

Crystal Screen 2 Cryo™ is a biased sparse matrix reagent kit designed to provide a rapid screening method for the crystallization of biological macromolecules in the presence of a cryoprotectant. 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 2 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 2 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.

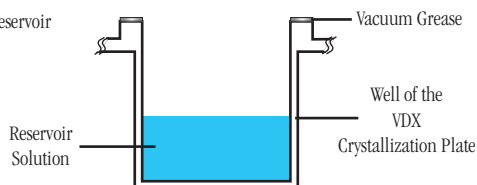
Performing The Screen

The following procedure describes the use of Crystal Screen 2 Cryo with the Hanging Drop Vapor Diffusion method. Crystal Screen 2 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). Forty eight reservoirs are to be prepared for a complete Crystal Screen 2 Cryo. See Figure 1.

Figure 1

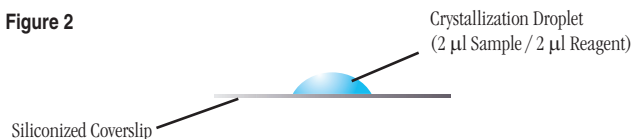
Cross section of a reservoir in the VDX plate.



2. Using a clean pipet tip, pipet 1 ml of Crystal Screen 2 Cryo reagent 1 into reservoir A1. Discard the pipet tip, add a new pipet tip and pipet 1 ml of Cryst-

tal Screen 2 Cryo reagent 2 into reservoir A2. Repeat the procedure for the remaining 46 Crystal Screen 2 Cryo reagents using a clean pipet tip for each reagent so as to avoid reagent contamination and carry over.

Figure 2



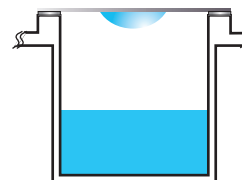
3. Pipet 2 µl of the sample to the center of a clean, siliconized 22 mm diameter circle or square cover slide. See Figure 2.

4. Pipet 2 µl of Crystal Screen 2 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.



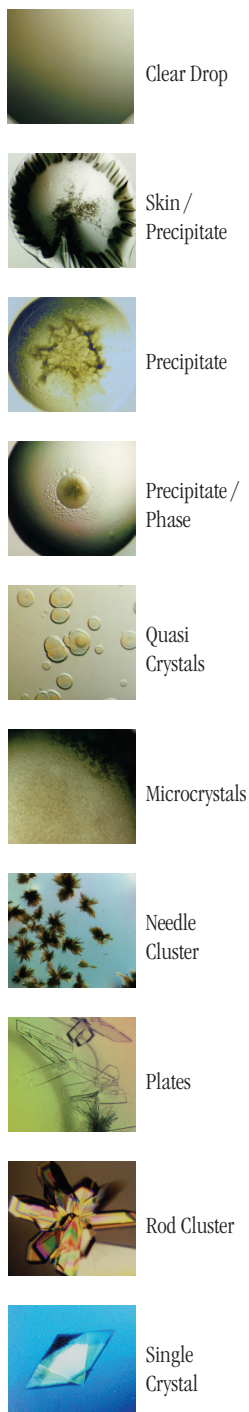
6. Repeat operations 3 through 5 for the remaining 47 Crystal Screen 2 Cryo reagents.

7. If the quantity of sample permits, perform Crystal Screen 2 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 thereafter. 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.

Figure 4
Typical observations in a crystallization experiment



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

Crystal Screen 2 Cryo reagents are formulated using the high-

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

Crystal Screen 2 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 2 Cryo reagents are stable at room temperature and are best if used within 12 months of receipt. To enhance reagent stability it is recommended that Crystal Screen 2 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 2 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 2 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.

