Crystal Screen™

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Solutions for Crystal Growth

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

Crystal Screen is 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 micro-filtration prior to use (1, 2, 4).

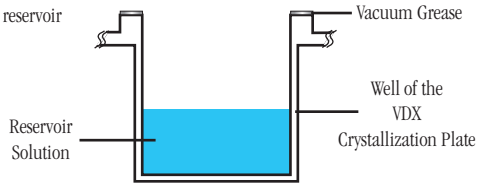
The recommended sample concentration is 5 to 25 mg/ml in water. Initially, the sample should be free of any unnecessary additives in order to observe the effect of the Crystal Screen variables. Ideally, the initial screen should be performed with a sample which has been dialyzed against water although ligands, ions, reducing agents, or other additives may be present as required by the sample for solubility, stability, or activity.

Performing The Screen

Since it is the most frequently reported method of crystallization, the following procedure describes the use of Crystal Screen with the Hanging Drop Vapor Diffusion method. Crystal Screen is also very compatible with the Sitting Drop, Sandwich Drop, MicroBatch, and Microdialysis methods. A complete description of the Hanging, Sitting, Sandwich Drop, Dialysis and other crystallization methods are available from the Hampton Research Crystal Growth 101 Library.

1. Prepare a VDX Plate (HR3-140) 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 (HR3-170). Fifty reservoirs are to be prepared for a complete Crystal Screen. See Figure 1.

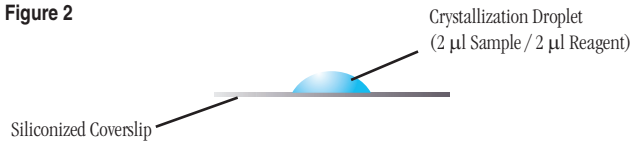
Figure 1
Cross section of a reservoir in the VDX plate.



2. Using a clean pipet tip, pipet 1 ml of Crystal Screen reagent 1 into reservoir A1. Discard the pipet tip, add a new pipet tip and pipet 1 ml of Crystal Screen reagent 2 into reservoir A2. Repeat the procedure for the remaining 48 Crystal 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.

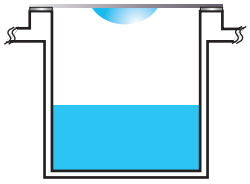
Figure 2



4. Pipet 2 µl of Crystal Screen reagent 1 from reservoir A1 into the sample droplet 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 49 Crystal Screen reagents.

7. If the quantity of sample permits, perform Crystal Screen in duplicate and incubate one set of plates at 4°C and the second set at room temperature. Incubate and store the crystallization plates in a stable temperature environment free of vibration.

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 4 (on page 2) shows typical examples of what one might observe in a crystallization experiment.

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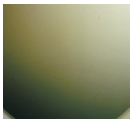
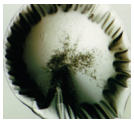
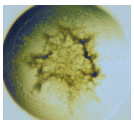
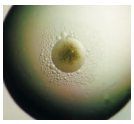
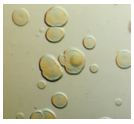
Figure 4
Typical observations in a crystallization experiment

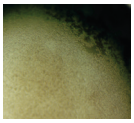

Clear Drop


Skin / Precipitate

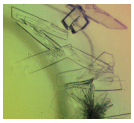

Precipitate


Precipitate / Phase

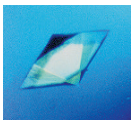

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

Interpreting Crystal 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 Crystal Screen condition and doubling the sample concentration. If more than 35 of the 50 Crystal Screen drops are clear consider doubling the sample concentration and repeating the entire screen.

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 Crystal Screen condition. If more than 35 of the 50 Crystal 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 good. 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 Formulation

Crystal Screen 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 containers (no preservatives added).

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

Crystal Screen 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.

Crystal Screen reagents are stable at room temperature and are best if used within 12 months of receipt. To enhance reagent stability it is strongly recommended that Crystal Screen 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 Crystal Screen 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.

