Crystal Screen 2™

HAMPTON RESEARCH
Solutions for Crystal Growth

User Guide **HR2-112 (pg 1)**

Crystal Screen 2™ is a complete reagent kit designed to provide a rapid screening method for the crystallization of biological macromolecules. Crystal Screen 2 is a straightforward, effective, and practical kit for determining preliminary crystallization conditions. Crystal Screen 2 is also effective in determining the solubility of a macromolecule in a wide range of precipitants and pH.

Crystal Screen 2 is a sparse matrix an extension 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).

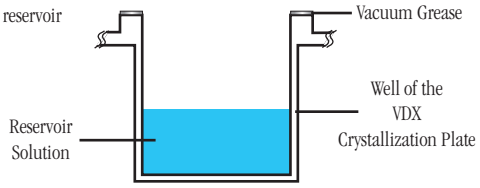
The recommended sample concentration is 5 to 25 mg/ml in water. Initially, the sample should be free of any unnecessary additives in order to observe the effect of the Crystal Screen 2 variables. Ideally, the initial screen should be performed with a sample which has been dialyzed against water although 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 2 with the Hanging Drop Vapor Diffusion method. Crystal Screen 2 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). Forty-eight reservoirs are to be prepared for a complete Crystal Screen 2. See Figure 1.

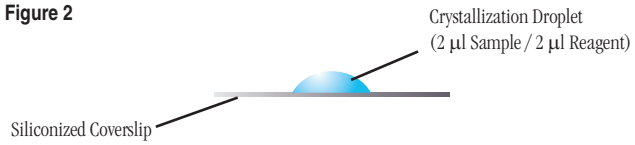
Figure 1
Cross section of a reservoir in the VDX plate.



2. Using a clean pipet tip, pipet 1 ml of Crystal Screen 2 reagent 1 into reservoir A1. Discard the pipet tip, add a new pipet tip and pipet 1 ml of Crystal Screen 2 reagent 2 into reservoir A2. Repeat the procedure for the remaining 46 Crystal Screen 2 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.

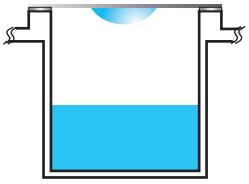
Figure 2



4. Pipet 2 µl of Crystal Screen 2 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 47 Crystal Screen 2 reagents.

7. If the quantity of sample permits, perform Crystal Screen 2 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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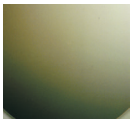
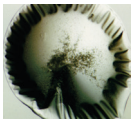
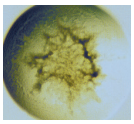
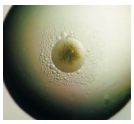
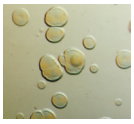
Figure 4
Typical observations in a crystallization experiment

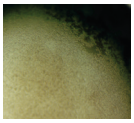

Clear Drop


Skin / Precipitate

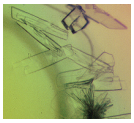

Precipitate


Precipitate / Phase

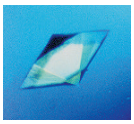

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

Interpreting Crystal Screen 2

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 2 condition and doubling the sample concentration. If more than 33 of the 48 Crystal Screen 2 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 2 condition. If more than 33 of the 48 Crystal Screen 2 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 2 Formulation

Crystal Screen 2 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 2 reagents are readily reproduced using Hampton Research Optimize™ stock solutions of salts, polymers and buffers. Optimize stock reagents make reproducing Crystal Screen 2 reagents fast, convenient and easy. Dilutions can be performed directly into the crystallization plate using Optimize stock reagents.

