

Application

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 is a general sparse matrix screen. 67 core reagents are based upon a statistical analysis performed by Page¹ 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 Index², an expansion of the JCSG selected by Newman² to fill out the pH profile of the screen and to enhance the range of precipitants by including neutralized organic acids³ and Tacsimate⁴. 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.

Performing The Screen

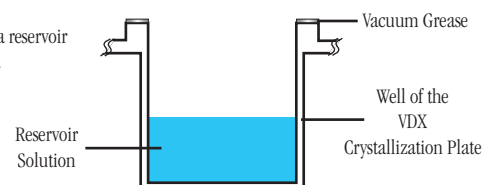
Since it is the most frequently reported method of crystallization, the following procedure describes the use of JCSG Plus with the Hanging Drop Vapor Diffusion method. JCSG Plus is also compatible with the Sitting Drop, Sand-

wich 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 (HR3-141) 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 (HR3-171). Ninety-six reservoirs are to be prepared for a complete JCSG Plus. See Figure 1.

Figure 1

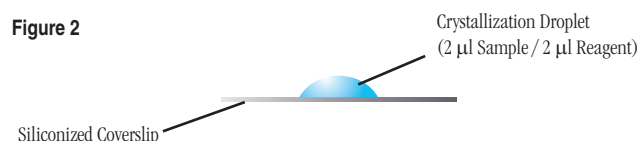
Cross section of a reservoir in the VDX plate.



2. Using a clean pipet tip, pipet 1 ml of JCSG Plus reagent 1 into reservoir A1. Discard the pipet tip, add a new pipet tip and pipet 1 ml of JCSG Plus reagent 2 into reservoir A2. Repeat the procedure for the remaining 94 JCSG Plus 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

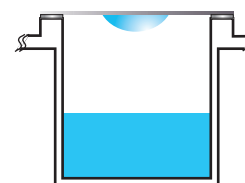


4. Pipet 2 µl of JCSG Plus reagent 1 from reservoir A1 into the sample droplet and mix by aspirating and dispensing the droplet several times, keeping the tip in the drop during mixing to avoid foaming. See Figure 2.

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.

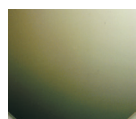
Figure 3

Inverted siliconized coverslip placed over the reservoir.

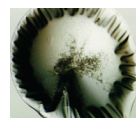


6. Repeat operations 3 through 5 for the remaining 95 JCSG Plus reagents.
7. If the quantity of sample permits, perform JCSG Plus in duplicate and incubate one set of plates at 4°C and the second set at room temperature. Incubate and store the crystallization plates in a stable temperature environment free of vibration.

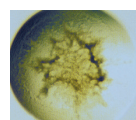
Figure 4
Typical observations in a crystallization experiment



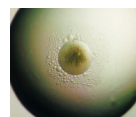
Clear Drop



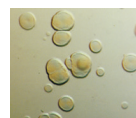
Skin /
Precipitate



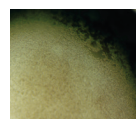
Precipitate



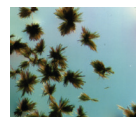
Precipitate /
Phase



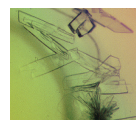
Quasi
Crystals



Microcrystals



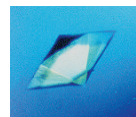
Needle
Cluster



Plates



Rod Cluster



Single
Crystal

Examine The Drop

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

Interpreting JCSG Plus

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 JCSG Plus condition and doubling the sample concentration. If more than 70 of the 96 JCSG Plus drops are clear consider doubling the sample concentration and repeating the entire screen.

Drops containing precipitate indicate that either the relative supersaturation of the sample and reagent is too high, the sample has denatured, or the sample is heterogeneous. To reduce the relative supersaturation, dilute the sample twofold and repeat the JCSG Plus condition. If more than 70 of the 96 JCSG Plus 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.

JCSG Plus Formulation

JCSG Plus 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).

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

JCSG Plus 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.

JCSG Plus 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 JCSG Plus 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 JCSG Plus 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

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.

Technical Support

Inquiries regarding JCSG Plus 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

HR2-145

How to Reproduce JCSG Plus Reagents

JCSG Plus reagents and optimization conditions based on JCSG Plus 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 reagent 83 in a crystallization plate.

Solution Composition: 0.1 M BIS-TRIS pH 5.5
2.0 M Ammonium sulfate

- 329 µl water³
- 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 reagent 4.

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 water³
- 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 reagent 61.

