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

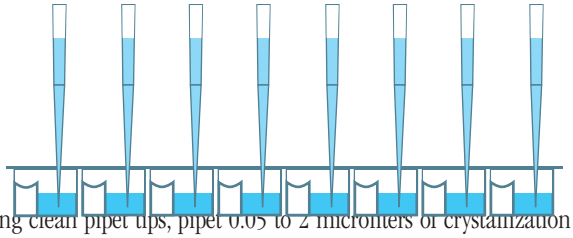
It is recommended the Deep Well block be centrifuged before removing the sealing film. Centrifugation at 500 rpm for five minutes will remove stray reagent from the sealing film. Removing the reagent from the film prevents stray reagent droplets from falling into neighboring wells during film removal. 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

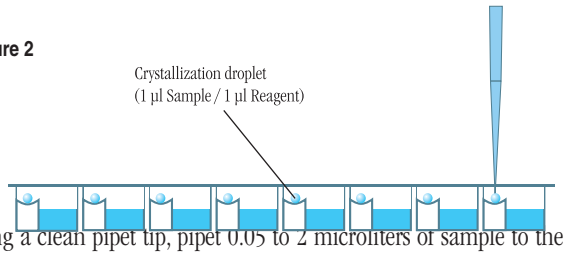
1. Using a 96 well sitting drop vapor diffusion plate, pipet the recommended volume (typically 50 to 100 microliters) of crystallization reagent from the Deep Well block into the reservoirs of the crystallization plate. The Deep Well block is compatible with 8 and 12 channel pipets as well as many automated 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



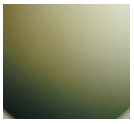
2. Using clean pipet tips, pipet 0.05 to 2 microliters of crystallization reagent from the crystallization plate reservoir to the sitting drop well. Some 96 well crystallization plates allow this procedure to be performed using a multichannel pipet where other plates require the use of a single channel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

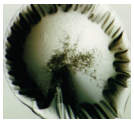
Figure 2

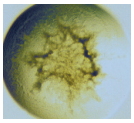


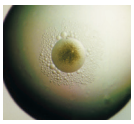
3. Using a clean pipet tip, pipet 0.05 to 2 microliters of sample to the reagent drop in the sitting drop well. One may choose to simply dispense the sample

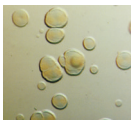
Figure 6
Typical observations in a crystallization experiment

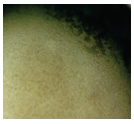

Clear Drop


Skin / Precipitate

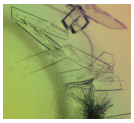

Precipitate


Precipitate / Phase

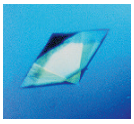

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

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 Hydrochloric 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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9. Chayen, N. et al, J. Appl. Cryst. (1990) 23, 297.
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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 4:00 p.m. USA Pacific Standard Time.

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SaltRx HT™

HAMPTON RESEARCH
Solutions for Crystal Growth

How to Reproduce SaltRx HT Reagents

SaltRx HT reagents and optimization conditions based on SaltRx HT 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 HT reagent 1 (well A1) 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 HT reagent 57 (well E9).
Solution Composition: 0.63 M Sodium phosphate monobasic monohydrate,
1.17 M Potassium phosphate dibasic /pH 6.9

- 550 µl water[3]
- 293 µl 4.0 M Potassium phosphate dibasic
(CAS # 7758-11-4, Catalog # HR2-635)
- 157 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 HT reagent 27 (well C3).
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 HT Reagents

1. No additional pH adjustment is made to any reagent after formulation. Use the buffers in Table 1 to reproduce a SaltRx HT 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 HT 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 HT 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 HT 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 HT 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 HT 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 4:00 p.m. USA Pacific Standard Time.

