Grid Screen Salt HT™

HAMPTON RESEARCH
Solutions for Crystal Growth

User Guide **HR2-248 (pg 1)**

Applications

Preliminary screen for the crystallization of soluble proteins as well as soluble biological macromolecules.

Features

- Single 96 Deep Well block format
- Compatible with robotics and multi-channel pipets
- Primary screen variables : salt, salt concentration, and pH
- Samples pH 4.0 – 9.0
- Preformulated, ready to screen

General Description

Grid Screen Salt HT™ is a 96 reagent crystallization screen that combines the strategies of Grid Screen™ Ammonium Sulfate, Grid Screen™ Sodium Malonate, Quik Screen™, and Grid Screen™ Sodium Chloride into a highly effective and efficient format. This kit allows one to evaluate a large variety of potential crystallization conditions with the 96 unique reagents.

Grid Screen Salt HT is supplied in a sterile, polypropylene 96 Deep Well block, each reservoir containing 1 ml of sterile filtered reagent. The block is compatible with robotic and multi-channel pipet liquid handling systems and is heat sealed using a special polypropylene backed film. Each Grid Screen Salt HT kit is supplied with an adhesive sealing film which can be used to seal the block after removing the heat seal. Additional adhesive sealing films can be obtained from Hampton Research.

Within the 96 Deep Well block, rows A through B feature the 24 reagents of Grid Screen Ammonium Sulfate (HR2-211). These reagent variables consist of a single precipitant screened at four unique concentrations (0.8, 1.6, 2.4, 3.0 M) versus six precise levels of pH 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0. The concentration of the precipitant and the buffer are based upon those that are most frequently reported to offer success. The pH of each Grid Screen Ammonium Sulfate is the actual final measured pH of the solution.

Rows C through D feature the 24 reagents of Grid Screen Sodium Malonate (HR2-247). This kit systematically screens four pH levels (4.0, 5.0, 6.0, 7.0) versus six concentrations of Sodium Malonate (1.0, 1.5, 2.4, 2.9, and 3.4 M). Using Grid Screen Sodium Malonate gives you the exact concentration and pH that is being screened.

Rows E through F feature the 24 reagents of Quick Screen™ (HR2-221). The Quik Screen kit screens two of the most successful phosphate reagents for the crystallization of biological molecules. Quik Screen is composed of high purity Sodium phosphate monobasic monohydrate (NaH_2PO_4, M_r 137.99), Potassium phosphate dibasic(K_2HPO_4, M_r 174.18), and ultrapure water. Reagent pH is determined by the ratio of the two salts in solution. The concentration range of the kit is 0.8 to 1.8 M (0.8, 1.0, 1.4, and 1.8 M) in phosphate. The pH range of the screen is 5.0 to 8.2 (5.0, 5.6, 6.3, 6.9, 7.5, and 8.2).

The Quik Screen formulation has been used successfully in both small and large scale crystallization of biological macromolecules. The phosphate system utilized by Quik Screen is stable, safe, versatile, easy to reproduce, cost-effective, and easy to scale up for large scale batch crystallization. Quik Screen was developed by Macrocrystal Oy (Olarinluoma 16, Fin-02200 Espoo Finland) and is manufactured and distributed exclusively by Hampton Research.

Rows G through H feature the 24 reagents of Grid Screen Sodium Chloride (HR2-219). Screen four concentrations of Sodium chloride at 1.0, 2.0, 3.0, and 4.0 M versus six precise pH levels of 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0. The pH of each Grid Screen Sodium Chloride reagent is the actual final measured pH of the solution.

Refer to the enclosed Grid Screen Salt HT reagent formulation for additional information on all 96 reagents.

Sample Preparation

The sample should be as pure as is practically possible (>95%) and free of amorphous and particulate material. Remove amorphous material by centrifugation prior to use.

Since divalent cations can complex with the phosphate in Quik Screen and form inorganic salt crystals, the sample should be free of and contain less than 10 mM of divalent cations such as: magnesium, calcium, and zinc. The presence of any level of divalent cations can lead to inorganic salt crystals.

