# JCSG Plus HT™

HAMPTON RESEARCH
*Solutions for Crystal Growth*

## User Guide HR2-150

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

### Features
- Data mined sparse matrix screen
- Efficiently samples salts, polymers, organics & pH
- PEG & Salt versus pH
- PEG & Salt
- Low ionic strength versus pH
- High [polymer] with low [salt]
- Tube or Deep Well block format

### General Description
The JCSG Plus HT is a general sparse matrix screen. 67 core reagents are based upon a statistical analysis performed by Page[1] using results of the Joint Center for Structural Genomics (JCSG), in which a large number (473) of proteins from Thermotoga maritima were each set up against 480 reagents from 12 commercially available screens. 40 of the resulting 67 reagents (60%) are Hampton Research crystallization screen reagents (Crystal Screen, Crystal Screen 2, Crystal Screen Cryo, PEG/Ion Screen, Grid Screen Ammonium Sulfate, Grid Screen PEG 6000, Grid Screen PEG/LiCl, and Grid Screen MPD). The core 67 reagents are complemented with solutions from Index[2], an expansion of the JCSG selected by Newman[2] to fill out the pH profile of the screen and to enhance the range of precipitants by including neutralized organic acids[3] and Tacsimate[4]. Therefore JCSG Plus is composed of 96 reagents, 69 (72%) of which are based on Hampton Research screens. If one wishes to screen beyond the reagents offered in JCSG Plus one should consider PEG/Ion or PEGRx for polymer biased reagents or SaltRx for salt biased reagents.

### 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.

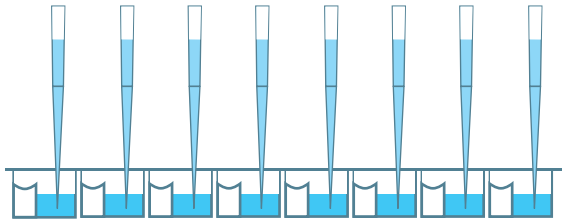
The recommended sample concentration is 5 to 25 mg/ml in dilute buffer (10 to 25 mM). The sample should be free of any unnecessary additives in order to observe the effect of the JCSG Plus variables. Ideally, the initial screen should be performed with a sample which has been dialyzed against dilute 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.

### Preparing the Deep Well Block for Use
It is recommended the Deep Well block be centrifuged before removing the sealing film. Centrifugation at 500 rpm for five minutes will remove stray reagent from the sealing film. Removing the reagent from the film prevents stray reagent droplets from falling into neighboring wells during film removal. After centrifugation the film can be removed by grasping a corner of the film and gently peeling the film from the plate. Alternatively, the film can be left intact and the pierced for reagent access.

### Performing the Screen
Manual Method - Sitting Drop Vapor Diffusion

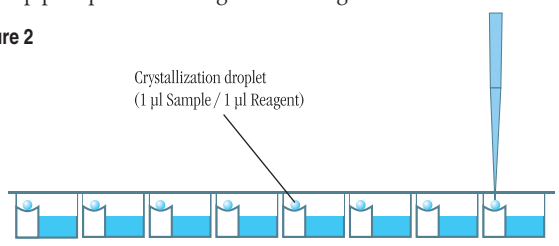
1. Using a 96 well sitting drop vapor diffusion plate, pipet the recommended volume (typically 50 to 100 microliters) of crystallization reagent from the Deep Well block into the reservoirs of the crystallization plate. The Deep Well block is compatible with 8 and 12 channel pipets as well as many automated liquid handling systems. Use clean pipet tips for each reagent set transfer and change pipet tips when changing reagents. For an 8 channel pipet, transfer reagents A1-H1 to reservoirs A1-H1 of the crystallization plate. Repeat this procedure for reagent columns B through H. Change pipet tips when moving between reagent columns. For a 12 channel pipet, transfer reagents A1-A12 to reservoirs A1-A12 of the crystallization plate. Repeat this procedure for reagent rows 1 through 12. See Figure 1. Time and pipet tips can be conserved by batch pipetting multiple plates with the same (row or column) of reagent before changing reagent and pipet tips.

**Figure 1**



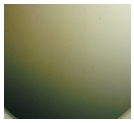
2. Using clean pipet tips, pipet 0.05 to 2 microliters of crystallization reagent from the crystallization plate reservoir to the sitting drop well. Some 96 well crystallization plates allow this procedure to be performed using a multichannel pipet where other plates require the use of a single channel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

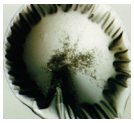
**Figure 2**

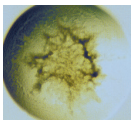


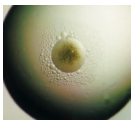

3. Using a clean pipet tip, pipet 0.05 to 2 microliters of sample to the reagent drop in the sitting drop well. One may choose to simply dispense the sample with no mixing or dispense with mixing by gently aspirating and dispensing the sample several times, keeping the tip in the drop during mixing to avoid foaming. Work carefully but quickly to minimize evaporation from the crystallization plate. See Figure 2 above.

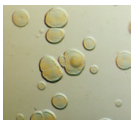
**Figure 6**
Typical observations in a crystallization experiment

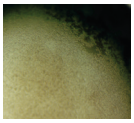

Clear Drop


Skin / Precipitate

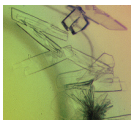

Precipitate


Precipitate / Phase

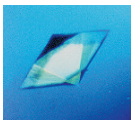

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

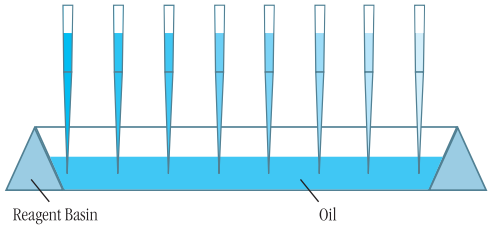
4. Seal the crystallization plate as per the manufacturer's recommendation. Most 96 well crystallization plates are sealed using a clear sealing tape or film. View and score the experiment as desired. See Hampton Research technical bulletin Crystal Growth 101 - Viewing Crystallization Experiments for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

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

Manual Method – Micro Batch 96 well format

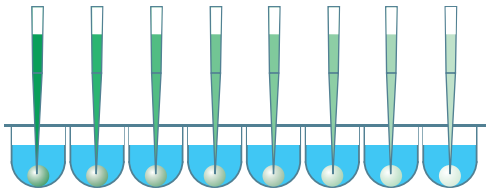
1. Using a 96 well clear polystyrene microplate (U-bottom recommended for best drop centering, flat-bottom recommended for best optics) pipet 50 to 150 microliters of microbatch compatible oil into each of the 96 reservoirs. This can be accomplished using an 8 or 12 channel pipet and pipetting the oil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

**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.

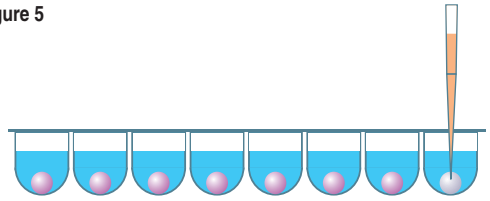
**Figure 4**



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.

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.

**Figure 5**



Note: If the drops appear flat or is fragmented into multiple drops, the centrifugation speed is too high and the centrifugation time is 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.

JCSG Plus 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 8x12 96 well assay blocks. Follow the manufacturer's recommendation for handling deep well microplates.

