SaltRx™ 1

HAMPTON RESEARCH
Solutions for Crystal Growth

User Guide **HR2-107 (pg 1)**

Application
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 4.6 – 8.5
- 22 unique salts versus concentration and pH
- Preformulated, ready to screen

General Description
SaltRx™ 1 was developed by Hampton Research as a salt only crystallization screen matrix. Salt is the single primary crystallization reagent (precipitant) utilized in SaltRx 1. Based on a design of 96 conditions (SaltRx 1 and SaltRx 2), 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 1 is to be used as a primary crystallization screen when salt and ionic strength is desired or suspected as an appropriate crystallization reagent. SaltRx 1 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 1 does not contain volatile organics the screen is compatible with Microbatch, Vapor Diffusion, Liquid and Gel diffusion crystallization methods. SaltRx 1 may also be used for microdialysis crystallization in conjunction with Dialysis Buttons.

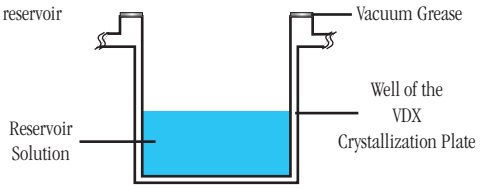
Sample Preparation
The macromolecular sample should be homogenous, as pure as is practically possible (>95%) and free of amorphous and particulate material. Remove amorphous material by centrifugation or micro-filtration prior to use.

The recommended sample concentration is 5 to 25 mg/ml in dilute buffer (10 to 25 mM). The sample should be free of any unnecessary additives in order to observe the effect of the SaltRx 1 variables. Ideally, the initial screen should be performed with a sample which has been dialyzed against dilute buffer (such as 25 mM HEPES sodium pH 7.0) 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 SaltRx 1 with the Hanging Drop Vapor Diffusion method. SaltRx 1 is also compatible with the Sitting Drop, Sandwich Drop, Microbatch, and Dialysis 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 SaltRx 1. See Figure 1.

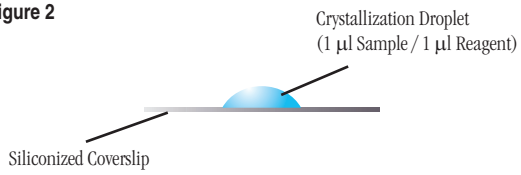
Figure 1
Cross section of a reservoir in the VDX plate.



2. Using a clean pipet tip, pipet 1 ml of SaltRx 1 reagent 1 into reservoir A1. Discard the pipet tip, add a new pipet tip and pipet 1 ml of SaltRx 1 reagent 2 into reservoir A2. Repeat the procedure for the remaining 46 SaltRx 1 reagents using a clean pipet tip for each reagent so as to avoid reagent contamination and carry over.

3. Pipet 1 µl of the sample to the center of a clean, siliconized 22 mm diameter circle or square cover slide. See Figure 2.

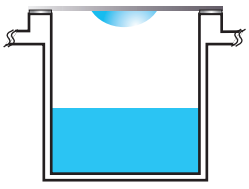
Figure 2



4. Pipet 1 µl of SaltRx 1 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 SaltRx 1 reagents.

7. If the quantity of sample permits, perform the SaltRx 1 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.

SaltRx™ 1

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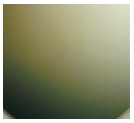
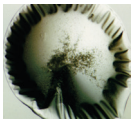
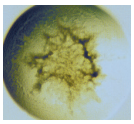
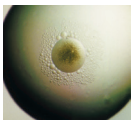
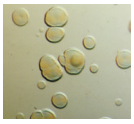
Figure 4
Typical observations in a crystallization experiment

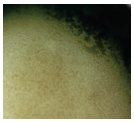

Clear Drop


Skin / Precipitate

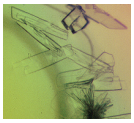

Precipitate


Precipitate / Phase

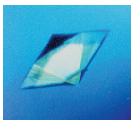

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

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 shows typical examples of what one might observe in a crystallization experiment.

Interpreting SaltRx 1

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 SaltRx 1 condition and doubling the sample concentration. If more than 70 of the 96 SaltRx 1 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 SaltRx 1 condition. If more than 70 of the 96 SaltRx 1 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 crystallization. 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 1 Formulation

SaltRx 1 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).

SaltRx 1 reagents are readily reproduced using Hampton Research Optimize™ stock solutions of salts, polymers and buffers. Optimize stock reagents make reproducing SaltRx 1 reagents fast, convenient and easy. Dilutions can be performed directly into the crystallization plate using Optimize stock reagents.

SaltRx 1 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.

SaltRx 1 reagents are stable at room temperature and are best if used within 12 months of receipt.

If the sample contains phosphate, borate, or carbonate buffers it is possible to obtain inorganic crystals (false positives) when using SaltRx 1 reagents containing divalent cations. 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 such as HEPES sodium.

References and Readings

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Technical Support

Inquiries regarding SaltRx 1 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.

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