Crystal Screen Cryo HT™

HAMPTON RESEARCH
Solutions for Crystal Growth

User Guide **HR2-133 (pg 1)**

Applications
Crystallization screen for proteins, peptides, nucleic acids and water soluble small molecules in the presence of a cryoprotectant.

Features
Crystal Screen Cryo HT™ is designed to provide a biased sparse matrix of trial containing conditions developed by Hampton Research as well as published crystallization conditions - optimized for cryo cooling. The reagent parameter variables are:

- pH
- Buffer material
- Salt
- Precipitant
- Cryoprotectant

Seven different pH's 4.5, 4.6, 5.6, 6.5, 7.5, 8.5, and 9.0 are utilized with nine buffers:

- BICINE
- HEPES
- HEPES sodium
- MES monohydrate
- Sodium acetate trihydrate
- Sodium cacodylate trihydrate
- Sodium citrate tribasic dihydrate
- Tris
- TRIS hydrochloride

The four categories of precipitating agents utilized are:

- Volatile organics
- Non-volatile organics
- Polymers
- Salts

Refer to the enclosed Crystal Screen Cryo HT reagent formulation for additional information.

General Description
Crystal Screen Cryo HT is supplied in a sterile, polypropylene 96 Deep Well block, each reservoir containing 1 ml of sterile filtered reagent. The block is heat sealed using a special polypropylene backed film.

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 microfiltration prior to use.

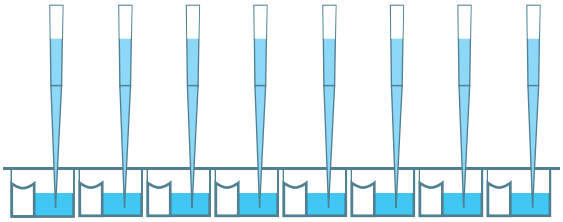
The recommended sample concentration is 5 to 25 mg/ml in sterile filtered, dilute (25 mM or less) buffer. For initial screens, the sample should be free of unnecessary additives in order to observe the effect of the Crystal Screen Cryo HT variables. However, agents that promote and preserve sample stability and homogeneity can and should be included in the sample. For additional sample preparation recommendation see Crystal Growth 101 - Preliminary Sample Preparation bulletin from Hampton Research.

Preparing the Deep Well Block for Use
Allow the block to equilibrate to room temperature. To remove stray reagent from the sealing film, centrifuge the block at 500 rpm for 5 minutes. To remove film, grasp a corner of the film and gently peel film from the block. Alternatively, the film can be pierced to access reagents.

Performing the Screen
Manual Method - Sitting Drop Vapor Diffusion

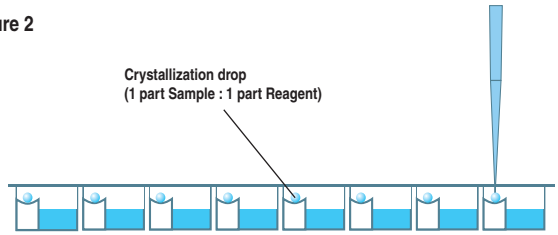
1. Using a 96 well sitting drop vapor diffusion plate, pipet the recommended volume (typically 50 to 100 microliters) of crystallization reagent from the Deep Well block into the reservoirs of the crystallization plate. The Deep Well block is compatible with 8 and 12 channel pipets as well as many automated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



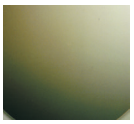
2. Using clean pipet tips, pipet 0.05 to 2 microliters of crystallization reagent from the crystallization plate reservoir to the sitting drop well. Change the pipet tip between reagents. See Figure 2.

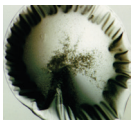
Figure 2

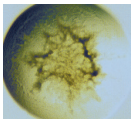


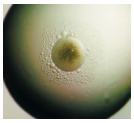
3. Using a clean pipet tip, pipet 0.05 to 2 microliters of sample to the reagent drop in the sitting drop well. One may choose to simply dispense the sample with no mixing or dispense with mixing by gently aspirating and dispensing the sample several times, keeping the tip in the drop during mixing to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

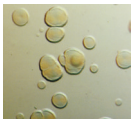
Figure 3
Typical observations in a crystallization experiment

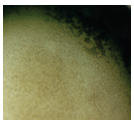

Clear Drop


Skin / Precipitate

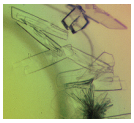

Precipitate


Precipitate / Phase

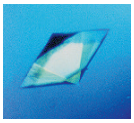

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

4. Seal the crystallization plate as per the manufacturer's recommendation. View and score the experiment as desired.

5. Seal the remaining reagent in the Deep Well block using sealing film.

Crystal Screen Cryo HT Deep Well Block and Automated Liquid Handling Systems

The polypropylene Deep Well block is designed to be compatible with the SBS standard 96 microwell format and is therefore compatible with numerous automated liquid handling systems that accept 8 x 12 96 well assay blocks. Follow the manufacturer's recommendation for handling 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 3, on the left side of page 2 shows typical examples of what one might observe in a crystallization experiment.

Interpreting Crystal Screen Cryo 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.

Crystal Screen Cryo 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.