Crystal Screen 2 Cryo™



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 2 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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Tube #	Salt	Tube #	Buffer ◇	Tube #	Precipitant	Tube #	Glycerol
1.	1.6 M Sodium chloride	1.		1.	8% w/v Polyethylene glycol 6,000	1.	20% v/v
2.	0.3 M Sodium chloride, 0.006 M Magnesium chloride hexahydrate	2.		2.	0.006 M Hexadecyltrimethylammonium bromide	2.	40% v/v
3.		3.		3.	21.25% v/v Ethylene glycol	3.	15% v/v
4.		4.		4.	26.25% v/v 1,4-Dioxane	4.	25% v/v
5.	1.5 M Ammonium sulfate	5.		5.	3.75% v/v 2-Propanol	5.	25% v/v
6.		6.		6.	0.65 M Imidazole pH 7.0	6.	35% v/v
7.		7.		7.	8% w/v Polyethylene glycol 1,000, 8% w/v Polyethylene glycol 8,000	7.	20% v/v
8.	1.05 M Sodium chloride	8.		8.	7% v/v Ethanol	8.	30% v/v
9.		9.	0.075 M Sodium acetate trihydrate pH 4.6	9.	1.5 M Sodium chloride	9.	25% v/v
10.	0.2 M Sodium chloride	10.	0.1 M Sodium acetate trihydrate pH 4.6	10.	30% v/v (+/-)-2-Methyl-2,4-pentanediol	10.	None
11.	0.008 M Cobalt(II) chloride hexahydrate	11.	0.08 M Sodium acetate trihydrate pH 4.6	11.	0.8 M 1,6-Hexanediol	11.	20% v/v
12.	0.095 M Cadmium chloride hydrate	12.	0.095 M Sodium acetate trihydrate pH 4.6	12.	28.5% v/v Polyethylene glycol 400	12.	5% v/v
13.	0.18 M Ammonium sulfate	13.	0.09 M Sodium acetate trihydrate pH 4.6	13.	27% w/v Polyethylene glycol monomethyl ether 2,000	13.	10% v/v
14.	0.15 M Potassium sodium tartrate tetrahydrate	14.	0.075 M Sodium citrate tribasic dihydrate pH 5.6	14.	1.5 M Ammonium sulfate	14.	25% v/v
15.	0.375 M Ammonium sulfate	15.	0.075 M Sodium citrate tribasic dihydrate pH 5.6	15.	0.75 M Lithium sulfate monohydrate	15.	25% v/v
16.	0.3 M Sodium chloride	16.	0.06 M Sodium citrate tribasic dihydrate pH 5.6	16.	1.2% v/v Ethylene imine polymer	16.	40% v/v
17.		17.	0.08 M Sodium citrate tribasic dihydrate pH 5.6	17.	28% v/v tert-Butanol	17.	20% v/v
18.	0.007 M Iron(III) chloride hexahydrate	18.	0.07 M Sodium citrate tribasic dihydrate pH 5.6	18.	7% v/v Jeffamine® M-600®	18.	30% v/v
19.		19.	0.095 M Sodium citrate tribasic dihydrate pH 5.6	19.	2.375 M 1,6-Hexanediol	19.	5% v/v
20.		20.	0.08 M MES monohydrate pH 6.5	20.	1.28 M Magnesium sulfate heptahydrate	20.	20% v/v
21.	0.075 M Sodium phosphate monobasic monohydrate, 0.075 M Potassium phosphate monobasic	21.	0.075 M MES monohydrate pH 6.5	21.	1.5 M Sodium chloride	21.	25% v/v
22.		22.	0.065 M MES monohydrate pH 6.5	22.	7.8% w/v Polyethylene glycol 20,000	22.	35% v/v
23.	1.2 M Ammonium sulfate	23.	0.075 M MES monohydrate pH 6.5	23.	7.5% v/v 1,4-Dioxane	23.	25% v/v
