

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 BMCD¹⁰, 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.

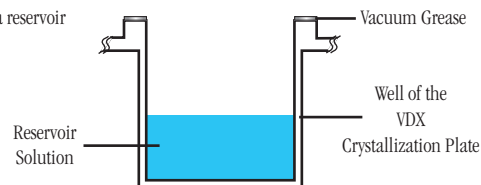
Performing The Screen

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

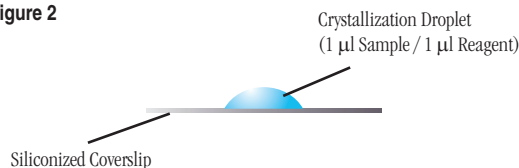
Cross section of a reservoir in the VDX plate.



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

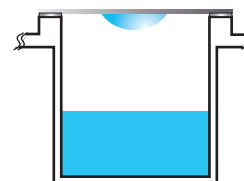


4. Pipet 1 µl of SaltRx 1 reagent 1 from reservoir A1 into the sample droplet and mix by aspirating and dispensing the droplet several times, keeping the tip in the drop during mixing to avoid foaming. See Figure 2.

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.

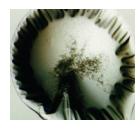


6. Repeat operations 3 through 5 for the remaining 47 SaltRx 1 reagents.
7. If the quantity of sample permits, perform the SaltRx 1 in duplicate and incubate one set of plates at 4°C and the second set at room temperature. Incubate and store the crystallization plates in a stable temperature environment free of vibration.

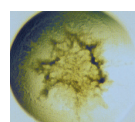
Figure 4
Typical observations in a crystallization experiment



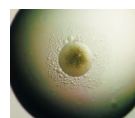
Clear Drop



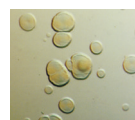
Skin/
Precipitate



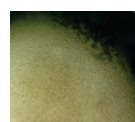
Precipitate



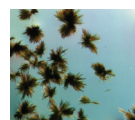
Precipitate/
Phase



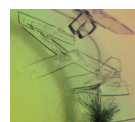
Quasi
Crystals



Microcrystals



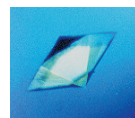
Needle
Cluster



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 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 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

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

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5. A comparison of salts for the crystallization of macromolecules. McPherson, A. *Protein Science*, 10:418-422, 2001.

6. Entering a new phase: Using solvent halide ions in protein structure determination. Dauter, Z. and Dauter, M. *Structure*, Vol 9, R21-26, Feb 2001.

7. Efficiency Analysis of Screening Protocols Used in Protein Crystallization, B. W. Segelke, *Journal of Crystal Growth* 232 : 553-562 (2001).

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10. Gilliland, G.L., Tung, M., Blakeslee, D.M. and Ladner, J. 1994. The Biological Macromolecule Crystallization Database, Version 3.0: New Features, Data, and the NASA Archive for Protein Crystal Growth Data. *Acta Crystallogr. D*50 408-413.

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.

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How to Reproduce SaltRx Reagents

SaltRx reagents and optimization conditions based on SaltRx hits can be formulated using volumetric methods and carefully prepared reagent stocks (Table 1). Note the examples below.

Example 1. To prepare 1.0 milliliter of SaltRx reagent 1 in a crystallization plate.

Solution Composition: 1.8 M Sodium acetate trihydrate pH 7.0
0.1 M BIS-TRIS propane pH 7.0

- 450 µl water³
- 100 µl 1.0 M BIS-TRIS propane pH 7.0
(CAS # 64431-96-5, Catalog # HR2-795)
- 450 µl 4.0 M Sodium acetate trihydrate pH 7.0
(CAS # 6131-90-4, Catalog # HR2-763)

Make no pH adjustments. Mix well by aspirating and dispensing the solution multiple times.

Example 2. To prepare 1.0 milliliter of SaltRx reagent 57.

Solution Composition: 0.63 M Sodium phosphate monobasic monohydrate,
1.17 M Potassium phosphate dibasic /pH 6.9

- 550 µl water³
- 157 µl 4.0 M Potassium phosphate dibasic
(CAS # 7758-11-4, Catalog # HR2-635)
- 293 µl 4.0 M Sodium phosphate monobasic monohydrate
(CAS # 10049-21-5, Catalog # HR2-551)

Make no pH adjustments. Mix well. Final pH will be 6.9

Example 3. To prepare 10 milliliters of SaltRx reagent 27.

