SaltRx™ 2

HAMPTON RESEARCH
Solutions for Crystal Growth

User Guide **HR2-109 (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™ 2 was developed by Hampton Research as a salt only crystallization screen matrix. Salt is the single primary crystallization reagent (precipitant) utilized in SaltRx 2. 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 2 is to be used as a primary crystallization screen when salt and ionic strength is desired or suspected as an appropriate crystallization reagent. SaltRx 2 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 2 does not contain volatile organics the screen is compatible with Microbatch, Vapor Diffusion, Liquid and Gel diffusion crystallization methods. SaltRx 2 may also be used for microdialysis crystallization in conjunction with Dialysis Buttons.

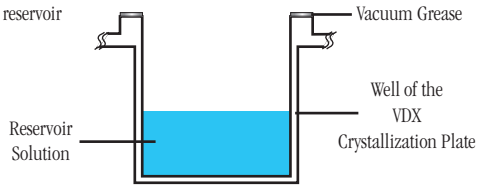
Sample Preparation
The macromolecular sample should be homogenous, as pure as is practically possible (>95%) and free of amorphous and particulate material. Remove amorphous material by centrifugation or micro-filtration prior to use.

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 2 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 2 with the Hanging Drop Vapor Diffusion method. SaltRx 2 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 2. See Figure 1.

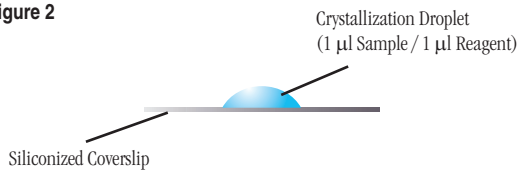
Figure 1
Cross section of a reservoir in the VDX plate.



2. Using a clean pipet tip, pipet 1 ml of SaltRx 2 reagent 1 into reservoir A1. Discard the pipet tip, add a new pipet tip and pipet 1 ml of SaltRx 2 reagent 2 into reservoir A2. Repeat the procedure for the remaining 46 SaltRx 2 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.

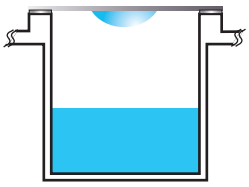
Figure 2



4. Pipet 1 µl of SaltRx 2 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 2 reagents.

7. If the quantity of sample permits, perform the SaltRx 2 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™ 2

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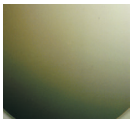
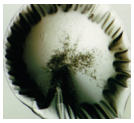
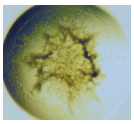
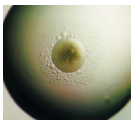
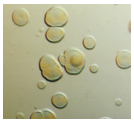
Figure 4
Typical observations in a crystallization experiment

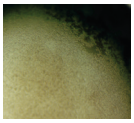

Clear Drop


Skin / Precipitate

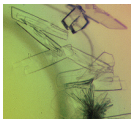

Precipitate


Precipitate / Phase

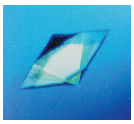

Quasi Crystals


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 2

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 2 condition and doubling the sample concentration. If more than 70 of the 96 SaltRx 2 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 2 condition. If more than 70 of the 96 SaltRx 2 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 2 Formulation

SaltRx 2 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 2 reagents are readily reproduced using Hampton Research Optimize™ stock solutions of salts, polymers and buffers. Optimize stock reagents make reproducing SaltRx 2 reagents fast, convenient and easy. Dilutions can be performed directly into the crystallization plate using Optimize stock reagents.

SaltRx 2 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 2 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 2 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. Giege, The Practical Approach Series, Oxford Univ. Press, 1992.

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

4. Protein and Nucleic Acid Crystallization. Methods, A Companion to Methods in Enzymology, Academic Press, Volume 1, Number 1, August 1990.

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).

8. A novel approach to crystallizing proteins under oil. D'Arcy, A. et al. Journal of Crystal Growth, (1996) 168, 175-180.

9. Chayen, N. et al, J. Appl. Cryst. (1990) 23, 297.

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. D50 408-413.

Technical Support

Inquiries regarding SaltRx 2 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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SaltRx™

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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 water[3]
- 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 water[3]
- 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 water[3]
- 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