The recommended sample concentration is 5 to 25 mg/ml in water or dilute (less than 50 mM) buffer. Initially, the sample should be free of any unnecessary additives in order to observe the effect of the Grid Screen Salt HT variables. However, common sense requires this to be balanced with the need to maintain the stability and solubility of the sample, so include those reagents crucial for sample stability, solubility, and homogeneity. Ideally, the initial screen should be preformed with a sample which has the minimal concentrations of ligands, ions, reducing agents, or other additives required by the sample for solubility, stability, activity, and homogeneity.

For additional sample preparation recommendation see Crystal Growth 101 - Preliminary Sample Preparation bulletin from Hampton Research.

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Preparing the Deep Well Block for Use

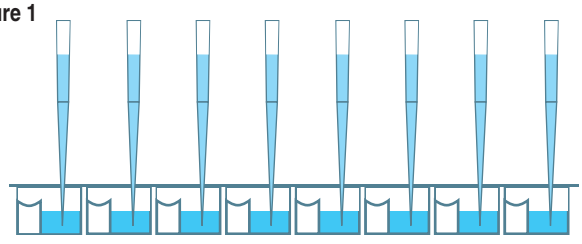
It is recommended the Deep Well block be centrifuged and at 25°C before removing the sealing film. Centrifugation at 500 rpm for five minutes will remove stray reagent from the sealing film. Removing the reagent from the film prevents stray reagent droplets from falling into neighboring wells during 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 the pierced for reagent access. It is recommended film removal only be performed when the Deep Well block is at 25°C (room temperature).

Performing The Screen

Manual Method - Sitting Drop Vapor Diffusion

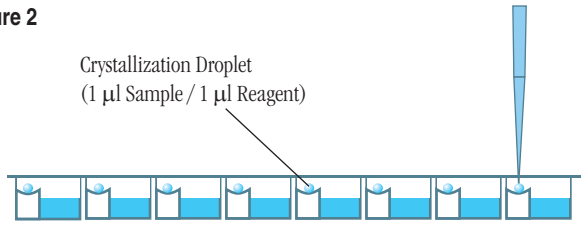
1. Using a 96 well sitting drop vapor diffusion plate, pipet the recommended volume (typically 50 to 100 microliters) of crystallization reagent from the Deep Well block into the reservoirs of the crystallization plate. The Deep Well block is compatible with 8 and 12 channel pipets as well as many automated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

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 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.

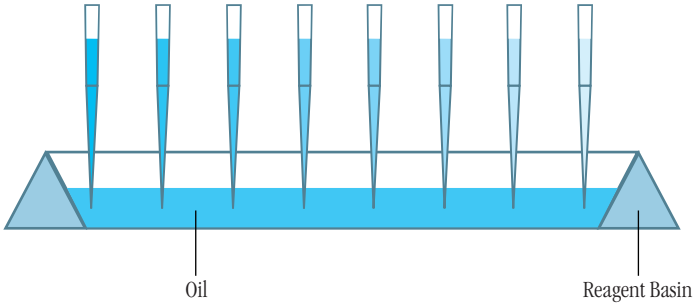
4. Seal the crystallization plate as per the manufacturers recommendation. Most 96 well crystallization plates are sealed using a clear sealing tape or film. View and score the experiment as desired. See Hampton Research technical bulletin Crystal Growth 101 - Viewing Crystallization Experiments for additional information on viewing drops.

5. Seal the remaining reagent in the Deep Well block using either clear sealing tape, film, cap mat, or AlumaSeal™ II.

Manual Method - Microbatch 96 well format

1. Using a 96 well clear polystyrene microplate (U-bottom recommended for best drop centering, flat-bottom recommended for best optics) pipet 50 to 150 microliters of Microbatch compatible oil into each of the 96 reservoirs. This can be accomplished using an 8 or 12 channel pipet and pipetting the oil from a reagent basin. See Figure 3 below.

Figure 3



2. Once the plate is oiled, use an 8 or 12 channel pipet to aspirate reagent from the Deep Well block and dispense the reagent under the oil in the Microbatch plate. Change tips when changing reagent to prevent cross reagent contamination. To save time and pipet tips, set multiple plates at one time. See Figure 4 below.