### Examine the Drop

Carefully examine the drops under a stereo microscope (10 to 100x magnification) immediately after setting up the screen. Record all observations and be particularly careful to scan the focal plane for small crystals. Observe the drops once each day for the first week, then once a week there after. Records should indicate whether the drop is clear, contains precipitate, and or crystals. It is helpful to describe the drop contents using descriptive terms. Adding magnitude is also helpful. Example: 4+ yellow/brown fine precipitate, 2+ small bipyramid crystals, clear drop, 3+ needle shaped crystals in 1+ white precipitate. One may also employ a standard numerical scoring scheme (Clear = 0, Precipitate = 1, Crystal = 10, etc). Figure 6, on the left side of page 3 shows typical examples of what one might observe in a crystallization experiment.

# JCSG Plus HT™

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### Interpreting JCSG Plus 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 super saturation of the sample and reagent is too high, the sample has denatured, or the sample is heterogeneous. To reduce the relative supersaturation, dilute the sample twofold and repeat the screen condition. If more than 70 of the 96 screen drops contain precipitate and no crystals are present, consider diluting the sample concentration in half and repeating the entire screen. If sample denaturation is suspect, take measures to stabilize the sample (add reducing agent, ligands, glycerol, salt, or other stabilizing agents). If the sample is impure, aggregated, or heterogeneous take measures to pursue homogeneity. It is possible to obtain crystals from precipitate so do not discard nor ignore a drop containing precipitate. If possible, examine drops containing precipitate under polarizing optics to differentiate precipitate from microcrystalline material.

If the drop contains a macromolecular crystal the relative supersaturation of the sample and reagent is appropriate for crystal nucleation and growth. The next step is to optimize the preliminary conditions (pH, salt type, salt concentration, precipitant type, precipitant concentration, sample concentration, temperature, additives, and other crystallization variables) which produced the crystal in order to improve crystal size and quality.

Compare the observations between the 4°C and room temperature incubation to determine the effect of temperature on sample solubility. Different results in the same drops at different temperatures indicate that sample solubility is temperature dependent and that one should include temperature as a variable in subsequent screens and optimization experiments.

Retain and observe plates until the drops are dried out. Crystal growth can occur within 15 minutes or one year.

### JCSG Plus HT Formulation

Crystallization reagents are formulated using the highest purity chemicals, ultrapure water (18.2 Megohm-cm, 5 ppb TOC) and are sterile filtered using 0.22 micron filters into sterile Deep Well blocks (no preservatives added).

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

Crystallization reagents containing buffers are formulated by creating a 1.0 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.

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. 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 crystallization reagents containing divalent cations such as magnesium, calcium, or zinc. To avoid false positives use phosphate, borate, or carbonate buffers at concentrations of 10 mM or less or exchange the phosphate, borate, or carbonate buffer with a more soluble buffer that does not complex with divalent cations.

### References and Readings


1. Page et al (2003). Shotgun crystallization strategy for structural genomics: an optimized two-tiered crystallization screen against the Thermotoga maritima proteome. Acta Cryst. D59, 1028-1037.

2. Newman et al (2005). Towards rationalization of crystallization screening for small- to medium-sized academic laboratories: the PACT/JCSG+ strategy. Acta Cryst. D61, 1426-1431.

3. A comparison of salts for the crystallization of macromolecules. Alexander McPherson. Protein Science (2001), 10:418-422.

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. Polymers as nucleants under high salt conditions. McPherson, A. Oral presentation at RAMC 2001, San Diego, CA. USA.

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

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### Technical Support

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

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## Fundamentals HR2-150

### How to Reproduce JCSG Plus HT Reagents

JCSG Plus HT reagents and optimization conditions based on JCSG Plus HT hits can be formulated using volumetric methods and carefully prepared reagent stocks (Table 1). Note the examples below.

**Example 1**. To prepare 1.0 milliliter of JCSG Plus HT reagent 83 (G11) in a crystallization plate.
Solution Composition: 0.1 M BIS-TRIS pH 5.5
2.0 M Ammonium sulfate

- 329 µl water[3]
- 100 µl 1.0 M BIS-TRIS pH 5.5
  (CAS # 6976-37-0, Catalog # HR2-781)
- 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 JCSG Plus HT reagent 4 (A4).
Solution Composition: 0.02 M Calcium chloride dihydrate,
0.1 M Sodium acetate trihydrate pH 4.6,
30% v/v (+/-)-2-Methyl-2,4-pentanediol

- 580 µl water[3]
- 20 µl 1.0 M Calcium chloride dihydrate
  (CAS # 10035-04-8, Catalog # HR2-557)
- 100 µl 1.0 M Sodium acetate trihydrate pH 4.6
  (CAS # 6131-90-4, Catalog # HR2-731)
- 300 µl 100% (+/-)-2-Methyl-2,4-pentanediol
  (CAS # 107-41-5, Catalog # HR2-627)

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

**Example 3**. To prepare 10 milliliters of JCSG Plus HT reagent 61 (F1).
Solution Composition: 30% v/v Jeffamine® M-600® pH 7.0
0.1 M MES monohydrate pH 6.5
0.05 M Cesium chloride

- 2.5 ml water[3]
- 0.5 ml 1.0 M Cesium chloride
  (CAS # 7647-17-8, Catalog # HR2-719)
- 1.0 ml 1.0 M MES monohydrate pH 6.5
  (CAS # 145224-94-8, Catalog # HR2-787)
- 6.0 ml 50% v/v Jeffamine® M-600® pH 7.0
  (CAS # 77110-54-4, Catalog # HR2-501)

Make no pH adjustments. Mix well.

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

### Formulation Notes for JCSG Plus HT Reagents

1. No additional pH adjustment is made to any reagent after formulation. Use the buffers in Table 1 to reproduce an JCSG Plus HT reagent.
2. All Optimize solutions and screen reagents are sterile filtered using 0.22 µm filters into sterile containers.
3. Add water first as this will help maintain the solubility of subsequently added reagents.
4. When formulating reagents using a pipet, add the largest volume last (except water). Use this larger volume setting to aspirate and dispense the reagent until the solution is mixed.
5. When formulating reagents using a pipet, use a clean, sterile pipet tip for each reagent added to the solution.
6. Use the buffers in Table 2 to systematically vary the pH as a crystallization variable.

### pH as a Crystallization Variable

The buffers listed in Table 2, can be used to vary the pH as a crystallization variable and are recommended when optimizing a crystal grown from an JCSG Plus HT kit.

Optimize™ buffer stocks are supplied as a 100 milliliters sterile filtered solution. Optimize buffers are available as an acid-base pair or titrated to a specific pH.

StockOptions™ buffer kits contain 10 milliliters each of ready to pipet buffers, titrated in 0.1 pH increments over the indicated pH range. The number of reagents offered in a StockOptions buffer kit depends upon the pH range of the buffer. The broader the pH range, the more buffers in the kit.

### Online Information

Visit www.hamptonresearch.com and enter one of the following:

- Reagent Catalog Number
- Kit Catalog Number
- CAS Number
- Reagent Name

To obtain reagent specifications, pH titration tables, user guides, certificates of analysis, material safety data sheets (SDS), and any other additional information.

### MakeTray™

MakeTray is a free, web based program at www.hamptonresearch.com which generates both a pipetting worksheet and a reagent formulation document for crystallization set ups. MakeTray allows one to enter general information about the sample and experiment, which is then printed on the pipet worksheet and the reagent formulation document. The plate size can be customized for any number of wells, so MakeTray works for: 24, 48, and 96 well plates. MakeTray is especially useful for the design and formulation of crystal optimization experiments.