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Well #	Salt	Well #	Buffer ◊
1. (A1)	1.8 M Sodium acetate trihydrate pH 7.0	1. (A1)	0.1 M BIS-TRIS propane pH 7.0
2. (A2)	2.8 M Sodium acetate trihydrate pH 7.0	2. (A2)	0.1 M BIS-TRIS propane pH 7.0
3. (A3)	1.5 M Ammonium chloride	3. (A3)	0.1 M Sodium acetate trihydrate pH 4.6
4. (A4)	1.5 M Ammonium chloride	4. (A4)	0.1 M BIS-TRIS propane pH 7.0
5. (A5)	1.5 M Ammonium chloride	5. (A5)	0.1 M Tris pH 8.5
6. (A6)	3.5 M Ammonium chloride	6. (A6)	0.1 M Sodium acetate trihydrate pH 4.6
7. (A7)	3.5 M Ammonium chloride	7. (A7)	0.1 M BIS-TRIS propane pH 7.0
8. (A8)	3.5 M Ammonium chloride	8. (A8)	0.1 M Tris pH 8.5
9. (A9)	2.2 M Sodium chloride	9. (A9)	0.1 M Sodium acetate trihydrate pH 4.6
10. (A10)	2.2 M Sodium chloride	10. (A10)	0.1 M BIS-TRIS propane pH 7.0
11. (A11)	2.2 M Sodium chloride	11. (A11)	0.1 M Tris pH 8.5
12. (A12)	3.2 M Sodium chloride	12. (A12)	0.1 M Sodium acetate trihydrate pH 4.6
13. (B1)	3.2 M Sodium chloride	13. (B1)	0.1 M BIS-TRIS propane pH 7.0
14. (B2)	3.2 M Sodium chloride	14. (B2)	0.1 M Tris pH 8.5
15. (B3)	1.0 M Ammonium citrate dibasic	15. (B3)	0.1 M Sodium acetate trihydrate pH 4.6
16. (B4)	1.8 M Ammonium citrate dibasic	16. (B4)	0.1 M Sodium acetate trihydrate pH 4.6
17. (B5)	1.0 M Ammonium citrate tribasic pH 7.0	17. (B5)	0.1 M BIS-TRIS propane pH 7.0
18. (B6)	2.0 M Ammonium citrate tribasic pH 7.0	18. (B6)	0.1 M BIS-TRIS propane pH 7.0
19. (B7)	0.7 M Sodium citrate tribasic dihydrate	19. (B7)	0.1 M BIS-TRIS propane pH 7.0
20. (B8)	0.7 M Sodium citrate tribasic dihydrate	20. (B8)	0.1 M Tris pH 8.5
21. (B9)	1.2 M Sodium citrate tribasic dihydrate	21. (B9)	0.1 M BIS-TRIS propane pH 7.0
22. (B10)	1.2 M Sodium citrate tribasic dihydrate	22. (B10)	0.1 M Tris pH 8.5
23. (B11)	0.4 M Magnesium formate dihydrate	23. (B11)	0.1 M Sodium acetate trihydrate pH 4.6
24. (B12)	0.4 M Magnesium formate dihydrate	24. (B12)	0.1 M BIS-TRIS propane pH 7.0