Figure 4

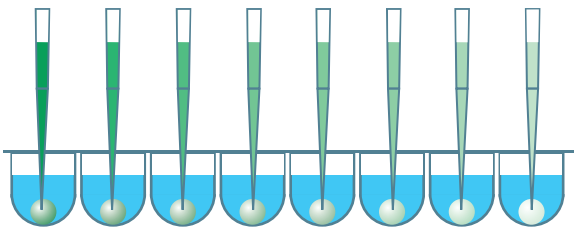
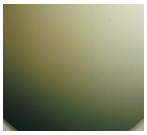
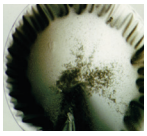
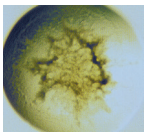
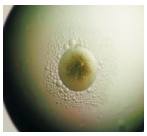


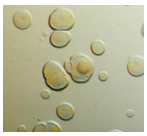
Figure 6
Typical observations in a crystallization experiment

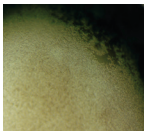

Clear Drop


Skin/Precipitate

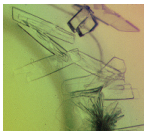

Precipitate


Precipitate/Phase

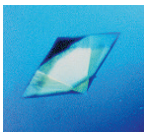

Quasi Crystals


Microcrystals


Needle Cluster

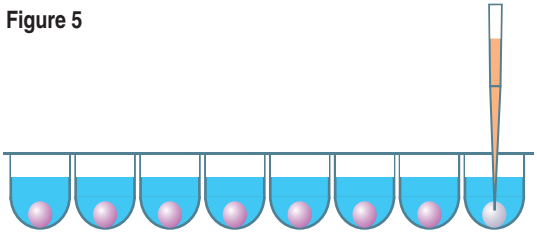

Plates


Rod Cluster


Single Crystal

3. Using a single channel pipet, aspirate the sample and dispense the sample under oil in the Microbatch plate. It is not necessary to dispense the sample drop into the reagent drop or mix the drops. See Figure 5 below.

Figure 5



4. After all reagent and sample drops have been dispensed to the Microbatch plate, place the loose fitting clear cover on the Microbatch plate and centrifuge the plate for 10 minutes at 500 rpm. Centrifugation will cause the drops to coalesce into a single drop.

Note: If the drops appear flat or is fragmented into multiple drops, the centrifugation speed is too high and the centrifugation time it too long - adjust to obtain a spherical single drop in the center of the well.

5. Store the plates with the loose fitting clear polystyrene cover and observe for crystals. See Hampton Research technical bulletin Crystal Growth 101 - Viewing Crystallization Experiments for additional information on viewing drops.

Grid Screen Salt 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 and sealing of the crystallization 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 3) shows typical examples of what one might observe in a crystallization experiment.

Interpreting Grid Screen Salt 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.

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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.

Grid Screen Salt 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 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.

Related Products

HR2-211 - Grid Screen™ Ammonium Sulfate Kit - 10 ml tube format
HR2-219 - Grid Screen™ Sodium Chloride Kit - 10 ml tube format
HR2-221 - Quik Screen™ Kit - 10 ml tube format
HR2-247 - Grid Screen™ Sodium Malonate Kit - 10 ml tube format

HR3-609 - Crystal Clear Sealing Film - 100 pack
HR3-511 - 1.88" wide Crystal Clear Sealing Tape - 1 roll
HR4-506 - 3" wide Crystal Clear Sealing Tape - 1 roll
HR8-069 - AlumaSeal™ II Sealing Film - 100 pack

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.

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.

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. A protein crystallization strategy using automated grid searches on successively finer grids. Patricia C. Weber. Methods: A Companion to Methods in Enzymology Vol. 1, No. 1, August, pp. 31-37, 1990.

Technical Support

Inquiries regarding Grid Screen Salt 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 4:30 p.m. USA Pacific Standard Time.