Crystal Screen Cryo HT™

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Crystallization reagents are stable at room temperature and are best if used within 12 months of receipt. To enhance reagent stability the crystallization reagents can 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. Cudney, R., Patel, S., Weisgraber, K., Newhouse, Y., and McPherson, A., Acta Cryst. (1994) D50, 414-423.

2. The development and application of a method to quantify the quality of cryoprotectant solutions using standard area-detector X-ray images. McFerrin and Snell, J. Appl. Cryst. (2002). 35, 538-545.

3. Crystallization of nucleic acids and proteins, Edited by A. Ducruix and R. Giege, The Practical Approach Series, Oxford Univ. Press, 1992.

4. Current approaches to macromolecular crystallization. McPherson, A. Eur. J. Biochem. 189, 1-23, 1990.

5. Sparse Matrix Sampling: a screening method for crystallization of proteins. Jancarik, J. and Kim, S.H. J. Appl. Cryst., 24,409-411, 1991.

Technical Support

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

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Well #	Salt	Well #	Buffer ◊	Well #	Precipitant	Well #	Glycerol
1. (A1)	0.02 M Calcium chloride dihydrate	1. (A1)	0.1 M Sodium acetate trihydrate pH 4.6	1. (A1)	30% v/v (+/-)-2-Methyl-2,4-pentanediol	1. (A1)	None
2. (A2)		2. (A2)		2. (A2)	0.26 M Potassium sodium tartrate tetrahydrate	2. (A2)	35% v/v
3. (A3)		3. (A3)		3. (A3)	0.26 M Ammonium phosphate monobasic	3. (A3)	35% v/v
4. (A4)		4. (A4)	0.075 M TRIS hydrochloride pH 8.5	4. (A4)	1.5 M Ammonium sulfate	4. (A4)	25% v/v
5. (A5)	0.2 M Sodium citrate tribasic dihydrate	5. (A5)	0.1 M HEPES sodium pH 7.5	5. (A5)	30% v/v (+/-)-2-Methyl-2,4-pentanediol	5. (A5)	None
6. (A6)	0.16 M Magnesium chloride hexahydrate	6. (A6)	0.08 M TRIS hydrochloride pH 8.5	6. (A6)	24% w/v Polyethylene glycol 4,000	6. (A6)	20% v/v
7. (A7)		7. (A7)	0.07 M Sodium cacodylate trihydrate pH 6.5	7. (A7)	0.98 M Sodium acetate trihydrate	7. (A7)	30% v/v
8. (A8)	0.14 M Sodium citrate tribasic dihydrate	8. (A8)	0.07 M Sodium cacodylate trihydrate pH 6.5	8. (A8)	21% v/v 2-Propanol	8. (A8)	30% v/v
9. (A9)	0.17 M Ammonium acetate	9. (A9)	0.085 M Sodium citrate tribasic dihydrate pH 5.6	9. (A9)	25.5% w/v Polyethylene glycol 4,000	9. (A9)	15% v/v
10. (A10)	0.17 M Ammonium acetate	10. (A10)	0.085 M Sodium acetate trihydrate pH 4.6	10. (A10)	25.5% w/v Polyethylene glycol 4,000	10. (A10)	15% v/v
11. (A11)		11. (A11)	0.07 M Sodium citrate tribasic dihydrate pH 5.6	11. (A11)	0.7 M Ammonium phosphate monobasic	11. (A11)	30% v/v
12. (A12)	0.18 M Magnesium chloride hexahydrate	12. (A12)	0.09 M HEPES sodium pH 7.5	12. (A12)	27% v/v 2-Propanol	12. (A12)	10% v/v
13. (B1)	0.2 M Sodium citrate tribasic dihydrate	13. (B1)	0.1 M TRIS hydrochloride pH 8.5	13. (B1)	30% v/v Polyethylene glycol 400	13. (B1)	None
14. (B2)	0.19 M Calcium chloride dihydrate	14. (B2)	0.095 M HEPES sodium pH 7.5	14. (B2)	26.6% v/v Polyethylene glycol 400	14. (B2)	5% v/v
15. (B3)	0.17 M Ammonium sulfate	15. (B3)	0.085 M Sodium cacodylate trihydrate pH 6.5	15. (B3)	25.5% w/v Polyethylene glycol 8,000	15. (B3)	15% v/v
16. (B4)		16. (B4)	0.075 M HEPES sodium pH 7.5	16. (B4)	1.125 M Lithium sulfate monohydrate	16. (B4)	25% v/v
17. (B5)	0.17 M Lithium sulfate monohydrate	17. (B5)	0.085 M TRIS hydrochloride pH 8.5	17. (B5)	25.5% w/v Polyethylene glycol 4,000	17. (B5)	15% v/v
18. (B6)	0.16 M Magnesium acetate tetrahydrate	18. (B6)	0.08 M Sodium cacodylate trihydrate pH 6.5	18. (B6)	16% w/v Polyethylene glycol 8,000	18. (B6)	20% v/v
19. (B7)	0.16 M Ammonium acetate	19. (B7)	0.08 M TRIS hydrochloride pH 8.5	19. (B7)	24% v/v 2-Propanol	19. (B7)	20% v/v