25. (C1)	0.4 M Magnesium formate dihydrate	25. (C1)	0.1 M Tris pH 8.5
26. (C2)	0.7 M Magnesium formate dihydrate	26. (C2)	0.1 M BIS-TRIS propane pH 7.0
27. (C3)	2.0 M Sodium formate	27. (C3)	0.1 M Sodium acetate trihydrate pH 4.6
28. (C4)	2.0 M Sodium formate	28. (C4)	0.1 M BIS-TRIS propane pH 7.0
29. (C5)	2.0 M Sodium formate	29. (C5)	0.1 M Tris pH 8.5
30. (C6)	3.5 M Sodium formate	30. (C6)	0.1 M Sodium acetate trihydrate pH 4.6
31. (C7)	3.5 M Sodium formate	31. (C7)	0.1 M BIS-TRIS propane pH 7.0
32. (C8)	3.5 M Sodium formate	32. (C8)	0.1 M Tris pH 8.5
33. (C9)	1.2 M DL-Malic acid pH 7.0	33. (C9)	0.1 M BIS-TRIS propane pH 7.0
34. (C10)	2.2 M DL-Malic acid pH 7.0	34. (C10)	0.1 M BIS-TRIS propane pH 7.0
35. (C11)	1.4 M Sodium malonate pH 7.0	35. (C11)	0.1 M BIS-TRIS propane pH 7.0
36. (C12)	2.4 M Sodium malonate pH 7.0	36. (C12)	0.1 M BIS-TRIS propane pH 7.0
37. (D1)	2.5 M Ammonium nitrate	37. (D1)	0.1 M Sodium acetate trihydrate pH 4.6
38. (D2)	2.5 M Ammonium nitrate	38. (D2)	0.1 M BIS-TRIS propane pH 7.0
39. (D3)	2.5 M Ammonium nitrate	39. (D3)	0.1 M Tris pH 8.5
40. (D4)	6.0 M Ammonium nitrate	40. (D4)	0.1 M Sodium acetate trihydrate pH 4.6
41. (D5)	6.0 M Ammonium nitrate	41. (D5)	0.1 M BIS-TRIS propane pH 7.0
42. (D6)	6.0 M Ammonium nitrate	42. (D6)	0.1 M Tris pH 8.5
43. (D7)	1.5 M Sodium nitrate	43. (D7)	0.1 M Sodium acetate trihydrate pH 4.6
44. (D8)	1.5 M Sodium nitrate	44. (D8)	0.1 M BIS-TRIS propane pH 7.0
45. (D9)	1.5 M Sodium nitrate	45. (D9)	0.1 M Tris pH 8.5
46. (D10)	4.0 M Sodium nitrate	46. (D10)	0.1 M Sodium acetate trihydrate pH 4.6
47. (D11)	4.0 M Sodium nitrate	47. (D11)	0.1 M BIS-TRIS propane pH 7.0
48. (D12)	4.0 M Sodium nitrate	48. (D12)	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 HT (Deep Well Block) contains ninety-six unique reagents beginning at position A1. To determine the formulation of each reagent, simply read across the page.

