Index™

HAMPTON RESEARCH
Solutions for Crystal Growth

User Guide **HR2-144 (pg 1)**

Application
Crystallization screen for proteins, peptides, nucleic acids and water soluble small molecules.

Features
Screens classic, contemporary and new crystallization reagent systems Compatible with MicroBatch, Vapor Diffusion and Liquid Diffusion methods Samples pH 3.5 to 8.5 Specially formulated reagent zones

- Classic salts versus pH
- Neutralized organic acids
- High [salt] with low [polymer]
- High [polymer] with low [salt]
- PEG & Salt versus pH
- PEG & Salt
- Low ionic strength versus pH

General Description
Index™ is a kit of 96 preformulated reagents designed to provide a rapid screening method for the crystallization of biological macromolecules including proteins, peptides, and nucleic acids. Index is a straightforward, effective, and practical kit for determining preliminary crystallization conditions. Index is also effective in determining the solubility of a macromolecule in a wide range of precipitants and pH.

Background
Index is designed as a 96 reagent crystallization screen than combines the strategies of the Grid Screen, Sparse Matrix, and Incomplete Factorial with classical, contemporary and new crystallization reagent systems into a highly effective and efficient format. Index was designed, developed and evaluated to 1) be compatible with MicroBatch, Vapor Diffusion, and Liquid Diffusion crystallization methodologies, 2) evaluate classical, contemporary and new crystallization reagent systems, 3) efficiently sample crystallization reagent, concentration and pH space using 96 conditions, 4) combine the most effective features of the Grid Screen, Sparse Matrix and Incomplete Factorial methodologies, and 5) demonstrate that each condition is effective as producing crystals of biological macromolecules.

Index crystallization reagents are compatible with Paraffin (mineral) and Silicon based crystallization oils. Index is the first commercially available crystallization screen specifically designed and available to be compatible with MicroBatch, Vapor or Liquid Diffusion crystallization methodologies.

Index utilizes a broad, yet selective portfolio of crystallization reagent systems which encompasses the following: Classic reagents such as ammonium sulfate and sodium potassium phosphate. Contemporary reagents such as polyethylene glycols and MPD. New crystallization reagent systems such as the neutralized Organic Acids Sodium Malonate and Succinate along with Tacsimate and the Pentaerythritols. These reagent systems are formulated across a sparse matrix and incomplete factorial of concentration ranges, a pH range of 3.5 to 8.5, low ionic strength, high ionic strength, and mixed polymer/salt including halides for potential phasing.

Index samples the classical and often effective simple reagent ammonium sulfate in a Grid Screen format across the pH range 3.5 to 8.5. Classical salts Sodium Chloride, Phosphate and Formate are also sampled across a broad range of pH. Successful crystallization screening in this zone of Index might indicate a Grid Screen optimization using a simple salt versus pH approach might be useful for optimization and production of crystals.

Neutralized organic acids have recently been reported as highly effective crystallization reagents. These Index salts include Malonate, Citrate, Succinate, Malate, Formate, Acetate, Tartrate, and Tacsimate.

Relatively high supersaturation levels of salts combined with low concentration of polymers are reported as effective crystallization reagent systems and are found in the Index reagents 30-36.

Some proteins, especially intact and fragmented antibodies respond well to low ionic strength crystallization reagent systems which are found in Index reagents 37-48.

Non-volatile organics such as MPD as well as polyols such as PEG 400 and low molecular weight PEG MME are frequently reported in the literature as useful reagents in the crystallization of nucleic acids and these reagents are also effective with proteins, especially when combined with salts. More recently, a class of reagents, pentaerythritols, has been reported as effective crystallization reagents.

Currently, if one were to select the most reported precipitant system successful in producing single crystals of biological macromolecules, it would be the combination of high purity polyethylene glycols with salts. Between 1999 and 2002 60% of the crystallization reported in the literature utilized a polyethylene glycol/salt reagent formulation. More than 30 Index conditions evaluate this highly effective reagent combination across a broad pH range.

Index is formulated in zones. Classic salts versus pH. Neutralized organic acids. High [salt] with low [polymer]. High [polymer] with low [salt]. Low ionic strength versus pH. PEG & Salt versus pH. PEG & Salt. If one zone is more effective at producing crystals than another zone, then further crystallization screening and/or optimization could or should focus on this reagent system zone. Zone formulation makes interpretation of screen results a bit easier and much faster.

Many of the Index reagent formulations were selected from the literature based on the reagent's relative efficacy in producing crystals of biological macromolecular crystals. Other reagents were selected and in a single case, synthesized based on their unique chemical properties, their compatibility with MicroBatch, Vapor and Liquid Diffusion methods, and their ability to produce crystals where at times, the other reagent systems failed. These reagent systems, not strongly represented in the literature were evaluated

at Hampton Research using a portfolio of more than 40 biological macromolecules. Finally, a sampling of the Index formulations were designed using the Incomplete Factorial approach and again evaluated at Hampton Research using a portfolio of more than 40 biological macromolecules. Further evaluation of Index was performed in collaboration with academic and industrial crystallography labs in order to test and refine the Index formulation. Each reagent formulation in Index has produced a crystal of a biological macromolecule.

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.