**Table 1. Recommended reagents for the formulation of JCSG Plus HT and Optimization reagents.**

Each of these reagents are available as an Optimize™ crystallization grade reagent from Hampton Research. Table 1 provides the common chemical name, the Hampton Research catalog number, supplied stock concentration, the supplied volume, and the CAS number for each reagent. For more information on a specific Optimize reagent, go to www.hamptonresearch.com. Using Search, enter either the catalog number, CAS number, or chemical name to obtain additional information for the Optimize reagent, including a Certificate of Analysis and SDS (where applicable).

| Salts | Hampton Research Catalog # | Supplied [ Stock ] | Supplied Volume | CAS # |
|---|---|---|---|---|
| Ammonium acetate | HR2-565 | 1.0 M | 100 ml | 631-61-8 |
| Ammonium chloride | HR2-691 | 5.0 M | 200 ml | 12125-02-9 |
| Ammonium citrate dibasic | HR2-685 | 2.5 M | 200 ml | 3012-65-5 |
| Ammonium formate | HR2-659 | 10.0 M | 200 ml | 540-69-2 |
| Ammonium nitrate | HR2-665 | 10.0 M | 200 ml | 6484-52-2 |
| Ammonium phosphate dibasic | HR2-629 | 3.5 M | 200 ml | 7783-28-0 |
| Ammonium phosphate monobasic | HR2-555 | 2.5 M | 200 ml | 7722-76-1 |
| Ammonium sulfate | HR2-541 | 3.5 M | 200 ml | 7783-20-2 |
| Cadmium chloride hydrate | HR2-715 | 1.0 M | 100 ml | 654054-66-7 |
| Calcium acetate hydrate | HR2-567 | 1.0 M | 100 ml | 114460-21-8 |
| Calcium chloride dihydrate | HR2-557 | 2.0 M | 100 ml | 10035-04-8 |
| Cesium chloride | HR2-719 | 1.0 M | 100 ml | 7647-17-8 |
| Cobalt(II) chloride hexahydrate | HR2-713 | 1.0 M | 100 ml | 7791-13-1 |
| Lithium chloride | HR2-631 | 10.0 M | 200 ml | 7447-41-8 |
| 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 |
| Magnesium formate dihydrate | HR2-537 | 1.0 M | 200 ml | 6150-82-9 |
| Magnesium sulfate heptahydrate | HR2-821 | 2.0 M | 100 ml | 10034-99-8 |
| 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 | 100 ml | 7791-20-0 |
| Potassium bromide | HR2-779 | 4.0 M | 100 ml | 7758-02-3 |
| Potassium citrate tribasic monohydrate | HR2-683 | 2.5 M | 200 ml | 6100-05-6 |
| Potassium formate | HR2-667 | 14.0 M | 200 ml | 590-29-4 |
| Potassium nitrate | HR2-663 | 2.0 M | 200 ml | 7757-79-1 |
| Potassium phosphate dibasic | HR2-635 | 4.0 M | 200 ml | 7758-11-4 |
| Potassium phosphate monobasic | HR2-553 | 1.5 M | 200 ml | 7778-77-0 |
| Potassium thiocyanate | HR2-695 | 8.0 M | 200 ml | 333-20-0 |
| 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 citrate tribasic dihydrate pH 6.5 | HR2-912-28 | 1.6 M | 185 ml | 6132-04-3 |

**Table 1 (Continued).** **Recommended reagents for the formulation of JCSG Plus HT and Optimization reagents.**

| Salts | Hampton Research Catalog # | Supplied [ Stock ] | Supplied Volume | CAS # |
|---|---|---|---|---|
| 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 |
| Sodium thiocyanate | HR2-693 | 8.0 M | 200 ml | 540-72-7 |
| Succinic acid pH 7.0 | HR2-709 | 1.2 M | 200 ml | 110-15-6 |
| 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 |
| | | | | |
| **Polymers** | **Hampton Research Catalog #** | **Supplied [ Stock ]** | **Supplied Volume** | **CAS #** |
| Jeffamine ED-2001 ® pH 7.0 | HR2-597 | 50% w/v | 200 ml | 65605-36-9 |
| Jeffamine M-600 ® pH 7.0 | HR2-501 | 50% v/v | 200 ml | 83713-01-3 |
| Poly(acrylic acid sodium salt) 5,100 | HR2-773 | 50% w/v | 200 ml | 9003-04-7 |
| Polyethylene glycol 200 | HR2-601 | 100% | 200 ml | 25322-68-3 |
| Polyethylene glycol 300 | HR2-517 | 100% | 200 ml | 25322-68-3 |
| Polyethylene glycol 400 | HR2-603 | 100% | 200 ml | 25322-68-3 |
| Polyethylene glycol 1,000 | HR2-523 | 50% w/v | 200 ml | 25322-68-3 |
| Polyethylene glycol 1,500 | HR2-525 | 50% w/v | 200 ml | 25322-68-3 |
| Polyethylene glycol 3,000 | HR2-604 | 50% w/v | 200 ml | 25322-68-3 |
| Polyethylene glycol 3,350 | HR2-527 | 50% w/v | 200 ml | 25322-68-3 |
| Polyethylene glycol 4,000 | HR2-529 | 50% w/v | 200 ml | 25322-68-3 |
| Polyethylene glycol 6,000 | HR2-533 | 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 20,000 | HR2-609 | 30% w/v | 200 ml | 25322-68-3 |
| Polyethylene glycol monomethyl ether 2,000 | HR2-613 | 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 #** |
| Ethylene glycol | HR2-621 | 100% | 200 ml | 107-21-1 |
| Glycerol | HR2-623 | 100% | 100 ml | 56-81-5 |
| (+/-)-2-Methyl-2,4-pentanediol | HR2-627 | 100% | 200 ml | 107-41-5 |
| 1,2-Propanediol | N/A | N/A | N/A | 57-55-6 |
| | | | | |

**Table 1 (Continued).** **Recommended reagents for the formulation of JCSG Plus HT and Optimization reagents.**

| Organics (volatile) | Hampton Research Catalog # | Supplied [ Stock ] | Supplied Volume | CAS # |
|---|---|---|---|---|
| 1,4-Dioxane | N/A | N/A | N/A | 123-91-1 |
| 2-Propanol | N/A | N/A | N/A | 67-63-0 |
| Ethanol | N/A | N/A | N/A | 64-17-5 |
| | | | | |
| **Buffers** | **Hampton Research Catalog #** | **Supplied [ Stock ]** | **Supplied Volume** | **CAS #** |
| BICINE pH 9.0 [2] | HR2-723 | 1.0 M | 100 ml | 150-25-4 |
| BIS-TRIS pH 5.5 [1] | HR2-781 | 1.0 M | 100 ml | 6976-37-0 |
| CAPS pH 10.5 [2] | HR2-941-42-5 | 1.0 M | 185 ml | 1135-40-6 |
| CHES pH 9.5 [1] | HR2-256-10 | 1.0 M | 185 ml | 103-47-9 |
| Citric acid pH 4.0 [2] | HR2-904-19 | 1.0 M | 185 ml | 77-92-9 |
| Citric acid pH 5.0 [2] | HR2-904-29 | 1.0 M | 185 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 |
| HEPES sodium pH 7.5 [1] | HR2-733 | 1.0 M | 100 ml | 75277-39-3 |
| Imidazole pH 8.0 [1] | HR2-995-19 | 1.0 M | 185 ml | 288-32-4 |
| MES monohydrate pH 6.0 [2] | HR2-943-09 | 1.0 M | 185 ml | 145224-94-8 |
| MES monohydrate pH 6.5 [2] | HR2-787 | 1.0 M | 100 ml | 145224-94-8 |
| Sodium acetate trihydrate pH 4.5 [1] | HR2-789 | 1.0 M | 100 ml | 6131-90-4 |
| Sodium acetate trihydrate pH 4.6 [1] | HR2-731 | 1.0 M | 100 ml | 6131-90-4 |
| Sodium cacodylate trihydrate pH 6.5 [1] | HR2-737 | 1.0 M | 100 ml | 6131-99-3 |
| Sodium citrate tribasic dihydrate pH 4.2 [1] | HR2-935-01 | 1.0 M | 185 ml | 6132-04-3 |
| Sodium citrate tribasic dihydrate pH 5.5 [1] | HR2-935-14 | 1.0 M | 185 ml | 6132-04-3 |
| Tris pH 7.0 [1] | HR2-900-01 | 1.0 M | 185 ml | 77-86-1 |
| 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 JCSG Plus HT and Optimization reagents.**