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 water³
- 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 Reagents

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

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Table 1. Recommended reagents for the formulation of JCSG Plus 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

Fundamentals

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Table 1 (Continued). Recommended reagents for the formulation of JCSG Plus 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

Fundamentals

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

Buffer Solution <u>or</u> 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 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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Tube #	Salt	Tube #	Buffer ◇	Tube #	Precipitant
1.	0.2 M Lithium sulfate monohydrate	1.	0.1 M Sodium acetate trihydrate pH 4.5	1.	50% v/v Polyethylene glycol 400
2.	None	2.	0.1 M Sodium citrate tribasic dihydrate pH 5.5	2.	20% w/v Polyethylene glycol 3,000
3.	0.2 M Ammonium citrate dibasic	3.	None	3.	20% w/v Polyethylene glycol 3,350
4.	0.02 M Calcium chloride dihydrate	4.	0.1 M Sodium acetate trihydrate pH 4.6	4.	30% v/v (+/-)-2-Methyl-2,4-pentanediol
5.	0.2 M Magnesium formate dihydrate	5.	None	5.	20% w/v Polyethylene glycol 3,350
6.	0.2 M Lithium sulfate monohydrate	6.	0.1 M Sodium citrate tribasic dihydrate pH 4.2	6.	20% w/v Polyethylene glycol 1,000
7.	None	7.	0.1 M CHES pH 9.5	7.	20% w/v Polyethylene glycol 8,000
8.	0.2 M Ammonium formate	8.	None	8.	20% w/v Polyethylene glycol 3,350
9.	0.2 M Ammonium chloride	9.	None	9.	20% w/v Polyethylene glycol 3,350
10.	0.2 M Potassium formate	10.	None	10.	20% w/v Polyethylene glycol 3,350
11.	0.2 M Ammonium phosphate monobasic	11.	0.1 M Tris pH 8.5	11.	50% v/v (+/-)-2-Methyl-2,4-pentanediol
12.	0.2 M Potassium nitrate	12.	None	12.	20% w/v Polyethylene glycol 3,350
13.	None	13.	0.1 M Citric acid pH 4.0	13.	0.8 M Ammonium sulfate
14.	0.2 M Sodium thiocyanate	14.	None	14.	20% w/v Polyethylene glycol 3,350
15.	None	15.	0.1 M BICINE pH 9.0	15.	20% w/v Polyethylene glycol 6,000
16.	None	16.	0.1 M HEPES pH 7.5	16.	10% w/v Polyethylene glycol 8,000, 8% v/v Ethylene glycol
17.	None	17.	0.1 M Sodium cacodylate trihydrate pH 6.5	17.	5% w/v Polyethylene glycol 8,000, 40% v/v (+/-)-2-Methyl-2,4-pentanediol
18.	None	18.	0.1 M Sodium citrate tribasic dihydrate pH 4.2	18.	5% w/v Polyethylene glycol 1,000, 40% v/v Ethanol
19.	None	19.	0.1 M Sodium acetate trihydrate pH 4.6	19.	8% w/v Polyethylene glycol 4,000
20.	0.2 M Magnesium chloride hexahydrate	20.	0.1 M Tris pH 7.0	20.	10% w/v Polyethylene glycol 8,000
21.	None	21.	0.1 M Citric acid pH 5.0	21.	20% w/v Polyethylene glycol 6,000