Solution Composition: 2.0 M Sodium formate
0.1 M Sodium acetate trihydrate pH 4.6

- 6.1 ml water³
- 1.0 ml 1.0 M Sodium acetate trihydrate pH 4.6
(CAS # 6131-90-4, Catalog # HR2-731)
- 2.9 ml 7.0 M Sodium formate
(CAS # 141-53-7, Catalog # HR2-547)

Make no pH adjustments. Mix well.

³ ASTM Type II (laboratory grade) or Type III (analytical grade) water.

Formulation Notes for SaltRx Reagents

1. No additional pH adjustment is made to any reagent after formulation. Use the buffers in Table 1 to reproduce a SaltRx reagent.

2. All Optimize solutions and screen reagents are sterile filtered using 0.22 µm filters into sterile containers.
3. Add water first as this will help maintain the solubility of subsequently added reagents.
4. When formulating reagents using a pipet, add the largest volume last (except water). Use this larger volume setting to aspirate and dispense the reagent until the solution is mixed.
5. When formulating reagents using a pipet, use a clean, sterile pipet tip for each reagent added to the solution.
6. Use the buffers in Table 2 to systematically vary the pH as a crystallization variable.

pH as a Crystallization Variable

The buffers listed in Table 2, can be used to vary the pH as a crystallization variable and are recommended when optimizing a crystal grown from a SaltRx kit.

Optimize™ buffer stocks are supplied as a 100 milliliters sterile filtered solution. Optimize buffers are available as an acid-base pair or titrated to a specific pH.

StockOptions™ buffer kits contain 10 milliliters each of ready to pipet buffers, titrated in 0.1 pH increments over the indicated pH range. The number of reagents offered in a StockOptions buffer kit depends upon the pH range of the buffer. The broader the pH range, the more buffers in the kit.

Online Information

Visit www.hamptonresearch.com and enter one of the following:

- Reagent Catalog Number
- Kit Catalog Number
- CAS Number
- Reagent Name

To obtain reagent specifications, pH titration tables, user guides, certificates of analysis, material safety data sheets (MSDS), and any other additional information.

MakeTray™

MakeTray is a free, web based program at www.hamptonresearch.com which generates both a pipetting worksheet and a reagent formulation document for crystallization set ups. MakeTray allows one to enter general information about the sample and experiment, which is then printed on the pipet worksheet and the reagent formulation document. The plate size can be customized for any number of wells, so MakeTray works for: 24, 48, and 96 well plates. MakeTray is especially useful for the design and formulation of crystal optimization experiments.

Table 1. Recommended reagents for the formulation of SaltRx and Optimization reagents.

Each of these reagents are available as an Optimize™ crystallization grade reagent from Hampton Research. Table 1 provides the common chemical name, the Hampton Research catalog number, supplied stock concentration, the supplied volume, and the CAS number for each reagent. For more information on a specific Optimize reagent, go to

www.hamptonresearch.com. Using Search, enter either the catalog number, CAS number, or chemical name to obtain additional information for the Optimize reagent, including a Certificate of Analysis and MSDS (where applicable).

Salts	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
Ammonium acetate	HR2-565	1.0 M	100 ml	631-61-8
	HR2-799	8.0 M	200 ml	631-61-8
Ammonium chloride	HR2-691	5.0 M	200 ml	12125-02-9
Ammonium citrate dibasic	HR2-685	2.5 M	200 ml	3012-65-5
Ammonium citrate tribasic pH 7.0	HR2-759	2.5 M	200 ml	3458-72-8
Ammonium nitrate	HR2-665	10.0 M	200 ml	6484-52-2
Ammonium phosphate dibasic	HR2-629	3.5 M	200 ml	7783-28-0
Ammonium phosphate monobasic	HR2-555	2.5 M	200 ml	7722-76-1
Ammonium sulfate	HR2-541	3.5 M	200 ml	7783-20-2
Ammonium tartrate dibasic	HR2-679	2.0 M	200 ml	3164-29-2
Lithium sulfate monohydrate	HR2-545	2.0 M	200 ml	10377-48-7
Magnesium formate dihydrate	HR2-537	1.0 M	200 ml	557-39-1
Magnesium sulfate hydrate	HR2-633	2.5 M	200 ml	22189-08-8
DL-Malic acid pH 7.0	HR2-761	3.0 M	200 ml	6915-15-7
Potassium phosphate dibasic	HR2-635	4.0 M	200 ml	7758-11-4
Potassium sodium tartrate tetrahydrate	HR2-539	1.5 M	200 ml	6381-59-5
Potassium thiocyanate	HR2-695	8.0 M	200 ml	333-20-0
Sodium acetate trihydrate pH 7.0	HR2-763	4.0 M	200 ml	6131-90-4
Sodium chloride	HR2-637	5.0 M	200 ml	7647-14-5
Sodium citrate tribasic dihydrate	HR2-549	1.6 M	200 ml	6132-04-3
Sodium formate	HR2-547	7.0 M	200 ml	141-53-7
Sodium malonate pH 7.0	HR2-707	3.4 M	200 ml	141-82-2
Sodium nitrate	HR2-661	7.0 M	200 ml	7631-99-4
Sodium phosphate monobasic monohydrate	HR2-551	4.0 M	200 ml	10049-21-5
Succinic acid pH 7.0	HR2-709	1.2 M	200 ml	110-15-6
Tacsimate pH 7.0	HR2-755	100 %	200 ml	N/A

