

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 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 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.

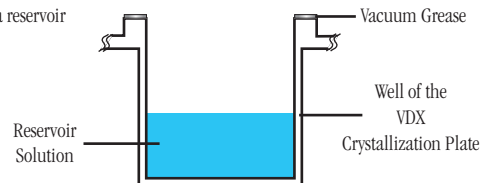
Performing The Screen

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

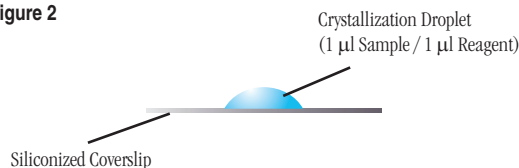
Cross section of a reservoir in the VDX plate.



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

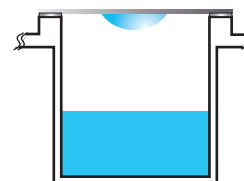


4. Pipet 1 µl of SaltRx 2 reagent 1 from reservoir A1 into the sample droplet and mix by aspirating and dispensing the droplet several times, keeping the tip in the drop during mixing to avoid foaming. See Figure 2.

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.

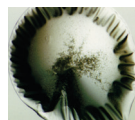
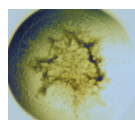


6. Repeat operations 3 through 5 for the remaining 47 SaltRx 2 reagents.
7. If the quantity of sample permits, perform the SaltRx 2 in duplicate and incubate one set of plates at 4°C and the second set at room temperature. Incubate and store the crystallization plates in a stable temperature environment free of vibration.

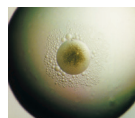
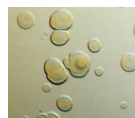
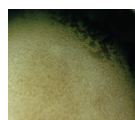
Figure 4
Typical observations in a crystallization experiment



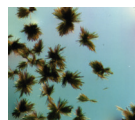
Clear Drop

Skin/
Precipitate

Precipitate

Precipitate/
PhaseQuasi
Crystals

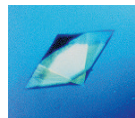
Microcrystals

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 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

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

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

Solutions for Crystal Growth

HAMPTON
RESEARCH

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			

34 Journey
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SaltRx Technical and Frequently Asked Questions

How should one reproduce or optimize SaltRx HT reagents E5-E10 or SaltRx 2 reagents 5-10 which contain sodium/potassium phosphate?

These reagents are a mixture of Sodium phosphate monobasic monohydrate and Potassium phosphate dibasic. For the stock reagents use a 4.0 M Sodium phosphate monobasic monohydrate (catalog number HR2-551) and a 4.0 M Potassium phosphate dibasic (catalog number HR2-635). These are also available together in a kit form called Quik Optimize (catalog number HR2-223). Then use the Sodium/Potassium Phosphate Dilution Table available on the SaltRx web page which shows how much of each stock to mix with water to create the desired pH and salt concentration. Using the stock and table one can screen a concentration range of 0.2 to 4.0 M Sodium potassium phosphate and a pH range of 5.0 to 8.2.

SaltRx REAGENT NUMBER 53 (SaltRx HT E5, SaltRx 2 reagent 5)

1.0 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 5.0
(0.98 M Sodium phosphate monobasic monohydrate, 0.02 M Potassium phosphate dibasic)

To make one milliliter, pipette 750 microliters of water, 245 microliters of Sodium phosphate monobasic monohydrate and 5 microliters of Potassium phosphate dibasic.

SaltRx REAGENT NUMBER 54 (SaltRx HT E6, SaltRx 2 reagent 6)

1.0 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 6.9
(0.35 M Sodium phosphate monobasic monohydrate, 0.65 M Potassium phosphate dibasic)

To make one milliliter, pipette 750 microliters of water, 88 microliters of Sodium phosphate monobasic monohydrate and 162 microliters of Potassium phosphate dibasic.

SaltRx REAGENT NUMBER 55 (SaltRx HT E7, SaltRx 2 reagent 7)

1.0 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 8.2

(0.04 M Sodium phosphate monobasic monohydrate, 0.96 M Potassium phosphate dibasic)

To make one milliliter, pipette 750 microliters of water, 10 microliters of Sodium phosphate monobasic monohydrate and 240 microliters of Potassium phosphate dibasic.

SaltRx REAGENT NUMBER 56 (SaltRx HT E8, SaltRx 2 reagent 8)

1.8 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 5.0

(1.764 M Sodium phosphate monobasic monohydrate, 0.036 M Potassium phosphate dibasic)

To make one milliliter, pipette 550 microliters of water, 441 microliters of Sodium phosphate monobasic monohydrate and 9 microliters of Potassium phosphate dibasic.

SaltRx REAGENT NUMBER 57 (SaltRx HT E9, SaltRx 2 reagent 9)

1.8 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 6.9

(0.63 M Sodium phosphate monobasic monohydrate, 1.17 M Potassium phosphate dibasic)

To make one milliliter, pipette 550 microliters of water, 157 microliters of Sodium phosphate monobasic monohydrate and 293 microliters of Potassium phosphate dibasic.

SaltRx REAGENT NUMBER 58 (SaltRx HT E10, SaltRx 2 reagent 10)

1.8 M Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic / pH 8.2

(0.072 M Sodium phosphate monobasic monohydrate, 1.728 M Potassium phosphate dibasic)

To make one milliliter, pipette 550 microliters of water, 18 microliters of Sodium phosphate monobasic monohydrate and 432 microliters of Potassium phosphate dibasic.

HR2-221 Quik Screen 10 ml, tube format

HR2-223 Quik Optimize 100 ml, 2 bottles

HR2-551 Sodium phosphate monobasic monohydrate - 4.0 M 200 ml - solution

HR2-635 Potassium phosphate dibasic - 4.0 M solution 200 ml