22.	0.2 M Magnesium chloride hexahydrate	22.	0.1 M Sodium cacodylate trihydrate pH 6.5	22.	50% v/v Polyethylene glycol 200
23.	None	23.	None	23.	1.6 M Sodium citrate tribasic dihydrate pH 6.5
24.	0.2 M Potassium citrate tribasic monohydrate	24.	None	24.	20% w/v Polyethylene glycol 3,350
25.	0.2 M Sodium chloride	25.	0.1 M Sodium citrate tribasic dihydrate pH 4.2	25.	20% w/v Polyethylene glycol 8,000
26.	1.0 M Lithium chloride	26.	0.1 M Citric acid pH 4.0	26.	20% w/v Polyethylene glycol 6,000
27.	0.2 M Ammonium nitrate	27.	None	27.	20% w/v Polyethylene glycol 3,350
28.	None	28.	0.1 M HEPES pH 7.0	28.	10% w/v Polyethylene glycol 6,000
29.	None	29.	0.1 M HEPES sodium pH 7.5	29.	0.8 M Sodium phosphate monobasic monohydrate 0.8 M Potassium phosphate monobasic
30.	None	30.	0.1 M Sodium citrate tribasic dihydrate pH 4.2	30.	40% v/v Polyethylene glycol 300
31.	0.2 M Zinc acetate dihydrate	31.	0.1 M Sodium acetate trihydrate pH 4.5	31.	10% w/v Polyethylene glycol 3,000
32.	None	32.	0.1 M Tris pH 8.5	32.	20% v/v Ethanol
33.	None	33.	0.068 M Sodium phosphate monobasic monohydrate 0.032 M Potassium phosphate dibasic pH 6.2	33.	10% v/v Glycerol, 25% v/v 1,2-Propanediol
34.	None	34.	0.1 M BICINE pH 9.0	34.	2% v/v 1,4-Dioxane 10% w/v Polyethylene glycol 20,000
35.	None	35.	0.1 M Sodium acetate trihydrate pH 4.6	35.	2.0 M Ammonium sulfate
36.	None	36.	None	36.	10% w/v Polyethylene glycol 1,000 10% w/v Polyethylene glycol 8,000
37.	None	37.	None	37.	24% w/v Polyethylene glycol 1,500, 20% v/v Glycerol
38.	0.2 M Magnesium chloride hexahydrate	38.	0.1 M HEPES sodium pH 7.5	38.	30% v/v Polyethylene glycol 400
39.	0.2 M Sodium chloride	39.	0.068 M Sodium phosphate monobasic monohydrate 0.032 M Potassium phosphate dibasic pH 6.2	39.	50% v/v Polyethylene glycol 200
40.	0.2 M Lithium sulfate monohydrate	40.	0.1 M Sodium acetate trihydrate pH 4.5	40.	30% w/v Polyethylene glycol 8,000
41.	None	41.	0.1 M HEPES pH 7.5	41.	70% v/v (+/-)-2-Methyl-2,4-pentanediol
42.	0.2 M Magnesium chloride hexahydrate	42.	0.1 M Tris pH 8.5	42.	20% w/v Polyethylene glycol 8,000
43.	0.2 M Lithium sulfate monohydrate	43.	0.1 M Tris pH 8.5	43.	40% v/v Polyethylene glycol 400
44.	None	44.	0.1 M Tris pH 8.0	44.	40% v/v (+/-)-2-Methyl-2,4-pentanediol
45.	0.17 M Ammonium sulfate	45.	None	45.	25.5% w/v Polyethylene glycol 4,000, 15% v/v Glycerol
46.	0.2 M Calcium acetate hydrate	46.	0.1 M Sodium cacodylate trihydrate pH 6.5	46.	40% v/v Polyethylene glycol 300
47.	0.14 M Calcium chloride dihydrate	47.	0.07 M Sodium acetate trihydrate pH 4.6	47.	14% v/v 2-Propanol, 30% v/v Glycerol
48.	0.04 M Potassium phosphate monobasic	48.	None	48.	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.

