Crystal Screen HT™

HAMPTON RESEARCH
Solutions for Crystal Growth

Features

Crystal Screen HT™ is a high throughput reagent kit designed to provide a rapid screening method for the crystallization of biological macromolecules. The kit is straightforward, effective, and practical for the determination of preliminary crystallization conditions. The kit is also effective in determining the solubility of a macromolecule in a wide range of reagents and pH.

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

Crystal Screen™ and Crystal Screen 2™ offer a sparse matrix of trial crystallization reagent conditions based upon the original Jancarik and Kim screen.[3] The primary screen variables are salt, pH, and precipitant (salts, polymers, volatile organics, and non-volatile organics).

General Description

Crystal Screen 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.

Each Crystal Screen HT kit is supplied with an adhesive sealing film which can be used to seal the block after removing the heat seal. Additional adhesive sealing films can be obtained from Hampton Research or laboratory supply companies which offer high throughput plates and seals.

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. [1,2,4]

The recommended sample concentration is 5 to 25 mg/ml in sterile filtered, deionized water or 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 and Crystal Screen 2 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

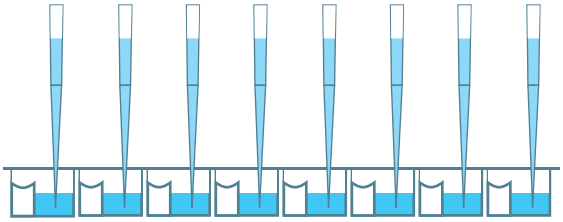
Allow the block to equilibrate to room temperature. To remove stray reagent from the sealing film, centrifuge the block at 500 rpm for 5 minutes. To remove film, grasp a corner of the film and gently peel film from the block. Alternatively, the film can be pierced to access reagents.

Performing the Screen

Manual Method - Sitting Drop Vapor Diffusion

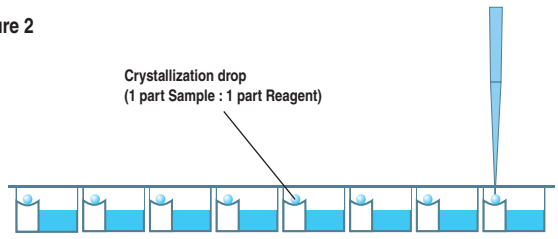
1. Using a 96 well sitting drop vapor diffusion plate, pipet the recommended volume (typically 100 microliters) of crystallization reagent from the Deep Well block into the reservoirs of the crystallization plate. The Deep Well block is compatible with 8 and 12 channel pipets as well as many automated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

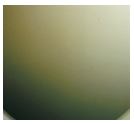
Figure 2

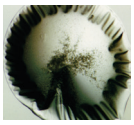


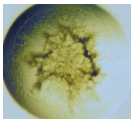
3. Using a clean pipet tip, pipet 0.05 to 2 microliters of sample to the reagent drop in the sitting drop well. One may choose to simply dispense the sample with no mixing or dispense with mixing by gently aspirating and dispensing the sample several times, keeping the tip in the drop during mixing to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

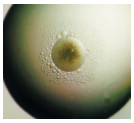
4. Seal the crystallization plate as per the manufacturer's recommendation. Most 96 well crystallization plates are sealed using a clear sealing tape, film, or cap mat. View and score the experiment as desired. See Hampton

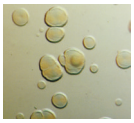
Figure 3
Typical observations in a crystallization experiment

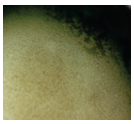

Clear Drop


Skin / Precipitate

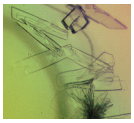

Precipitate


Precipitate / Phase

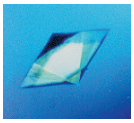

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

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 sealing film.

Crystal Screen 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 HT

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

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

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

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

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

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

4. Protein and Nucleic Acid Crystallization. Methods, A Companion to Methods in Enzymology, Academic Press, Volume 1, Number 1, August 1990.