Crystal Screen 2 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 2 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 2 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 2 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 2 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 2 Reagents

Crystal Screen 2 reagents and optimization conditions based on Crystal Screen 2 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 2 reagent 1 in a crystallization plate.
Solution Composition: 10 % w/v Polyethylene glycol 6,000
2.0 M Sodium chloride

- 400 µl water[3]
- 200 µl 50 % w/v Polyethylene glycol 6,000 (CAS # 25322-68-3, Catalog # HR2-533)
- 400 µl 5.0 M Sodium chloride (CAS # 7647-14-5, Catalog # HR2-637)

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

Example 2. To prepare 10 milliliters of Crystal Screen 2 reagent 24.
Solution Composition: 30% v/v Jeffamine® M-600®
0.1 M MES monohydrate pH 6.5
0.05 M Cesium chloride

- 2.5 ml water[3]
- 0.5 ml 1.0 M Cesium chloride (CAS # 7647-17-8, Catalog # HR2-719)
- 1.0 ml 1.0 M MES monohydrate pH 6.5 (CAS # 145224-94-8, Catalog # HR2-787)
- 6.0 ml 50% v/v Jeffamine® M-600® pH 7.0 (CAS # 77110-54-4, Catalog # HR2-501)

Make no pH adjustments. Mix well.

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

Formulation Notes for Crystal Screen 2 Reagents

1. No additional pH adjustment is made to any reagent after formulation. Use the buffers in Table 1 to reproduce a Crystal Screen 2 reagent.
2. All Optimize solutions and screen reagents are sterile filtered using 0.22 um 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 2 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 2 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 formate	HR2-659	10.0 M	200 ml	540-69-2
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
Cadmium chloride hydrate	HR2-715	1.0 M	100 ml	654054-66-7
Cadmium sulfate hydrate	HR2-721	1.0 M	100 ml	7790-84-3
Cesium chloride	HR2-719	1.0 M	100 ml	7647-17-8
Cobalt(II) chloride hexahydrate	HR2-713	1.0 M	100 ml	7791-13-1
Hexadecyltrimethylammonium bromide	HR2-711	0.05 M	200 ml	57-09-0
Iron(III) chloride hexahydrate	HR2-717	1.0 M	100 ml	10025-77-1
Lithium sulfate monohydrate	HR2-545	2.0 M	200 ml	10377-48-7
Magnesium chloride hexahydrate	HR2-559	2.0 M	100 ml	7791-18-6
	HR2-803	5.0 M	200 ml	7791-18-6
Magnesium sulfate heptahydrate	HR2-821	3.0 M	200 ml	10034-99-8
Nickel(II) chloride hexahydrate	HR2-687	4.0 M	200 ml	7791-20-0
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 chloride	HR2-637	5.0 M	200 ml	7647-14-5
Sodium citrate tribasic dihydrate	HR2-549	1.6 M	200 ml	6132-04-3
Sodium phosphate monobasic monohydrate	HR2-551	4.0 M	200 ml	10049-21-5
Zinc sulfate heptahydrate	HR2-641	2.0 M	200 ml	7446-20-0
Polymers	**Hampton Research Catalog #**	**Supplied [Stock]**	**Supplied Volume**	**CAS #**
Ethylene imine polymer	HR2-599	50 %	200 ml	9002-98-6
Jeffamine® M-600® pH 7.0	HR2-501	50 % v/v	200 ml	77110-54-4
Polyethylene glycol 400	HR2-603	100 %	200 ml	25322-68-3
Polyethylene glycol 1,000	HR2-523	50 % w/v	200 ml	25322-68-3
Polyethylene glycol 6,000	HR2-533	50 % w/v	200 ml	25322-68-3

(Polymers continued on page 3)

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Table 1 (Continued). **Recommended reagents for the formulation of Crystal Screen 2 and Optimization reagents.**