20. (B8)	0.16 M Ammonium sulfate	20. (B8)	0.08 M Sodium acetate trihydrate pH 4.6	20. (B8)	20% w/v Polyethylene glycol 4,000	20. (B8)	20% v/v
21. (B9)	0.2 M Magnesium acetate tetrahydrate	21. (B9)	0.1 M Sodium cacodylate trihydrate pH 6.5	21. (B9)	30% v/v (+/-)-2-Methyl-2,4-pentanediol	21. (B9)	None
22. (B10)	0.17 M Sodium acetate trihydrate	22. (B10)	0.085 M TRIS hydrochloride pH 8.5	22. (B10)	25.5% w/v Polyethylene glycol 4,000	22. (B10)	15% v/v
23. (B11)	0.2 M Magnesium chloride hexahydrate	23. (B11)	0.1 M HEPES sodium pH 7.5	23. (B11)	30% v/v Polyethylene glycol 400	23. (B11)	None
24. (B12)	0.14 M Calcium chloride dihydrate	24. (B12)	0.07 M Sodium acetate trihydrate pH 4.6	24. (B12)	14% v/v 2-Propanol	24. (B12)	30% v/v
25. (C1)		25. (C1)	0.07 M Imidazole pH 6.5	25. (C1)	0.7 M Sodium acetate trihydrate	25. (C1)	30% v/v
26. (C2)	0.2 M Ammonium acetate	26. (C2)	0.1 M Sodium citrate tribasic dihydrate pH 5.6	26. (C2)	30% v/v (+/-)-2-Methyl-2,4-pentanediol	26. (C2)	None
27. (C3)	0.14 M Sodium citrate tribasic dihydrate	27. (C3)	0.07 M HEPES sodium pH 7.5	27. (C3)	14% v/v 2-Propanol	27. (C3)	30% v/v
28. (C4)	0.17 M Sodium acetate trihydrate	28. (C4)	0.085 M Sodium cacodylate trihydrate pH 6.5	28. (C4)	25.5% w/v Polyethylene glycol 8,000	28. (C4)	15% v/v
29. (C5)		29. (C5)	0.065 M HEPES sodium pH 7.5	29. (C5)	0.52 M Potassium sodium tartrate tetrahydrate	29. (C5)	35% v/v
30. (C6)	0.17 M Ammonium sulfate	30. (C6)		30. (C6)	25.5% w/v Polyethylene glycol 8,000	30. (C6)	15% v/v
31. (C7)	0.17 M Ammonium sulfate	31. (C7)		31. (C7)	25.5% w/v Polyethylene glycol 4,000	31. (C7)	15% v/v
32. (C8)		32. (C8)		32. (C8)	1.5 M Ammonium sulfate	32. (C8)	25% v/v
33. (C9)		33. (C9)		33. (C9)	3.6 M Sodium formate	33. (C9)	10% v/v
34. (C10)		34. (C10)	0.07 M Sodium acetate trihydrate pH 4.6	34. (C10)	1.4 M Sodium formate	34. (C10)	30% v/v
35. (C11)		35. (C11)	0.075 M HEPES sodium pH 7.5	35. (C11)	0.6 M Sodium phosphate monobasic monohydrate, 0.6 M Potassium phosphate monobasic	35. (C11)	25% v/v
36. (C12)		36. (C12)	0.065 M TRIS hydrochloride pH 8.5	36. (C12)	5.2% w/v Polyethylene glycol 8,000	36. (C12)	35% v/v
37. (D1)		37. (D1)	0.07 M Sodium acetate trihydrate pH 4.6	37. (D1)	5.6% w/v Polyethylene glycol 4,000	37. (D1)	30% v/v
38. (D2)		38. (D2)	0.09 M HEPES sodium pH 7.5	38. (D2)	1.26 M Sodium citrate tribasic dihydrate	38. (D2)	10% v/v
39. (D3)		39. (D3)	0.085 M HEPES sodium pH 7.5	39. (D3)	1.7% v/v Polyethylene glycol 400 1.7 M Ammonium sulfate	39. (D3)	15% v/v
40. (D4)		40. (D4)	0.095 M Sodium citrate tribasic dihydrate pH 5.6	40. (D4)	19% v/v 2-Propanol, 19% w/v Polyethylene glycol 4,000	40. (D4)	5% v/v
41. (D5)		41. (D5)	0.085 M HEPES sodium pH 7.5	41. (D5)	8.5% v/v 2-Propanol, 17% w/v Polyethylene glycol 4,000	41. (D5)	15% v/v
42. (D6)	0.04 M Potassium phosphate monobasic	42. (D6)		42. (D6)	16% w/v Polyethylene glycol 8,000	42. (D6)	20% v/v
43. (D7)		43. (D7)		43. (D7)	24% w/v Polyethylene glycol 1,500	43. (D7)	20% v/v
44. (D8)		44. (D8)		44. (D8)	0.1 M Magnesium formate dihydrate	44. (D8)	50% v/v
45. (D9)	0.16 M Zinc acetate dihydrate	45. (D9)	0.08 M Sodium cacodylate trihydrate pH 6.5	45. (D9)	14.4% w/v Polyethylene glycol 8,000	45. (D9)	20% v/v
46. (D10)	0.16 M Calcium acetate hydrate	46. (D10)	0.08 M Sodium cacodylate trihydrate pH 6.5	46. (D10)	14.4% w/v Polyethylene glycol 8,000	46. (D10)	20% v/v
47. (D11)		47. (D11)	0.08 M Sodium acetate trihydrate pH 4.6	47. (D11)	1.6 M Ammonium sulfate	47. (D11)	20% v/v
48. (D12)		48. (D12)	0.08 M TRIS hydrochloride pH 8.5	48. (D12)	1.6 M Ammonium phosphate monobasic	48. (D12)	20% v/v

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

Crystal Screen Cryo HT contains ninety-six unique reagents. To determine the formulation of each reagent, simply read across the page.