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Technical Support

Inquiries regarding Crystal Screen 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 4:30 p.m. USA Pacific Standard Time.

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How to Reproduce Crystal Screen Reagents

Crystal Screen reagents and optimization conditions based on Crystal Screen hits can be formulated using volumetric methods and carefully prepared reagent stocks (Table 1). Note the examples below.

Example 1. To prepare 1.0 milliliter of Crystal Screen reagent 1 in a crystallization plate.

Solution Composition: 30% v/v (+/-)-2-Methyl-2,4-pentanediol
0.1 M Sodium acetate trihydrate pH 4.6
0.02 M Calcium chloride dihydrate

- 580 µl water[3]
- 20 µl 1.0 M Calcium chloride dihydrate (CAS # 10035-04-8, Catalog # HR2-557)
- 100 µl 1.0 M Sodium acetate trihydrate pH 4.6 (CAS # 6131-90-4, Catalog # HR2-731)
- 300 µl 100% (+/-)-2-Methyl-2,4-pentanediol (CAS # 107-41-5, Catalog # HR2-627)

Make no pH adjustments. Mix well by aspirating and dispensing the solution multiple times.

Example 2. To prepare 10 milliliters of Crystal Screen reagent 4.

Solution Composition: 2.0 M Ammonium sulfate
0.1 M TRIS hydrochloride pH 8.5

- 3.3 ml water[3]
- 1.0 ml 1.0 M TRIS hydrochloride pH 8.5 (CAS # 1185-53-1, Catalog # HR2-727)
- 5.7 ml 3.5 M Ammonium sulfate (CAS # 7783-20-2, Catalog # HR2-541)

Make no pH adjustments. Mix well.

[3] ASTM Type II (laboratory grade) or Type III (analytical grade) water.

Formulation Notes for Crystal Screen Reagents

1. No additional pH adjustment is made to any reagent after formulation. Use the buffers in Table 1 to reproduce a Crystal Screen reagent.
2. All Optimize solutions and screen reagents are sterile filtered using 0.22 µm filters into sterile containers.
3. Add water first as this will help maintain the solubility of subsequently added reagents.
4. When formulating reagents using a pipet, add the largest volume last (except water). Use this larger volume setting to aspirate and dispense the reagent until the solution is mixed.
5. When formulating reagents using a pipet, use a clean, sterile pipet tip for each reagent added to the solution.
6. Use the buffers in Table 2 to systematically vary the pH as a crystallization variable.

pH as a Crystallization Variable

The buffers listed in Table 2, can be used to vary the pH as a crystallization variable and are recommended when optimizing a crystal grown from a Crystal Screen kit.

Optimize™ buffer stocks are supplied as a 100 milliliters sterile filtered solution. The pH can be adjusted to the indicated pH range using either HCl or NaOH and the supplied titration tables.

StockOptions™ buffer kits contain 10 milliliters each of ready to pipet buffers, titrated in 0.1 pH increments over the indicated pH range. The number of reagents offered in a StockOptions buffer kit depends upon the pH range of the buffer. The broader the pH range, the more buffers in the kit.

Online Information

Visit www.hamptonresearch.com and enter one of the following:

- Reagent Catalog Number
- Kit Catalog Number
- CAS Number
- Reagent Name

To obtain reagent specifications, pH titration tables, user guides, certificates of analysis, material safety data sheets (MSDS), and any other additional information.

MakeTray™

MakeTray is a free, web based program at www.hamptonresearch.com which generates both a pipetting worksheet and a reagent formulation document for crystallization set ups. MakeTray allows one to enter general information about the sample and experiment, which is then printed on the pipet worksheet and the reagent formulation document. The plate size can be customized for any number of wells, so MakeTray works for: 24, 48, and 96 well plates. MakeTray is especially useful for the design and formulation of crystal optimization experiments.

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Table 1. Recommended reagents for the formulation of Crystal Screen and optimization reagents.