Polymers (Continued from page 2)	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
Polyethylene glycol 8,000	HR2-535	50 % w/v	200 ml	25322-68-3
Polyethylene glycol 10,000	HR2-607	50 % w/v	200 ml	25322-68-3
Polyethylene glycol 20,000	HR2-609	30 % w/v	200 ml	25322-68-3
Polyethylene glycol monomethyl ether 550	HR2-611	100 %	200 ml	9004-74-4
Polyethylene glycol monomethyl ether 2,000	HR2-613	50 % w/v	200 ml	9004-74-4
Polyethylene glycol monomethyl ether 5,000	HR2-615	50 % w/v	200 ml	9004-74-4
Organics (volatile)	**Hampton Research Catalog #**	**Supplied [Stock]**	**Supplied Volume**	**CAS #**
1,4-Dioxane	HR2-617	100 %	200 ml	123-91-1
Ethanol (available from Aldrich)	45,983-6	100 %	—	64-17-5
2-Propanol	HR2-619	100 %	200 ml	67-63-0
tert-Butanol (available from Fluka)	19460	100 %	—	75-65-0
Organics (non-volatile)	**Hampton Research Catalog #**	**Supplied [Stock]**	**Supplied Volume**	**CAS #**
1,6-Hexanediol	HR2-625	6.0 M	200 ml	629-11-8
Ethylene glycol	HR2-621	100 %	100 ml	107-21-1
Glycerol	HR2-623	100 %	100 ml	56-81-5
(+/-)-2-Methyl-2,4-pentanediol	HR2-627	100 %	200 ml	107-41-5
Buffers	**Hampton Research Catalog #**	**Supplied [Stock]**	**Supplied Volume**	**CAS #**
BICINE pH 9.0 [2]	HR2-723	1.0 M	100 ml	150-25-4
HEPES pH 7.5 [2]	HR2-729	1.0 M	100 ml	7365-45-9
Imidazole pH 7.0 [1]	HR2-819	1.0 M	100 ml	288-32-4
MES monohydrate pH 6.5 [2]	HR2-787	1.0 M	100 ml	145224-94-8
Sodium acetate trihydrate pH 4.6 [1]	HR2-731	1.0 M	100 ml	6131-90-4
Sodium citrate tribasic dihydrate pH 5.6 [1]	HR2-735	1.0 M	100 ml	6132-04-3
Tris pH 8.5 [1]	HR2-725	1.0 M	100 ml	77-86-1

