Crystal Screen Cryo™

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Solutions for Crystal Growth

Crystal Screen Cryo™ is a complete sparse matrix reagent kit designed to provide a rapid screening method for the crystallization of biological macromolecules in the presence of glycerol. Crystal Screen Cryo utilizes the original Crystal Screen protocol (3) but is optimized to include the appropriate concentration of glycerol required to form an amorphous glass at 100K. The primary screen variables are salt, pH, and precipitant (salts, polymers, volatile organics, and non-volatile organics) and cryoprotectant. The screen is a straightforward, effective, and practical kit for determining preliminary crystallization conditions and provides a good starting point for finding suitable cryoprotectant conditions for macromolecular crystals grown in a wide range of reagents. Crystal Screen Cryo is also effective in determining the solubility of a macromolecule in a wide range of precipitants and pH.

Sample Preparation

The macromolecular sample should be homogenous, as pure as practically possible (> 95%) and free of amorphous and particulate material. Remove amorphous material by centrifugation (or micro-filtration) prior to use.

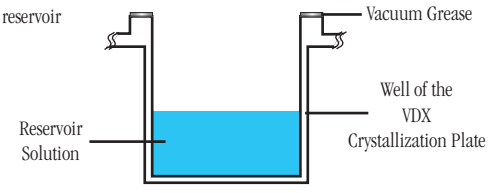
The recommended sample concentration is 5 to 25 mg/ml in water. Initially, the sample should be free of any unnecessary additives in order to observe the effect of the Crystal Screen Cryo variables. The initial screen should be performed with the sample in dilute buffer with ligands, ions, reducing agents, or other additives as required by the sample for solubility, stability, or activity.

Performing The Screen

The following procedure describes the use of Crystal Screen Cryo with the Hanging Drop Vapor Diffusion method. Crystal Screen Cryo is also compatible with the Sitting Drop, Sandwich Drop, MicroBatch, Free Interface Diffusion, 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.

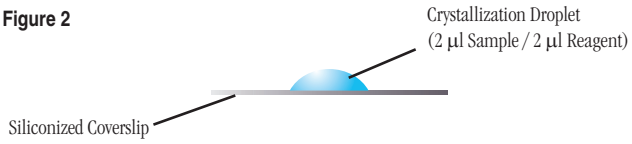
1. Prepare a VDX Plate (HR3-140) for Hanging Drop Vapor Diffusion by applying a thin bead of cover slide sealant to the upper edge of each of the 24 reservoirs. One may also use a Greased VDX Plate (HR3-170). Fifty reservoirs are to be prepared for a complete Crystal Screen Cryo. See Figure 1.

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



2. Using a clean pipet tip, pipet 1 ml of Crystal Screen Cryo reagent 1 into reservoir A1. Discard the pipet tip, add a new pipet tip and pipet 1 ml of Crystal Screen Cryo reagent 2 into reservoir A2. Repeat the procedure for the remaining 48 Crystal Screen Cryo reagents using a clean pipet tip for each reagent so as to avoid reagent contamination and carry over.

Figure 2

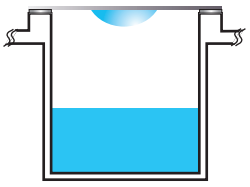


3. Pipet 2 µl of the sample to the center of a clean, siliconized 22 mm diameter circle or square cover slide. See Figure 2.

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

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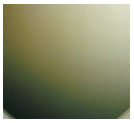
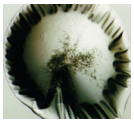
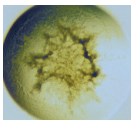
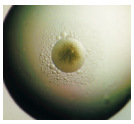
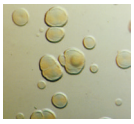
Figure 4
Typical observations in a crystallization experiment

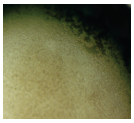

Clear Drop


Skin / Precipitate

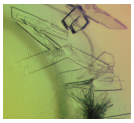

Precipitate


Precipitate / Phase

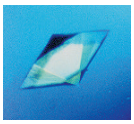

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

Interpreting Crystal Screen Cryo

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 Cryo condition and doubling the sample concentration. If more than 35 of the 50 Crystal Screen Cryo 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 Cryo condition. If more than 35 of the 50 Crystal Screen Cryo 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 Cryo Formulation

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

Crystal Screen Cryo 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 Cryo reagents are stable at room temperature and are best used before the "Best If Used By" date on the kit tubes. To enhance reagent stability it is recommended that Crystal Screen Cryo 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 Cryo 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.