Tube #	Salt	Tube #	Buffer ◇	Tube #	Precipitant
49.	None	49.	0.1 M Sodium cacodylate trihydrate pH 6.5	49.	1.0 M Sodium citrate tribasic dihydrate
50.	0.2 M Sodium chloride	50.	0.1 M Sodium cacodylate trihydrate pH 6.5	50.	2.0 M Ammonium sulfate
51.	0.2 M Sodium chloride	51.	0.1 M HEPES pH 7.5	51.	10% v/v 2-Propanol
52.	0.2 M Lithium sulfate monohydrate	52.	0.1 M Tris pH 8.5	52.	1.26 M Ammonium sulfate
53.	None	53.	0.1 M CAPS pH 10.5	53.	40% v/v (+/-)-2-Methyl-2,4-pentanediol
54.	0.2 M Zinc acetate dihydrate	54.	0.1 M Imidazole pH 8.0	54.	20% w/v Polyethylene glycol 3,000
55.	0.2 M Zinc acetate dihydrate	55.	0.1 M Sodium cacodylate trihydrate pH 6.5	55.	10% v/v 2-Propanol
56.	None	56.	0.1 M Sodium acetate trihydrate pH 4.5	56.	1.0 M Ammonium phosphate dibasic
57.	None	57.	0.1 M MES monohydrate pH 6.5	57.	1.6 M Magnesium sulfate heptahydrate
58.	None	58.	0.1 M BICINE pH 9.0	58.	10% w/v Polyethylene glycol 6,000
59.	0.16 M Calcium acetate hydrate	59.	0.08 M Sodium cacodylate trihydrate pH 6.5	59.	14.4% w/v Polyethylene glycol 8,000, 20% v/v Glycerol
60.	None	60.	0.1 M Imidazole pH 8.0	60.	10% w/v Polyethylene glycol 8,000
61.	0.05 M Cesium chloride	61.	0.1 M MES monohydrate pH 6.5	61.	30% v/v Jeffamine® M-600®
62.	None	62.	0.1 M Citric acid pH 5.0	62.	3.0 M Ammonium sulfate
63.	None	63.	0.1 M Tris pH 8.0	63.	20% v/v (+/-)-2-Methyl-2,4-pentanediol
64.	None	64.	0.1 M HEPES pH 7.5	64.	20% v/v Jeffamine® M-600®
65.	0.2 M Magnesium chloride hexahydrate	65.	0.1 M Tris pH 8.5	65.	50% v/v Ethylene glycol
66.	None	66.	0.1 M BICINE pH 9.0	66.	10% v/v (+/-)-2-Methyl-2,4-pentanediol
67.	None	67.	None	67.	0.8 M Succinic acid pH 7.0
68.	None	68.	None	68.	2.1 M DL-Malic acid pH 7.0
69.	None	69.	None	69.	2.4 M Sodium malonate pH 7.0
70.	None	70.	0.1 M HEPES pH 7.0	70.	1.1 M Sodium malonate pH 7.0 0.5% v/v Jeffamine® ED-2001 pH 7.0
71.	None	71.	0.1 M HEPES pH 7.0	71.	1.0 M Succinic acid pH 7.0 1% w/v Polyethylene glycol monomethyl ether 2,000
72.	None	72.	0.1 M HEPES pH 7.0	72.	30% v/v Jeffamine® M-600® pH 7.0
73.	None	73.	0.1 M HEPES pH 7.0	73.	30% v/v Jeffamine® ED-2001 pH 7.0
74.	0.02 M Magnesium chloride hexahydrate	74.	0.1 M HEPES pH 7.5	74.	22% w/v Poly(acrylic acid sodium salt) 5,100
75.	0.01 M Cobalt(II) chloride hexahydrate	75.	0.1 M Tris pH 8.5	75.	20% w/v Polyvinylpyrrolidone K 15
76.	0.2 M Trimethylamine N-oxide dihydrate	76.	0.1 M Tris pH 8.5	76.	20% w/v Polyethylene glycol monomethyl ether 2,000
77.	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	77.	0.1 M HEPES pH 7.5	77.	12% w/v Polyethylene glycol 3,350
78.	0.2 M Sodium malonate pH 7.0	78.	None	78.	20% w/v Polyethylene glycol 3,350
79.	0.1 M Succinic acid pH 7.0	79.	None	79.	15% w/v Polyethylene glycol 3,350
80.	0.15 M DL-Malic acid pH 7.0	80.	None	80.	20% w/v Polyethylene glycol 3,350
81.	0.1 M Potassium thiocyanate	81.	None	81.	30% w/v Polyethylene glycol monomethyl ether 2,000
82.	0.15 M Potassium bromide	82.	None	82.	30% w/v Polyethylene glycol monomethyl ether 2,000
83.	None	83.	0.1 M BIS-TRIS pH 5.5	83.	2.0 M Ammonium sulfate
84.	None	84.	0.1 M BIS-TRIS pH 5.5	84.	3.0 M Sodium chloride
85.	None	85.	0.1 M BIS-TRIS pH 5.5	85.	0.3 M Magnesium formate dihydrate
86.	None	86.	0.1 M BIS-TRIS pH 5.5	86.	1.0 M Ammonium sulfate 1% w/v Polyethylene glycol 3,350
87.	None	87.	0.1 M BIS-TRIS pH 5.5	87.	25% w/v Polyethylene glycol 3,350
88.	0.2 M Calcium chloride dihydrate	88.	0.1 M BIS-TRIS pH 5.5	88.	45% v/v (+/-)-2-Methyl-2,4-pentanediol
89.	0.2 M Ammonium acetate	89.	0.1 M BIS-TRIS pH 5.5	89.	45% v/v (+/-)-2-Methyl-2,4-pentanediol
90.	0.1 M Ammonium acetate	90.	0.1 M BIS-TRIS pH 5.5	90.	17% w/v Polyethylene glycol 10,000
91.	0.2 M Ammonium sulfate	91.	0.1 M BIS-TRIS pH 5.5	91.	25% w/v Polyethylene glycol 3,350
92.	0.2 M Sodium chloride	92.	0.1 M BIS-TRIS pH 5.5	92.	25% w/v Polyethylene glycol 3,350
93.	0.2 M Lithium sulfate monohydrate	93.	0.1 M BIS-TRIS pH 5.5	93.	25% w/v Polyethylene glycol 3,350
94.	0.2 M Ammonium acetate	94.	0.1 M BIS-TRIS pH 5.5	94.	25% w/v Polyethylene glycol 3,350
95.	0.2 M Magnesium chloride hexahydrate	95.	0.1 M BIS-TRIS pH 5.5	95.	25% w/v Polyethylene glycol 3,350
96.	0.2 M Ammonium acetate	96.	0.1 M HEPES pH 7.5	96.	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.

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™ - HR2-145 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			

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™ - HR2-145 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			