HAMPTON RESEARCH
Solutions for Crystal Growth

Well #	Salt	Well #	Buffer ◊
49. (E1)	1.0 M Ammonium phosphate monobasic	49. (E1)	0.1 M Sodium acetate trihydrate pH 4.6
50. (E2)	1.8 M Ammonium phosphate monobasic	50. (E2)	0.1 M Sodium acetate trihydrate pH 4.6
51. (E3)	1.5 M Ammonium phosphate dibasic	51. (E3)	0.1 M Tris pH 8.5
52. (E4)	2.4 M Ammonium phosphate dibasic	52. (E4)	0.1 M Tris pH 8.5
53. (E5)	1.0 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 5.0	53. (E5)	None
54. (E6)	1.0 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 6.9	54. (E6)	None
55. (E7)	1.0 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 8.2	55. (E7)	None
56. (E8)	1.8 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 5.0	56. (E8)	None
57. (E9)	1.8 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 6.9	57. (E9)	None
58. (E10)	1.8 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 8.2	58. (E10)	None
59. (E11)	0.5 M Succinic acid pH 7.0	59. (E11)	0.1 M BIS-TRIS propane pH 7.0
60. (E12)	1.0 M Succinic acid pH 7.0	60. (E12)	0.1 M BIS-TRIS propane pH 7.0
61. (F1)	1.5 M Ammonium sulfate	61. (F1)	0.1 M Sodium acetate trihydrate pH 4.6
62. (F2)	1.5 M Ammonium sulfate	62. (F2)	0.1 M BIS-TRIS propane pH 7.0
63. (F3)	1.5 M Ammonium sulfate	63. (F3)	0.1 M Tris pH 8.5
64. (F4)	2.5 M Ammonium sulfate	64. (F4)	0.1 M Sodium acetate trihydrate pH 4.6
65. (F5)	2.5 M Ammonium sulfate	65. (F5)	0.1 M BIS-TRIS propane pH 7.0
66. (F6)	2.5 M Ammonium sulfate	66. (F6)	0.1 M Tris pH 8.5
67. (F7)	0.8 M Lithium sulfate monohydrate	67. (F7)	0.1 M Sodium acetate trihydrate pH 4.6
68. (F8)	0.8 M Lithium sulfate monohydrate	68. (F8)	0.1 M BIS-TRIS propane pH 7.0
69. (F9)	0.8 M Lithium sulfate monohydrate	69. (F9)	0.1 M Tris pH 8.5
70. (F10)	1.5 M Lithium sulfate monohydrate	70. (F10)	0.1 M Sodium acetate trihydrate pH 4.6
71. (F11)	1.5 M Lithium sulfate monohydrate	71. (F11)	0.1 M BIS-TRIS propane pH 7.0
72. (F12)	1.5 M Lithium sulfate monohydrate	72. (F12)	0.1 M Tris pH 8.5
73. (G1)	1.0 M Magnesium sulfate hydrate	73. (G1)	0.1 M Sodium acetate trihydrate pH 4.6