Each of these reagents are available as an Optimize™ crystallization grade reagent from Hampton Research. Table 1 provides the common chemical name, the Hampton Research catalog number, supplied stock concentration, the supplied volume, and the CAS number for each reagent. For more information on a specific Optimize reagent, go to www.hamptonresearch.com. Using Search, enter either the catalog number, CAS number, or chemical name to obtain additional information for the Optimize reagent, including a Certificate of Analysis and MSDS (where applicable).

Salts	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
Ammonium acetate	HR2-565	1.0 M	100 ml	631-61-8
	HR2-799	8.0 M	200 ml	631-61-8
Ammonium phosphate monobasic	HR2-555	2.5 M	200 ml	7722-76-1
Ammonium sulfate	HR2-541	3.5 M	200 ml	7783-20-2
Calcium acetate hydrate	HR2-567	1.0 M	100 ml	62-54-4
Calcium chloride dihydrate	HR2-557	2.0 M	100 ml	10035-04-8
Lithium sulfate monohydrate	HR2-545	2.0 M	200 ml	10377-48-7
Magnesium acetate tetrahydrate	HR2-561	1.0 M	100 ml	16674-78-5
Magnesium chloride hexahydrate	HR2-559	2.0 M	100 ml	7791-18-6
	HR2-803	5.0 M	200 ml	7791-18-6
Magnesium formate dihydrate	HR2-537	1.0 M	200 ml	557-39-1
Potassium phosphate monobasic	HR2-553	1.5 M	200 ml	7778-77-0
Potassium sodium tartrate tetrahydrate	HR2-539	1.5 M	200 ml	6381-59-5
Sodium acetate trihydrate	HR2-543	3.0 M	200 ml	6131-90-4
Sodium citrate tribasic dihydrate	HR2-549	1.6 M	200 ml	6132-04-3
Sodium formate	HR2-547	7.0 M	200 ml	141-53-7
Sodium phosphate monobasic monohydrate	HR2-551	4.0 M	200 ml	10049-21-5
Zinc acetate dihydrate	HR2-563	1.0 M	100 ml	5970-45-6
Polymers	**Hampton Research Catalog #**	**Supplied [Stock]**	**Supplied Volume**	**CAS #**
Polyethylene glycol 400	HR2-603	100 %	200 ml	25322-68-3
Polyethylene glycol 1,500	HR2-525	50 % w/v	200 ml	25322-68-3
Polyethylene glycol 4,000	HR2-529	50 % w/v	200 ml	25322-68-3
Polyethylene glycol 8,000	HR2-535	50 % w/v	200 ml	25322-68-3
Organics (volatile)	**Hampton Research Catalog #**	**Supplied [Stock]**	**Supplied Volume**	**CAS #**
2-Propanol	HR2-619	100 %	200 ml	67-63-0

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Table 1 (Continued). **Recommended reagents for the formulation of Crystal Screen and optimization reagents.**

Organics (non-volatile)	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
(+/-)-2-Methyl-2,4-pentanediol	HR2-627	100 %	200 ml	107-41-5
Buffers	**Hampton Research Catalog #**	**Supplied [Stock]**	**Supplied Volume**	**CAS #**
HEPES sodium pH 7.5 [1]	HR2-733	1.0 M	100 ml	75277-39-3
Imidazole	HR2-573	1.0 M	100 ml	288-32-4
Sodium acetate trihydrate pH 4.6 [1]	HR2-731	1.0 M	100 ml	6131-90-4
Sodium cacodylate trihydrate pH 6.5 [1]	HR2-737	1.0 M	100 ml	6131-99-3
Sodium citrate tribasic dihydrate pH 5.6 [1]	HR2-735	1.0 M	100 ml	6132-04-3
TRIS hydrochloride pH 8.5 [2]	HR2-727	1.0 M	100 ml	1185-53-1
[1] pH titrated using Hydrochloric acid (HR2-581) CAS # 7647-01-0				
[2] pH titrated using Sodium hydroxide (HR2-583) CAS # 1310-73-2				