| Buffer Solution or Kit | Hampton Research Catalog # | Supplied [Stock] | Supplied Volume | CAS # | pH range |
|---|---|---|---|---|---|
| StockOptions™ Bicine kit [4] | HR2-101 | 1.0 M | 10 ml each | 150-25-4 | 7.4 - 9.3 |
| StockOptions™ Bis-Tris kit [4] | HR2-106 | 1.0 M | 10 ml each | 6976-37-0 | 5.5 - 7.5 |
| StockOptions™ CHES kit [4] | HR2-256 | 1.0 M | 10 ml each | 103-47-9 | 8.6 - 10.0 |
| StockOptions™ Citric Acid kit [4] | HR2-104 | 1.0 M | 10 ml each | 77-92-9 | 2.2 - 6.5 |
| StockOptions™ HEPES kit [4] | HR2-102 | 1.0 M | 10 ml each | 7365-45-9 | 6.8 - 8.2 |
| StockOptions™ Imidazole kit [4] | HR2-095 | 1.0 M | 10 ml each | 288-32-4 | 6.2 - 7.8 |
| StockOptions™ MES kit [4] | HR2-243 | 1.0 M | 10 ml each | 145224-94-8 | 5.2 - 7.1 |
| StockOptions™ Sodium Acetate kit [4] | HR2-233 | 1.0 M | 10 ml each | 6131-90-4 | 3.6 - 5.6 |
| StockOptions™ Sodium Cacodylate kit [4] | HR2-239 | 1.0 M | 10 ml each | 6131-99-3 | 5.1 - 7.4 |
| StockOptions™ Sodium Citrate kit [4] | HR2-235 | 1.0 M | 10 ml each | 6132-04-3 | 4.2 - 6.5 |
| 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 JCSG Plus HT Fundamentals, interpretation of screen results, optimization strategies and general inquiries regarding crystallization are welcome. Please e-mail, fax, or telephone your request to Hampton Research. Fax and e-mail Technical Support are available 24 hours a day. Telephone technical support is available 8:00 a.m. to 4:00 p.m. USA Pacific Standard Time.

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M-600 ® is a registered trademark of Texaco.