[1] pH titrated using Hydrochloric acid (HR2-581) CAS # 7647-01-0

[2] pH titrated using Sodium hydroxide (HR2-583) CAS # 1310-73-2

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

Buffer Solution or Kit	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #	pH range
BICINE untitrated	HR2-509	1.0 M	100 ml	150-25-4	7.4 - 9.3
Titrate with NaOH	HR2-583	1.0 M	100 ml	1310-73-2	—
HEPES untitrated	HR2-585	1.0 M	100 ml	7365-45-9	6.6 - 8.5
Titrate with NaOH	HR2-583	1.0 M	100 ml	1310-73-2	—
StockOptions™ Hepes kit [4]	HR2-102	1.0 M	10 ml each	7365-45-9	6.8 - 8.2
MES monohydrate untitrated	HR2-587	0.5 M	100 ml	145224-94-8	5.2 - 7.1
Titrate with NaOH	HR2-583	1.0 M	100 ml	1310-73-2	—
StockOptions™ MES monohydrate kit [4]	HR2-243	1.0 M	10 ml each	145224-94-8	5.2 - 7.1
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 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
Tris untitrated	HR2-589	1.0 M	100 ml	77-86-1	7.0 - 9.0
Titrate with HCl	HR2-581	1.0 M	100 ml	7647-01-0	—
StockOptions™ Tris kit [4]	HR2-100	1.0 M	10 ml each	77-86-1	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 2 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.	2.0 M Sodium chloride	None	10% w/v Polyethylene glycol 6,000
2.	0.5 M Sodium chloride 0.01 M Magnesium chloride hexahydrate	None	0.01 M Hexadecyltrimethylammonium bromide
3.	None	None	25% v/v Ethylene glycol
4.	None	None	35% v/v 1,4-Dioxane
5.	2.0 M Ammonium sulfate	None	5% v/v 2-Propanol
6.	None	None	1.0 M Imidazole pH 7.0
7.	None	None	10% w/v Polyethylene glycol 1,000 10% w/v Polyethylene glycol 8,000
8.	1.5 M Sodium chloride	None	10% v/v Ethanol
9.	None	0.1 M Sodium acetate trihydrate pH 4.6	2.0 M Sodium chloride
10.	0.2 M Sodium chloride	0.1 M Sodium acetate trihydrate pH 4.6	30% v/v (+/-)-2-Methyl-2,4-pentanediol
11.	0.01 M Cobalt(II) chloride hexahydrate	0.1 M Sodium acetate trihydrate pH 4.6	1.0 M 1,6-Hexanediol
12.	0.1 M Cadmium chloride hydrate	0.1 M Sodium acetate trihydrate pH 4.6	30% v/v Polyethylene glycol 400
13.	0.2 M Ammonium sulfate	0.1 M Sodium acetate trihydrate pH 4.6	30% w/v Polyethylene glycol monomethyl ether 2,000
14.	0.2 M Potassium sodium tartrate tetrahydrate	0.1 M Sodium citrate tribasic dihydrate pH 5.6	2.0 M Ammonium sulfate
15.	0.5 M Ammonium sulfate	0.1 M Sodium citrate tribasic dihydrate pH 5.6	1.0 M Lithium sulfate monohydrate
16.	0.5 M Sodium chloride	0.1 M Sodium citrate tribasic dihydrate pH 5.6	2% v/v Ethylene imine polymer
17.	None	0.1 M Sodium citrate tribasic dihydrate pH 5.6	35% v/v tert-Butanol