24.	0.05 M Cesium chloride	24.	0.1 M MES monohydrate pH 6.5	24.	30% v/v Jeffamine® M-600®	24.	None
25.	0.0075 M Cobalt(II) chloride hexahydrate	25.	0.075 M MES monohydrate pH 6.5	25.	1.35 M Ammonium sulfate	25.	25% v/v
26.	0.18 M Ammonium sulfate	26.	0.09 M MES monohydrate pH 6.5	26.	27% w/v Polyethylene glycol monomethyl ether 5,000	26.	10% v/v
27.	0.009 M Zinc sulfate heptahydrate	27.	0.09 M MES monohydrate pH 6.5	27.	22.5% v/v Polyethylene glycol monomethyl ether 550	27.	10% v/v
28.		28.		28.	1.6 M Sodium citrate tribasic dihydrate pH 6.5	28.	None
29.	0.5 M Ammonium sulfate	29.	0.1 M HEPES pH 7.5	29.	30% v/v (+/-)-2-Methyl-2,4-pentanediol	29.	None
30.		30.	0.08 M HEPES pH 7.5	30.	8% w/v Polyethylene glycol 6,000, 4% v/v (+/-)-2-Methyl-2,4-pentanediol	30.	20% v/v
31.		31.	0.085 M HEPES pH 7.5	31.	17% v/v Jeffamine® M-600®	31.	15% v/v
32.	0.075 M Sodium chloride	32.	0.075 M HEPES pH 7.5	32.	1.2 M Ammonium sulfate	32.	25% v/v
33.		33.	0.07 M HEPES pH 7.5	33.	1.4 M Ammonium formate	33.	30% v/v
34.	0.0375 M Cadmium sulfate hydrate	34.	0.075 M HEPES pH 7.5	34.	0.75 M Sodium acetate trihydrate	34.	25% v/v
35.		35.	0.1 M HEPES pH 7.5	35.	70% v/v (+/-)-2-Methyl-2,4-pentanediol	35.	None
36.		36.	0.085 M HEPES pH 7.5	36.	3.655 M Sodium chloride	36.	15% v/v
37.		37.	0.075 M HEPES pH 7.5	37.	7.5% w/v Polyethylene glycol 8,000, 6% v/v Ethylene glycol	37.	25% v/v
38.		38.	0.075 M HEPES pH 7.5	38.	15% w/v Polyethylene glycol 10,000	38.	25% v/v
39.	0.2 M Magnesium chloride hexahydrate	39.	0.1 M Tris pH 8.5	39.	3.4 M 1,6-Hexanediol	39.	None
40.		40.	0.075 M Tris pH 8.5	40.	18.75% v/v tert-Butanol	40.	25% v/v
41.	0.0075 M Nickel(II) chloride hexahydrate	41.	0.075 M Tris pH 8.5	41.	0.75 M Lithium sulfate monohydrate	41.	25% v/v
42.	1.275 M Ammonium sulfate	42.	0.085 M Tris pH 8.5	42.		42.	25.2% v/v
43.	0.2 M Ammonium phosphate monobasic	43.	0.1 M Tris pH 8.5	43.	50% v/v (+/-)-2-Methyl-2,4-pentanediol	43.	None
44.		44.	0.075 M Tris pH 8.5	44.	15% v/v Ethanol	44.	25% v/v
45.	0.008 M Nickel(II) chloride hexahydrate	45.	0.08 M Tris pH 8.5	45.	16% w/v Polyethylene glycol monomethyl ether 2,000	45.	20% v/v
46.	0.085 M Sodium chloride	46.	0.085 M BICINE pH 9.0	46.	17% v/v Polyethylene glycol monomethyl ether 550	46.	15% v/v
47.		47.	0.095 M BICINE pH 9.0	47.	1.9 M Magnesium chloride hexahydrate	47.	5% v/v
48.		48.	0.07 M BICINE pH 9.0	48.	1.4% v/v 1,4-Dioxane, 7% w/v Polyethylene glycol 20,000	48.	30% 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 2 Cryo contains forty-eight unique reagents. To determine the formulation of each reagent, simply read across the page.