Table 2. **Recommended buffers for screening the pH of Crystal Screen and optimization reagents.**

Buffer Solution or Kit	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #	pH range
Hepes sodium untitrated	HR2-577	1.0 M	100 ml	75277-39-3	6.6 - 8.5
Titrate with HCl	HR2-581	1.0 M	100 ml	7647-01-0	—
StockOptions™ Sodium Hepes kit [4]	HR2-231	1.0 M	10 ml each	75277-39-3	6.8 - 8.2
Imidazole untitrated	HR2-573	1.0 M	100 ml	288-32-4	6.2 - 7.8
Titrate with HCl	HR2-581	1.0 M	100 ml	7647-01-0	—
Sodium acetate trihydrate untitrated	HR2-569	1.0 M	100 ml	6131-90-4	3.6 - 5.6
Titrate with HCl	HR2-581	1.0 M	100 ml	7647-01-07	—
StockOptions™ Sodium Acetate kit [4]	HR2-233	1.0 M	10 ml each	6131-90-4	3.6 - 5.6
Sodium cacodylate trihydrate untitrated	HR2-575	1.0 M	100 ml	6131-99-3	5.0 - 7.4
Titrate with HCl	HR2-581	1.0 M	100 ml	7647-01-0	—
StockOptions™ Sodium Cacodylate kit [4]	HR2-239	1.0 M	10 ml each	6131-99-3	5.1 - 7.4
Sodium citrate tribasic dihydrate untitrated	HR2-571	1.0 M	100 ml	6132-04-3	3.0 - 6.2
Titrate with HCl	HR2-581	1.0 M	100 ml	7647-01-0	—
StockOptions™ Sodium Citrate kit [4]	HR2-235	1.0 M	10 ml each	6132-04-3	4.2 - 6.5

(Continued on page 4)

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Crystal Screen Fundamentals — HR2-110 (pg 4)

Buffer Solution or Kit	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #	pH range
Tris hydrochloride untitrated	HR2-579	1.0 M	100 ml	1185-53-1	7.0 - 9.0
Titrate with NaOH	HR2-583	1.0 M	100 ml	1310-73-2	—
StockOptions™ Tris Hydrochloride kit [4]	HR2-237	1.0 M	10 ml each	1185-53-1	7.0 - 9.0

[4] Individual StockOptions buffers titrated to any pH within the kit's pH range are available in 185 ml volumes from the Hampton Research Custom Shop

Technical Support

Inquiries regarding Crystal Screen Fundamentals, 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 4:30 p.m. USA Pacific Standard Time.