18.	0.01 M Iron(III) chloride hexahydrate	0.1 M Sodium citrate tribasic dihydrate pH 5.6	10% v/v Jeffamine® M-600®
19.	None	0.1 M Sodium citrate tribasic dihydrate pH 5.6	2.5 M 1,6-Hexanediol
20.	None	0.1 M MES monohydrate pH 6.5	1.6 M Magnesium sulfate heptahydrate
21.	0.1 M Sodium phosphate monobasic monohydrate 0.1 M Potassium phosphate monobasic	0.1 M MES monohydrate pH 6.5	2.0 M Sodium chloride
22.	None	0.1 M MES monohydrate pH 6.5	12% w/v Polyethylene glycol 20,000
23.	1.6 M Ammonium sulfate	0.1 M MES monohydrate pH 6.5	10% v/v 1,4-Dioxane
24.	0.05 M Cesium chloride	0.1 M MES monohydrate pH 6.5	30% v/v Jeffamine® M-600®
25.	0.01 M Cobalt(II) chloride hexahydrate	0.1 M MES monohydrate pH 6.5	1.8 M Ammonium sulfate
26.	0.2 M Ammonium sulfate	0.1 M MES monohydrate pH 6.5	30% w/v Polyethylene glycol monomethyl ether 5,000
27.	0.01 M Zinc sulfate heptahydrate	0.1 M MES monohydrate pH 6.5	25% v/v Polyethylene glycol monomethyl ether 550
28.	None	None	1.6 M Sodium citrate tribasic dihydrate pH 6.5
29.	0.5 M Ammonium sulfate	0.1 M HEPES pH 7.5	30% v/v (+/-)-2-Methyl-2,4-pentanediol
30.	None	0.1 M HEPES pH 7.5	10% w/v Polyethylene glycol 6,000 5% v/v (+/-)-2-Methyl-2,4-pentanediol
31.	None	0.1 M HEPES pH 7.5	20% v/v Jeffamine® M-600®
32.	0.1 M Sodium chloride	0.1 M HEPES pH 7.5	1.6 M Ammonium sulfate
33.	None	0.1 M HEPES pH 7.5	2.0 M Ammonium formate
34.	0.05 M Cadmium sulfate hydrate	0.1 M HEPES pH 7.5	1.0 M Sodium acetate trihydrate
35.	None	0.1 M HEPES pH 7.5	70% v/v (+/-)-2-Methyl-2,4-pentanediol
36.	None	0.1 M HEPES pH 7.5	4.3 M Sodium chloride
37.	None	0.1 M HEPES pH 7.5	10% w/v Polyethylene glycol 8,000 8% v/v Ethylene glycol
38.	None	0.1 M HEPES pH 7.5	20% w/v Polyethylene glycol 10,000
39.	0.2 M Magnesium chloride hexahydrate	0.1 M Tris pH 8.5	3.4 M 1,6-Hexanediol
40.	None	0.1 M Tris pH 8.5	25% v/v tert-Butanol
41.	0.01 M Nickel(II) chloride hexahydrate	0.1 M Tris pH 8.5	1.0 M Lithium sulfate monohydrate
42.	1.5 M Ammonium sulfate	0.1 M Tris pH 8.5	12% v/v Glycerol
43.	0.2 M Ammonium phosphate monobasic	0.1 M Tris pH 8.5	50% v/v (+/-)-2-Methyl-2,4-pentanediol
44.	None	0.1 M Tris pH 8.5	20% v/v Ethanol
45.	0.01 M Nickel(II) chloride hexahydrate	0.1 M Tris pH 8.5	20% w/v Polyethylene glycol monomethyl ether 2,000
46.	0.1 M Sodium chloride	0.1 M BICINE pH 9.0	20% v/v Polyethylene glycol monomethyl ether 550
47.	None	0.1 M BICINE pH 9.0	2.0 M Magnesium chloride hexahydrate
48.	None	0.1 M BICINE pH 9.0	2% v/v 1,4-Dioxane 10% w/v Polyethylene glycol 20,000