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Tube #	Salt	Tube #	Buffer ◊
1.	1.0 M Ammonium phosphate monobasic	1.	0.1 M Sodium acetate trihydrate pH 4.6
2.	1.8 M Ammonium phosphate monobasic	2.	0.1 M Sodium acetate trihydrate pH 4.6
3.	1.5 M Ammonium phosphate dibasic	3.	0.1 M Tris pH 8.5
4	2.4 M Ammonium phosphate dibasic	4	0.1 M Tris pH 8.5
5.	1.0 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 5.0	5.	None
6.	1.0 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 6.9	6.	None
7.	1.0 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 8.2	7.	None
8.	1.8 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 5.0	8.	None
9.	1.8 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 6.9	9.	None
10.	1.8 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 8.2	10.	None
11.	0.5 M Succinic acid pH 7.0	11.	0.1 M BIS-TRIS propane pH 7.0
12.	1.0 M Succinic acid pH 7.0	12.	0.1 M BIS-TRIS propane pH 7.0
13.	1.5 M Ammonium sulfate	13.	0.1 M Sodium acetate trihydrate pH 4.6
14.	1.5 M Ammonium sulfate	14.	0.1 M BIS-TRIS propane pH 7.0
15.	1.5 M Ammonium sulfate	15.	0.1 M Tris pH 8.5
16.	2.5 M Ammonium sulfate	16.	0.1 M Sodium acetate trihydrate pH 4.6
17.	2.5 M Ammonium sulfate	17.	0.1 M BIS-TRIS propane pH 7.0
18.	2.5 M Ammonium sulfate	18.	0.1 M Tris pH 8.5
19.	0.8 M Lithium sulfate monohydrate	19.	0.1 M Sodium acetate trihydrate pH 4.6
20.	0.8 M Lithium sulfate monohydrate	20.	0.1 M BIS-TRIS propane pH 7.0
21.	0.8 M Lithium sulfate monohydrate	21.	0.1 M Tris pH 8.5
22.	1.5 M Lithium sulfate monohydrate	22.	0.1 M Sodium acetate trihydrate pH 4.6
23.	1.5 M Lithium sulfate monohydrate	23.	0.1 M BIS-TRIS propane pH 7.0
24.	1.5 M Lithium sulfate monohydrate	24.	0.1 M Tris pH 8.5
25.	1.0 M Magnesium sulfate hydrate	25.	0.1 M Sodium acetate trihydrate pH 4.6
26.	1.0 M Magnesium sulfate hydrate	26.	0.1 M BIS-TRIS propane pH 7.0
27.	1.0 M Magnesium sulfate hydrate	27.	0.1 M Tris pH 8.5
28.	1.8 M Magnesium sulfate hydrate	28.	0.1 M Sodium acetate trihydrate pH 4.6
29.	1.8 M Magnesium sulfate hydrate	29.	0.1 M BIS-TRIS propane pH 7.0
30.	1.8 M Magnesium sulfate hydrate	30.	0.1 M Tris pH 8.5
31.	0.7 M Ammonium tartrate dibasic	31.	0.1 M Sodium acetate trihydrate pH 4.6
32.	0.7 M Ammonium tartrate dibasic	32.	0.1 M BIS-TRIS propane pH 7.0
33.	0.7 M Ammonium tartrate dibasic	33.	0.1 M Tris pH 8.5
34.	1.0 M Ammonium tartrate dibasic	34.	0.1 M Sodium acetate trihydrate pH 4.6
35.	1.3 M Ammonium tartrate dibasic	35.	0.1 M BIS-TRIS propane pH 7.0
36.	1.4 M Ammonium tartrate dibasic	36.	0.1 M Tris pH 8.5
37.	0.6 M Potassium sodium tartrate tetrahydrate	37.	0.1 M BIS-TRIS propane pH 7.0
38.	1.2 M Potassium sodium tartrate tetrahydrate	38.	0.1 M BIS-TRIS propane pH 7.0
39.	0.6 M Potassium sodium tartrate tetrahydrate	39.	0.1 M Tris pH 8.5
40.	1.2 M Potassium sodium tartrate tetrahydrate	40.	0.1 M Tris pH 8.5
41.	0.5 M Potassium thiocyanate	41.	0.1 M Sodium acetate trihydrate pH 4.6
42.	0.5 M Potassium thiocyanate	42.	0.1 M BIS-TRIS propane pH 7.0
43.	0.5 M Potassium thiocyanate	43.	0.1 M Tris pH 8.5
44.	4.0 M Ammonium acetate	44.	0.1 M Sodium acetate trihydrate pH 4.6
45.	4.0 M Ammonium acetate	45.	0.1 M BIS-TRIS propane pH 7.0
46.	4.0 M Ammonium acetate	46.	0.1 M Tris pH 8.5
47.	35% v/v Tacsimate pH 7.0	47.	0.1 M BIS-TRIS propane pH 7.0
48.	60% v/v Tacsimate pH 7.0	48.	0.1 M BIS-TRIS propane pH 7.0

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

SaltRx 2 contains forty-eight unique reagents. To determine the formulation of each reagent, simply read across the page.