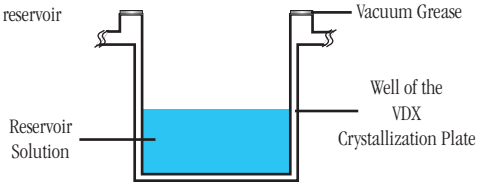
The recommended sample concentration is 5 to 25 mg/ml in dilute buffer (10 to 25 mM). The sample should be free of any unnecessary additives in order to observe the effect of the Index variables. Ideally, the initial screen should be performed with a sample which has been dialyzed against dilute buffer (such as 25 mM sodium Hepes 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 Index with the Hanging Drop Vapor Diffusion method. Index is also very compatible with the Sitting Drop, Sandwich Drop, Micro Batch, and Microdialysis 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 VDX™ Plate with sealant (HR3-170). Ninety-six reservoirs are to be prepared for a complete Index. See Figure 1.

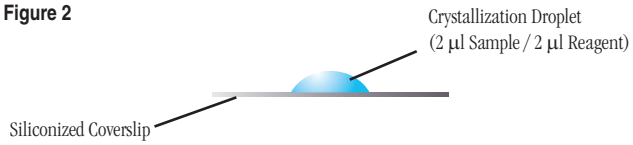
Figure 1
Cross section of a reservoir in the VDX plate.



2. Using a clean pipet tip, pipet 1 ml of Index reagent 1 into reservoir A1. Discard the pipet tip, add a new pipet tip and pipet 1 ml of Index reagent 2 into reservoir A2. Repeat the procedure for the remaining 94 Index reagents using a clean pipet tip for each reagent so as to avoid reagent contamination and carry over.

3. Pipet 2 µl of the sample to the center of a clean, siliconized 22 mm diameter circle or square cover slide. See Figure 2.

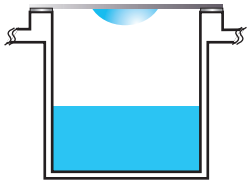
Figure 2



4. Pipet 2 µl of Index 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 95 Index reagents.

7. If the quantity of sample permits, perform Index 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.

Examine The Drop

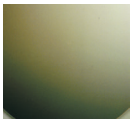
Carefully examine the drops under a stereo microscope (10 to 100x magnification) immediately after setting up the screen. Record all observations and be particularly careful to scan the focal plane for small crystals. Observe the drops once each day for the first week, then once a week there after. Records should indicate whether the drop is clear, contains precipitate, and or crystals. It is helpful to describe the drop contents using descriptive terms. Adding magnitude is also helpful. Example: 4+ yellow/brown fine precipitate, 2+ small bipyramid crystals, clear drop, 3+ needle shaped crystals in 1+ white precipitate. One may also employ a standard numerical scoring scheme (Clear = 0, Precipitate = 1, Crystal = 10, etc). Figure 4 (on page 3) shows typical examples of what one might observe in a crystallization experiment.

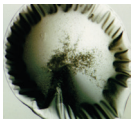
Interpreting Index

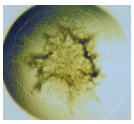
Clear drops indicate that either the relative supersaturation of the sample and reagent is too low or the drop has not yet completed equilibration. If the drop remains clear after 3 to 4 weeks consider repeating the Index condition and doubling the sample concentration. If more than 70 of the 96 Index drops are clear consider doubling the sample concentration and repeating the entire screen.

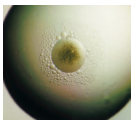
Drops containing precipitate indicate that either the relative supersaturation of the sample and reagent is too high, the sample has denatured, or the

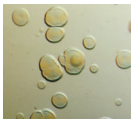
Figure 4
Typical observations in a crystallization experiment

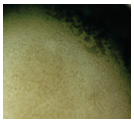

Clear Drop


Skin / Precipitate

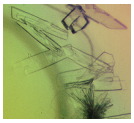

Precipitate


Precipitate / Phase

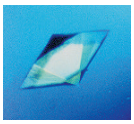

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

sample is heterogeneous. To reduce the relative supersaturation, dilute the sample twofold and repeat the Index condition. If more than 70 of the 96 Index 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.

Index Formulation

Index 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).

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

Index 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.

Index 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 Index be stored at 4°C or -20°C. Avoid ultraviolet light to preserve reagent stability.

If the sample contains phosphate, borate, or carbonate buffers it is possible to obtain inorganic crystals (false positives) when using Index 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 sodium HEPES.

References and Readings

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

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Technical Support

Inquiries regarding Crystal Screen reagent formulation, interpretation of screen results, optimization strategies and general inquiries regarding crystallization are welcome. Please e-mail, fax, or telephone your request to Hampton Research. Fax and e-mail Technical Support are available 24 hours a day. Telephone technical support is available 8:00 a.m. to 4:30 p.m. USA Pacific Standard Time.