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Well #	Salt	Well #	Buffer ◊	Well #	Precipitant	Well #	Glycerol
49. (E1)	1.6 M Sodium chloride	49. (E1)		49. (E1)	8% w/v Polyethylene glycol 6,000	49. (E1)	20% v/v
50. (E2)	0.3 M Sodium chloride, 0.006 M Magnesium chloride hexahydrate	50. (E2)		50. (E2)	0.006 M Hexadecyltrimethylammonium bromide	50. (E2)	40% v/v
51. (E3)		51. (E3)		51. (E3)	21.25% v/v Ethylene glycol	51. (E3)	15% v/v
52. (E4)		52. (E4)		52. (E4)	26.25% v/v 1,4-Dioxane	52. (E4)	25% v/v
53. (E5)	1.5 M Ammonium sulfate	53. (E5)		53. (E5)	3.75% v/v 2-Propanol	53. (E5)	25% v/v
54. (E6)		54. (E6)		54. (E6)	0.65 M Imidazole pH 7.0	54. (E6)	35% v/v
55. (E7)		55. (E7)		55. (E7)	8% w/v Polyethylene glycol 1,000, 8% w/v Polyethylene glycol 8,000	55. (E7)	20% v/v
56. (E8)	1.05 M Sodium chloride	56. (E8)		56. (E8)	7% v/v Ethanol	56. (E8)	30% v/v
57. (E9)		57. (E9)	0.075 M Sodium acetate trihydrate pH 4.6	57. (E9)	1.5 M Sodium chloride	57. (E9)	25% v/v
58. (E10)	0.2 M Sodium chloride	58. (E10)	0.1 M Sodium acetate trihydrate pH 4.6	58. (E10)	30% v/v (+/-)-2-Methyl-2,4-pentanediol	58. (E10)	None
59. (E11)	0.008 M Cobalt(II) chloride hexahydrate	59. (E11)	0.08 M Sodium acetate trihydrate pH 4.6	59. (E11)	0.8 M 1,6-Hexanediol	59. (E11)	20% v/v
60. (E12)	0.095 M Cadmium chloride hydrate	60. (E12)	0.095 M Sodium acetate trihydrate pH 4.6	60. (E12)	28.5% v/v Polyethylene glycol 400	60. (E12)	5% v/v
61. (F1)	0.18 M Ammonium sulfate	61. (F1)	0.09 M Sodium acetate trihydrate pH 4.6	61. (F1)	27% w/v Polyethylene glycol monomethyl ether 2,000	61. (F1)	10% v/v
62. (F2)	0.15 M Potassium sodium tartrate tetrahydrate	62. (F2)	0.075 M Sodium citrate tribasic dihydrate pH 5.6	62. (F2)	1.5 M Ammonium sulfate	62. (F2)	25% v/v
63. (F3)	0.375 M Ammonium sulfate	63. (F3)	0.075 M Sodium citrate tribasic dihydrate pH 5.6	63. (F3)	0.75 M Lithium sulfate monohydrate	63. (F3)	25% v/v
64. (F4)	0.3 M Sodium chloride	64. (F4)	0.06 M Sodium citrate tribasic dihydrate pH 5.6	64. (F4)	1.2% v/v Ethylene imine polymer	64. (F4)	40% v/v
65. (F5)		65. (F5)	0.08 M Sodium citrate tribasic dihydrate pH 5.6	65. (F5)	28% v/v tert-Butanol	65. (F5)	20% v/v
66. (F6)	0.007 M Iron(III) chloride hexahydrate	66. (F6)	0.07 M Sodium citrate tribasic dihydrate pH 5.6	66. (F6)	7% v/v Jeffamine® M-600®	66. (F6)	30% v/v
67. (F7)		67. (F7)	0.095 M Sodium citrate tribasic dihydrate pH 5.6	67. (F7)	2.375 M 1,6-Hexanediol	67. (F7)	5% v/v
68. (F8)		68. (F8)	0.08 M MES monohydrate pH 6.5	68. (F8)	1.28 M Magnesium sulfate heptahydrate	68. (F8)	20% v/v
69. (F9)	0.075 M Sodium phosphate monobasic monohydrate, 0.075 M Potassium phosphate monobasic	69. (F9)	0.075 M MES monohydrate pH 6.5	69. (F9)	1.5 M Sodium chloride	69. (F9)	25% v/v
70. (F10)		70. (F10)	0.065 M MES monohydrate pH 6.5	70. (F10)	7.8% w/v Polyethylene glycol 20,000	70. (F10)	35% v/v
71. (F11)	1.2 M Ammonium sulfate	71. (F11)	0.075 M MES monohydrate pH 6.5	71. (F11)	7.5% v/v 1,4-Dioxane	71. (F11)	25% v/v