74. (G2)	1.0 M Magnesium sulfate hydrate	74. (G2)	0.1 M BIS-TRIS propane pH 7.0
75. (G3)	1.0 M Magnesium sulfate hydrate	75. (G3)	0.1 M Tris pH 8.5
76. (G4)	1.8 M Magnesium sulfate hydrate	76. (G4)	0.1 M Sodium acetate trihydrate pH 4.6
77. (G5)	1.8 M Magnesium sulfate hydrate	77. (G5)	0.1 M BIS-TRIS propane pH 7.0
78. (G6)	1.8 M Magnesium sulfate hydrate	78. (G6)	0.1 M Tris pH 8.5
79. (G7)	0.7 M Ammonium tartrate dibasic	79. (G7)	0.1 M Sodium acetate trihydrate pH 4.6
80. (G8)	0.7 M Ammonium tartrate dibasic	80. (G8)	0.1 M BIS-TRIS propane pH 7.0
81. (G9)	0.7 M Ammonium tartrate dibasic	81. (G9)	0.1 M Tris pH 8.5
82. (G10)	1.0 M Ammonium tartrate dibasic	82. (G10)	0.1 M Sodium acetate trihydrate pH 4.6
83. (G11)	1.3 M Ammonium tartrate dibasic	83. (G11)	0.1 M BIS-TRIS propane pH 7.0
84. (G12)	1.4 M Ammonium tartrate dibasic	84. (G12)	0.1 M Tris pH 8.5
85. (H1)	0.6 M Potassium sodium tartrate tetrahydrate	85. (H1)	0.1 M BIS-TRIS propane pH 7.0
86. (H2)	1.2 M Potassium sodium tartrate tetrahydrate	86. (H2)	0.1 M BIS-TRIS propane pH 7.0
87. (H3)	0.6 M Potassium sodium tartrate tetrahydrate	87. (H3)	0.1 M Tris pH 8.5
88. (H4)	1.2 M Potassium sodium tartrate tetrahydrate	88. (H4)	0.1 M Tris pH 8.5
89. (H5)	0.5 M Potassium thiocyanate	89. (H5)	0.1 M Sodium acetate trihydrate pH 4.6
90. (H6)	0.5 M Potassium thiocyanate	90. (H6)	0.1 M BIS-TRIS propane pH 7.0
91. (H7)	0.5 M Potassium thiocyanate	91. (H7)	0.1 M Tris pH 8.5
92. (H8)	4.0 M Ammonium acetate	92. (H8)	0.1 M Sodium acetate trihydrate pH 4.6
93. (H9)	4.0 M Ammonium acetate	93. (H9)	0.1 M BIS-TRIS propane pH 7.0
94. (H10)	4.0 M Ammonium acetate	94. (H10)	0.1 M Tris pH 8.5
95. (H11)	35% v/v Tacsimate pH 7.0	95. (H11)	0.1 M BIS-TRIS propane pH 7.0
96. (H12)	60% v/v Tacsimate pH 7.0	96. (H12)	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 HT (Deep Well Block) contains ninety-six unique reagents beginning at position A1. To determine the formulation of each reagent, simply read across the page.