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Reagents A1 - B12 are equivalent to Hampton Research Grid Screen™ Ammonium Sulfate HR2-211

Well #	Precipitant	Well #	Buffer ◊	Well #	Custom Shop ◊
A1.	0.8 M Ammonium sulfate	A1.	0.1 M Citric acid pH 4.0	A1.	HR2-924-A1
A2.	0.8 M Ammonium sulfate	A2.	0.1 M Citric acid pH 5.0	A2.	HR2-924-A2
A3.	0.8 M Ammonium sulfate	A3.	0.1 M MES monohydrate pH 6.0	A3.	HR2-924-A3
A4.	0.8 M Ammonium sulfate	A4.	0.1 M HEPES pH 7.0	A4.	HR2-924-A4
A5.	0.8 M Ammonium sulfate	A5.	0.1 M Tris pH 8.0	A5.	HR2-924-A5
A6.	0.8 M Ammonium sulfate	A6.	0.1 M BICINE pH 9.0	A6.	HR2-924-A6
A7.	1.6 M Ammonium sulfate	A7.	0.1 M Citric acid pH 4.0	A7.	HR2-924-B1
A8.	1.6 M Ammonium sulfate	A8.	0.1 M Citric acid pH 5.0	A8.	HR2-924-B2
A9.	1.6 M Ammonium sulfate	A9.	0.1 M MES monohydrate pH 6.0	A9.	HR2-924-B3
A10.	1.6 M Ammonium sulfate	A10.	0.1 M HEPES pH 7.0	A10.	HR2-924-B4
A11.	1.6 M Ammonium sulfate	A11.	0.1 M Tris pH 8.0	A11.	HR2-924-B5
A12.	1.6 M Ammonium sulfate	A12.	0.1 M BICINE pH 9.0	A12.	HR2-924-B6
B1.	2.4 M Ammonium sulfate	B1.	0.1 M Citric acid pH 4.0	B1.	HR2-924-C1
B2.	2.4 M Ammonium sulfate	B2.	0.1 M Citric acid pH 5.0	B2.	HR2-924-C2
B3.	2.4 M Ammonium sulfate	B3.	0.1 M MES monohydrate pH 6.0	B3.	HR2-924-C3
B4.	2.4 M Ammonium sulfate	B4.	0.1 M HEPES pH 7.0	B4.	HR2-924-C4
B5.	2.4 M Ammonium sulfate	B5.	0.1 M Tris pH 8.0	B5.	HR2-924-C5
B6.	2.4 M Ammonium sulfate	B6.	0.1 M BICINE pH 9.0	B6.	HR2-924-C6
B7.	3.0 M Ammonium sulfate	B7.	0.1 M Citric acid pH 4.0	B7.	HR2-924-D1
B8.	3.0 M Ammonium sulfate	B8.	0.1 M Citric acid pH 5.0	B8.	HR2-924-D2
B9.	3.0 M Ammonium sulfate	B9.	0.1 M MES monohydrate pH 6.0	B9.	HR2-924-D3
B10.	3.0 M Ammonium sulfate	B10.	0.1 M HEPES pH 7.0	B10.	HR2-924-D4
B11.	3.0 M Ammonium sulfate	B11.	0.1 M Tris pH 8.0	B11.	HR2-924-D5
B12.	3.0 M Ammonium sulfate	B12.	0.1 M BICINE pH 9.0	B12.	HR2-924-D6

Reagents C1 - D12 are equivalent to Hampton Research Grid Screen™ Sodium Malonate HR2-247