72. (F12)	0.05 M Cesium chloride	72. (F12)	0.1 M MES monohydrate pH 6.5	72. (F12)	30% v/v Jeffamine® M-600®	72. (F12)	None
73. (G1)	0.0075 M Cobalt(II) chloride hexahydrate	73. (G1)	0.075 M MES monohydrate pH 6.5	73. (G1)	1.35 M Ammonium sulfate	73. (G1)	25% v/v
74. (G2)	0.18 M Ammonium sulfate	74. (G2)	0.09 M MES monohydrate pH 6.5	74. (G2)	27% w/v Polyethylene glycol monomethyl ether 5,000	74. (G2)	10% v/v
75. (G3)	0.009 M Zinc sulfate heptahydrate	75. (G3)	0.09 M MES monohydrate pH 6.5	75. (G3)	22.5% v/v Polyethylene glycol monomethyl ether 550	75. (G3)	10% v/v
76. (G4)		76. (G4)		76. (G4)	1.6 M Sodium citrate tribasic dihydrate pH 6.5	76. (G4)	None
77. (G5)	0.5 M Ammonium sulfate	77. (G5)	0.1 M HEPES pH 7.5	77. (G5)	30% v/v (+/-)-2-Methyl-2,4-pentanediol	77. (G5)	None
78. (G6)		78. (G6)	0.08 M HEPES pH 7.5	78. (G6)	8% w/v Polyethylene glycol 6,000, 4% v/v (+/-)-2-Methyl-2,4-pentanediol	78. (G6)	20% v/v
79. (G7)		79. (G7)	0.085 M HEPES pH 7.5	79. (G7)	17% v/v Jeffamine® M-600®	79. (G7)	15% v/v
80. (G8)	0.075 M Sodium chloride	80. (G8)	0.075 M HEPES pH 7.5	80. (G8)	1.2 M Ammonium sulfate	80. (G8)	25% v/v
81. (G9)		81. (G9)	0.07 M HEPES pH 7.5	81. (G9)	1.4 M Ammonium formate	81. (G9)	30% v/v
82. (G10)	0.0375 M Cadmium sulfate hydrate	82. (G10)	0.075 M HEPES pH 7.5	82. (G10)	0.75 M Sodium acetate trihydrate	82. (G10)	25% v/v
83. (G11)		83. (G11)	0.1 M HEPES pH 7.5	83. (G11)	70% v/v (+/-)-2-Methyl-2,4-pentanediol	83. (G11)	None
84. (G12)		84. (G12)	0.085 M HEPES pH 7.5	84. (G12)	3.655 M Sodium chloride	84. (G12)	15% v/v
85. (H1)		85. (H1)	0.075 M HEPES pH 7.5	85. (H1)	7.5% w/v Polyethylene glycol 8,000, 6% v/v Ethylene glycol	85. (H1)	25% v/v
86. (H2)		86. (H2)	0.075 M HEPES pH 7.5	86. (H2)	15% w/v Polyethylene glycol 10,000	86. (H2)	25% v/v
87. (H3)	0.2 M Magnesium chloride hexahydrate	87. (H3)	0.1 M Tris pH 8.5	87. (H3)	3.4 M 1,6-Hexanediol	87. (H3)	None
88. (H4)		88. (H4)	0.075 M Tris pH 8.5	88. (H4)	18.75% v/v tert-Butanol	88. (H4)	25% v/v
89. (H5)	0.0075 M Nickel(II) chloride hexahydrate	89. (H5)	0.075 M Tris pH 8.5	89. (H5)	0.75 M Lithium sulfate monohydrate	89. (H5)	25% v/v
90. (H6)	1.275 M Ammonium sulfate	90. (H6)	0.085 M Tris pH 8.5	90. (H6)		90. (H6)	25.2% v/v
91. (H7)	0.2 M Ammonium phosphate monobasic	91. (H7)	0.1 M Tris pH 8.5	91. (H7)	50% v/v (+/-)-2-Methyl-2,4-pentanediol	91. (H7)	None
92. (H8)		92. (H8)	0.075 M Tris pH 8.5	92. (H8)	15% v/v Ethanol	92. (H8)	25% v/v
93. (H9)	0.008 M Nickel(II) chloride hexahydrate	93. (H9)	0.08 M Tris pH 8.5	93. (H9)	16% w/v Polyethylene glycol monomethyl ether 2,000	93. (H9)	20% v/v
94. (H10)	0.085 M Sodium chloride	94. (H10)	0.085 M BICINE pH 9.0	94. (H10)	17% v/v Polyethylene glycol monomethyl ether 550	94. (H10)	15% v/v
95. (H11)		95. (H11)	0.095 M BICINE pH 9.0	95. (H11)	1.9 M Magnesium chloride hexahydrate	95. (H11)	5% v/v
96. (H12)		96. (H12)	0.07 M BICINE pH 9.0	96. (H12)	1.4% v/v 1,4-Dioxane, 7% w/v Polyethylene glycol 20,000	96. (H12)	30% v/v