Refining Cryoprotectant Concentration

The ideal cryoprotectant concentration will allow the drop to freeze as an amorphous glass to avoid diffraction from ordered ice and damage to the crystal. Crystal Screen Cryo is designed to determine both preliminary crystallization conditions and cryoprotectant concentration. If a crystal reacts poorly to the reagent (cracks) or the drop has a milky appearance upon freezing, one should try higher concentrations of cryoprotectant in the drop. Alternatively one may adjust the concentration of the screen reagent components.

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.

5. Garman, E.F. and Mitchell, E.P., J. Appl. Cryst. (1996) 29, 584-587.

Technical Support

Inquiries regarding Crystal Screen Cryo 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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Crystal Screen Cryo Fundamentals HR2-122

How to Reproduce Crystal Screen Cryo Reagents

Crystal Screen Cryo reagents and optimization conditions based on Crystal Screen Cryo 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 Cryo reagent 2 in a crystallization plate.
Solution Composition: 0.26 M Potassium sodium tartrate tetrahydrate
35% v/v Glycerol

- 477 µl water[3]
- 173 µl 1.5 M Potassium sodium tartrate tetrahydrate (CAS # 6381-59-5, Catalog # HR2-539)
- 350 µl 100% Glycerol (CAS # 56-81-5, Catalog # HR2-623)

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

Example 2. To prepare 1.0 milliliter of Crystal Screen Cryo reagent 29.
Solution Composition: 0.065 M HEPES sodium pH 7.5
0.52 M Potassium sodium tartrate tetrahydrate
35% v/v Glycerol

- 238 µl water[3]
- 65 µl 1.0 M HEPES sodium pH 7.5 (CAS # 75277-39-3, Catalog # HR2-733)
- 345 µl 1.5 M Potassium sodium tartrate tetrahydrate (CAS # 6381-59-5, Catalog # HR2-539)
- 350 µl 100% Glycerol (CAS # 56-81-5, Catalog # HR2-623)

Make no pH adjustments. Mix well.

Example 3. To prepare 10 milliliters of Crystal Screen Cryo reagent 30.
Solution Composition: 0.17 M Ammonium sulfate
25.5% w/v Polyethylene glycol 4,000
15 % v/v Glycerol

- 2,914 µl water[3]
- 486 µl 3.5 M Ammonium sulfate (CAS # 7783-20-2, Catalog # HR2-541)
- 1,500 µl 100% Glycerol (CAS # 56-81-5, Catalog # HR2-623)
- 5,100 µl 50% w/v Polyethylene glycol 4,000 (CAS # 25322-68-3, Catalog # HR2-529)

Make no pH adjustments. Mix well.

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

Formulation Notes for Crystal Screen Cryo Reagents

1. No additional pH adjustment is made to any reagent after formulation. Use the buffers in Table 1 to reproduce a Crystal Screen Cryo 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 Cryo 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.

Crystal Screen Cryo™

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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.

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

Each of these reagents are available as an Optimize™ crystallization grade reagent from Hampton Research. Table 1 below 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

(Continued on page 3)

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

Organics (volatile)	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
2-Propanol	HR2-619	100 %	200 ml	67-63-0
Organics (non-volatile)	**Hampton Research Catalog #**	**Supplied [Stock]**	**Supplied Volume**	**CAS #**
(+/-)-2-Methyl-2,4-pentanediol	HR2-627	100 %	200 ml	107-41-5
Glycerol	HR2-623	100 %	200 ml	56-81-5
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 Cryo 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

(Continued on page 4)