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Tube #	Salt	Buffer ◊	Precipitant
1.	0.02 M Calcium chloride dihydrate	0.1 M Sodium acetate trihydrate pH 4.6	30% v/v (+/-)-2-Methyl-2,4-pentanediol
2.	None	None	0.4 M Potassium sodium tartrate tetrahydrate
3.	None	None	0.4 M Ammonium phosphate monobasic
4.	None	0.1 M TRIS hydrochloride pH 8.5	2.0 M Ammonium sulfate
5.	0.2 M Sodium citrate tribasic dihydrate	0.1 M HEPES sodium pH 7.5	30% v/v (+/-)-2-Methyl-2,4-pentanediol
6.	0.2 M Magnesium chloride hexahydrate	0.1 M TRIS hydrochloride pH 8.5	30% w/v Polyethylene glycol 4,000
7.	None	0.1 M Sodium cacodylate trihydrate pH 6.5	1.4 M Sodium acetate trihydrate
8.	0.2 M Sodium citrate tribasic dihydrate	0.1 M Sodium cacodylate trihydrate pH 6.5	30% v/v 2-Propanol
9.	0.2 M Ammonium acetate	0.1 M Sodium citrate tribasic dihydrate pH 5.6	30% w/v Polyethylene glycol 4,000
10.	0.2 M Ammonium acetate	0.1 M Sodium acetate trihydrate pH 4.6	30% w/v Polyethylene glycol 4,000
11.	None	0.1 M Sodium citrate tribasic dihydrate pH 5.6	1.0 M Ammonium phosphate monobasic
12.	0.2 M Magnesium chloride hexahydrate	0.1 M HEPES sodium pH 7.5	30% v/v 2-Propanol
13.	0.2 M Sodium citrate tribasic dihydrate	0.1 M TRIS hydrochloride pH 8.5	30% v/v Polyethylene glycol 400
14.	0.2 M Calcium chloride dihydrate	0.1 M HEPES sodium pH 7.5	28% v/v Polyethylene glycol 400
15.	0.2 M Ammonium sulfate	0.1 M Sodium cacodylate trihydrate pH 6.5	30% w/v Polyethylene glycol 8,000
16.	None	0.1 M HEPES sodium pH 7.5	1.5 M Lithium sulfate monohydrate
17.	0.2 M Lithium sulfate monohydrate	0.1 M TRIS hydrochloride pH 8.5	30% w/v Polyethylene glycol 4,000
18.	0.2 M Magnesium acetate tetrahydrate	0.1 M Sodium cacodylate trihydrate pH 6.5	20% w/v Polyethylene glycol 8,000
19.	0.2 M Ammonium acetate	0.1 M TRIS hydrochloride pH 8.5	30% v/v 2-Propanol
20.	0.2 M Ammonium sulfate	0.1 M Sodium acetate trihydrate pH 4.6	25% w/v Polyethylene glycol 4,000
21.	0.2 M Magnesium acetate tetrahydrate	0.1 M Sodium cacodylate trihydrate pH 6.5	30% v/v (+/-)-2-Methyl-2,4-pentanediol
22.	0.2 M Sodium acetate trihydrate	0.1 M TRIS hydrochloride pH 8.5	30% w/v Polyethylene glycol 4,000
23.	0.2 M Magnesium chloride hexahydrate	0.1 M HEPES sodium pH 7.5	30% v/v Polyethylene glycol 400