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| Well # | Salt | Well # | Buffer ◊ | Well # | Precipitant |
|---|---|---|---|---|---|
| 1. (A1) | 0.2 M Lithium sulfate monohydrate | 1. (A1) | 0.1 M Sodium acetate trihydrate pH 4.5 | 1. (A1) | 50% v/v Polyethylene glycol 400 |
| 2. (A2) | None | 2. (A2) | 0.1 M Sodium citrate tribasic dihydrate pH 5.5 | 2. (A2) | 20% w/v Polyethylene glycol 3,000 |
| 3. (A3) | 0.2 M Ammonium citrate dibasic | 3. (A3) | None | 3. (A3) | 20% w/v Polyethylene glycol 3,350 |
| 4. (A4) | 0.02 M Calcium chloride dihydrate | 4. (A4) | 0.1 M Sodium acetate trihydrate pH 4.6 | 4. (A4) | 30% v/v (+/-)-2-Methyl-2,4-pentanediol |
| 5. (A5) | 0.2 M Magnesium formate dihydrate | 5. (A5) | None | 5. (A5) | 20% w/v Polyethylene glycol 3,350 |
| 6. (A6) | 0.2 M Lithium sulfate monohydrate | 6. (A6) | 0.1 M Sodium citrate tribasic dihydrate pH 4.2 | 6. (A6) | 20% w/v Polyethylene glycol 1,000 |
| 7. (A7) | None | 7. (A7) | 0.1 M CHES pH 9.5 | 7. (A7) | 20% w/v Polyethylene glycol 8,000 |
| 8. (A8) | 0.2 M Ammonium formate | 8. (A8) | None | 8. (A8) | 20% w/v Polyethylene glycol 3,350 |
| 9. (A9) | 0.2 M Ammonium chloride | 9. (A9) | None | 9. (A9) | 20% w/v Polyethylene glycol 3,350 |
| 10. (A10) | 0.2 M Potassium formate | 10. (A10) | None | 10. (A10) | 20% w/v Polyethylene glycol 3,350 |
| 11. (A11) | 0.2 M Ammonium phosphate monobasic | 11. (A11) | 0.1 M Tris pH 8.5 | 11. (A11) | 50% v/v (+/-)-2-Methyl-2,4-pentanediol |
| 12. (A12) | 0.2 M Potassium nitrate | 12. (A12) | None | 12. (A12) | 20% w/v Polyethylene glycol 3,350 |
| 13. (B1) | None | 13. (B1) | 0.1 M Citric acid pH 4.0 | 13. (B1) | 0.8 M Ammonium sulfate |
| 14. (B2) | 0.2 M Sodium thiocyanate | 14. (B2) | None | 14. (B2) | 20% w/v Polyethylene glycol 3,350 |
| 15. (B3) | None | 15. (B3) | 0.1 M BICINE pH 9.0 | 15. (B3) | 20% w/v Polyethylene glycol 6,000 |
| 16. (B4) | None | 16. (B4) | 0.1 M HEPES pH 7.5 | 16. (B4) | 10% w/v Polyethylene glycol 8,000, 8% v/v Ethylene glycol |
| 17. (B5) | None | 17. (B5) | 0.1 M Sodium cacodylate trihydrate pH 6.5 | 17. (B5) | 5% w/v Polyethylene glycol 8,000, 40% v/v (+/-)-2-Methyl-2,4-pentanediol |
| 18. (B6) | None | 18. (B6) | 0.1 M Sodium citrate tribasic dihydrate pH 4.2 | 18. (B6) | 5% w/v Polyethylene glycol 1,000, 40% v/v Ethanol |
| 19. (B7) | None | 19. (B7) | 0.1 M Sodium acetate trihydrate pH 4.6 | 19. (B7) | 8% w/v Polyethylene glycol 4,000 |
| 20. (B8) | 0.2 M Magnesium chloride hexahydrate | 20. (B8) | 0.1 M Tris pH 7.0 | 20. (B8) | 10% w/v Polyethylene glycol 8,000 |
| 21. (B9) | None | 21. (B9) | 0.1 M Citric acid pH 5.0 | 21. (B9) | 20% w/v Polyethylene glycol 6,000 |
| 22. (B10) | 0.2 M Magnesium chloride hexahydrate | 22. (B10) | 0.1 M Sodium cacodylate trihydrate pH 6.5 | 22. (B10) | 50% v/v Polyethylene glycol 200 |
| 23. (B11) | None | 23. (B11) | None | 23. (B11) | 1.6 M Sodium citrate tribasic dihydrate pH 6.5 |
| 24. (B12) | 0.2 M Potassium citrate tribasic monohydrate | 24. (B12) | None | 24. (B12) | 20% w/v Polyethylene glycol 3,350 |
| 25. (C1) | 0.2 M Sodium chloride | 25. (C1) | 0.1 M Sodium citrate tribasic dihydrate pH 4.2 | 25. (C1) | 20% w/v Polyethylene glycol 8,000 |
| 26. (C2) | 1.0 M Lithium chloride | 26. (C2) | 0.1 M Citric acid pH 4.0 | 26. (C2) | 20% w/v Polyethylene glycol 6,000 |
| 27. (C3) | 0.2 M Ammonium nitrate | 27. (C3) | None | 27. (C3) | 20% w/v Polyethylene glycol 3,350 |
| 28. (C4) | None | 28. (C4) | 0.1 M HEPES pH 7.0 | 28. (C4) | 10% w/v Polyethylene glycol 6,000 |
| 29. (C5) | None | 29. (C5) | 0.1 M HEPES sodium pH 7.5 | 29. (C5) | 0.8 M Sodium phosphate monobasic monohydrate 0.8 M Potassium phosphate monobasic |
| 30. (C6) | None | 30. (C6) | 0.1 M Sodium citrate tribasic dihydrate pH 4.2 | 30. (C6) | 40% v/v Polyethylene glycol 300 |
| 31. (C7) | 0.2 M Zinc acetate dihydrate | 31. (C7) | 0.1 M Sodium acetate trihydrate pH 4.5 | 31. (C7) | 10% w/v Polyethylene glycol 3,000 |
| 32. (C8) | None | 32. (C8) | 0.1 M Tris pH 8.5 | 32. (C8) | 20% v/v Ethanol |
| 33. (C9) | None | 33. (C9) | 0.068 M Sodium phosphate monobasic monohydrate 0.032 M Potassium phosphate dibasic pH 6.2 | 33. (C9) | 10% v/v Glycerol, 25% v/v 1,2-Propanediol |
| 34. (C10) | None | 34. (C10) | 0.1 M BICINE pH 9.0 | 34. (C10) | 2% v/v 1,4-Dioxane 10% w/v Polyethylene glycol 20,000 |
| 35. (C11) | None | 35. (C11) | 0.1 M Sodium acetate trihydrate pH 4.6 | 35. (C11) | 2.0 M Ammonium sulfate |
| 36. (C12) | None | 36. (C12) | None | 36. (C12) | 10% w/v Polyethylene glycol 1,000 10% w/v Polyethylene glycol 8,000 |
| 37. (D1) | None | 37. (D1) | None | 37. (D1) | 24% w/v Polyethylene glycol 1,500, 20% v/v Glycerol |
| 38. (D2) | 0.2 M Magnesium chloride hexahydrate | 38. (D2) | 0.1 M HEPES sodium pH 7.5 | 38. (D2) | 30% v/v Polyethylene glycol 400 |
| 39. (D3) | 0.2 M Sodium chloride | 39. (D3) | 0.068 M Sodium phosphate monobasic monohydrate 0.032 M Potassium phosphate dibasic pH 6.2 | 39. (D3) | 50% v/v Polyethylene glycol 200 |
| 40. (D4) | 0.2 M Lithium sulfate monohydrate | 40. (D4) | 0.1 M Sodium acetate trihydrate pH 4.5 | 40. (D4) | 30% w/v Polyethylene glycol 8,000 |
| 41. (D5) | None | 41. (D5) | 0.1 M HEPES pH 7.5 | 41. (D5) | 70% v/v (+/-)-2-Methyl-2,4-pentanediol |
| 42. (D6) | 0.2 M Magnesium chloride hexahydrate | 42. (D6) | 0.1 M Tris pH 8.5 | 42. (D6) | 20% w/v Polyethylene glycol 8,000 |
| 43. (D7) | 0.2 M Lithium sulfate monohydrate | 43. (D7) | 0.1 M Tris pH 8.5 | 43. (D7) | 40% v/v Polyethylene glycol 400 |
| 44. (D8) | None | 44. (D8) | 0.1 M Tris pH 8.0 | 44. (D8) | 40% v/v (+/-)-2-Methyl-2,4-pentanediol |
| 45. (D9) | 0.17 M Ammonium sulfate | 45. (D9) | None | 45. (D9) | 25.5% w/v Polyethylene glycol 4,000, 15% v/v Glycerol |
| 46. (D10) | 0.2 M Calcium acetate hydrate | 46. (D10) | 0.1 M Sodium cacodylate trihydrate pH 6.5 | 46. (D10) | 40% v/v Polyethylene glycol 300 |
| 47. (D11) | 0.14 M Calcium chloride dihydrate | 47. (D11) | 0.07 M Sodium acetate trihydrate pH 4.6 | 47. (D11) | 14% v/v 2-Propanol, 30% v/v Glycerol |
| 48. (D12) | 0.04 M Potassium phosphate monobasic | 48. (D12) | None | 48. (D12) | 16% w/v Polyethylene glycol 8,000, 20% v/v Glycerol |

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

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*Solutions for Crystal Growth*