Crystal Screen Cryo™

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

Buffer Solution or Kit	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #	pH range
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 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 Cryo 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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Crystal Screen Cryo™

HR2-122 Reagent Formulation

Tube #	Salt	Tube #	Buffer ◊	Tube #	Precipitant	Tube #	Glycerol
1.	0.02 M Calcium chloride dihydrate	1.	0.1 M Sodium acetate trihydrate pH 4.6	1.	30% v/v (+/-)-2-Methyl-2,4-pentanediol	1.	None
2.	None	2.	None	2.	0.26 M Potassium sodium tartrate tetrahydrate	2.	35% v/v
3.	None	3.	None	3.	0.26 M Ammonium phosphate monobasic	3.	35% v/v
4.	None	4.	0.075 M TRIS hydrochloride pH 8.5	4.	1.5 M Ammonium sulfate	4.	25% v/v
5.	0.2 M Sodium citrate tribasic dihydrate	5.	0.1 M HEPES sodium pH 7.5	5.	30% v/v (+/-)-2-Methyl-2,4-pentanediol	5.	None
6.	0.16 M Magnesium chloride hexahydrate	6.	0.08 M TRIS hydrochloride pH 8.5	6.	24% w/v Polyethylene glycol 4,000	6.	20% v/v
7.	None	7.	0.07 M Sodium cacodylate trihydrate pH 6.5	7.	0.98 M Sodium acetate trihydrate	7.	30% v/v
8.	0.14 M Sodium citrate tribasic dihydrate	8.	0.07 M Sodium cacodylate trihydrate pH 6.5	8.	21% v/v 2-Propanol	8.	30% v/v
9.	0.17 M Ammonium acetate	9.	0.085 M Sodium citrate tribasic dihydrate pH 5.6	9.	25.5% w/v Polyethylene glycol 4,000	9.	15% v/v
10.	0.17 M Ammonium acetate	10.	0.085 M Sodium acetate trihydrate pH 4.6	10.	25.5% w/v Polyethylene glycol 4,000	10.	15% v/v
11.	None	11.	0.07 M Sodium citrate tribasic dihydrate pH 5.6	11.	0.7 M Ammonium phosphate monobasic	11.	30% v/v
12.	0.18 M Magnesium chloride hexahydrate	12.	0.09 M HEPES sodium pH 7.5	12.	27% v/v 2-Propanol	12.	10% v/v
13.	0.2 M Sodium citrate tribasic dihydrate	13.	0.1 M TRIS hydrochloride pH 8.5	13.	30% v/v Polyethylene glycol 400	13.	None
14.	0.19 M Calcium chloride dihydrate	14.	0.095 M HEPES sodium pH 7.5	14.	26.6% v/v Polyethylene glycol 400	14.	5% v/v
15.	0.17 M Ammonium sulfate	15.	0.085 M Sodium cacodylate trihydrate pH 6.5	15.	25.5% w/v Polyethylene glycol 8,000	15.	15% v/v
16.	None	16.	0.075 M HEPES sodium pH 7.5	16.	1.125 M Lithium sulfate monohydrate	16.	25% v/v
17.	0.17 M Lithium sulfate monohydrate	17.	0.085 M TRIS hydrochloride pH 8.5	17.	25.5% w/v Polyethylene glycol 4,000	17.	15% v/v
18.	0.16 M Magnesium acetate tetrahydrate	18.	0.08 M Sodium cacodylate trihydrate pH 6.5	18.	16% w/v Polyethylene glycol 8,000	18.	20% v/v
19.	0.16 M Ammonium acetate	19.	0.08 M TRIS hydrochloride pH 8.5	19.	24% v/v 2-Propanol	19.	20% v/v
20.	0.16 M Ammonium sulfate	20.	0.08 M Sodium acetate trihydrate pH 4.6	20.	20% w/v Polyethylene glycol 4,000	20.	20% v/v
21.	0.2 M Magnesium acetate tetrahydrate	21.	0.1 M Sodium cacodylate trihydrate pH 6.5	21.	30% v/v (+/-)-2-Methyl-2,4-pentanediol	21.	None