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How to Reproduce Index Reagents

Index reagents and optimization conditions based on Index 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 Index reagent 4 in a crystallization plate.
Solution Composition: 0.1 M BIS-TRIS pH 6.5
2.0 M Ammonium sulfate

- 329 µl water[3]
- 100 µl 1.0 M BIS-TRIS pH 6.5
(CAS # 6976-37-0, Catalog # HR2-783)
- 571 µl 3.5 M Ammonium sulfate
(CAS # 7783-20-2, Catalog # HR2-541)

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

Example 2. To prepare 1.0 milliliter of Index reagent 17.
Solution Composition: 1.26 M Sodium phosphate monobasic monohydrate,
0.14 M Potassium phosphate dibasic, pH 5.6

- 650 µl water[3]
- 35 µl 4.0 M Potassium phosphate dibasic
(CAS # 7758-11-4, Catalog # HR2-635)
- 315 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 5.6

Example 3. To prepare 10 milliliters of Index reagent 25.
Solution Composition: 3.5 M Sodium formate pH 7.0

- 3.0 ml water[3]
- 7.0 ml 5.0 M Sodium formate pH 7.0
(CAS # 141-53-7, Catalog # HR2-765)

Make no pH adjustments. Mix well.

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

Formulation Notes for Index Reagents

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

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Table 1. Recommended reagents for the formulation of Index 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 citrate tribasic pH 7.0	HR2-759	2.5 M	200 ml	3458-72-8
Ammonium sulfate	HR2-541	3.5 M	200 ml	7783-20-2
Ammonium tartrate dibasic pH 7.0	HR2-767	1.6 M	200 ml	3164-29-2
Cadmium chloride hydrate	HR2-715	1.0 M	100 ml	654054-66-7
Calcium chloride dihydrate	HR2-557	2.0 M	100 ml	10035-04-8
Cobalt (II) chloride hexahydrate	HR2-713	1.0 M	100 ml	7791-13-1
Lithium sulfate monohydrate	HR2-545	2.0 M	200 ml	10377-48-7
Magnesium chloride hexahydrate	HR2-559	2.0 M	100 ml	7791-18-6
	HR2-803	5.0 M	200 ml	7791-18-6
Magnesium formate dihydrate	HR2-537	1.0 M	200 ml	557-39-1
DL-Malic acid pH 7.0	HR2-761	3.0 M	200 ml	6915-15-7
Nickel (II) chloride hexahydrate	HR2-687	4.0 M	200 ml	7791-20-0
Potassium bromide	HR2-779	4.0 M	100 ml	7758-02-3
Potassium chloride	HR2-649	4.0 M	200 ml	7447-40-7
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
L-Proline	HR2-775	1.0 M	100 ml	147-85-3
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 formate pH 7.0	HR2-765	5.0 M	200 ml	141-53-7
Sodium malonate pH 7.0	HR2-707	3.4 M	200 ml	141-82-2
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
Trimethylamine N-oxide dihydrate	HR2-777	1.0 M	100 ml	62637-93-8
Zinc acetate dihydrate	HR2-563	1.0 M	100 ml	5970-45-6

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

Polymers	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
Jeffamine M-600 ® pH 7.0	HR2-501	50 % v/v	200 ml	77110-54-4
Jeffamine ED-2001 ® pH 7.0	HR2-597	50 % w/v	200 ml	65605-36-9
Pentaerythritol propoxylate (5/4 PO/OH)	HR2-739	50 % v/v	200 ml	9051-49-4
Pentaerythritol ethoxylate (15/4 EO/OH)	HR2-745	50 % v/v	200 ml	30599-15-6
Polyethylene glycol P 400	HR2-771	100 %	200 ml	25322-69-4
Poly(acrylic acid sodium salt) 5,100	HR2-773	50 % w/v	200 ml	9003-04-7
Polyethylene glycol 1,500	HR2-525	50 % w/v	200 ml	25322-68-3
Polyethylene glycol 3,350	HR2-527	50 % w/v	200 ml	25322-68-3
Polyethylene glycol 8,000	HR2-535	50 % w/v	200 ml	25322-68-3
Polyethylene glycol 10,000	HR2-607	50 % w/v	200 ml	25322-68-3
Polyethylene glycol monomethyl ether 550	HR2-611	100 %	200 ml	9004-74-4
Polyethylene glycol monomethyl ether 2,000	HR2-613	50 % w/v	200 ml	9004-74-4
Polyethylene glycol monomethyl ether 5,000	HR2-615	50 % w/v	200 ml	9004-74-4
Polyvinylpyrrolidone K 15	HR2-769	50 % w/v	200 ml	9003-39-8
Organics (non-volatile)	**Hampton Research Catalog #**	**Supplied [Stock]**	**Supplied Volume**	**CAS #**
(+/-)-2-Methyl-2,4-pentanediol	HR2-627	100 %	200 ml	107-41-5
Buffers	**Hampton Research Catalog #**	**Supplied [Stock]**	**Supplied Volume**	**CAS #**
BIS-TRIS pH 5.5 [1]	HR2-781	1.0 M	100 ml	6976-37-0
BIS-TRIS pH 6.5 [1]	HR2-783	1.0 M	100 ml	6976-37-0
Citric acid pH 3.5 [2]	HR2-757	1.0 M	100 ml	77-92-9
HEPES pH 7.0 [2]	HR2-785	1.0 M	100 ml	7365-45-9
HEPES pH 7.5 [2]	HR2-729	1.0 M	100 ml	7365-45-9
Sodium acetate trihydrate pH 4.5 [1]	HR2-789	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				
[2] pH titrated using Sodium hydroxide (HR2-583) CAS # 1310-73-2				