Well #	Precipitant	Well #	Buffer ◊	Well #	Custom Shop ◊
C1.	1.0 M Sodium malonate pH 4.0	C1.	None	C1.	HR2-947-A1
C2.	1.5 M Sodium malonate pH 4.0	C2.	None	C2.	HR2-947-A2
C3.	1.9 M Sodium malonate pH 4.0	C3.	None	C3.	HR2-947-A3
C4.	2.4 M Sodium malonate pH 4.0	C4.	None	C4.	HR2-947-A4
C5.	2.9 M Sodium malonate pH 4.0	C5.	None	C5.	HR2-947-A5
C6.	3.4 M Sodium malonate pH 4.0	C6.	None	C6.	HR2-947-A6
C7.	1.0 M Sodium malonate pH 5.0	C7.	None	C7.	HR2-947-B1
C8.	1.5 M Sodium malonate pH 5.0	C8.	None	C8.	HR2-947-B2
C9.	1.9 M Sodium malonate pH 5.0	C9.	None	C9.	HR2-947-B3
C10.	2.4 M Sodium malonate pH 5.0	C10.	None	C10.	HR2-947-B4
C11.	2.9 M Sodium malonate pH 5.0	C11.	None	C11.	HR2-947-B5
C12.	3.4 M Sodium malonate pH 5.0	C12.	None	C12.	HR2-947-B6
D1.	1.0 M Sodium malonate pH 6.0	D1.	None	D1.	HR2-947-C1
D2.	1.5 M Sodium malonate pH 6.0	D2.	None	D2.	HR2-947-C2
D3.	1.9 M Sodium malonate pH 6.0	D3.	None	D3.	HR2-947-C3
D4.	2.4 M Sodium malonate pH 6.0	D4.	None	D4.	HR2-947-C4
D5.	2.9 M Sodium malonate pH 6.0	D5.	None	D5.	HR2-947-C5
D6.	3.4 M Sodium malonate pH 6.0	D6.	None	D6.	HR2-947-C6
D7.	1.0 M Sodium malonate pH 7.0	D7.	None	D7.	HR2-947-D1
D8.	1.5 M Sodium malonate pH 7.0	D8.	None	D8.	HR2-947-D2
D9.	1.9 M Sodium malonate pH 7.0	D9.	None	D9.	HR2-947-D3
D10.	2.4 M Sodium malonate pH 7.0	D10.	None	D10.	HR2-947-D4
D11.	2.9 M Sodium malonate pH 7.0	D11.	None	D11.	HR2-947-D5
D12.	3.4 M Sodium malonate pH 7.0	D12.	None	D12.	HR2-947-D6

◊ Hampton Research Custom Shop™ individual reagents available in 185 ml volumes.

Grid Screen Salt 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.

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Reagents E1 - F12 are equivalent to Hampton Research Quik Screen™ HR2-221

Well #	Sodium phosphate monobasic monohydrate [M]	Well #	Potassium phosphate dibasic [M]	Well #	Combined Concentration and pH	Well #	Custom Shop ◊
E1.	0.784	E1.	0.016	E1.	0.8 M Sodium / Potassium phosphate pH 5.0	E1.	HR2-921-A1
E2.	0.72	E2.	0.080	E2.	0.8 M Sodium / Potassium phosphate pH 5.6	E2.	HR2-921-A2
E3.	0.52	E3.	0.28	E3.	0.8 M Sodium / Potassium phosphate pH 6.3	E3.	HR2-921-A3
E4.	0.28	E4.	0.52	E4.	0.8 M Sodium / Potassium phosphate pH 6.9	E4.	HR2-921-A4
E5.	0.12	E5.	0.68	E5.	0.8 M Sodium / Potassium phosphate pH 7.5	E5.	HR2-921-A5
E6.	0.032	E6.	0.768	E6.	0.8 M Sodium / Potassium phosphate pH 8.2	E6.	HR2-921-A6
E7.	0.980	E7.	0.020	E7.	1.0 M Sodium / Potassium phosphate pH 5.0	E7.	HR2-921-B1
E8.	0.90	E8.	0.10	E8.	1.0 M Sodium / Potassium phosphate pH 5.6	E8.	HR2-921-B2
E9.	0.65	E9.	0.35	E9.	1.0 M Sodium / Potassium phosphate pH 6.3	E9.	HR2-921-B3
E10.	0.35	E10.	0.65	E10.	1.0 M Sodium / Potassium phosphate pH 6.9	E10.	HR2-921-B4
E11.	0.15	E11.	0.85	E11.	1.0 M Sodium / Potassium phosphate pH 7.5	E11.	HR2-921-B5
E12.	0.04	E12.	0.96	E12.	1.0 M Sodium / Potassium phosphate pH 8.2	E12.	HR2-921-B6
F1.	1.372	F1.	0.028	F1.	1.4 M Sodium / Potassium phosphate pH 5.0	F1.	HR2-921-C1
F2.	1.26	F2.	0.14	F2.	1.4 M Sodium / Potassium phosphate pH 5.6	F2.	HR2-921-C2
F3.	0.91	F3.	0.49	F3.	1.4 M Sodium / Potassium phosphate pH 6.3	F3.	HR2-921-C3
F4.	0.49	F4.	0.91	F4.	1.4 M Sodium / Potassium phosphate pH 6.9	F4.	HR2-921-C4
F5.	0.21	F5.	1.19	F5.	1.4 M Sodium / Potassium phosphate pH 7.5	F5.	HR2-921-C5
F6.	0.056	F6.	1.344	F6.	1.4 M Sodium / Potassium phosphate pH 8.2	F6.	HR2-921-C6
F7.	1.764	F7.	0.036	F7.	1.8 M Sodium / Potassium phosphate pH 5.0	F7.	HR2-921-D1
F8.	1.62	F8.	0.18	F8.	1.8 M Sodium / Potassium phosphate pH 5.6	F8.	HR2-921-D2
F9.	1.17	F9.	0.63	F9.	1.8 M Sodium / Potassium phosphate pH 6.3	F9.	HR2-921-D3
F10.	0.63	F10.	1.17	F10.	1.8 M Sodium / Potassium phosphate pH 6.9	F10.	HR2-921-D4
F11.	0.27	F11.	1.53	F11.	1.8 M Sodium / Potassium phosphate pH 7.5	F11.	HR2-921-D5
F12.	0.072	F12.	1.728	F12.	1.8 M Sodium / Potassium phosphate pH 8.2	F12.	HR2-921-D6