(Table 1 continued on page 3)

Table 1 (Continued). **Recommended reagents for the formulation of SaltRx and Optimization reagents.**

Buffers	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
BIS-TRIS propane pH 7.0 ¹	HR2-795	1.0 M	100 ml	64431-96-5
Sodium acetate trihydrate pH 4.6 ¹	HR2-731	1.0 M	100 ml	6131-90-4
Tris pH 8.5 ¹	HR2-725	1.0 M	100 ml	77-86-1
¹ pH titrated using Hydrochloric acid (HR2-581) CAS # 7647-01-0				

Table 2. **Recommended buffers for screening the pH of SaltRx and Optimization reagents.**

Buffer Solution or Kit	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #	pH range
StockOptions™ Bis-Tris propane	HR2-993-**	1.0 M	185 ml	64431-96-5	6.3 - 9.5
StockOptions™ Sodium Acetate kit ⁴	HR2-233	1.0 M	10 ml each	6131-90-4	3.6 - 5.6
StockOptions™ Tris ⁴	HR2-100	1.0 M	10 ml each	77-86-1	7.0 - 9.0
⁴ Individual StockOptions buffers titrated to any pH within the kit's pH range are available in 185 ml volumes from the Hampton Research Custom Shop					
** Refers to the reagent number in the kit. For example, reagent number 1 = HR2-993-01 (pH 6.3)					

Technical Support

Inquiries regarding SaltRx Fundamentals, 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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Website: www.hamptonresearch.com