| Well # | Salt | Well # | Buffer ◊ | Well # | Precipitant |
|---|---|---|---|---|---|
| 49. (E1) | None | 49. (E1) | 0.1 M Sodium cacodylate trihydrate pH 6.5 | 49. (E1) | 1.0 M Sodium citrate tribasic dihydrate |
| 50. (E2) | 0.2 M Sodium chloride | 50. (E2) | 0.1 M Sodium cacodylate trihydrate pH 6.5 | 50. (E2) | 2.0 M Ammonium sulfate |
| 51. (E3) | 0.2 M Sodium chloride | 51. (E3) | 0.1 M HEPES pH 7.5 | 51. (E3) | 10% v/v 2-Propanol |
| 52. (E4) | 0.2 M Lithium sulfate monohydrate | 52. (E4) | 0.1 M Tris pH 8.5 | 52. (E4) | 1.26 M Ammonium sulfate |
| 53. (E5) | None | 53. (E5) | 0.1 M CAPS pH 10.5 | 53. (E5) | 40% v/v (+/-)-2-Methyl-2,4-pentanediol |
| 54. (E6) | 0.2 M Zinc acetate dihydrate | 54. (E6) | 0.1 M Imidazole pH 8.0 | 54. (E6) | 20% w/v Polyethylene glycol 3,000 |
| 55. (E7) | 0.2 M Zinc acetate dihydrate | 55. (E7) | 0.1 M Sodium cacodylate trihydrate pH 6.5 | 55. (E7) | 10% v/v 2-Propanol |
| 56. (E8) | None | 56. (E8) | 0.1 M Sodium acetate trihydrate pH 4.5 | 56. (E8) | 1.0 M Ammonium phosphate dibasic |
| 57. (E9) | None | 57. (E9) | 0.1 M MES monohydrate pH 6.5 | 57. (E9) | 1.6 M Magnesium sulfate heptahydrate |
| 58. (E10) | None | 58. (E10) | 0.1 M BICINE pH 9.0 | 58. (E10) | 10% w/v Polyethylene glycol 6,000 |
| 59. (E11) | 0.16 M Calcium acetate hydrate | 59. (E11) | 0.08 M Sodium cacodylate trihydrate pH 6.5 | 59. (E11) | 14.4% w/v Polyethylene glycol 8,000, 20% v/v Glycerol |
| 60. (E12) | None | 60. (E12) | 0.1 M Imidazole pH 8.0 | 60. (E12) | 10% w/v Polyethylene glycol 8,000 |
| 61. (F1) | 0.05 M Cesium chloride | 61. (F1) | 0.1 M MES monohydrate pH 6.5 | 61. (F1) | 30% v/v Jeffamine® M-600® |
| 62. (F2) | None | 62. (F2) | 0.1 M Citric acid pH 5.0 | 62. (F2) | 3.0 M Ammonium sulfate |
| 63. (F3) | None | 63. (F3) | 0.1 M Tris pH 8.0 | 63. (F3) | 20% v/v (+/-)-2-Methyl-2,4-pentanediol |
| 64. (F4) | None | 64. (F4) | 0.1 M HEPES pH 7.5 | 64. (F4) | 20% v/v Jeffamine® M-600® |
| 65. (F5) | 0.2 M Magnesium chloride hexahydrate | 65. (F5) | 0.1 M Tris pH 8.5 | 65. (F5) | 50% v/v Ethylene glycol |
| 66. (F6) | None | 66. (F6) | 0.1 M BICINE pH 9.0 | 66. (F6) | 10% v/v (+/-)-2-Methyl-2,4-pentanediol |
| 67. (F7) | None | 67. (F7) | None | 67. (F7) | 0.8 M Succinic acid pH 7.0 |
| 68. (F8) | None | 68. (F8) | None | 68. (F8) | 2.1 M DL-Malic acid pH 7.0 |
| 69. (F9) | None | 69. (F9) | None | 69. (F9) | 2.4 M Sodium malonate pH 7.0 |
| 70. (F10) | None | 70. (F10) | 0.1 M HEPES pH 7.0 | 70. (F10) | 1.1 M Sodium malonate pH 7.0<br>0.5% v/v Jeffamine® ED-2001 pH 7.0 |
| 71. (F11) | None | 71. (F11) | 0.1 M HEPES pH 7.0 | 71. (F11) | 1.0 M Succinic acid pH 7.0<br>1% w/v Polyethylene glycol monomethyl ether 2,000 |
| 72. (F12) | None | 72. (F12) | 0.1 M HEPES pH 7.0 | 72. (F12) | 30% v/v Jeffamine® M-600® pH 7.0 |
| 73. (G1) | None | 73. (G1) | 0.1 M HEPES pH 7.0 | 73. (G1) | 30% v/v Jeffamine® ED-2001 pH 7.0 |
| 74. (G2) | 0.02 M Magnesium chloride hexahydrate | 74. (G2) | 0.1 M HEPES pH 7.5 | 74. (G2) | 22% w/v Poly(acrylic acid sodium salt) 5,100 |
| 75. (G3) | 0.01 M Cobalt(II) chloride hexahydrate | 75. (G3) | 0.1 M Tris pH 8.5 | 75. (G3) | 20% w/v Polyvinylpyrrolidone K 15 |
| 76. (G4) | 0.2 M Trimethylamine N-oxide dihydrate | 76. (G4) | 0.1 M Tris pH 8.5 | 76. (G4) | 20% w/v Polyethylene glycol monomethyl ether 2,000 |
| 77. (G5) | 0.005 M Cobalt(II) chloride hexahydrate<br>0.005 M Nickel(II) chloride hexahydrate<br>0.005 M Cadmium chloride hydrate<br>0.005 M Magnesium chloride hexahydrate | 77. (G5) | 0.1 M HEPES pH 7.5 | 77. (G5) | 12% w/v Polyethylene glycol 3,350 |
| 78. (G6) | 0.2 M Sodium malonate pH 7.0 | 78. (G6) | None | 78. (G6) | 20% w/v Polyethylene glycol 3,350 |
| 79. (G7) | 0.1 M Succinic acid pH 7.0 | 79. (G7) | None | 79. (G7) | 15% w/v Polyethylene glycol 3,350 |
| 80. (G8) | 0.15 M DL-Malic acid pH 7.0 | 80. (G8) | None | 80. (G8) | 20% w/v Polyethylene glycol 3,350 |
| 81. (G9) | 0.1 M Potassium thiocyanate | 81. (G9) | None | 81. (G9) | 30% w/v Polyethylene glycol monomethyl ether 2,000 |
| 82. (G10) | 0.15 M Potassium bromide | 82. (G10) | None | 82. (G10) | 30% w/v Polyethylene glycol monomethyl ether 2,000 |
| 83. (G11) | None | 83. (G11) | 0.1 M BIS-TRIS pH 5.5 | 83. (G11) | 2.0 M Ammonium sulfate |
| 84. (G12) | None | 84. (G12) | 0.1 M BIS-TRIS pH 5.5 | 84. (G12) | 3.0 M Sodium chloride |
| 85. (H1) | None | 85. (H1) | 0.1 M BIS-TRIS pH 5.5 | 85. (H1) | 0.3 M Magnesium formate dihydrate |
| 86. (H2) | None | 86. (H2) | 0.1 M BIS-TRIS pH 5.5 | 86. (H2) | 1.0 M Ammonium sulfate<br>1% w/v Polyethylene glycol 3,350 |
| 87. (H3) | None | 87. (H3) | 0.1 M BIS-TRIS pH 5.5 | 87. (H3) | 25% w/v Polyethylene glycol 3,350 |
| 88. (H4) | 0.2 M Calcium chloride dihydrate | 88. (H4) | 0.1 M BIS-TRIS pH 5.5 | 88. (H4) | 45% v/v (+/-)-2-Methyl-2,4-pentanediol |
| 89. (H5) | 0.2 M Ammonium acetate | 89. (H5) | 0.1 M BIS-TRIS pH 5.5 | 89. (H5) | 45% v/v (+/-)-2-Methyl-2,4-pentanediol |
| 90. (H6) | 0.1 M Ammonium acetate | 90. (H6) | 0.1 M BIS-TRIS pH 5.5 | 90. (H6) | 17% w/v Polyethylene glycol 10,000 |
| 91. (H7) | 0.2 M Ammonium sulfate | 91. (H7) | 0.1 M BIS-TRIS pH 5.5 | 91. (H7) | 25% w/v Polyethylene glycol 3,350 |
| 92. (H8) | 0.2 M Sodium chloride | 92. (H8) | 0.1 M BIS-TRIS pH 5.5 | 92. (H8) | 25% w/v Polyethylene glycol 3,350 |
| 93. (H9) | 0.2 M Lithium sulfate monohydrate | 93. (H9) | 0.1 M BIS-TRIS pH 5.5 | 93. (H9) | 25% w/v Polyethylene glycol 3,350 |
| 94. (H10) | 0.2 M Ammonium acetate | 94. (H10) | 0.1 M BIS-TRIS pH 5.5 | 94. (H10) | 25% w/v Polyethylene glycol 3,350 |
| 95. (H11) | 0.2 M Magnesium chloride hexahydrate | 95. (H11) | 0.1 M BIS-TRIS pH 5.5 | 95. (H11) | 25% w/v Polyethylene glycol 3,350 |
| 96. (H12) | 0.2 M Ammonium acetate | 96. (H12) | 0.1 M HEPES pH 7.5 | 96. (H12) | 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.