Reagents G1 - H12 are equivalent to Hampton Research Grid Screen™ Sodium Chloride HR2-219

Well #	Precipitant	Well #	Buffer ◊	Well #	Custom Shop ◊
G1.	1.0 M Sodium chloride	G1.	0.1 M Citric acid pH 4.0	G1.	HR2-932-A1
G2.	1.0 M Sodium chloride	G2.	0.1 M Citric acid pH 5.0	G2.	HR2-932-A2
G3.	1.0 M Sodium chloride	G3.	0.1 M MES monohydrate pH 6.0	G3.	HR2-932-A3
G4.	1.0 M Sodium chloride	G4.	0.1 M HEPES pH 7.0	G4.	HR2-932-A4
G5.	1.0 M Sodium chloride	G5.	0.1 M Tris pH 8.0	G5.	HR2-932-A5
G6.	1.0 M Sodium chloride	G6.	0.1 M BICINE pH 9.0	G6.	HR2-932-A6
G7.	2.0 M Sodium chloride	G7.	0.1 M Citric acid pH 4.0	G7.	HR2-932-B1
G8.	2.0 M Sodium chloride	G8.	0.1 M Citric acid pH 5.0	G8.	HR2-932-B2
G9.	2.0 M Sodium chloride	G9.	0.1 M MES monohydrate pH 6.0	G9.	HR2-932-B3
G10.	2.0 M Sodium chloride	G10.	0.1 M HEPES pH 7.0	G10.	HR2-932-B4
G11.	2.0 M Sodium chloride	G11.	0.1 M Tris pH 8.0	G11.	HR2-932-B5
G12.	2.0 M Sodium chloride	G12.	0.1 M BICINE pH 9.0	G12.	HR2-932-B6
H1.	3.0 M Sodium chloride	H1.	0.1 M Citric acid pH 4.0	H1.	HR2-932-C1
H2.	3.0 M Sodium chloride	H2.	0.1 M Citric acid pH 5.0	H2.	HR2-932-C2
H3.	3.0 M Sodium chloride	H3.	0.1 M MES monohydrate pH 6.0	H3.	HR2-932-C3
H4.	3.0 M Sodium chloride	H4.	0.1 M HEPES pH 7.0	H4.	HR2-932-C4
H5.	3.0 M Sodium chloride	H5.	0.1 M Tris pH 8.0	H5.	HR2-932-C5
H6.	3.0 M Sodium chloride	H6.	0.1 M BICINE pH 9.0	H6.	HR2-932-C6
H7.	4.0 M Sodium chloride	H7.	0.1 M Citric acid pH 4.0	H7.	HR2-932-D1
H8.	4.0 M Sodium chloride	H8.	0.1 M Citric acid pH 5.0	H8.	HR2-932-D2
H9.	4.0 M Sodium chloride	H9.	0.1 M MES monohydrate pH 6.0	H9.	HR2-932-D3
H10.	4.0 M Sodium chloride	H10.	0.1 M HEPES pH 7.0	H10.	HR2-932-D4
H11.	4.0 M Sodium chloride	H11.	0.1 M Tris pH 8.0	H11.	HR2-932-D5
H12.	4.0 M Sodium chloride	H12.	0.1 M BICINE pH 9.0	H12.	HR2-932-D6