34 Journey
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Tel: (949) 425-1321 • Fax: (949) 425-1611
E-mail: tech@hrmail.com
Website: www.hamptonresearch.com

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)

HAMPTON RESEARCH
Solutions for Crystal Growth

34 Journey
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e-mail: tech@hrmail.com
Website: www.hamptonresearch.com

SaltRx HT™ - HR2-136 Scoring Sheet	Date:	Date:	Date:
1. (A1) 1.8 M Sodium acetate trihydrate pH 7.0, 0.1 M BIS-TRIS propane pH 7.0			
2. (A2) 2.8 M Sodium acetate trihydrate pH 7.0, 0.1 M BIS-TRIS propane pH 7.0			
3. (A3) 1.5 M Ammonium chloride, 0.1 M Sodium acetate trihydrate pH 4.6			
4. (A4) 1.5 M Ammonium chloride, 0.1 M BIS-TRIS propane pH 7.0			
5. (A5) 1.5 M Ammonium chloride, 0.1 M Tris pH 8.5			
6. (A6) 3.5 M Ammonium chloride, 0.1 M Sodium acetate trihydrate pH 4.6			
7. (A7) 3.5 M Ammonium chloride, 0.1 M BIS-TRIS propane pH 7.0			
8. (A8) 3.5 M Ammonium chloride, 0.1 M Tris pH 8.5			
9. (A9) 2.2 M Sodium chloride, 0.1 M Sodium acetate trihydrate pH 4.6			
10. (A10) 2.2 M Sodium chloride, 0.1 M BIS-TRIS propane pH 7.0			
11. (A11) 2.2 M Sodium chloride, 0.1 M Tris pH 8.5			
12. (A12) 3.2 M Sodium chloride, 0.1 M Sodium acetate trihydrate pH 4.6			
13. (B1) 3.2 M Sodium chloride, 0.1 M BIS-TRIS propane pH 7.0			
14. (B2) 3.2 M Sodium chloride, 0.1 M Tris pH 8.5			
15. (B3) 1.0 M Ammonium citrate dibasic, 0.1 M Sodium acetate trihydrate pH 4.6			
16. (B4) 1.8 M Ammonium citrate dibasic, 0.1 M Sodium acetate trihydrate pH 4.6			
17. (B5) 1.0 M Ammonium citrate tribasic pH 7.0, 0.1 M BIS-TRIS propane pH 7.0			
18. (B6) 2.0 M Ammonium citrate tribasic pH 7.0, 0.1 M BIS-TRIS propane pH 7.0			
19. (B7) 0.7 M Sodium citrate tribasic dihydrate, 0.1 M BIS-TRIS propane pH 7.0			
20. (B8) 0.7 M Sodium citrate tribasic dihydrate, 0.1 M Tris pH 8.5			
21. (B9) 1.2 M Sodium citrate tribasic dihydrate, 0.1 M BIS-TRIS propane pH 7.0			
22. (B10) 1.2 M Sodium citrate tribasic dihydrate, 0.1 M Tris pH 8.5			
23. (B11) 0.4 M Magnesium formate dihydrate, 0.1 M Sodium acetate trihydrate pH 4.6			
24. (B12) 0.4 M Magnesium formate dihydrate, 0.1 M BIS-TRIS propane pH 7.0			
25. (C1) 0.4 M Magnesium formate dihydrate, 0.1 M Tris pH 8.5			
26. (C2) 0.7 M Magnesium formate dihydrate, 0.1 M BIS-TRIS propane pH 7.0			
27. (C3) 2.0 M Sodium formate, 0.1 M Sodium acetate trihydrate pH 4.6			
28. (C4) 2.0 M Sodium formate, 0.1 M BIS-TRIS propane pH 7.0			
29. (C5) 2.0 M Sodium formate, 0.1 M Tris pH 8.5			
30. (C6) 3.5 M Sodium formate, 0.1 M Sodium acetate trihydrate pH 4.6			
31. (C7) 3.5 M Sodium formate, 0.1 M BIS-TRIS propane pH 7.0			
32. (C8) 3.5 M Sodium formate, 0.1 M Tris pH 8.5			
33. (C9) 1.2 M DL-Malic acid pH 7.0, 0.1 M BIS-TRIS propane pH 7.0			
34. (C10) 2.2 M DL-Malic acid pH 7.0, 0.1 M BIS-TRIS propane pH 7.0			
35. (C11) 1.4 M Sodium malonate pH 7.0, 0.1 M BIS-TRIS propane pH 7.0			
36. (C12) 2.4 M Sodium malonate pH 7.0, 0.1 M BIS-TRIS propane pH 7.0			
37. (D1) 2.5 M Ammonium nitrate, 0.1 M Sodium acetate trihydrate pH 4.6			
38. (D2) 2.5 M Ammonium nitrate, 0.1 M BIS-TRIS propane pH 7.0			
39. (D3) 2.5 M Ammonium nitrate, 0.1 M Tris pH 8.5			
40. (D4) 6.0 M Ammonium nitrate, 0.1 M Sodium acetate trihydrate pH 4.6			
41. (D5) 6.0 M Ammonium nitrate, 0.1 M BIS-TRIS propane pH 7.0			
42. (D6) 6.0 M Ammonium nitrate, 0.1 M Tris pH 8.5			
43. (D7) 1.5 M Sodium nitrate, 0.1 M Sodium acetate trihydrate pH 4.6			
44. (D8) 1.5 M Sodium nitrate, 0.1 M BIS-TRIS propane pH 7.0			
45. (D9) 1.5 M Sodium nitrate, 0.1 M Tris pH 8.5			
46. (D10) 4.0 M Sodium nitrate, 0.1 M Sodium acetate trihydrate pH 4.6			
47. (D11) 4.0 M Sodium nitrate, 0.1 M BIS-TRIS propane pH 7.0			
48. (D12) 4.0 M Sodium nitrate, 0.1 M Tris pH 8.5			