22.	0.17 M Sodium acetate trihydrate	22.	0.085 M TRIS hydrochloride pH 8.5	22.	25.5% w/v Polyethylene glycol 4,000	22.	15% v/v
23.	0.2 M Magnesium chloride hexahydrate	23.	0.1 M HEPES sodium pH 7.5	23.	30% v/v Polyethylene glycol 400	23.	None
24.	0.14 M Calcium chloride dihydrate	24.	0.07 M Sodium acetate trihydrate pH 4.6	24.	14% v/v 2-Propanol	24.	30% v/v
25.	None	25.	0.07 M Imidazole pH 6.5	25.	0.7 M Sodium acetate trihydrate	25.	30% v/v
26.	0.2 M Ammonium acetate	26.	0.1 M Sodium citrate tribasic dihydrate pH 5.6	26.	30% v/v (+/-)-2-Methyl-2,4-pentanediol	26.	None
27.	0.14 M Sodium citrate tribasic dihydrate	27.	0.07 M HEPES sodium pH 7.5	27.	14% v/v 2-Propanol	27.	30% v/v
28.	0.17 M Sodium acetate trihydrate	28.	0.085 M Sodium cacodylate trihydrate pH 6.5	28.	25.5% w/v Polyethylene glycol 8,000	28.	15% v/v
29.	None	29.	0.065 M HEPES sodium pH 7.5	29.	0.52 M Potassium sodium tartrate tetrahydrate	29.	35% v/v
30.	0.17 M Ammonium sulfate	30.	None	30.	25.5% w/v Polyethylene glycol 8,000	30.	15% v/v
31.	0.17 M Ammonium sulfate	31.	None	31.	25.5% w/v Polyethylene glycol 4,000	31.	15% v/v
32.	None	32.	None	32.	1.5 M Ammonium sulfate	32.	25% v/v
33.	None	33.	None	33.	3.6 M Sodium formate	33.	10% v/v
34.	None	34.	0.07 M Sodium acetate trihydrate pH 4.6	34.	1.4 M Sodium formate	34.	30% v/v
35.	None	35.	0.075 M HEPES sodium pH 7.5	35.	0.6 M Sodium phosphate monobasic monohydrate 0.6 M Potassium phosphate monobasic	35.	25% v/v
36.	None	36.	0.065 M TRIS hydrochloride pH 8.5	36.	5.2% w/v Polyethylene glycol 8,000	36.	35% v/v
37.	None	37.	0.07 M Sodium acetate trihydrate pH 4.6	37.	5.6% w/v Polyethylene glycol 4,000	37.	30% v/v
38.	None	38.	0.09 M HEPES sodium pH 7.5	38.	1.26 M Sodium citrate tribasic dihydrate	38.	10% v/v
39.	None	39.	0.085 M HEPES sodium pH 7.5	39.	1.7% v/v Polyethylene glycol 400 1.7 M Ammonium sulfate	39.	15% v/v
40.	None	40.	0.095 M Sodium citrate tribasic dihydrate pH 5.6	40.	19% v/v 2-Propanol 19% w/v Polyethylene glycol 4,000	40.	5% v/v
41.	None	41.	0.085 M HEPES sodium pH 7.5	41.	8.5% v/v 2-Propanol 17% w/v Polyethylene glycol 4,000	41.	15% v/v
42.	0.04 M Potassium phosphate monobasic	42.	None	42.	16% w/v Polyethylene glycol 8,000	42.	20% v/v
43.	None	43.	None	43.	24% w/v Polyethylene glycol 1,500	43.	20% v/v
44.	None	44.	None	44.	0.1 M Magnesium formate dihydrate	44.	50% v/v
45.	0.16 M Zinc acetate dihydrate	45.	0.08 M Sodium cacodylate trihydrate pH 6.5	45.	14.4% w/v Polyethylene glycol 8,000	45.	20% v/v
46.	0.16 M Calcium acetate hydrate	46.	0.08 M Sodium cacodylate trihydrate pH 6.5	46.	14.4% w/v Polyethylene glycol 8,000	46.	20% v/v
47.	None	47.	0.08 M Sodium acetate trihydrate pH 4.6	47.	1.6 M Ammonium sulfate	47.	20% v/v
48.	None	48.	0.08 M TRIS hydrochloride pH 8.5	48.	1.6 M Ammonium phosphate monobasic	48.	20% v/v
49.	0.8 M Lithium sulfate monohydrate	49.	None	49.	1.6% w/v Polyethylene glycol 8,000	49.	20% v/v
50.	0.4 M Lithium sulfate monohydrate	50.	None	50.	12% w/v Polyethylene glycol 8,000	50.	20% v/v