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HAMPTON RESEARCH
*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)

| JCSG Plus HT™ - HR2-150 Scoring Sheet | Date: | Date: | Date: |
|---|---|---|---|
| 1. (A1) 0.2 M Lithium sulfate monohydrate, 0.1 M Sodium acetate trihydrate pH 4.5, 50% v/v Polyethylene glycol 400 | | | |
| 2. (A2) 0.1 M Sodium citrate tribasic dihydrate pH 5.5, 20% w/v Polyethylene glycol 3,000 | | | |
| 3. (A3) 0.2 M Ammonium citrate dibasic, 20% w/v Polyethylene glycol 3,350 | | | |
| 4. (A4) 0.02 M Calcium chloride dihydrate, 0.1 M Sodium acetate trihydrate pH 4.6, 30% v/v (+/-)-2-Methyl-2,4-pentanediol | | | |
| 5. (A5) 0.2 M Magnesium formate dihydrate, 20% w/v Polyethylene glycol 3,350 | | | |
| 6. (A6) 0.2 M Lithium sulfate monohydrate, 0.1 M Sodium citrate tribasic dihydrate pH 4.2, 20% w/v Polyethylene glycol 1,000 | | | |
| 7. (A7) 0.1 M CHES pH 9.5, 20% w/v Polyethylene glycol 8,000 | | | |
| 8. (A8) 0.2 M Ammonium formate, 20% w/v Polyethylene glycol 3,350 | | | |
| 9. (A9) 0.2 M Ammonium chloride, 20% w/v Polyethylene glycol 3,350 | | | |
| 10. (A10) 0.2 M Potassium formate, 20% w/v Polyethylene glycol 3,350 | | | |
| 11. (A11) 0.2 M Ammonium phosphate monobasic, 0.1 M Tris pH 8.5, 50% v/v (+/-)-2-Methyl-2,4-pentanediol | | | |
| 12. (A12) 0.2 M Potassium nitrate, 20% w/v Polyethylene glycol 3,350 | | | |
| 13. (B1) 0.1 M Citric acid pH 4.0, 0.8 M Ammonium sulfate | | | |
| 14. (B2) 0.2 M Sodium thiocyanate, 20% w/v Polyethylene glycol 3,350 | | | |
| 15. (B3) 0.1 M BICINE pH 9.0, 20% w/v Polyethylene glycol 6,000 | | | |
| 16. (B4) 0.1 M HEPES pH 7.5, 10% w/v Polyethylene glycol 8,000, 8% v/v Ethylene glycol | | | |
| 17. (B5) 0.1 M Sodium cacodylate trihydrate pH 6.5, 5% w/v Polyethylene glycol 8,000, 40% v/v (+/-)-2-Methyl-2,4-pentanediol | | | |
| 18. (B6) 0.1 M Sodium citrate tribasic dihydrate pH 4.2, 5% w/v Polyethylene glycol 1,000, 40% v/v Ethanol | | | |
| 19. (B7) 0.1 M Sodium acetate trihydrate pH 4.6, 8% w/v Polyethylene glycol 4,000 | | | |
| 20. (B8) 0.2 M Magnesium chloride hexahydrate, 0.1 M Tris pH 7.0, 10% w/v Polyethylene glycol 8,000 | | | |
| 21. (B9) 0.1 M Citric acid pH 5.0, 20% w/v Polyethylene glycol 6,000 | | | |
| 22. (B10) 0.2 M Magnesium chloride hexahydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 50% v/v Polyethylene glycol 200 | | | |
| 23. (B11) 1.6 M Sodium citrate tribasic dihydrate pH 6.5 | | | |
| 24. (B12) 0.2 M Potassium citrate tribasic monohydrate, 20% w/v Polyethylene glycol 3,350 | | | |
| 25. (C1) 0.2 M Sodium chloride, 0.1 M Sodium citrate tribasic dihydrate pH 4.2, 20% w/v Polyethylene glycol 8,000 | | | |
| 26. (C2) 1.0 M Lithium chloride, 0.1 M Citric acid pH 4.0, 20% w/v Polyethylene glycol 6,000 | | | |
| 27. (C3) 0.2 M Ammonium nitrate, 20% w/v Polyethylene glycol 3,350 | | | |
| 28. (C4) 0.1 M HEPES pH 7.0, 10% w/v Polyethylene glycol 6,000 | | | |
| 29. (C5) 0.1 M HEPES sodium pH 7.5, 0.8 M Sodium phosphate monobasic monohydrate, 0.8 M Potassium phosphate monobasic | | | |
| 30. (C6) 0.1 M Sodium citrate tribasic dihydrate pH 4.2, 40% v/v Polyethylene glycol 300 | | | |
| 31. (C7) 0.2 M Zinc acetate dihydrate, 0.1 M Sodium acetate trihydrate pH 4.5, 10% w/v Polyethylene glycol 3,000 | | | |
| 32. (C8) 0.1 M Tris pH 8.5, 20% v/v Ethanol | | | |
| 33. (C9) 0.1 M Sodium/Potassium phosphate pH 6.2, 10% v/v Glycerol, 25% v/v 1,2-Propanediol | | | |
| 34. (C10) 0.1 M BICINE pH 9.0, 2% v/v 1,4-Dioxane, 10% w/v Polyethylene glycol 20,000 | | | |
| 35. (C11) 0.1 M Sodium acetate trihydrate pH 4.6, 2.0 M Ammonium sulfate | | | |
| 36. (C12) 10% w/v Polyethylene glycol 1,000, 10% w/v Polyethylene glycol 8,000 | | | |
| 37. (D1) 24% w/v Polyethylene glycol 1,500, 20% v/v Glycerol | | | |
| 38. (D2) 0.2 M Magnesium chloride hexahydrate, 0.1 M HEPES sodium pH 7.5, 30% v/v Polyethylene glycol 400 | | | |
| 39. (D3) 0.2 M Sodium chloride, 0.1 M Sodium/Potassium phosphate pH 6.2, 50% v/v Polyethylene glycol 200 | | | |
| 40. (D4) 0.2 M Lithium sulfate monohydrate, 0.1 M Sodium acetate trihydrate pH 4.5, 30% w/v Polyethylene glycol 8,000 | | | |
| 41. (D5) 0.1 M HEPES pH 7.5, 70% v/v (+/-)-2-Methyl-2,4-pentanediol | | | |
| 42. (D6) 0.2 M Magnesium chloride hexahydrate, 0.1 M Tris pH 8.5, 20% w/v Polyethylene glycol 8,000 | | | |
| 43. (D7) 0.2 M Lithium sulfate monohydrate, 0.1 M Tris pH 8.5, 40% v/v Polyethylene glycol 400 | | | |
| 44. (D8) 0.1 M Tris pH 8.0, 40% v/v (+/-)-2-Methyl-2,4-pentanediol | | | |
| 45. (D9) 0.17 M Ammonium sulfate, 25.5% w/v Polyethylene glycol 4,000, 15% v/v Glycerol | | | |
| 46. (D10) 0.2 M Calcium acetate hydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 40% v/v Polyethylene glycol 300 | | | |
| 47. (D11) 0.14 M Calcium chloride dihydrate, 0.07 M Sodium acetate trihydrate pH 4.6, 14% v/v 2-Propanol, 30% v/v Glycerol | | | |
| 48. (D12) 0.04 M Potassium phosphate monobasic, 16% w/v Polyethylene glycol 8,000, 20% v/v Glycerol | | | |

