 0.1 M Bicine pH 9.0, 0.1 M Sodium Chloride					
H11. 2.0 M Magnesium Chloride, 0.1 M Bicine pH 9.0					
H12. 10% PEG 20,000, 0.1 M Bicine pH 9.0, 2% Dioxane					

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