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

Buffer Solution or Kit	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #	pH range
StockOptions™ Bis-Tris kit [4]	HR2-106	1.0 M	10 ml each	6976-37-0	5.5 - 7.5
StockOptions™ Citric Acid kit [4]	HR2-104	1.0 M	10 ml each	77-92-9	2.2 - 6.5
HEPES untitrated	HR2-585	1.0 M	100 ml	7365-45-9	6.6 - 8.5
Titrate with NaOH	HR2-583	1.0 M	100 ml	1310-73-2	—
StockOptions™ Hepes kit [4]	HR2-102	1.0 M	10 ml each	7365-45-9	6.8 - 8.2
Sodium acetate trihydrate untitrated	HR2-569	1.0 M	100 ml	6131-90-4	3.6 - 5.6
Titrate with HCl	HR2-581	1.0 M	100 ml	7647-01-07	—
StockOptions™ Sodium Acetate kit [4]	HR2-233	1.0 M	10 ml each	6131-90-4	3.6 - 5.6
Tris untitrated	HR2-589	1.0 M	100 ml	77-86-1	7.0 - 9.0
Titrate with HCl	HR2-581	1.0 M	100 ml	7647-01-0	—
StockOptions™ Tris kit [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

Technical Support

Inquiries regarding Index Fundamentals, interpretation of screen results, optimization strategies and general inquiries regarding crystallization are welcome. Please e-mail, fax, or telephone your request to Hampton Research. Fax and e-mail Technical Support are available 24 hours a day. Telephone technical support is available 8:00 a.m. to 4:30 p.m. USA Pacific Standard Time.

Jeffamine ® is a registered trademark of the Huntsman Corporation.

M-600 ® is a registered trademark of Texaco.

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Tube #	Salt	Buffer ◊	Precipitant
1.	None	0.1 M Citric acid pH 3.5	2.0 M Ammonium sulfate
2.	None	0.1 M Sodium acetate trihydrate pH 4.5	2.0 M Ammonium sulfate
3.	None	0.1 M BIS-TRIS pH 5.5	2.0 M Ammonium sulfate
4.	None	0.1 M BIS-TRIS pH 6.5	2.0 M Ammonium sulfate
5.	None	0.1 M HEPES pH 7.5	2.0 M Ammonium sulfate
6.	None	0.1 M Tris pH 8.5	2.0 M Ammonium sulfate
7.	None	0.1 M Citric acid pH 3.5	3.0 M Sodium chloride
8.	None	0.1 M Sodium acetate trihydrate pH 4.5	3.0 M Sodium chloride
9.	None	0.1 M BIS-TRIS pH 5.5	3.0 M Sodium chloride
10.	None	0.1 M BIS-TRIS pH 6.5	3.0 M Sodium chloride
11.	None	0.1 M HEPES pH 7.5	3.0 M Sodium chloride
12.	None	0.1 M Tris pH 8.5	3.0 M Sodium chloride
13.	None	0.1 M BIS-TRIS pH 5.5	0.3 M Magnesium formate dihydrate
14.	None	0.1 M BIS-TRIS pH 6.5	0.5 M Magnesium formate dihydrate
15.	None	0.1 M HEPES pH 7.5	0.5 M Magnesium formate dihydrate
16.	None	0.1 M Tris pH 8.5	0.3 M Magnesium formate dihydrate
17.	None	None - pH 5.6	1.26 M Sodium phosphate monobasic monohydrate 0.14 M Potassium phosphate dibasic
18.	None	None - pH 6.9	0.49 M Sodium phosphate monobasic monohydrate 0.91 M Potassium phosphate dibasic
19.	None	None - pH 8.2	0.056 M Sodium phosphate monobasic monohydrate 1.344 M Potassium phosphate dibasic
20.	None	0.1 M HEPES pH 7.5	1.4 M Sodium citrate tribasic dihydrate
21.	None	None	1.8 M Ammonium citrate tribasic pH 7.0
22.	None	None	0.8 M Succinic acid pH 7.0
23.	None	None	2.1 M DL-Malic acid pH 7.0
24.	None	None	2.8 M Sodium acetate trihydrate pH 7.0
25.	None	None	3.5 M Sodium formate pH 7.0
26.	None	None	1.1 M Ammonium tartrate dibasic pH 7.0
27.	None	None	2.4 M Sodium malonate pH 7.0
28.	None	None	35% v/v Tacsimate™ pH 7.0
29.	None	None	60% v/v Tacsimate™ pH 7.0
30.	0.1 M Sodium chloride	0.1 M BIS-TRIS pH 6.5	1.5 M Ammonium sulfate
31.	0.8 M Potassium sodium tartrate tetrahydrate	0.1 M Tris pH 8.5	0.5% w/v Polyethylene glycol monomethyl ether 5,000
32.	1.0 M Ammonium sulfate	0.1 M BIS-TRIS pH 5.5	1% w/v Polyethylene glycol 3,350
33.	1.1 M Sodium malonate pH 7.0	0.1 M HEPES pH 7.0	0.5% v/v Jeffamine® ED-2001 pH 7.0
34.	1.0 M Succinic acid pH 7.0	0.1 M HEPES pH 7.0	1% w/v Polyethylene glycol monomethyl ether 2,000
35.	1.0 M Ammonium sulfate	0.1 M HEPES pH 7.0	0.5% w/v Polyethylene glycol 8,000
36.	15% v/v Tacsimate™ pH 7.0	0.1 M HEPES pH 7.0	2% w/v Polyethylene glycol 3,350
37.	None	None	25% w/v Polyethylene glycol 1,500
38.	None	0.1 M HEPES pH 7.0	30% v/v Jeffamine® M-600® pH 7.0
39.	None	0.1 M HEPES pH 7.0	30% v/v Jeffamine® ED-2001 pH 7.0
40.	None	0.1 M Citric acid pH 3.5	25% w/v Polyethylene glycol 3,350
41.	None	0.1 M Sodium acetate trihydrate pH 4.5	25% w/v Polyethylene glycol 3,350
42.	None	0.1 M BIS-TRIS pH 5.5	25% w/v Polyethylene glycol 3,350
43.	None	0.1 M BIS-TRIS pH 6.5	25% w/v Polyethylene glycol 3,350
44.	None	0.1 M HEPES pH 7.5	25% w/v Polyethylene glycol 3,350
45.	None	0.1 M Tris pH 8.5	25% w/v Polyethylene glycol 3,350
46.	None	0.1 M BIS-TRIS pH 6.5	20% w/v Polyethylene glycol monomethyl ether 5,000
47.	None	0.1 M BIS-TRIS pH 6.5	28% w/v Polyethylene glycol monomethyl ether 2,000
48.	0.2 M Calcium chloride dihydrate	0.1 M BIS-TRIS pH 5.5	45% v/v (+/-)-2-Methyl-2,4-pentanediol