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

Crystal Screen Cryo contains fifty 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 Cryo™ - HR2-122 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. 0.26 M Potassium sodium tartrate tetrahydrate, 35% v/v Glycerol			
3. 0.26 M Ammonium phosphate monobasic, 35% v/v Glycerol			
4. 0.075 M TRIS hydrochloride pH 8.5, 1.5 M Ammonium sulfate, 25% v/v Glycerol			
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. 0.16 M Magnesium chloride hexahydrate, 0.08 M TRIS hydrochloride pH 8.5, 24% w/v Polyethylene glycol 4,000, 20% v/v Glycerol			
7. 0.07 M Sodium cacodylate trihydrate pH 6.5, 0.98 M Sodium acetate trihydrate, 30% v/v Glycerol			
8. 0.14 M Sodium citrate tribasic dihydrate, 0.07 M Sodium cacodylate trihydrate pH 6.5, 21% v/v 2-Propanol, 30% v/v Glycerol			
9. 0.17 M Ammonium acetate, 0.085 M Sodium citrate tribasic dihydrate pH 5.6, 25.5% w/v Polyethylene glycol 4,000, 15% v/v Glycerol			
10. 0.17 M Ammonium acetate, 0.085 M Sodium acetate trihydrate pH 4.6, 25.5% w/v Polyethylene glycol 4,000, 15% v/v Glycerol			
11. 0.07 M Sodium citrate tribasic dihydrate pH 5.6, 0.7 M Ammonium phosphate monobasic, 30% v/v Glycerol			
12. 0.18 M Magnesium chloride hexahydrate, 0.09 M HEPES sodium pH 7.5, 27% v/v 2-Propanol, 10% v/v Glycerol			
13. 0.2 M Sodium citrate tribasic dihydrate, 0.1 M TRIS hydrochloride pH 8.5, 30% v/v Polyethylene glycol 400			
14. 0.19 M Calcium chloride dihydrate, 0.095 M HEPES sodium pH 7.5, 26.6% v/v Polyethylene glycol 400, 5% v/v Glycerol			
15. 0.17 M Ammonium sulfate, 0.085 M Sodium cacodylate trihydrate pH 6.5, 25.5% w/v Polyethylene glycol 8,000, 15% v/v Glycerol			
16. 0.075 M HEPES sodium pH 7.5, 1.125 M Lithium sulfate monohydrate, 25% v/v Glycerol			
17. 0.17 M Lithium sulfate monohydrate, 0.085 M TRIS hydrochloride pH 8.5, 25.5% w/v Polyethylene glycol 4,000, 15% v/v Glycerol			
18. 0.16 M Magnesium acetate tetrahydrate, 0.08 M Sodium cacodylate trihydrate pH 6.5, 16% w/v Polyethylene glycol 8,000, 20% v/v Glycerol			
19. 0.16 M Ammonium acetate, 0.08 M TRIS hydrochloride pH 8.5, 24% v/v 2-Propanol, 20% v/v Glycerol			
20. 0.16 M Ammonium sulfate, 0.08 M Sodium acetate trihydrate pH 4.6, 20% w/v Polyethylene glycol 4,000, 20% v/v Glycerol			
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. 0.17 M Sodium acetate trihydrate, 0.085 M TRIS hydrochloride pH 8.5, 25.5% w/v Polyethylene glycol 4,000, 15% v/v Glycerol			
23. 0.2 M Magnesium chloride hexahydrate, 0.1 M HEPES sodium pH 7.5, 30% v/v Polyethylene glycol 400			
24. 0.14 M Calcium chloride dihydrate, 0.07 M Sodium acetate trihydrate pH 4.6, 14% v/v 2-Propanol, 30% v/v Glycerol			
