Crystal Screen Cryo HT™

HAMPTON RESEARCH
Solutions for Crystal Growth

User Guide **HR2-133 (pg 1)**

Applications

Crystallization screen for proteins, peptides, nucleic acids and water soluble small molecules in the presence of a cryoprotectant.

Features

Crystal Screen Cryo HT™ is designed to provide a biased sparse matrix of trial containing conditions developed by Hampton Research as well as published crystallization conditions - optimized for cryo cooling. The reagent parameter variables are:

- pH
- Buffer material
- Salt
- Precipitant
- Cryoprotectant

Seven different pH's 4.5, 4.6, 5.6, 6.5, 7.5, 8.5, and 9.0 are utilized with nine buffers:

- BICINE
- HEPES
- HEPES sodium
- MES monohydrate
- Sodium acetate trihydrate
- Sodium cacodylate trihydrate
- Sodium citrate tribasic dihydrate
- Tris
- TRIS hydrochloride

The four categories of precipitating agents utilized are:

- Volatile organics
- Non-volatile organics
- Polymers
- Salts

Refer to the enclosed Crystal Screen Cryo HT reagent formulation for additional information.

General Description

Crystal Screen Cryo HT is supplied in a sterile, polypropylene 96 Deep Well block, each reservoir containing 1 ml of sterile filtered reagent. The block is heat sealed using a special polypropylene backed film.

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 microfiltration prior to use.

The recommended sample concentration is 5 to 25 mg/ml in sterile filtered, dilute (25 mM or less) buffer. For initial screens, the sample should be free of unnecessary additives in order to observe the effect of the Crystal Screen Cryo HT variables. However, agents that promote and preserve sample stability and homogeneity can and should be included in the sample. For additional sample preparation recommendation see Crystal Growth 101 - Preliminary Sample Preparation bulletin from Hampton Research.

Preparing the Deep Well Block for Use

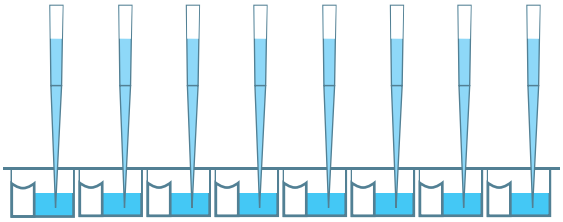
Allow the block to equilibrate to room temperature. To remove stray reagent from the sealing film, centrifuge the block at 500 rpm for 5 minutes. To remove film, grasp a corner of the film and gently peel film from the block. Alternatively, the film can be pierced to access reagents.

Performing the Screen

Manual Method - Sitting Drop Vapor Diffusion

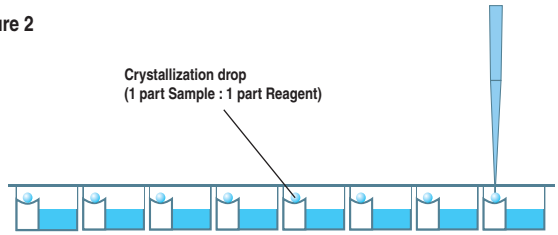
1. Using a 96 well sitting drop vapor diffusion plate, pipet the recommended volume (typically 50 to 100 microliters) of crystallization reagent from the Deep Well block into the reservoirs of the crystallization plate. The Deep Well block is compatible with 8 and 12 channel pipets as well as many automated liquid handling systems. Use clean pipet tips for each reagent set transfer and change pipet tips when changing reagents. For an 8 channel pipet, transfer reagents A1-H1 to reservoirs A1-H1 of the crystallization plate. Repeat this procedure for reagent columns B through H. Change pipet tips when moving between reagent columns. See Figure 1. Time and pipet tips can be conserved by batch pipetting multiple plates with the same (row or column) of reagent before changing reagent and pipet tips.

Figure 1



2. Using clean pipet tips, pipet 0.05 to 2 microliters of crystallization reagent from the crystallization plate reservoir to the sitting drop well. Change the pipet tip between reagents. See Figure 2.

Figure 2

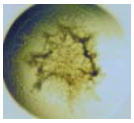


3. Using a clean pipet tip, pipet 0.05 to 2 microliters of sample to the reagent drop in the sitting drop well. One may choose to simply dispense the sample with no mixing or dispense with mixing by gently aspirating and dispensing the sample several times, keeping the tip in the drop during mixing to avoid foaming. Work carefully but quickly to minimize evaporation from the crystallization plate. See Figure 2 above.

Figure 3
Typical observations in a crystallization experiment


Clear Drop


Skin / Precipitate


Precipitate


Precipitate / Phase

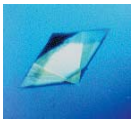

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

4. Seal the crystallization plate as per the manufacturer's recommendation. View and score the experiment as desired.

5. Seal the remaining reagent in the Deep Well block using sealing film.

Crystal Screen Cryo HT Deep Well Block and Automated Liquid Handling Systems

The polypropylene Deep Well block is designed to be compatible with the SBS standard 96 microwell format and is therefore compatible with numerous automated liquid handling systems that accept 8 x 12 96 well assay blocks. Follow the manufacturer's recommendation for handling deep well microplates.

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 3, on the left side of page 2 shows typical examples of what one might observe in a crystallization experiment.

Interpreting Crystal Screen Cryo HT

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 screen condition and doubling the sample concentration. If more than 70 of the 96 screen drops are clear consider doubling the sample concentration and repeating the entire screen.

Drops containing precipitate indicate either the relative super saturation 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 screen condition. If more than 70 of the 96 screen drops contain precipitate and no crystals are present, consider diluting the sample concentration in half and repeating the entire screen. If sample denaturation is suspect, take measures to stabilize the sample (add reducing agent, ligands, glycerol, salt, or other stabilizing agents). If the sample is impure, aggregated, or heterogeneous take measures to pursue homogeneity. It is possible to obtain crystals from precipitate so do not discard nor ignore a drop containing precipitate. If possible, examine drops containing precipitate under polarizing optics to differentiate precipitate from microcrystalline material.

If the drop contains a macromolecular crystal the relative supersaturation of the sample and reagent is appropriate for crystal nucleation and growth. 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 HT Formulation

Crystallization 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 Deep Well blocks (no preservatives added).

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

Crystallization 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.

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Crystallization reagents are stable at room temperature and are best used before the "Best If Used By" date. To enhance reagent stability the crystallization reagents can 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 crystallization 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. Cudney, R., Patel, S., Weisgraber, K., Newhouse, Y., and McPherson, A., Acta Cryst. (1994) D50, 414-423.

2. The development and application of a method to quantify the quality of cryoprotectant solutions using standard area-detector X-ray images. McFerrin and Snell, J. Appl. Cryst. (2002). 35, 538-545.

3. Crystallization of nucleic acids and proteins, Edited by A. Ducruix and R. Giege, The Practical Approach Series, Oxford Univ. Press, 1992.

4. Current approaches to macromolecular crystallization. McPherson, A. Eur. J. Biochem. 189, 1-23, 1990.

5. Sparse Matrix Sampling: a screening method for crystallization of proteins. Jancarik, J. and Kim, S.H. J. Appl. Cryst., 24,409-411, 1991.

Technical Support

Inquiries regarding Crystal Screen Cryo HT 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.

Hampton Research
34 Journey
Aliso Viejo, CA 92656-3317 U.S.A.
Tel: (949) 425-1321 • Fax: (949) 425-1611
Technical Support e-mail: tech@hrmail.com
Website: www.hamptonresearch.com