34 Journey
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HAMPTON RESEARCH
Solutions for Crystal Growth

Sample: _____ **Sample Concentration:** _____

Sample Buffer: _____ **Date:** _____

Reservoir Volume: _____ **Temperature:** _____

Drop Volume: **Total** _____ µl **Sample** _____ µl **Reservoir** _____ µl **Additive** _____ µl

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™ 2 - HR2-109 Scoring Sheet	Date:	Date:	Date:
1. 1.0 M Ammonium phosphate monobasic, 0.1 M Sodium acetate trihydrate pH 4.6			
2. 1.8 M Ammonium phosphate monobasic, 0.1 M Sodium acetate trihydrate pH 4.6			
3. 1.5 M Ammonium phosphate dibasic, 0.1 M Tris pH 8.5			
4. 2.4 M Ammonium phosphate dibasic, 0.1 M Tris pH 8.5			
5. 1.0 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 5.0			
6. 1.0 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 6.9			
7. 1.0 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 8.2			
8. 1.8 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 5.0			
9. 1.8 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 6.9			
10. 1.8 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 8.2			
11. 0.5 M Succinic acid pH 7.0, 0.1 M BIS-TRIS propane pH 7.0			
12. 1.0 M Succinic acid pH 7.0, 0.1 M BIS-TRIS propane pH 7.0			
13. 1.5 M Ammonium sulfate, 0.1 M Sodium acetate trihydrate pH 4.6			
14. 1.5 M Ammonium sulfate, 0.1 M BIS-TRIS propane pH 7.0			
15. 1.5 M Ammonium sulfate, 0.1 M Tris pH 8.5			
16. 2.5 M Ammonium sulfate, 0.1 M Sodium acetate trihydrate pH 4.6			
17. 2.5 M Ammonium sulfate, 0.1 M BIS-TRIS propane pH 7.0			
18. 2.5 M Ammonium sulfate, 0.1 M Tris pH 8.5			
19. 0.8 M Lithium sulfate monohydrate, 0.1 M Sodium acetate trihydrate pH 4.6			
20. 0.8 M Lithium sulfate monohydrate, 0.1 M BIS-TRIS propane pH 7.0			
21. 0.8 M Lithium sulfate monohydrate, 0.1 M Tris pH 8.5			
22. 1.5 M Lithium sulfate monohydrate, 0.1 M Sodium acetate trihydrate pH 4.6			
23. 1.5 M Lithium sulfate monohydrate, 0.1 M BIS-TRIS propane pH 7.0			
24. 1.5 M Lithium sulfate monohydrate, 0.1 M Tris pH 8.5			
25. 1.0 M Magnesium sulfate hydrate, 0.1 M Sodium acetate trihydrate pH 4.6			
26. 1.0 M Magnesium sulfate hydrate, 0.1 M BIS-TRIS propane pH 7.0			
27. 1.0 M Magnesium sulfate hydrate, 0.1 M Tris pH 8.5			
28. 1.8 M Magnesium sulfate hydrate, 0.1 M Sodium acetate trihydrate pH 4.6			
29. 1.8 M Magnesium sulfate hydrate, 0.1 M BIS-TRIS propane pH 7.0			
30. 1.8 M Magnesium sulfate hydrate, 0.1 M Tris pH 8.5			
31. 0.7 M Ammonium tartrate dibasic, 0.1 M Sodium acetate trihydrate pH 4.6			
32. 0.7 M Ammonium tartrate dibasic, 0.1 M BIS-TRIS propane pH 7.0			
33. 0.7 M Ammonium tartrate dibasic, 0.1 M Tris pH 8.5			
34. 1.0 M Ammonium tartrate dibasic, 0.1 M Sodium acetate trihydrate pH 4.6			
35. 1.3 M Ammonium tartrate dibasic, 0.1 M BIS-TRIS propane pH 7.0			
36. 1.4 M Ammonium tartrate dibasic, 0.1 M Tris pH 8.5			
37. 0.6 M Potassium sodium tartrate tetrahydrate, 0.1 M BIS-TRIS propane pH 7.0			
38. 1.2 M Potassium sodium tartrate tetrahydrate, 0.1 M BIS-TRIS propane pH 7.0			
39. 0.6 M Potassium sodium tartrate tetrahydrate, 0.1 M Tris pH 8.5			
40. 1.2 M Potassium sodium tartrate tetrahydrate, 0.1 M Tris pH 8.5			
41. 0.5 M Potassium thiocyanate, 0.1 M Sodium acetate trihydrate pH 4.6			
42. 0.5 M Potassium thiocyanate, 0.1 M BIS-TRIS propane pH 7.0			
43. 0.5 M Potassium thiocyanate, 0.1 M Tris pH 8.5			
44. 4.0 M Ammonium acetate, 0.1 M Sodium acetate trihydrate pH 4.6			
45. 4.0 M Ammonium acetate, 0.1 M BIS-TRIS propane pH 7.0			
46. 4.0 M Ammonium acetate, 0.1 M Tris pH 8.5			
47. 35% v/v Tacsimate pH 7.0, 0.1 M BIS-TRIS propane pH 7.0			
48. 60% v/v Tacsimate pH 7.0, 0.1 M BIS-TRIS propane pH 7.0			

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