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

Index contains ninety-six unique reagents. To determine the formulation of each reagent, simply read across the page.

Tube #	Salt	Buffer ◊	Precipitant
49.	0.2 M Calcium chloride dihydrate	0.1 M BIS-TRIS pH 6.5	45% v/v (+/-)-2-Methyl-2,4-pentanediol
50.	0.2 M Ammonium acetate	0.1 M BIS-TRIS pH 5.5	45% v/v (+/-)-2-Methyl-2,4-pentanediol
51.	0.2 M Ammonium acetate	0.1 M BIS-TRIS pH 6.5	45% v/v (+/-)-2-Methyl-2,4-pentanediol
52.	0.2 M Ammonium acetate	0.1 M HEPES pH 7.5	45% v/v (+/-)-2-Methyl-2,4-pentanediol
53.	0.2 M Ammonium acetate	0.1 M Tris pH 8.5	45% v/v (+/-)-2-Methyl-2,4-pentanediol
54.	0.05 M Calcium chloride dihydrate	0.1 M BIS-TRIS pH 6.5	30% v/v Polyethylene glycol monomethyl ether 550
55.	0.05 M Magnesium chloride hexahydrate	0.1 M HEPES pH 7.5	30% v/v Polyethylene glycol monomethyl ether 550
56.	0.2 M Potassium chloride	0.05 M HEPES pH 7.5	35% v/v Pentaerythritol propoxylate (5/4 PO/OH)
57.	0.05 M Ammonium sulfate	0.05 M BIS-TRIS pH 6.5	30% v/v Pentaerythritol ethoxylate (15/4 EO/OH)
58.	None	0.1 M BIS-TRIS pH 6.5	45% v/v Polypropylene glycol P 400
59.	0.02 M Magnesium chloride hexahydrate	0.1 M HEPES pH 7.5	22% w/v Poly(acrylic acid sodium salt) 5,100
60.	0.01 M Cobalt(II) chloride hexahydrate	0.1 M Tris pH 8.5	20% w/v Polyvinylpyrrolidone K 15
61.	0.2 M L-Proline	0.1 M HEPES pH 7.5	10% w/v Polyethylene glycol 3,350
62.	0.2 M Trimethylamine N-oxide dihydrate	0.1 M Tris pH 8.5	20% w/v Polyethylene glycol monomethyl ether 2,000
63.	5% v/v Tacsimate™ pH 7.0	0.1 M HEPES pH 7.0	10% w/v Polyethylene glycol monomethyl ether 5,000
64.	0.005 M Cobalt(II) chloride hexahydrate 0.005 M Nickel(II) chloride hexahydrate 0.005 M Cadmium chloride hydrate 0.005 M Magnesium chloride hexahydrate	0.1 M HEPES pH 7.5	12% w/v Polyethylene glycol 3,350
65.	0.1 M Ammonium acetate	0.1 M BIS-TRIS pH 5.5	17% w/v Polyethylene glycol 10,000
66.	0.2 M Ammonium sulfate	0.1 M BIS-TRIS pH 5.5	25% w/v Polyethylene glycol 3,350
67.	0.2 M Ammonium sulfate	0.1 M BIS-TRIS pH 6.5	25% w/v Polyethylene glycol 3,350
68.	0.2 M Ammonium sulfate	0.1 M HEPES pH 7.5	25% w/v Polyethylene glycol 3,350
69.	0.2 M Ammonium sulfate	0.1 M Tris pH 8.5	25% w/v Polyethylene glycol 3,350
70.	0.2 M Sodium chloride	0.1 M BIS-TRIS pH 5.5	25% w/v Polyethylene glycol 3,350
71.	0.2 M Sodium chloride	0.1 M BIS-TRIS pH 6.5	25% w/v Polyethylene glycol 3,350
72.	0.2 M Sodium chloride	0.1 M HEPES pH 7.5	25% w/v Polyethylene glycol 3,350
73.	0.2 M Sodium chloride	0.1 M Tris pH 8.5	25% w/v Polyethylene glycol 3,350
74.	0.2 M Lithium sulfate monohydrate	0.1 M BIS-TRIS pH 5.5	25% w/v Polyethylene glycol 3,350
75.	0.2 M Lithium sulfate monohydrate	0.1 M BIS-TRIS pH 6.5	25% w/v Polyethylene glycol 3,350
76.	0.2 M Lithium sulfate monohydrate	0.1 M HEPES pH 7.5	25% w/v Polyethylene glycol 3,350
77.	0.2 M Lithium sulfate monohydrate	0.1 M Tris pH 8.5	25% w/v Polyethylene glycol 3,350
78.	0.2 M Ammonium acetate	0.1 M BIS-TRIS pH 5.5	25% w/v Polyethylene glycol 3,350
79.	0.2 M Ammonium acetate	0.1 M BIS-TRIS pH 6.5	25% w/v Polyethylene glycol 3,350
80.	0.2 M Ammonium acetate	0.1 M HEPES pH 7.5	25% w/v Polyethylene glycol 3,350
81.	0.2 M Ammonium acetate	0.1 M Tris pH 8.5	25% w/v Polyethylene glycol 3,350
82.	0.2 M Magnesium chloride hexahydrate	0.1 M BIS-TRIS pH 5.5	25% w/v Polyethylene glycol 3,350
83.	0.2 M Magnesium chloride hexahydrate	0.1 M BIS-TRIS pH 6.5	25% w/v Polyethylene glycol 3,350
84.	0.2 M Magnesium chloride hexahydrate	0.1 M HEPES pH 7.5	25% w/v Polyethylene glycol 3,350
85.	0.2 M Magnesium chloride hexahydrate	0.1 M Tris pH 8.5	25% w/v Polyethylene glycol 3,350
86.	0.2 M Potassium sodium tartrate tetrahydrate	None	20% w/v Polyethylene glycol 3,350
87.	0.2 M Sodium malonate pH 7.0	None	20% w/v Polyethylene glycol 3,350
88.	0.2 M Ammonium citrate tribasic pH 7.0	None	20% w/v Polyethylene glycol 3,350
89.	0.1 M Succinic acid pH 7.0	None	15% w/v Polyethylene glycol 3,350
90.	0.2 M Sodium formate	None	20% w/v Polyethylene glycol 3,350
91.	0.15 M DL-Malic acid pH 7.0	None	20% w/v Polyethylene glycol 3,350
92.	0.1 M Magnesium formate dihydrate	None	15% w/v Polyethylene glycol 3,350
93.	0.05 M Zinc acetate dihydrate	None	20% w/v Polyethylene glycol 3,350
94.	0.2 M Sodium citrate tribasic dihydrate	None	20% w/v Polyethylene glycol 3,350
95.	0.1 M Potassium thiocyanate	None	30% w/v Polyethylene glycol monomethyl ether 2,000
96.	0.15 M Potassium bromide	None	30% w/v Polyethylene glycol monomethyl ether 2,000