24.	0.2 M Calcium chloride dihydrate	0.1 M Sodium acetate trihydrate pH 4.6	20% v/v 2-Propanol
25.	None	0.1 M Imidazole pH 6.5	1.0 M Sodium acetate trihydrate
26.	0.2 M Ammonium acetate	0.1 M Sodium citrate tribasic dihydrate pH 5.6	30% v/v (+/-)-2-Methyl-2,4-pentanediol
27.	0.2 M Sodium citrate tribasic dihydrate	0.1 M HEPES sodium pH 7.5	20% v/v 2-Propanol
28.	0.2 M Sodium acetate trihydrate	0.1 M Sodium cacodylate trihydrate pH 6.5	30% w/v Polyethylene glycol 8,000
29.	None	0.1 M HEPES sodium pH 7.5	0.8 M Potassium sodium tartrate tetrahydrate
30.	0.2 M Ammonium sulfate	None	30% w/v Polyethylene glycol 8,000
31.	0.2 M Ammonium sulfate	None	30% w/v Polyethylene glycol 4,000
32.	None	None	2.0 M Ammonium sulfate
33.	None	None	4.0 M Sodium formate
34.	None	0.1 M Sodium acetate trihydrate pH 4.6	2.0 M Sodium formate
35.	None	0.1 M HEPES sodium pH 7.5	0.8 M Sodium phosphate monobasic monohydrate 0.8 M Potassium phosphate monobasic
36.	None	0.1 M TRIS hydrochloride pH 8.5	8% w/v Polyethylene glycol 8,000
37.	None	0.1 M Sodium acetate trihydrate pH 4.6	8% w/v Polyethylene glycol 4,000
38.	None	0.1 M HEPES sodium pH 7.5	1.4 M Sodium citrate tribasic dihydrate
39.	None	0.1 M HEPES sodium pH 7.5	2% v/v Polyethylene glycol 400 2.0 M Ammonium sulfate
40.	None	0.1 M Sodium citrate tribasic dihydrate pH 5.6	20% v/v 2-Propanol 20% w/v Polyethylene glycol 4,000
41.	None	0.1 M HEPES sodium pH 7.5	10% v/v 2-Propanol 20% w/v Polyethylene glycol 4,000
42.	0.05 M Potassium phosphate monobasic	None	20% w/v Polyethylene glycol 8,000
43.	None	None	30% w/v Polyethylene glycol 1,500
44.	None	None	0.2 M Magnesium formate dihydrate
45.	0.2 M Zinc acetate dihydrate	0.1 M Sodium cacodylate trihydrate pH 6.5	18% w/v Polyethylene glycol 8,000
46.	0.2 M Calcium acetate hydrate	0.1 M Sodium cacodylate trihydrate pH 6.5	18% w/v Polyethylene glycol 8,000
47.	None	0.1 M Sodium acetate trihydrate pH 4.6	2.0 M Ammonium sulfate
48.	None	0.1 M TRIS hydrochloride pH 8.5	2.0 M Ammonium phosphate monobasic
49.	1.0 M Lithium sulfate monohydrate	None	2% w/v Polyethylene glycol 8,000
50.	0.5 M Lithium sulfate monohydrate	None	15% w/v Polyethylene glycol 8,000