25. 0.07 M Imidazole pH 6.5, 0.7 M Sodium acetate trihydrate, 30% v/v Glycerol			
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. 0.14 M Sodium citrate tribasic dihydrate, 0.07 M HEPES sodium pH 7.5, 14% v/v 2-Propanol, 30% v/v Glycerol			
28. 0.17 M Sodium acetate trihydrate, 0.085 M Sodium cacodylate trihydrate pH 6.5, 25.5% w/v Polyethylene glycol 8,000, 15% v/v Glycerol			
29. 0.065 M HEPES sodium pH 7.5, 0.52 M Potassium sodium tartrate tetrahydrate, 35% v/v Glycerol			
30. 0.17 M Ammonium sulfate, 25.5% w/v Polyethylene glycol 8,000, 15% v/v Glycerol			
31. 0.17 M Ammonium sulfate, 25.5% w/v Polyethylene glycol 4,000, 15% v/v Glycerol			
32. 1.5 M Ammonium sulfate, 25% v/v Glycerol			
33. 3.6 M Sodium formate, 10% v/v Glycerol			
34. 0.07 M Sodium acetate trihydrate pH 4.6, 1.4 M Sodium formate, 30% v/v Glycerol			
35. 0.075 M HEPES sodium pH 7.5, 0.6 M Sodium phosphate monobasic monohydrate, 0.6 M Potassium phosphate monobasic, 25% v/v Glycerol			
36. 0.065 M TRIS hydrochloride pH 8.5, 5.2% w/v Polyethylene glycol 8,000, 35% v/v Glycerol			
37. 0.07 M Sodium acetate trihydrate pH 4.6, 5.6% w/v Polyethylene glycol 4,000, 30% v/v Glycerol			
38. 0.09 M HEPES sodium pH 7.5, 1.26 M Sodium citrate tribasic dihydrate, 10% v/v Glycerol			
39. 0.085 M HEPES sodium pH 7.5, 1.7% v/v Polyethylene glycol 400, 1.7 M Ammonium sulfate, 15% v/v Glycerol			
40. 0.095 M Sodium citrate tribasic dihydrate pH 5.6, 19% v/v 2-Propanol, 19% w/v Polyethylene glycol 4,000, 5% v/v Glycerol			
41. 0.085 M HEPES sodium pH 7.5, 8.5% v/v 2-Propanol, 17% w/v Polyethylene glycol 4,000, 15% v/v Glycerol			
42. 0.04 M Potassium phosphate monobasic, 16% w/v Polyethylene glycol 8,000, 20% v/v Glycerol			
43. 24% w/v Polyethylene glycol 1,500, 20% v/v Glycerol			
44. 0.1 M Magnesium formate dihydrate, 50% v/v Glycerol			
45. 0.16 M Zinc acetate dihydrate, 0.08 M Sodium cacodylate trihydrate pH 6.5, 14.4% w/v Polyethylene glycol 8,000, 20% v/v Glycerol			
46. 0.16 M Calcium acetate hydrate, 0.08 M Sodium cacodylate trihydrate pH 6.5 , 14.4% w/v Polyethylene glycol 8,000, 20% v/v Glycerol			
47. 0.08 M Sodium acetate trihydrate pH 4.6, 1.6 M Ammonium sulfate, 20% v/v Glycerol			
48. 0.08 M TRIS hydrochloride pH 8.5, 1.6 M Ammonium phosphate monobasic, 20% v/v Glycerol			
49. 0.8 M Lithium sulfate monohydrate, 1.6% w/v Polyethylene glycol 8,000, 20% v/v Glycerol			
50. 0.4 M Lithium sulfate monohydrate, 12% w/v Polyethylene glycol 8,000, 20% v/v Glycerol			

HAMPTON RESEARCH
Solutions for Crystal Growth

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