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

Index contains ninety-six unique reagents. To determine the formulation of each reagent, simply read across the page.

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HAMPTON RESEARCH
Solutions for Crystal Growth

Sample: _____ **Sample Concentration:** _____

Sample Buffer: _____ **Date:** _____

Reservoir Volume: _____ **Temperature:** _____

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

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

HAMPTON RESEARCH
Solutions for Crystal Growth

Index™ - HR2-144 Scoring Sheet	Date:	Date:	Date:
1. 0.1 M Citric acid pH 3.5, 2.0 M Ammonium sulfate			
2. 0.1 M Sodium acetate trihydrate pH 4.5, 2.0 M Ammonium sulfate			
3. 0.1 M BIS-TRIS pH 5.5, 2.0 M Ammonium sulfate			
4. 0.1 M BIS-TRIS pH 6.5, 2.0 M Ammonium sulfate			
5. 0.1 M HEPES pH 7.5, 2.0 M Ammonium sulfate			
6. 0.1 M Tris pH 8.5, 2.0 M Ammonium sulfate			
7. 0.1 M Citric acid pH 3.5, 3.0 M Sodium chloride			
8. 0.1 M Sodium acetate trihydrate pH 4.5, 3.0 M Sodium chloride			
9. 0.1 M BIS-TRIS pH 5.5, 3.0 M Sodium chloride			
10. 0.1 M BIS-TRIS pH 6.5, 3.0 M Sodium chloride			
11. 0.1 M HEPES pH 7.5, 3.0 M Sodium chloride			
12. 0.1 M Tris pH 8.5, 3.0 M Sodium chloride			
13. 0.1 M BIS-TRIS pH 5.5, 0.3 M Magnesium formate dihydrate			
14. 0.1 M BIS-TRIS pH 6.5, 0.5 M Magnesium formate dihydrate			
15. 0.1 M HEPES pH 7.5, 0.5 M Magnesium formate dihydrate			
16. 0.1 M TRIS pH 8.5, 0.3 M Magnesium formate dihydrate			
17. 1.26 M Sodium phosphate monobasic monohydrate, 0.14 M Potassium phosphate dibasic, pH 5.6			
18. 0.49 M Sodium phosphate monobasic monohydrate, 0.91 M Potassium phosphate dibasic, pH 6.9			
19. 0.056 M Sodium phosphate monobasic monohydrate, 1.344 M Potassium phosphate dibasic, pH 8.2			
20. 0.1 M HEPES pH 7.5, 1.4 M Sodium citrate tribasic dihydrate			
21. 1.8 M Ammonium citrate tribasic pH 7.0			
22. 0.8 M Succinic acid pH 7.0			
23. 2.1 M DL-Malic acid pH 7.0			
24. 2.8 M Sodium acetate trihydrate pH 7.0			
25. 3.5 M Sodium formate pH 7.0			
26. 1.1 M Ammonium tartrate dibasic pH 7.0			
27. 2.4 M Sodium malonate pH 7.0			
28. 35% v/v Tacsimate™ pH 7.0			
29. 60% v/v Tacsimate™ pH 7.0			
30. 0.1 M Sodium chloride, 0.1 M BIS-TRIS pH 6.5, 1.5 M Ammonium sulfate			
31. 0.8 M Potassium sodium tartrate tetrahydrate, 0.1 M Tris pH 8.5, 0.5% w/v Polyethylene glycol monomethyl ether 5,000			
32. 1.0 M Ammonium sulfate, 0.1 M BIS-TRIS pH 5.5, 1% w/v Polyethylene glycol 3,350			
33. 1.1 M Sodium malonate pH 7.0, 0.1 M HEPES pH 7.0, 0.5% v/v Jeffamine® ED-2001 pH 7.0			
34. 1.0 M Succinic acid pH 7.0, 0.1 M HEPES pH 7.0, 1% w/v Polyethylene glycol monomethyl ether 2,000			
35. 1.0 M Ammonium sulfate, 0.1 M HEPES pH 7.0, 0.5% w/v Polyethylene glycol 8,000			
36. 15% v/v Tacsimate™ pH 7.0, 0.1 M HEPES pH 7.0, 2% w/v Polyethylene glycol 3,350			
37. 25% w/v Polyethylene glycol 1,500			
38. 0.1 M HEPES pH 7.0, 30% v/v Jeffamine® M-600® pH 7.0			
39. 0.1 M HEPES pH 7.0, 30% v/v Jeffamine® ED-2001 pH 7.0			
40. 0.1 M Citric acid pH 3.5, 25% w/v Polyethylene glycol 3,350			
41. 0.1 M Sodium acetate trihydrate pH 4.5, 25% w/v Polyethylene glycol 3,350			
42. 0.1 M BIS-TRIS pH 5.5, 25% w/v Polyethylene glycol 3,350			
43. 0.1 M BIS-TRIS pH 6.5, 25% w/v Polyethylene glycol 3,350			
44. 0.1 M HEPES pH 7.5, 25% w/v Polyethylene glycol 3,350			
45. 0.1 M Tris pH 8.5, 25% w/v Polyethylene glycol 3,350			
46. 0.1 M BIS-TRIS pH 6.5, 20% w/v Polyethylene glycol monomethyl ether 5,000			
47. 0.1 M BIS-TRIS pH 6.5, 28% w/v Polyethylene glycol monomethyl ether 2,000			
48. 0.2 M Calcium chloride dihydrate, 0.1 M BIS-TRIS pH 5.5, 45% v/v (+/-)-2-Methyl-2,4-pentanediol			

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)