◊ Hampton Research Custom Shop™
individual reagents available in 185 ml volumes.

Grid Screen Salt 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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E-mail: tech@hrmail.com
Website: www.hamptonresearch.com

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)

Grid Screen Salt HT™ - HR2-248 Scoring Sheet	Date:	Date:	Date:
A1. 0.8 M Ammonium sulfate, 0.1 M Citric acid pH 4.0			
A2. 0.8 M Ammonium sulfate, 0.1 M Citric acid pH 5.0			
A3. 0.8 M Ammonium sulfate, 0.1 M MES monohydrate pH 6.0			
A4. 0.8 M Ammonium sulfate, 0.1 M HEPES pH 7.0			
A5. 0.8 M Ammonium sulfate, 0.1 M Tris pH 8.0			
A6. 0.8 M Ammonium sulfate, 0.1 M BICINE pH 9.0			
A7. 1.6 M Ammonium sulfate, 0.1 M Citric acid pH 4.0			
A8. 1.6 M Ammonium sulfate, 0.1 M Citric acid pH 5.0			
A9. 1.6 M Ammonium sulfate, 0.1 M MES monohydrate pH 6.0			
A10. 1.6 M Ammonium sulfate, 0.1 M HEPES pH 7.0			
A11. 1.6 M Ammonium sulfate, 0.1 M Tris pH 8.0			
A12. 1.6 M Ammonium sulfate, 0.1 M BICINE pH 9.0			
B1. 2.4 M Ammonium sulfate, 0.1 M Citric acid pH 4.0			
B2. 2.4 M Ammonium sulfate, 0.1 M Citric acid pH 5.0			
B3. 2.4 M Ammonium sulfate, 0.1 M MES monohydrate pH 6.0			
B4. 2.4 M Ammonium sulfate, 0.1 M HEPES pH 7.0			
B5. 2.4 M Ammonium sulfate, 0.1 M Tris pH 8.0			
B6. 2.4 M Ammonium sulfate, 0.1 M BICINE pH 9.0			
B7. 3.0 M Ammonium sulfate, 0.1 M Citric acid pH 4.0			
B8. 3.0 M Ammonium sulfate, 0.1 M Citric acid pH 5.0			
B9. 3.0 M Ammonium sulfate, 0.1 M MES monohydrate pH 6.0			
B10. 3.0 M Ammonium sulfate, 0.1 M HEPES pH 7.0			
B11. 3.0 M Ammonium sulfate, 0.1 M Tris pH 8.0			
B12. 3.0 M Ammonium sulfate, 0.1 M BICINE pH 9.0			
C1. 1.0 M Sodium malonate pH 4.0			
C2. 1.5 M Sodium malonate pH 4.0			
C3. 1.9 M Sodium malonate pH 4.0			
C4. 2.4 M Sodium malonate pH 4.0			
C5. 2.9 M Sodium malonate pH 4.0			
C6. 3.4 M Sodium malonate pH 4.0			
C7. 1.0 M Sodium malonate pH 5.0			
C8. 1.5 M Sodium malonate pH 5.0			
C9. 1.9 M Sodium malonate pH 5.0			
C10. 2.4 M Sodium malonate pH 5.0			
C11. 2.9 M Sodium malonate pH 5.0			
C12. 3.4 M Sodium malonate pH 5.0			
D1. 1.0 M Sodium malonate pH 6.0			
D2. 1.5 M Sodium malonate pH 6.0			
D3. 1.9 M Sodium malonate pH 6.0			
D4. 2.4 M Sodium malonate pH 6.0			
D5. 2.9 M Sodium malonate pH 6.0			
D6 3.4 M Sodium malonate pH 6.0			
D7. 1.0 M Sodium malonate pH 7.0			
D8. 1.5 M Sodium malonate pH 7.0			
D9. 1.9 M Sodium malonate pH 7.0			
D10. 2.4 M Sodium malonate pH 7.0			
D11. 2.9 M Sodium malonate pH 7.0			
D12. 3.4 M Sodium malonate pH 7.0			