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

Crystal Screen contains fifty unique reagents. To determine the formulation of each reagent, simply read across the page.

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HAMPTON RESEARCH
Solutions for Crystal Growth

Sample: _____ Sample Concentration: _____

Sample Buffer: _____ Date: _____

Reservoir Volume: _____ Temperature: _____

Drop Volume: Total _____ µl Sample _____ µl Reservoir _____ 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 < 0.2 mm)
9 Single Crystals (3D Growth > 0.2 mm)

Crystal Screen™ - HR2-110 Scoring Sheet

	Date:	Date:	Date:
1. 0.02 M Calcium chloride dihydrate, 0.1 M Sodium acetate trihydrate pH 4.6, 30% v/v (+/-)-2-Methyl-2,4-pentanediol			
2. 0.4 M Potassium sodium tartrate tetrahydrate			
3. 0.4 M Ammonium phosphate monobasic			
4. 0.1 M TRIS hydrochloride pH 8.5, 2.0 M Ammonium sulfate			
5. 0.2 M Sodium citrate tribasic dihydrate, 0.1 M HEPES sodium pH 7.5, 30% v/v (+/-)-2-Methyl-2,4-pentanediol			
6. 0.2 M Magnesium chloride hexahydrate, 0.1 M TRIS hydrochloride pH 8.5, 30% w/v Polyethylene glycol 4,000			
7. 0.1 M Sodium cacodylate trihydrate pH 6.5, 1.4 M Sodium acetate trihydrate			
8. 0.2 M Sodium citrate tribasic dihydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 30% v/v 2-Propanol			
9. 0.2 M Ammonium acetate, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 30% w/v Polyethylene glycol 4,000			
10. 0.2 M Ammonium acetate, 0.1 M Sodium acetate trihydrate pH 4.6, 30% w/v Polyethylene glycol 4,000			
11. 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 1.0 M Ammonium phosphate monobasic			
12. 0.2 M Magnesium chloride hexahydrate, 0.1 M HEPES sodium pH 7.5, 30% v/v 2-Propanol			
13. 0.2 M Sodium citrate tribasic dihydrate, 0.1 M TRIS hydrochloride pH 8.5, 30% v/v Polyethylene glycol 400			
14. 0.2 M Calcium chloride dihydrate, 0.1 M HEPES sodium pH 7.5, 28% v/v Polyethylene glycol 400			
15. 0.2 M Ammonium sulfate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 30% w/v Polyethylene glycol 8,000			
16. 0.1 M HEPES sodium pH 7.5, 1.5 M Lithium sulfate monohydrate			
17. 0.2 M Lithium sulfate monohydrate, 0.1 M TRIS hydrochloride pH 8.5, 30% w/v Polyethylene glycol 4,000			
18. 0.2 M Magnesium acetate tetrahydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 20% w/v Polyethylene glycol 8,000			
19. 0.2 M Ammonium acetate, 0.1 M TRIS hydrochloride pH 8.5, 30% v/v 2-Propanol			
20. 0.2 M Ammonium sulfate, 0.1 M Sodium acetate trihydrate pH 4.6, 25% w/v Polyethylene glycol 4,000			
21. 0.2 M Magnesium acetate tetrahydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 30% v/v (+/-)-2-Methyl-2,4-pentanediol			
22. 0.2 M Sodium acetate trihydrate, 0.1 M TRIS hydrochloride pH 8.5, 30% w/v Polyethylene glycol 4,000			
23. 0.2 M Magnesium chloride hexahydrate, 0.1 M HEPES sodium pH 7.5, 30% v/v Polyethylene glycol 400			
24. 0.2 M Calcium chloride dihydrate, 0.1 M Sodium acetate trihydrate pH 4.6, 20% v/v 2-Propanol			
25. 0.1 M Imidazole pH 6.5, 1.0 M Sodium acetate trihydrate			
26. 0.2 M Ammonium acetate, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 30% v/v (+/-)-2-Methyl-2,4-pentanediol			
27. 0.2 M Sodium citrate tribasic dihydrate, 0.1 M HEPES sodium pH 7.5, 20% v/v 2-Propanol			
28. 0.2 M Sodium acetate trihydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 30% w/v Polyethylene glycol 8,000			
29. 0.1 M HEPES sodium pH 7.5, 0.8 M Potassium sodium tartrate tetrahydrate			
30. 0.2 M Ammonium sulfate, 30% w/v Polyethylene glycol 8,000			
31. 0.2 M Ammonium sulfate, 30% w/v Polyethylene glycol 4,000			
32. 2.0 M Ammonium sulfate			
33. 4.0 M Sodium formate			
34. 0.1 M Sodium acetate trihydrate pH 4.6, 2.0 M Sodium formate			
35. 0.1 M HEPES sodium pH 7.5, 0.8 M Sodium phosphate monobasic monohydrate, 0.8 M Potassium phosphate monobasic			
36. 0.1 M TRIS hydrochloride pH 8.5, 8% w/v Polyethylene glycol 8,000			
37. 0.1 M Sodium acetate trihydrate pH 4.6, 8% w/v Polyethylene glycol 4,000			
38. 0.1 M HEPES sodium pH 7.5, 1.4 M Sodium citrate tribasic dihydrate			
39. 0.1 M HEPES sodium pH 7.5, 2% v/v Polyethylene glycol 400, 2.0 M Ammonium sulfate			
40. 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 20% v/v 2-Propanol, 20% w/v Polyethylene glycol 4,000			
41. 0.1 M HEPES sodium pH 7.5, 10% v/v 2-Propanol, 20% w/v Polyethylene glycol 4,000			
42. 0.05 M Potassium phosphate monobasic, 20% w/v Polyethylene glycol 8,000			
43. 30% w/v Polyethylene glycol 1,500			
44. 0.2 M Magnesium formate dihydrate			
45. 0.2 M Zinc acetate dihydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 18% w/v Polyethylene glycol 8,000			
46. 0.2 M Calcium acetate hydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5 , 18% w/v Polyethylene glycol 8,000			
47. 0.1 M Sodium acetate trihydrate pH 4.6, 2.0 M Ammonium sulfate			
48. 0.1 M TRIS hydrochloride pH 8.5, 2.0 M Ammonium phosphate monobasic			
49. 1.0 M Lithium sulfate monohydrate, 2% w/v Polyethylene glycol 8,000			
50. 0.5 M Lithium sulfate monohydrate, 15% w/v Polyethylene glycol 8,000			

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