Index™ - HR2-144 Scoring Sheet

	Date:	Date:	Date:
49. 0.2 M Calcium chloride dihydrate, 0.1 M BIS-TRIS pH 6.5, 45% v/v (+/-)-2-Methyl-2,4-pentanediol			
50. 0.2 M Ammonium acetate, 0.1 M BIS-TRIS pH 5.5, 45% v/v (+/-)-2-Methyl-2,4-pentanediol			
51. 0.2 M Ammonium acetate, 0.1 M BIS-TRIS pH 6.5, 45% v/v (+/-)-2-Methyl-2,4-pentanediol			
52. 0.2 M Ammonium acetate, 0.1 M HEPES pH 7.5, 45% v/v (+/-)-2-Methyl-2,4-pentanediol			
53. 0.2 M Ammonium acetate, 0.1 M Tris pH 8.5, 45% v/v (+/-)-2-Methyl-2,4-pentanediol			
54. 0.05 M Calcium chloride dihydrate, 0.1 M BIS-TRIS pH 6.5, 30% v/v Polyethylene glycol monomethyl ether 550			
55. 0.05 M Magnesium chloride hexahydrate, 0.1 M HEPES pH 7.5, 30% v/v Polyethylene glycol monomethyl ether 550			
56. 0.2 M Potassium chloride, 0.05 M HEPES pH 7.5, 35% v/v Pentaerythritol propoxylate (5/4 PO/OH)			
57. 0.05 M Ammonium sulfate, 0.05 M BIS-TRIS pH 6.5, 30% v/v Pentaerythritol ethoxylate (15/4 EO/OH)			
58. 0.1 M BIS-TRIS pH 6.5, 45% v/v Polypropylene glycol P 400			
59. 0.02 M Magnesium chloride hexahydrate, 0.1 M HEPES pH 7.5, 22% w/v Poly(acrylic acid sodium salt) 5,100			
60. 0.01 M Cobalt(II) chloride hexahydrate, 0.1 M Tris pH 8.5, 20% w/v Polyvinylpyrrolidone K 15			
61. 0.2 M L-Proline, 0.1 M HEPES pH 7.5, 10% w/v Polyethylene glycol 3,350			
62. 0.2 M Trimethylamine N-oxide dihydrate, 0.1 M Tris pH 8.5, 20% w/v Polyethylene glycol monomethyl ether 2,000			
63. 5% v/v Tacsimate™ pH 7.0, 0.1 M HEPES pH 7.0, 10% w/v Polyethylene glycol monomethyl ether 5,000			
64. 0.005 M Cobalt(II) chloride hexahydrate, 0.005 M Nickel(II) chloride hexahydrate, 0.005 M Cadmium chloride hydrate, 0.005 M Magnesium chloride hexahydrate, 0.1 M HEPES pH 7.5, 12% w/v Polyethylene glycol 3,350			
65. 0.1 M Ammonium acetate, 0.1 M BIS-TRIS pH 5.5, 17% w/v Polyethylene glycol 10,000			
66. 0.2 M Ammonium sulfate, 0.1 M BIS-TRIS pH 5.5, 25% w/v Polyethylene glycol 3,350			
67. 0.2 M Ammonium sulfate, 0.1 M BIS-TRIS pH 6.5, 25% w/v Polyethylene glycol 3,350			
68. 0.2 M Ammonium sulfate, 0.1 M HEPES pH 7.5, 25% w/v Polyethylene glycol 3,350			
69. 0.2 M Ammonium sulfate, 0.1 M Tris pH 8.5, 25% w/v Polyethylene glycol 3,350			
70. 0.2 M Sodium chloride, 0.1 M BIS-TRIS pH 5.5, 25% w/v Polyethylene glycol 3,350			