HAMPTON RESEARCH
*Solutions for Crystal Growth*

34 Journey
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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)

| JCSG Plus HT™ - HR2-150 Scoring Sheet | Date: | Date: | Date: |
|---|---|---|---|
| 49. (E1) 0.1 M Sodium cacodylate trihydrate pH 6.5, 1.0 M Sodium citrate tribasic dihydrate | | | |
| 50. (E2) 0.2 M Sodium chloride, 0.1 M Sodium cacodylate trihydrate pH 6.5, 2.0 M Ammonium sulfate | | | |
| 51. (E3) 0.2 M Sodium chloride, 0.1 M HEPES pH 7.5, 10% v/v 2-Propanol | | | |
| 52. (E4) 0.2 M Lithium sulfate monohydrate, 0.1 M Tris pH 8.5, 1.26 M Ammonium sulfate | | | |
| 53. (E5) 0.1 M CAPS pH 10.5, 40% v/v (+/-)-2-Methyl-2,4-pentanediol | | | |
| 54. (E6) 0.2 M Zinc acetate dihydrate, 0.1 M Imidazole pH 8.0, 20% w/v Polyethylene glycol 3,000 | | | |
| 55. (E7) 0.2 M Zinc acetate dihydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 10% v/v 2-Propanol | | | |
| 56. (E8) 0.1 M Sodium acetate trihydrate pH 4.5, 1.0 M Ammonium phosphate dibasic | | | |
| 57. (E9) 0.1 M MES monohydrate pH 6.5, 1.6 M Magnesium sulfate heptahydrate | | | |
| 58. (E10) 0.1 M BICINE pH 9.0, 10% w/v Polyethylene glycol 6,000 | | | |
| 59. (E11) 0.16 M Calcium acetate hydrate, 0.08 M Sodium cacodylate trihydrate pH 6.5, 14.4% w/v Polyethylene glycol 8,000, 20% v/v Glycerol | | | |
| 60. (E12) 0.1 M Imidazole pH 8.0, 10% w/v Polyethylene glycol 8,000 | | | |
| 61. (F1) 0.05 M Cesium chloride, 0.1 M MES monohydrate pH 6.5, 30% v/v Jeffamine® M-600® | | | |
| 62. (F2) 0.1 M Citric acid pH 5.0, 3.0 M Ammonium sulfate | | | |
| 63. (F3) 0.1 M Tris pH 8.0, 20% v/v (+/-)-2-Methyl-2,4-pentanediol | | | |
| 64. (F4) 0.1 M HEPES pH 7.5, 20% v/v Jeffamine® M-600® | | | |
| 65. (F5) 0.2 M Magnesium chloride hexahydrate, 0.1 M Tris pH 8.5, 50% v/v Ethylene glycol | | | |
| 66. (F6) 0.1 M BICINE pH 9.0, 10% v/v (+/-)-2-Methyl-2,4-pentanediol | | | |
| 67. (F7) 0.8 M Succinic acid pH 7.0 | | | |
| 68. (F8) 2.1 M DL-Malic acid pH 7.0 | | | |
| 69. (F9) 2.4 M Sodium malonate pH 7.0 | | | |
| 70. (F10) 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 | | | |
| 71. (F11) 1.0 M Succinic acid pH 7.0, 0.1 M HEPES pH 7.0, 1% w/v Polyethylene glycol monomethyl ether 2,000 | | | |
| 72. (F12) 0.1 M HEPES pH 7.0, 30% v/v Jeffamine® M-600® pH 7.0 | | | |
| 73. (G1) 0.1 M HEPES pH 7.0, 30% v/v Jeffamine® ED-2001 pH 7.0 | | | |
| 74. (G2) 0.02 M Magnesium chloride hexahydrate, 0.1 M HEPES pH 7.5, 22% w/v Poly(acrylic acid sodium salt) 5,100 | | | |
| 75. (G3) 0.01 M Cobalt(II) chloride hexahydrate, 0.1 M Tris pH 8.5, 20% w/v Polyvinylpyrrolidone K 15 | | | |
| 76. (G4) 0.2 M Trimethylamine N-oxide dihydrate, 0.1 M Tris pH 8.5, 20% w/v Polyethylene glycol monomethyl ether 2,000 | | | |
| 77. (G5) 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 | | | |
| 78. (G6) 0.2 M Sodium malonate pH 7.0, 20% w/v Polyethylene glycol 3,350 | | | |
| 79. (G7) 0.1 M Succinic acid pH 7.0, 15% w/v Polyethylene glycol 3,350 | | | |
| 80. (G8) 0.15 M DL-Malic acid pH 7.0, 20% w/v Polyethylene glycol 3,350 | | | |
| 81. (G9) 0.1 M Potassium thiocyanate, 30% w/v Polyethylene glycol monomethyl ether 2,000 | | | |
| 82. (G10) 0.15 M Potassium bromide, 30% w/v Polyethylene glycol monomethyl ether 2,000 | | | |
| 83. (G11) 0.1 M BIS-TRIS pH 5.5, 2.0 M Ammonium sulfate | | | |
| 84. (G12) 0.1 M BIS-TRIS pH 5.5, 3.0 M Sodium chloride | | | |
| 85. (H1) 0.1 M BIS-TRIS pH 5.5, 0.3 M Magnesium formate dihydrate | | | |
| 86. (H2) 1.0 M Ammonium sulfate, 0.1 M BIS-TRIS pH 5.5, 1% w/v Polyethylene glycol 3,350 | | | |
| 87. (H3) 0.1 M BIS-TRIS pH 5.5, 25% w/v Polyethylene glycol 3,350 | | | |
| 88. (H4) 0.2 M Calcium chloride dihydrate, 0.1 M BIS-TRIS pH 5.5, 45% v/v (+/-)-2-Methyl-2,4-pentanediol | | | |
| 89. (H5) 0.2 M Ammonium acetate, 0.1 M BIS-TRIS pH 5.5, 45% v/v (+/-)-2-Methyl-2,4-pentanediol | | | |
| 90. (H6) 0.1 M Ammonium acetate, 0.1 M BIS-TRIS pH 5.5, 17% w/v Polyethylene glycol 10,000 | | | |
| 91. (H7) 0.2 M Ammonium sulfate, 0.1 M BIS-TRIS pH 5.5, 25% w/v Polyethylene glycol 3,350 | | | |
| 92. (H8) 0.2 M Sodium chloride, 0.1 M BIS-TRIS pH 5.5, 25% w/v Polyethylene glycol 3,350 | | | |
| 93. (H9) 0.2 M Lithium sulfate monohydrate, 0.1 M BIS-TRIS pH 5.5, 25% w/v Polyethylene glycol 3,350 | | | |
| 94. (H10) 0.2 M Ammonium acetate, 0.1 M BIS-TRIS pH 5.5, 25% w/v Polyethylene glycol 3,350 | | | |
| 95. (H11) 0.2 M Magnesium chloride hexahydrate, 0.1 M BIS-TRIS pH 5.5, 25% w/v Polyethylene glycol 3,350 | | | |
| 96. (H12) 0.2 M Ammonium acetate, 0.1 M HEPES pH 7.5, 45% v/v (+/-)-2-Methyl-2,4-pentanediol | | | |

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