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Solutions for Crystal Growth

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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)

Grid Screen Salt HT™ - HR2-248 Scoring Sheet	Date:	Date:	Date:
E1. 0.8 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 5.0			
E2. 0.8 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 5.6			
E3. 0.8 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 6.3			
E4. 0.8 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 6.9			
E5. 0.8 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 7.5			
E6. 0.8 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 8.2			
E7. 1.0 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 5.0			
E8. 1.0 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 5.6			
E9. 1.0 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 6.3			
E10. 1.0 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 6.9			
E11. 1.0 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 7.5			
E12. 1.0 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 8.2			
F1. 1.4 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 5.0			
F2. 1.4 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 5.6			
F3. 1.4 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 6.3			
F4. 1.4 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 6.9			
F5. 1.4 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 7.5			
F6. 1.4 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 8.2			
F7. 1.8 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 5.0			
F8. 1.8 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 5.6			
F9. 1.8 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 6.3			
F10. 1.8 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 6.9			
F11. 1.8 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 7.5			
F12. 1.8 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 8.2			
G1. 1.0 M Sodium chloride, 0.1 M Citric acid pH 4.0			
G2. 1.0 M Sodium chloride, 0.1 M Citric acid pH 5.0			
G3. 1.0 M Sodium chloride, 0.1 M MES monohydrate pH 6.0			
G4. 1.0 M Sodium chloride, 0.1 M HEPES pH 7.0			
G5. 1.0 M Sodium chloride, 0.1 M Tris pH 8.0			
G6. 1.0 M Sodium chloride, 0.1 M BICINE pH 9.0			
G7. 2.0 M Sodium chloride, 0.1 M Citric acid pH 4.0			
G8. 2.0 M Sodium chloride, 0.1 M Citric acid pH 5.0			
G9. 2.0 M Sodium chloride, 0.1 M MES monohydrate pH 6.0			
G10. 2.0 M Sodium chloride, 0.1 M HEPES pH 7.0			
G11. 2.0 M Sodium chloride, 0.1 M Tris pH 8.0			
G12. 2.0 M Sodium chloride, 0.1 M BICINE pH 9.0			
H1. 3.0 M Sodium chloride, 0.1 M Citric acid pH 4.0			
H2. 3.0 M Sodium chloride, 0.1 M Citric acid pH 5.0			
H3. 3.0 M Sodium chloride, 0.1 M MES monohydrate pH 6.0			
H4. 3.0 M Sodium chloride, 0.1 M HEPES pH 7.0			
H5. 3.0 M Sodium chloride, 0.1 M Tris pH 8.0			
H6. 3.0 M Sodium chloride, 0.1 M BICINE pH 9.0			
H7. 4.0 M Sodium chloride, 0.1 M Citric acid pH 4.0			
H8. 4.0 M Sodium chloride, 0.1 M Citric acid pH 5.0			
H9. 4.0 M Sodium chloride, 0.1 M MES monohydrate pH 6.0			
H10. 4.0 M Sodium chloride, 0.1 M HEPES pH 7.0			
H11. 4.0 M Sodium chloride, 0.1 M Tris pH 8.0			
H12. 4.0 M Sodium chloride, 0.1 M BICINE pH 9.0			

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