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

Crystal Screen Cryo HT contains ninety-six unique reagents. To determine the formulation of each reagent, simply read across the page.

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HAMPTON RESEARCH
Solutions for Crystal Growth

Sample: _____ **Sample Concentration:** _____

Sample Buffer: _____ **Date:** _____

Reservoir Volume: _____ **Temperature:** _____

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

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

Crystal Screen Cryo HT™ - HR2-133 Scoring Sheet		Date:	Date:
1. (A1)	0.02 M Calcium chloride dihydrate, 0.1 M Sodium acetate trihydrate pH 4.6, 30% v/v (+/-)-2-Methyl-2,4-pentanediol		
2. (A2)	0.26 M Potassium sodium tartrate tetrahydrate, 35% v/v Glycerol		
3. (A3)	0.26 M Ammonium phosphate monobasic, 35% v/v Glycerol		
4. (A4)	0.075 M TRIS hydrochloride pH 8.5, 1.5 M Ammonium sulfate, 25% v/v Glycerol		
5. (A5)	0.2 M Sodium citrate tribasic dihydrate, 0.1 M HEPES sodium pH 7.5, 30% v/v (+/-)-2-Methyl-2,4-pentanediol		
6. (A6)	0.16 M Magnesium chloride hexahydrate, 0.08 M TRIS hydrochloride pH 8.5, 24% w/v Polyethylene glycol 4,000, 20% v/v Glycerol		
7. (A7)	0.07 M Sodium cacodylate trihydrate pH 6.5, 0.98 M Sodium acetate trihydrate, 30% v/v Glycerol		
8. (A8)	0.14 M Sodium citrate tribasic dihydrate, 0.07 M Sodium cacodylate trihydrate pH 6.5, 21% v/v 2-Propanol, 30% v/v Glycerol		
9. (A9)	0.17 M Ammonium acetate, 0.085 M Sodium citrate tribasic dihydrate pH 5.6, 25.5% w/v Polyethylene glycol 4,000, 15% v/v Glycerol		
10. (A10)	0.17 M Ammonium acetate, 0.085 M Sodium acetate trihydrate pH 4.6, 25.5% w/v Polyethylene glycol 4,000, 15% v/v Glycerol		
11. (A11)	0.07 M Sodium citrate tribasic dihydrate pH 5.6, 0.7 M Ammonium phosphate monobasic, 30% v/v Glycerol		
12. (A12)	0.18 M Magnesium chloride hexahydrate, 0.09 M HEPES sodium pH 7.5, 27% v/v 2-Propanol, 10% v/v Glycerol		
13. (B1)	0.2 M Sodium citrate tribasic dihydrate, 0.1 M TRIS hydrochloride pH 8.5, 30% v/v Polyethylene glycol 400		
14. (B2)	0.19 M Calcium chloride dihydrate, 0.095 M HEPES sodium pH 7.5, 26.6% v/v Polyethylene glycol 400, 5% v/v Glycerol		
15. (B3)	0.17 M Ammonium sulfate, 0.085 M Sodium cacodylate trihydrate pH 6.5, 25.5% w/v Polyethylene glycol 8,000, 15% v/v Glycerol		
16. (B4)	0.075 M HEPES sodium pH 7.5, 1.125 M Lithium sulfate monohydrate, 25% v/v Glycerol		
17. (B5)	0.17 M Lithium sulfate monohydrate, 0.085 M TRIS hydrochloride pH 8.5, 25.5% w/v Polyethylene glycol 4,000, 15% v/v Glycerol		
18. (B6)	0.16 M Magnesium acetate tetrahydrate, 0.08 M Sodium cacodylate trihydrate pH 6.5, 16% w/v Polyethylene glycol 8,000, 20% v/v Glycerol		
19. (B7)	0.16 M Ammonium acetate, 0.08 M TRIS hydrochloride pH 8.5, 24% v/v 2-Propanol, 20% v/v Glycerol		
20. (B8)	0.16 M Ammonium sulfate, 0.08 M Sodium acetate trihydrate pH 4.6, 20% w/v Polyethylene glycol 4,000, 20% v/v Glycerol		