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

Crystal Screen 2 contains forty-eight unique reagents. To determine the formulation of each reagent, simply read across the page.

34 Journey
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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 2™ - HR2-112 Scoring Sheet

	Date:	Date:	Date:
1. 2.0 M Sodium chloride, 10% w/v Polyethylene glycol 6,000			
2. 0.5 M Sodium chloride, 0.01 M Magnesium chloride hexahydrate, 0.01 M Hexadecyltrimethylammonium bromide			
3. 25% v/v Ethylene glycol			
4. 35% v/v 1,4-Dioxane			
5. 2.0 M Ammonium sulfate, 5% v/v 2-Propanol			
6. 1.0 M Imidazole pH 7.0			
7. 10% w/v Polyethylene glycol 1,000, 10% w/v Polyethylene glycol 8,000			
8. 1.5 M Sodium chloride, 10% v/v Ethanol			
9. 0.1 M Sodium acetate trihydrate pH 4.6, 2.0 M Sodium chloride			
10. 0.2 M Sodium chloride, 0.1 M Sodium acetate trihydrate pH 4.6, 30% v/v (+/-)-2-Methyl-2,4-pentanediol			
11. 0.01 M Cobalt(II) chloride hexahydrate, 0.1 M Sodium acetate trihydrate pH 4.6, 1.0 M 1,6-Hexanediol			
12. 0.1 M Cadmium chloride hydrate, 0.1 M Sodium acetate trihydrate pH 4.6, 30% v/v Polyethylene glycol 400			
13. 0.2 M Ammonium sulfate, 0.1 M Sodium acetate trihydrate pH 4.6, 30% w/v Polyethylene glycol monomethyl ether 2,000			
14. 0.2 M Potassium sodium tartrate tetrahydrate, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 2.0 M Ammonium sulfate			
15. 0.5 M Ammonium sulfate, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 1.0 M Lithium sulfate monohydrate			
16. 0.5 M Sodium chloride, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 2% v/v Ethylene imine polymer			
17. 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 35% v/v tert-Butanol			
18. 0.01 M Iron(III) chloride hexahydrate, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 10% v/v Jeffamine® M-600®			
19. 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 2.5 M 1,6-Hexanediol			
20. 0.1 M MES monohydrate pH 6.5, 1.6 M Magnesium sulfate heptahydrate			
21. 0.1 M Sodium phosphate monobasic monohydrate, 0.1 M Potassium phosphate monobasic			
0.1 M MES monohydrate pH 6.5, 2.0 M Sodium chloride			
22. 0.1 M MES monohydrate pH 6.5, 12% w/v Polyethylene glycol 20,000			
23. 1.6 M Ammonium sulfate, 0.1 M MES monohydrate pH 6.5, 10% v/v 1,4-Dioxane			
24. 0.05 M Cesium chloride, 0.1 M MES monohydrate pH 6.5, 30% v/v Jeffamine® M-600®			
25. 0.01 M Cobalt(II) chloride hexahydrate, 0.1 M MES monohydrate pH 6.5, 1.8 M Ammonium sulfate			
26. 0.2 M Ammonium sulfate, 0.1 M MES monohydrate pH 6.5, 30% w/v Polyethylene glycol monomethyl ether 5,000			
27. 0.01 M Zinc sulfate heptahydrate, 0.1 M MES monohydrate pH 6.5, 25% v/v Polyethylene glycol monomethyl ether 550			
28. 1.6 M Sodium citrate tribasic dihydrate pH 6.5			
29. 0.5 M Ammonium sulfate, 0.1 M HEPES pH 7.5, 30% v/v (+/-)-2-Methyl-2,4-pentanediol			
30. 0.1 M HEPES pH 7.5, 10% w/v Polyethylene glycol 6,000, 5% v/v (+/-)-2-Methyl-2,4-pentanediol			
31. 0.1 M HEPES pH 7.5, 20% v/v Jeffamine® M-600®			
32. 0.1 M Sodium chloride, 0.1 M HEPES pH 7.5, 1.6 M Ammonium sulfate			
33. 0.1 M HEPES pH 7.5, 2.0 M Ammonium formate			
34. 0.05 M Cadmium sulfate hydrate, 0.1 M HEPES pH 7.5, 1.0 M Sodium acetate trihydrate			
35. 0.1 M HEPES pH 7.5, 70% v/v (+/-)-2-Methyl-2,4-pentanediol			
36. 0.1 M HEPES pH 7.5, 4.3 M Sodium chloride			
37. 0.1 M HEPES pH 7.5, 10% w/v Polyethylene glycol 8,000, 8% v/v Ethylene glycol			
38. 0.1 M HEPES pH 7.5, 20% w/v Polyethylene glycol 10,000			
39. 0.2 M Magnesium chloride hexahydrate, 0.1 M Tris pH 8.5, 3.4 M 1,6-Hexanediol			
40. 0.1 M Tris pH 8.5, 25% v/v tert-Butanol			
41. 0.01 M Nickel(II) chloride hexahydrate, 0.1 M Tris pH 8.5, 1.0 M Lithium sulfate monohydrate			
42. 1.5 M Ammonium sulfate, 0.1 M Tris pH 8.5, 12% v/v Glycerol			
43. 0.2 M Ammonium phosphate monobasic, 0.1 M Tris pH 8.5, 50% v/v (+/-)-2-Methyl-2,4-pentanediol			
44. 0.1 M Tris pH 8.5, 20% v/v Ethanol			
45. 0.01 M Nickel(II) chloride hexahydrate, 0.1 M Tris pH 8.5, 20% w/v Polyethylene glycol monomethyl ether 2,000			
46. 0.1 M Sodium chloride, 0.1 M BICINE pH 9.0, 20% v/v Polyethylene glycol monomethyl ether 550			
47. 0.1 M BICINE pH 9.0, 2.0 M Magnesium chloride hexahydrate			
48. 0.1 M BICINE pH 9.0, 2% v/v 1,4-Dioxane, 10% w/v Polyethylene glycol 20,000			

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Solutions for Crystal Growth

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