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 HT™ - HR2-136 Scoring Sheet	Date:	Date:	Date:
49. (E1) 1.0 M Ammonium phosphate monobasic, 0.1 M Sodium acetate trihydrate pH 4.6			
50. (E2) 1.8 M Ammonium phosphate monobasic, 0.1 M Sodium acetate trihydrate pH 4.6			
51. (E3) 1.5 M Ammonium phosphate dibasic, 0.1 M Tris pH 8.5			
52. (E4) 2.4 M Ammonium phosphate dibasic, 0.1 M Tris pH 8.5			
53. (E5) 1.0 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 5.0			
54. (E6) 1.0 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 6.9			
55. (E7) 1.0 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 8.2			
56. (E8) 1.8 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 5.0			
57. (E9) 1.8 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 6.9			
58. (E10) 1.8 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 8.2			
59. (E11) 0.5 M Succinic acid pH 7.0, 0.1 M BIS-TRIS propane pH 7.0			
60. (E12) 1.0 M Succinic acid pH 7.0, 0.1 M BIS-TRIS propane pH 7.0			
61. (F1) 1.5 M Ammonium sulfate, 0.1 M Sodium acetate trihydrate pH 4.6			
62. (F2) 1.5 M Ammonium sulfate, 0.1 M BIS-TRIS propane pH 7.0			
63. (F3) 1.5 M Ammonium sulfate, 0.1 M Tris pH 8.5			
64. (F4) 2.5 M Ammonium sulfate, 0.1 M Sodium acetate trihydrate pH 4.6			
65. (F5) 2.5 M Ammonium sulfate, 0.1 M BIS-TRIS propane pH 7.0			
66. (F6) 2.5 M Ammonium sulfate, 0.1 M Tris pH 8.5			
67. (F7) 0.8 M Lithium sulfate monohydrate, 0.1 M Sodium acetate trihydrate pH 4.6			
68. (F8) 0.8 M Lithium sulfate monohydrate, 0.1 M BIS-TRIS propane pH 7.0			
69. (F9) 0.8 M Lithium sulfate monohydrate, 0.1 M Tris pH 8.5			
70. (F10) 1.5 M Lithium sulfate monohydrate, 0.1 M Sodium acetate trihydrate pH 4.6			
71. (F11) 1.5 M Lithium sulfate monohydrate, 0.1 M BIS-TRIS propane pH 7.0			
72. (F12) 1.5 M Lithium sulfate monohydrate, 0.1 M Tris pH 8.5			
73. (G1) 1.0 M Magnesium sulfate hydrate, 0.1 M Sodium acetate trihydrate pH 4.6			
74. (G2) 1.0 M Magnesium sulfate hydrate, 0.1 M BIS-TRIS propane pH 7.0			
75. (G3) 1.0 M Magnesium sulfate hydrate, 0.1 M Tris pH 8.5			
76. (G4) 1.8 M Magnesium sulfate hydrate, 0.1 M Sodium acetate trihydrate pH 4.6			
77. (G5) 1.8 M Magnesium sulfate hydrate, 0.1 M BIS-TRIS propane pH 7.0			
78. (G6) 1.8 M Magnesium sulfate hydrate, 0.1 M Tris pH 8.5			
79. (G7) 0.7 M Ammonium tartrate dibasic, 0.1 M Sodium acetate trihydrate pH 4.6			
80. (G8) 0.7 M Ammonium tartrate dibasic, 0.1 M BIS-TRIS propane pH 7.0			
81. (G9) 0.7 M Ammonium tartrate dibasic, 0.1 M Tris pH 8.5			
82. (G10) 1.0 M Ammonium tartrate dibasic, 0.1 M Sodium acetate trihydrate pH 4.6			
83. (G11) 1.3 M Ammonium tartrate dibasic, 0.1 M BIS-TRIS propane pH 7.0			
84. (G12) 1.4 M Ammonium tartrate dibasic, 0.1 M Tris pH 8.5			
85. (H1) 0.6 M Potassium sodium tartrate tetrahydrate, 0.1 M BIS-TRIS propane pH 7.0			
86. (H2) 1.2 M Potassium sodium tartrate tetrahydrate, 0.1 M BIS-TRIS propane pH 7.0			
87. (H3) 0.6 M Potassium sodium tartrate tetrahydrate, 0.1 M Tris pH 8.5			
88. (H4) 1.2 M Potassium sodium tartrate tetrahydrate, 0.1 M Tris pH 8.5			
89. (H5) 0.5 M Potassium thiocyanate, 0.1 M Sodium acetate trihydrate pH 4.6			
90. (H6) 0.5 M Potassium thiocyanate, 0.1 M BIS-TRIS propane pH 7.0			
91. (H7) 0.5 M Potassium thiocyanate, 0.1 M Tris pH 8.5			
92. (H8) 4.0 M Ammonium acetate, 0.1 M Sodium acetate trihydrate pH 4.6			
93. (H9) 4.0 M Ammonium acetate, 0.1 M BIS-TRIS propane pH 7.0			
94. (H10) 4.0 M Ammonium acetate, 0.1 M Tris pH 8.5			
95. (H11) 35% v/v Tacsimate pH 7.0, 0.1 M BIS-TRIS propane pH 7.0			
96. (H12) 60% v/v Tacsimate pH 7.0, 0.1 M BIS-TRIS propane pH 7.0			

HAMPTON RESEARCH
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34 Journey
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