21. (B9)	0.2 M Magnesium acetate tetrahydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 30% v/v (+/-)-2-Methyl-2,4-pentanediol		
22. (B10)	0.17 M Sodium acetate trihydrate, 0.085 M TRIS hydrochloride pH 8.5, 25.5% w/v Polyethylene glycol 4,000, 15% v/v Glycerol		
23. (B11)	0.2 M Magnesium chloride hexahydrate, 0.1 M HEPES sodium pH 7.5, 30% v/v Polyethylene glycol 400		
24. (B12)	0.14 M Calcium chloride dihydrate, 0.07 M Sodium acetate trihydrate pH 4.6, 14% v/v 2-Propanol, 30% v/v Glycerol		
25. (C1)	0.07 M Imidazole pH 6.5, 0.7 M Sodium acetate trihydrate, 30% v/v Glycerol		
26. (C2)	0.2 M Ammonium acetate, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 30% v/v (+/-)-2-Methyl-2,4-pentanediol		
27. (C3)	0.14 M Sodium citrate tribasic dihydrate, 0.07 M HEPES sodium pH 7.5, 14% v/v 2-Propanol, 30% v/v Glycerol		
28. (C4)	0.17 M Sodium acetate trihydrate, 0.085 M Sodium cacodylate trihydrate pH 6.5, 25.5% w/v Polyethylene glycol 8,000, 15% v/v Glycerol		
29. (C5)	0.065 M HEPES sodium pH 7.5, 0.52 M Potassium sodium tartrate tetrahydrate, 35% v/v Glycerol		
30. (C6)	0.17 M Ammonium sulfate, 25.5% w/v Polyethylene glycol 8,000, 15% v/v Glycerol		
31. (C7)	0.17 M Ammonium sulfate, 25.5% w/v Polyethylene glycol 4,000, 15% v/v Glycerol		
32. (C8)	1.5 M Ammonium sulfate, 25% v/v Glycerol		
33. (C9)	3.6 M Sodium formate, 10% v/v Glycerol		
34. (C10)	0.07 M Sodium acetate trihydrate pH 4.6, 1.4 M Sodium formate, 30% v/v Glycerol		
35. (C11)	0.075 M HEPES sodium pH 7.5, 0.6 M Sodium phosphate monobasic monohydrate, 0.6 M Potassium phosphate monobasic, 25% v/v Glycerol		
36. (C12)	0.065 M TRIS hydrochloride pH 8.5, 5.2% w/v Polyethylene glycol 8,000, 35% v/v Glycerol		
37. (D1)	0.07 M Sodium acetate trihydrate pH 4.6, 5.6% w/v Polyethylene glycol 4,000, 30% v/v Glycerol		
38. (D2)	0.09 M HEPES sodium pH 7.5, 1.26 M Sodium citrate tribasic dihydrate, 10% v/v Glycerol		
39. (D3)	0.085 M HEPES sodium pH 7.5, 1.7% v/v Polyethylene glycol 400, 1.7 M Ammonium sulfate, 15% v/v Glycerol		
40. (D4)	0.095 M Sodium citrate tribasic dihydrate pH 5.6, 19% v/v 2-Propanol, 19% w/v Polyethylene glycol 4,000, 5% v/v Glycerol		
41. (D5)	0.085 M HEPES sodium pH 7.5, 8.5% v/v 2-Propanol, 17% w/v Polyethylene glycol 4,000, 15% v/v Glycerol		
42. (D6)	0.04 M Potassium phosphate monobasic, 16% w/v Polyethylene glycol 8,000, 20% v/v Glycerol		
43. (D7)	24% w/v Polyethylene glycol 1,500, 20% v/v Glycerol		
44. (D8)	0.1 M Magnesium formate dihydrate, 50% v/v Glycerol		
45. (D9)	0.16 M Zinc acetate dihydrate, 0.08 M Sodium cacodylate trihydrate pH 6.5, 14.4% w/v Polyethylene glycol 8,000, 20% v/v Glycerol		
46. (D10)	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		
47. (D11)	0.08 M Sodium acetate trihydrate pH 4.6, 1.6 M Ammonium sulfate, 20% v/v Glycerol		
48. (D12)	0.08 M TRIS hydrochloride pH 8.5, 1.6 M Ammonium phosphate monobasic, 20% v/v Glycerol		