71. 0.2 M Sodium chloride, 0.1 M BIS-TRIS pH 6.5, 25% w/v Polyethylene glycol 3,350			
72. 0.2 M Sodium chloride, 0.1 M HEPES pH 7.5, 25% w/v Polyethylene glycol 3,350			
73. 0.2 M Sodium chloride, 0.1 M Tris pH 8.5, 25% w/v Polyethylene glycol 3,350			
74. 0.2 M Lithium sulfate monohydrate, 0.1 M BIS-TRIS pH 5.5, 25% w/v Polyethylene glycol 3,350			
75. 0.2 M Lithium sulfate monohydrate, 0.1 M BIS-TRIS pH 6.5, 25% w/v Polyethylene glycol 3,350			
76. 0.2 M Lithium sulfate monohydrate, 0.1 M HEPES pH 7.5, 25% w/v Polyethylene glycol 3,350			
77. 0.2 M Lithium sulfate monohydrate, 0.1 M Tris pH 8.5, 25% w/v Polyethylene glycol 3,350			
78. 0.2 M Ammonium acetate, 0.1 M BIS-TRIS pH 5.5, 25% w/v Polyethylene glycol 3,350			
79. 0.2 M Ammonium acetate, 0.1 M BIS-TRIS pH 6.5, 25% w/v Polyethylene glycol 3,350			
80. 0.2 M Ammonium acetate, 0.1 M HEPES pH 7.5, 25% w/v Polyethylene glycol 3,350			
81. 0.2 M Ammonium acetate, 0.1 M Tris pH 8.5, 25% w/v Polyethylene glycol 3,350			
82. 0.2 M Magnesium chloride hexahydrate, 0.1 M BIS-TRIS pH 5.5, 25% w/v Polyethylene glycol 3,350			
83. 0.2 M Magnesium chloride hexahydrate, 0.1 M BIS-TRIS pH 6.5, 25% w/v Polyethylene glycol 3,350			
84. 0.2 M Magnesium chloride hexahydrate, 0.1 M HEPES pH 7.5, 25% w/v Polyethylene glycol 3,350			
85. 0.2 M Magnesium chloride hexahydrate, 0.1 M Tris pH 8.5, 25% w/v Polyethylene glycol 3,350			
86. 0.2 M Potassium sodium tartrate tetrahydrate, 20% w/v Polyethylene glycol 3,350			
87. 0.2 M Sodium malonate pH 7.0, 20% w/v Polyethylene glycol 3,350			
88. 0.2 M Ammonium citrate tribasic pH 7.0, 20% w/v Polyethylene glycol 3,350			
89. 0.1 M Succinic acid pH 7.0, 15% w/v Polyethylene glycol 3,350			
90. 0.2 M Sodium formate, 20% w/v Polyethylene glycol 3,350			
91. 0.15 M DL-Malic acid pH 7.0, 20% w/v Polyethylene glycol 3,350			
92. 0.1 M Magnesium formate dihydrate, 15% w/v Polyethylene glycol 3,350			
93. 0.05 M Zinc acetate dihydrate, 20% w/v Polyethylene glycol 3,350			
94. 0.2 M Sodium citrate tribasic dihydrate, 20% w/v Polyethylene glycol 3,350			
95. 0.1 M Potassium thiocyanate, 30% w/v Polyethylene glycol monomethyl ether 2,000			
96. 0.15 M Potassium bromide, 30% w/v Polyethylene glycol monomethyl ether 2,000			

HAMPTON RESEARCH
Solutions for Crystal Growth

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