# SaltRx HT™

HAMPTON RESEARCH
*Solutions for Crystal Growth*

**User Guide** **HR2-136 (pg 1)**

## Applications

Crystallization screen for proteins, peptides, nucleic acids and water soluble small molecules where salt is the preferred primary crystallization reagent.

## Features

- Salt and pH only sparse matrix crystallization screen
- Samples pH 4.0 - 9.0
- 22 unique salts versus concentration and pH
- Preformulated, ready to screen
- Compatible with Microbatch, Vapor Diffusion, Liquid & Gel Diffusion methods

Refer to the enclosed SaltRx HT reagent formulation for additional information.

## General Description

SaltRx HT ™ was developed by Hampton Research as salt only crystallization screen matrix. SaltRx HT is supplied in a 96 Deep Well block format and is compatible with robotic and multi-channel pipet liquid handling systems. Salt is the only primary crystallization reagent (precipitant) utilized in SaltRx HT. Based on a design of 96 conditions, the screen evaluates a broad portfolio of crystallization salts of varying concentration and pH. The selection of salts, the concentration of salts and pH was determined by data mining the BMCD[10], additional crystallization reports in the literature and internal crystallization trials. Based on crystallization results in the BMCD and subsequent literature, up to 35% of protein crystallizations involve salt as the primary crystallization reagent. SaltRx HT is to be used as a primary crystallization screen when salt and ionic strength is desired or suspected as an appropriate crystallization reagent. SaltRx HT is also useful as a secondary screen when salt only reagents/conditions from screens such as Index™, Crystal Screen™, and Grid Screen™ Ammonium Sulfate produce crystals and further screening for additional salt conditions is desired. As SaltRx HT does not contain volatile organics the screen is compatible with Microbatch, Vapor Diffusion, Liquid and Gel diffusion crystallization methods.

SaltRx 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. Each SaltRx kit is supplied with an adhesive clear sealing film which can be used to seal the block after removing the heat seal. Additional adhesive clear sealing films can be obtained from Hampton Research or laboratory supply companies which offer high throughput plates and seals.

## 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 SaltRx 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

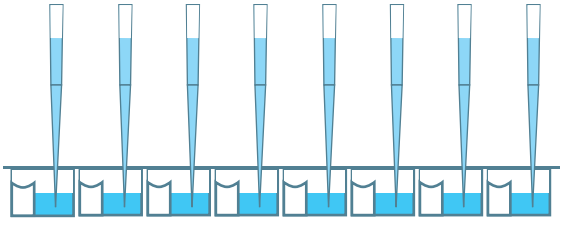
It is recommended the Deep Well block be centrifuged before removing the sealing film. Centrifugation at 500 rpm for five minutes will remove stray reagent from the sealing film. Removing the reagent from the film prevents stray reagent droplets from falling into neighboring wells during film removal. After centrifugation the film can be removed by grasping a corner of the film and gently peeling the film from the plate. Alternatively, the film can be left intact and then pierced for reagent access.

## Performing the Screen

Manual Method - Sitting Drop Vapor Diffusion

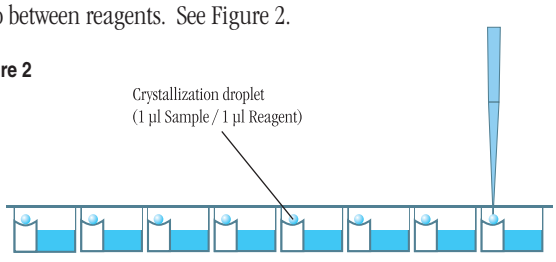
1. Using a 96 well sitting drop vapor diffusion plate, pipet the recommended volume (typically 50 to 100 microliters) of crystallization reagent from the Deep Well block into the reservoirs of the crystallization plate. The Deep Well block is compatible with 8 and 12 channel pipets as well as many automated 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. For a 12 channel pipet, transfer reagents A1-A12 to reservoirs A1-A12 of the crystallization plate. Repeat this procedure for reagent rows 1 through 12. See Figure 1 below. 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**



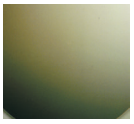
2. Using clean pipet tips, pipet 0.05 to 2 microliters of crystallization reagent from the crystallization plate reservoir to the sitting drop well. Some 96 well crystallization plates allow this procedure to be performed using a multichannel pipet where other plates require the use of a single channel pipet. Change the pipet tip between reagents. See Figure 2.

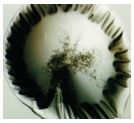
**Figure 2**

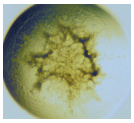


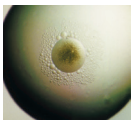

3. Using a clean pipet tip, pipet 0.05 to 2 microliters of sample to the reagent

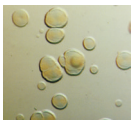
**Figure 6**
Typical observations in a crystallization experiment

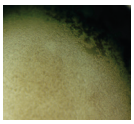

Clear Drop


Skin / Precipitate

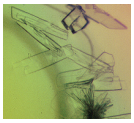

Precipitate


Precipitate / Phase

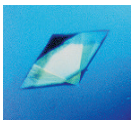

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

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 on page 1.

4. Seal the crystallization plate as per the manufacturer's recommendation. Most 96 well crystallization plates are sealed using a clear sealing tape, film, or cap mat. View and score the experiment as desired. See Hampton Research technical bulletin Crystal Growth 101 - Viewing Crystallization Experiments for additional information on viewing drops.

5. Seal the remaining reagent in the Deep Well block using either clear sealing tape, film, or cap mat.

SaltRx 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 6, on the left side of page 2 shows typical examples of what one might observe in a crystallization experiment.

### Interpreting SaltRx 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 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 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.

### SaltRx 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 1.0M stock buffer, titrated to the desired pH using Hy-

drochloric acid or Sodium hydroxide. The buffer is then diluted with the other reagent components and water. No further pH adjustment is required.

Crystallization 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 crystallization reagents be stored at 4°C or -20°C.

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

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### Technical Support

Inquiries regarding SaltRx 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 4:30 p.m. USA Pacific Standard Time.

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