34 Journey
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HAMPTON RESEARCH
Solutions for Crystal Growth

Sample: _____ **Sample Concentration:** _____

Sample Buffer: _____ **Date:** _____

Reservoir Volume: _____ **Temperature:** _____

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

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

Crystal Screen Cryo HT™ - HR2-133 Scoring Sheet	Date:	Date:
49. (E1) 1.6 M Sodium chloride, 8% w/v Polyethylene glycol 6,000, 20% v/v Glycerol		
50. (E2) 0.3 M Sodium chloride, 0.006 M Magnesium chloride hexahydrate, 0.006 M Hexadecyltrimethylammonium bromide, 40% v/v Glycerol		
51. (E3) 21.25% v/v Ethylene glycol, 15% v/v Glycerol		
52. (E4) 26.25% v/v 1,4-Dioxane, 25% v/v Glycerol		
53. (E5) 1.5 M Ammonium sulfate, 3.75% v/v 2-Propanol, 25% v/v Glycerol		
54. (E6) 0.65 M Imidazole pH 7.0, 35% v/v Glycerol		
55. (E7) 8% w/v Polyethylene glycol 1,000, 8% w/v Polyethylene glycol 8,000, 20% v/v Glycerol		
56. (E8) 1.05 M Sodium chloride, 7% v/v Ethanol, 30% v/v Glycerol		
57. (E9) 0.075 M Sodium acetate trihydrate pH 4.6, 1.5 M Sodium chloride, 25% v/v Glycerol		
58. (E10) 0.2 M Sodium chloride, 0.1 M Sodium acetate trihydrate pH 4.6, 30% v/v (+/-)-2-Methyl-2,4-pentanediol		
59. (E11) 0.008 M Cobalt(II) chloride hexahydrate, 0.08 M Sodium acetate trihydrate pH 4.6, 0.8 M 1,6-Hexanediol, 20% v/v Glycerol		
60. (E12) 0.095 M Cadmium chloride hydrate, 0.095 M Sodium acetate trihydrate pH 4.6, 28.5% v/v Polyethylene glycol 400, 5% v/v Glycerol		
61. (F1) 0.18 M Ammonium sulfate, 0.09 M Sodium acetate trihydrate pH 4.6, 27% w/v Polyethylene glycol monomethyl ether 2,000, 10% v/v Glycerol		
62. (F2) 0.15 M Potassium sodium tartrate tetrahydrate, 0.075 M Sodium citrate tribasic dihydrate pH 5.6, 1.5 M Ammonium sulfate , 25% v/v Glycerol		
63. (F3) 0.375 M Ammonium sulfate, 0.075 M Sodium citrate tribasic dihydrate pH 5.6, 0.75 M Lithium sulfate monohydrate, 25% v/v Glycerol		
64. (F4) 0.3 M Sodium chloride, 0.06 M Sodium citrate tribasic dihydrate pH 5.6, 1.2% v/v Ethylene imine polymer, 40% v/v Glycerol		
65. (F5) 0.08 M Sodium citrate tribasic dihydrate pH 5.6, 28% v/v tert-Butanol, 20% v/v Glycerol		
66. (F6) 0.007 M Iron(III) chloride hexahydrate, 0.07 M Sodium citrate tribasic dihydrate pH 5.6, 7% v/v Jeffamine M-600, 30% v/v Glycerol		
67. (F7) 0.095 M Sodium citrate tribasic dihydrate pH 5.6, 2.375 M 1,6-Hexanediol, 5% v/v Glycerol		
68. (F8) 0.08 M MES monohydrate pH 6.5, 1.28 M Magnesium sulfate heptahydrate, 20% v/v Glycerol		
69. (F9) 0.075 M Sodium phosphate monobasic monohydrate, 0.075 M Potassium phosphate monobasic, 0.075 M MES monohydrate pH 6.5,		
1.5 M Sodium chloride, 25% v/v Glycerol		
70. (F10) 0.065 M MES monohydrate pH 6.5, 7.8% w/v Polyethylene glycol 20,000, 35% v/v Glycerol		
71. (F11) 1.2 M Ammonium sulfate, 0.075 M MES monohydrate pH 6.5, 7.5% v/v 1,4-Dioxane, 25% v/v Glycerol		
72. (F12) 0.05 M Cesium chloride, 0.1 M MES monohydrate pH 6.5, 30% v/v Jeffamine M-600		
73. (G1) 0.0075 M Cobalt(II) chloride hexahydrate, 0.075 M MES monohydrate pH 6.5, 1.35 M Ammonium sulfate , 25% v/v Glycerol		
74. (G2) 0.18 M Ammonium sulfate, 0.09 M MES monohydrate pH 6.5, 27% w/v Polyethylene glycol monomethyl ether 5,000, 10% v/v Glycerol		
75. (G3) 0.009 M Zinc sulfate heptahydrate, 0.09 M MES monohydrate pH 6.5, 22.5% v/v Polyethylene glycol monomethyl ether 550, 10% v/v Glycerol		
76. (G4) 1.6 M Sodium citrate tribasic dihydrate pH 6.5		
77. (G5) 0.5 M Ammonium sulfate, 0.1 M HEPES pH 7.5, 30% v/v (+/-)-2-Methyl-2,4-pentanediol		
78. (G6) 0.08 M HEPES pH 7.5, 8% w/v Polyethylene glycol 6,000, 4% v/v (+/-)-2-Methyl-2,4-pentanediol, 20% v/v Glycerol		
79. (G7) 0.085 M HEPES pH 7.5, 17% v/v Jeffamine M-600, 15% v/v Glycerol		
80. (G8) 0.075 M Sodium chloride, 0.075 M HEPES pH 7.5, 1.2 M Ammonium sulfate , 25% v/v Glycerol		
81. (G9) 0.07 M HEPES pH 7.5, 1.4 M Ammonium formate, 30% v/v Glycerol		
82. (G10) 0.0375 M Cadmium sulfate hydrate, 0.075 M HEPES pH 7.5, 0.75 M Sodium acetate trihydrate, 25% v/v Glycerol		
83. (G11) 0.1 M HEPES pH 7.5, 70% v/v (+/-)-2-Methyl-2,4-pentanediol		
84. (G12) 0.085 M HEPES pH 7.5, 3.655 M Sodium chloride, 15% v/v Glycerol		
85. (H1) 0.075 M HEPES pH 7.5, 7.5% w/v Polyethylene glycol 8,000, 6% v/v Ethylene glycol, 25% v/v Glycerol		
86. (H2) 0.075 M HEPES pH 7.5, 15% w/v Polyethylene glycol 10,000, 25% v/v Glycerol		
87. (H3) 0.2 M Magnesium chloride hexahydrate, 0.1 M Tris pH 8.5, 3.4 M 1,6-Hexanediol		
88. (H4) 0.075 M Tris pH 8.5, 18.75% v/v tert-Butanol, 25% v/v Glycerol		
89. (H5) 0.0075 M Nickel(II) chloride hexahydrate, 0.075 M Tris pH 8.5, 0.75 M Lithium sulfate monohydrate, 25% v/v Glycerol		
90. (H6) 1.275 M Ammonium sulfate, 0.085 M Tris pH 8.5, 25.2% v/v Glycerol		
91. (H7) 0.2 M Ammonium phosphate monobasic, 0.1 M Tris pH 8.5, 50% v/v (+/-)-2-Methyl-2,4-pentanediol		
92. (H8) 0.075 M Tris pH 8.5, 15% v/v Ethanol, 25% v/v Glycerol		
93. (H9) 0.008 M Nickel(II) chloride hexahydrate, 0.08 M Tris pH 8.5, 16% w/v Polyethylene glycol monomethyl ether 2,000, 20% v/v Glycerol		
94. (H10) 0.085 M Sodium chloride, 0.085 M BICINE pH 9.0, 17% v/v Polyethylene glycol monomethyl ether 550, 15% v/v Glycerol		
95. (H11) 0.095 M BICINE pH 9.0, 1.9 M Magnesium chloride hexahydrate, 5% v/v Glycerol		
96. (H12) 0.07 M BICINE pH 9.0, 1.4% v/v 1,4-Dioxane, 7% w/v Polyethylene glycol 20,000, 30% v/v Glycerol		

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HAMPTON RESEARCH
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