# Crystal Screen Cryo™



## User Guide

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 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).

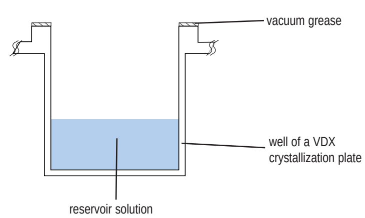
The recommended sample concentration is 5 to 25 mg/ml in water. Initially, the sample should be free of any unnecessary additives in order to observe the effect of the Crystal Screen Cryo variables. Ideally, the initial screen should be performed with a sample which has been dialyzed against water although 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 Cryo with the Hanging Drop Vapor Diffusion method. Crystal Screen Cryo 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.

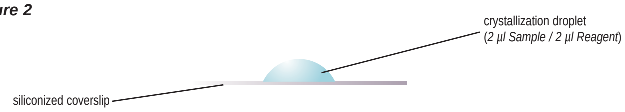
***figure 1***
Cross section of a reservoir in the VDX plate.




2. Using a clean pipet tip, pipet 1 ml of Crystal Screen 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.

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.

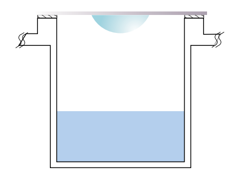
***figure 2***




4. Pipet 2 µl of Crystal Screen 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 reservior.



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.

### 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

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*Solutions for Crystal Growth*



*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

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 strongly 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 glycerol in the drop. Alternatively one may adjust the concentration of the screen reagent components. Finally one may substitute and evaluate other cryoprotectants such as MPD, PEG 400, ethylene glycol, xylitol, erythritol, glucose, or 2R,3R-(-)-Butane-2,3-diol.

**References and Readings**
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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 5:00 p.m. USA Pacific Standard Time.

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