Tube #	Salt	Tube #	Buffer ◇
1.	1.8 M Sodium acetate trihydrate pH 7.0	1.	0.1 M BIS-TRIS propane pH 7.0
2.	2.8 M Sodium acetate trihydrate pH 7.0	2.	0.1 M BIS-TRIS propane pH 7.0
3.	1.5 M Ammonium chloride	3.	0.1 M Sodium acetate trihydrate pH 4.6
4.	1.5 M Ammonium chloride	4.	0.1 M BIS-TRIS propane pH 7.0
5.	1.5 M Ammonium chloride	5.	0.1 M Tris pH 8.5
6.	3.5 M Ammonium chloride	6.	0.1 M Sodium acetate trihydrate pH 4.6
7.	3.5 M Ammonium chloride	7.	0.1 M BIS-TRIS propane pH 7.0
8.	3.5 M Ammonium chloride	8.	0.1 M Tris pH 8.5
9.	2.2 M Sodium chloride	9.	0.1 M Sodium acetate trihydrate pH 4.6
10.	2.2 M Sodium chloride	10.	0.1 M BIS-TRIS propane pH 7.0
11.	2.2 M Sodium chloride	11.	0.1 M Tris pH 8.5
12.	3.2 M Sodium chloride	12.	0.1 M Sodium acetate trihydrate pH 4.6
13.	3.2 M Sodium chloride	13.	0.1 M BIS-TRIS propane pH 7.0
14.	3.2 M Sodium chloride	14.	0.1 M Tris pH 8.5
15.	1.0 M Ammonium citrate dibasic	15.	0.1 M Sodium acetate trihydrate pH 4.6
16.	1.8 M Ammonium citrate dibasic	16.	0.1 M Sodium acetate trihydrate pH 4.6
17.	1.0 M Ammonium citrate tribasic pH 7.0	17.	0.1 M BIS-TRIS propane pH 7.0
18.	2.0 M Ammonium citrate tribasic pH 7.0	18.	0.1 M BIS-TRIS propane pH 7.0
19.	0.7 M Sodium citrate tribasic dihydrate	19.	0.1 M BIS-TRIS propane pH 7.0
20.	0.7 M Sodium citrate tribasic dihydrate	20.	0.1 M Tris pH 8.5
21.	1.2 M Sodium citrate tribasic dihydrate	21.	0.1 M BIS-TRIS propane pH 7.0
22.	1.2 M Sodium citrate tribasic dihydrate	22.	0.1 M Tris pH 8.5
23.	0.4 M Magnesium formate dihydrate	23.	0.1 M Sodium acetate trihydrate pH 4.6
24.	0.4 M Magnesium formate dihydrate	24.	0.1 M BIS-TRIS propane pH 7.0
25.	0.4 M Magnesium formate dihydrate	25.	0.1 M Tris pH 8.5
26.	0.7 M Magnesium formate dihydrate	26.	0.1 M BIS-TRIS propane pH 7.0
27.	2.0 M Sodium formate	27.	0.1 M Sodium acetate trihydrate pH 4.6
28.	2.0 M Sodium formate	28.	0.1 M BIS-TRIS propane pH 7.0
29.	2.0 M Sodium formate	29.	0.1 M Tris pH 8.5
30.	3.5 M Sodium formate	30.	0.1 M Sodium acetate trihydrate pH 4.6
31.	3.5 M Sodium formate	31.	0.1 M BIS-TRIS propane pH 7.0
32.	3.5 M Sodium formate	32.	0.1 M Tris pH 8.5
33.	1.2 M DL-Malic acid pH 7.0	33.	0.1 M BIS-TRIS propane pH 7.0
34.	2.2 M DL-Malic acid pH 7.0	34.	0.1 M BIS-TRIS propane pH 7.0
35.	1.4 M Sodium malonate pH 7.0	35.	0.1 M BIS-TRIS propane pH 7.0
36.	2.4 M Sodium malonate pH 7.0	36.	0.1 M BIS-TRIS propane pH 7.0
37.	2.5 M Ammonium nitrate	37.	0.1 M Sodium acetate trihydrate pH 4.6
38.	2.5 M Ammonium nitrate	38.	0.1 M BIS-TRIS propane pH 7.0
39.	2.5 M Ammonium nitrate	39.	0.1 M Tris pH 8.5
40.	6.0 M Ammonium nitrate	40.	0.1 M Sodium acetate trihydrate pH 4.6
41.	6.0 M Ammonium nitrate	41.	0.1 M BIS-TRIS propane pH 7.0
42.	6.0 M Ammonium nitrate	42.	0.1 M Tris pH 8.5
43.	1.5 M Sodium nitrate	43.	0.1 M Sodium acetate trihydrate pH 4.6
44.	1.5 M Sodium nitrate	44.	0.1 M BIS-TRIS propane pH 7.0
45.	1.5 M Sodium nitrate	45.	0.1 M Tris pH 8.5
46.	4.0 M Sodium nitrate	46.	0.1 M Sodium acetate trihydrate pH 4.6
47.	4.0 M Sodium nitrate	47.	0.1 M BIS-TRIS propane pH 7.0
48.	4.0 M Sodium nitrate	48.	0.1 M Tris pH 8.5

◇ Buffer pH is that of a 1.0 M stock prior to dilution with other reagent components: pH with HCl or NaOH.

SaltRx 1 contains forty-eight unique reagents. To determine the formulation of each reagent, simply read across the page.

Sample: _____ Sample Concentration: _____
 Sample Buffer: _____ Date: _____
 Reservoir Volume: _____ Temperature: _____
 Drop Volume: Total _____ μ l Sample _____ μ l Reservoir _____ μ l Additive _____ μ l

- 1 Clear Drop
- 2 Phase Separation
- 3 Regular Granular Precipitate
- 4 Birefringent Precipitate or Microcrystals
- 5 Posettes or Spherulites
- 6 Needles (1D Growth)
- 7 Plates (2D Growth)
- 8 Single Crystals (3D Growth < 0.2 mm)
- 9 Single Crystals (3D Growth > 0.2 mm)

SaltRx™ 1 - HR2-107 Scoring Sheet

Date: Date: Date:

1.	1.8 M Sodium acetate trihydrate pH 7.0, 0.1 M BIS-TRIS propane pH 7.0			
2.	2.8 M Sodium acetate trihydrate pH 7.0, 0.1 M BIS-TRIS propane pH 7.0			
3.	1.5 M Ammonium chloride, 0.1 M Sodium acetate trihydrate pH 4.6			
4.	1.5 M Ammonium chloride, 0.1 M BIS-TRIS propane pH 7.0			
5.	1.5 M Ammonium chloride, 0.1 M Tris pH 8.5			
6.	3.5 M Ammonium chloride, 0.1 M Sodium acetate trihydrate pH 4.6			
7.	3.5 M Ammonium chloride, 0.1 M BIS-TRIS propane pH 7.0			
8.	3.5 M Ammonium chloride, 0.1 M Tris pH 8.5			
9.	2.2 M Sodium chloride, 0.1 M Sodium acetate trihydrate pH 4.6			
10.	2.2 M Sodium chloride, 0.1 M BIS-TRIS propane pH 7.0			
11.	2.2 M Sodium chloride, 0.1 M Tris pH 8.5			
12.	3.2 M Sodium chloride, 0.1 M Sodium acetate trihydrate pH 4.6			
13.	3.2 M Sodium chloride, 0.1 M BIS-TRIS propane pH 7.0			
14.	3.2 M Sodium chloride, 0.1 M Tris pH 8.5			
15.	1.0 M Ammonium citrate dibasic, 0.1 M Sodium acetate trihydrate pH 4.6			
16.	1.8 M Ammonium citrate dibasic, 0.1 M Sodium acetate trihydrate pH 4.6			
17.	1.0 M Ammonium citrate tribasic pH 7.0, 0.1 M BIS-TRIS propane pH 7.0			
18.	2.0 M Ammonium citrate tribasic pH 7.0, 0.1 M BIS-TRIS propane pH 7.0			
19.	0.7 M Sodium citrate tribasic dihydrate, 0.1 M BIS-TRIS propane pH 7.0			
20.	0.7 M Sodium citrate tribasic dihydrate, 0.1 M Tris pH 8.5			
21.	1.2 M Sodium citrate tribasic dihydrate, 0.1 M BIS-TRIS propane pH 7.0			
22.	1.2 M Sodium citrate tribasic dihydrate, 0.1 M Tris pH 8.5			
23.	0.4 M Magnesium formate dihydrate, 0.1 M Sodium acetate trihydrate pH 4.6			
24.	0.4 M Magnesium formate dihydrate, 0.1 M BIS-TRIS propane pH 7.0			
25.	0.4 M Magnesium formate dihydrate, 0.1 M Tris pH 8.5			
26.	0.7 M Magnesium formate dihydrate, 0.1 M BIS-TRIS propane pH 7.0			
27.	2.0 M Sodium formate, 0.1 M Sodium acetate trihydrate pH 4.6			
28.	2.0 M Sodium formate, 0.1 M BIS-TRIS propane pH 7.0			
29.	2.0 M Sodium formate, 0.1 M Tris pH 8.5			
30.	3.5 M Sodium formate, 0.1 M Sodium acetate trihydrate pH 4.6			
31.	3.5 M Sodium formate, 0.1 M BIS-TRIS propane pH 7.0			
32.	3.5 M Sodium formate, 0.1 M Tris pH 8.5			
33.	1.2 M DL-Malic acid pH 7.0, 0.1 M BIS-TRIS propane pH 7.0			
34.	2.2 M DL-Malic acid pH 7.0, 0.1 M BIS-TRIS propane pH 7.0			
35.	1.4 M Sodium malonate pH 7.0, 0.1 M BIS-TRIS propane pH 7.0			
36.	2.4 M Sodium malonate pH 7.0, 0.1 M BIS-TRIS propane pH 7.0			
37.	2.5 M Ammonium nitrate, 0.1 M Sodium acetate trihydrate pH 4.6			
38.	2.5 M Ammonium nitrate, 0.1 M BIS-TRIS propane pH 7.0			
39.	2.5 M Ammonium nitrate, 0.1 M Tris pH 8.5			
40.	6.0 M Ammonium nitrate, 0.1 M Sodium acetate trihydrate pH 4.6			
41.	6.0 M Ammonium nitrate, 0.1 M BIS-TRIS propane pH 7.0			
42.	6.0 M Ammonium nitrate, 0.1 M Tris pH 8.5			
43.	1.5 M Sodium nitrate, 0.1 M Sodium acetate trihydrate pH 4.6			
44.	1.5 M Sodium nitrate, 0.1 M BIS-TRIS propane pH 7.0			
45.	1.5 M Sodium nitrate, 0.1 M Tris pH 8.5			
46.	4.0 M Sodium nitrate, 0.1 M Sodium acetate trihydrate pH 4.6			
47.	4.0 M Sodium nitrate, 0.1 M BIS-TRIS propane pH 7.0			
48.	4.0 M Sodium nitrate, 0.1 M Tris pH 8.5			