Technical Support

Inquiries regarding Crystal Screen 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
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
2. (A2)	None	2. (A2)	None	2. (A2)	0.4 M Potassium sodium tartrate tetrahydrate
3. (A3)	None	3. (A3)	None	3. (A3)	0.4 M Ammonium phosphate monobasic
4. (A4)	None	4. (A4)	0.1 M TRIS hydrochloride pH 8.5	4. (A4)	2.0 M Ammonium sulfate
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
6. (A6)	0.2 M Magnesium chloride hexahydrate	6. (A6)	0.1 M TRIS hydrochloride pH 8.5	6. (A6)	30% w/v Polyethylene glycol 4,000
7. (A7)	None	7. (A7)	0.1 M Sodium cacodylate trihydrate pH 6.5	7. (A7)	1.4 M Sodium acetate trihydrate
8. (A8)	0.2 M Sodium citrate tribasic dihydrate	8. (A8)	0.1 M Sodium cacodylate trihydrate pH 6.5	8. (A8)	30% v/v 2-Propanol
9. (A9)	0.2 M Ammonium acetate	9. (A9)	0.1 M Sodium citrate tribasic dihydrate pH 5.6	9. (A9)	30% w/v Polyethylene glycol 4,000
10. (A10)	0.2 M Ammonium acetate	10. (A10)	0.1 M Sodium acetate trihydrate pH 4.6	10. (A10)	30% w/v Polyethylene glycol 4,000
11. (A11)	None	11. (A11)	0.1 M Sodium citrate tribasic dihydrate pH 5.6	11. (A11)	1.0 M Ammonium phosphate monobasic
12. (A12)	0.2 M Magnesium chloride hexahydrate	12. (A12)	0.1 M HEPES sodium pH 7.5	12. (A12)	30% v/v 2-Propanol
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
14. (B2)	0.2 M Calcium chloride dihydrate	14. (B2)	0.1 M HEPES sodium pH 7.5	14. (B2)	28% v/v Polyethylene glycol 400
15. (B3)	0.2 M Ammonium sulfate	15. (B3)	0.1 M Sodium cacodylate trihydrate pH 6.5	15. (B3)	30% w/v Polyethylene glycol 8,000
16. (B4)	None	16. (B4)	0.1 M HEPES sodium pH 7.5	16. (B4)	1.5 M Lithium sulfate monohydrate
17. (B5)	0.2 M Lithium sulfate monohydrate	17. (B5)	0.1 M TRIS hydrochloride pH 8.5	17. (B5)	30% w/v Polyethylene glycol 4,000
18. (B6)	0.2 M Magnesium acetate tetrahydrate	18. (B6)	0.1 M Sodium cacodylate trihydrate pH 6.5	18. (B6)	20% w/v Polyethylene glycol 8,000
19. (B7)	0.2 M Ammonium acetate	19. (B7)	0.1 M TRIS hydrochloride pH 8.5	19. (B7)	30% v/v 2-Propanol
20. (B8)	0.2 M Ammonium sulfate	20. (B8)	0.1 M Sodium acetate trihydrate pH 4.6	20. (B8)	25% w/v Polyethylene glycol 4,000
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
22. (B10)	0.2 M Sodium acetate trihydrate	22. (B10)	0.1 M TRIS hydrochloride pH 8.5	22. (B10)	30% w/v Polyethylene glycol 4,000
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
24. (B12)	0.2 M Calcium chloride dihydrate	24. (B12)	0.1 M Sodium acetate trihydrate pH 4.6	24. (B12)	20% v/v 2-Propanol
25. (C1)	None	25. (C1)	0.1 M Imidazole pH 6.5	25. (C1)	1.0 M Sodium acetate trihydrate
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
27. (C3)	0.2 M Sodium citrate tribasic dihydrate	27. (C3)	0.1 M HEPES sodium pH 7.5	27. (C3)	20% v/v 2-Propanol
28. (C4)	0.2 M Sodium acetate trihydrate	28. (C4)	0.1 M Sodium cacodylate trihydrate pH 6.5	28. (C4)	30% w/v Polyethylene glycol 8,000
29. (C5)	None	29. (C5)	0.1 M HEPES sodium pH 7.5	29. (C5)	0.8 M Potassium sodium tartrate tetrahydrate
30. (C6)	0.2 M Ammonium sulfate	30. (C6)	None	30. (C6)	30% w/v Polyethylene glycol 8,000
31. (C7)	0.2 M Ammonium sulfate	31. (C7)	None	31. (C7)	30% w/v Polyethylene glycol 4,000
32. (C8)	None	32. (C8)	None	32. (C8)	2.0 M Ammonium sulfate
33. (C9)	None	33. (C9)	None	33. (C9)	4.0 M Sodium formate
34. (C10)	None	34. (C10)	0.1 M Sodium acetate trihydrate pH 4.6	34. (C10)	2.0 M Sodium formate
35. (C11)	None	35. (C11)	0.1 M HEPES sodium pH 7.5	35. (C11)	0.8 M Sodium phosphate monobasic monohydrate, 0.8 M Potassium phosphate monobasic
36. (C12)	None	36. (C12)	0.1 M TRIS hydrochloride pH 8.5	36. (C12)	8% w/v Polyethylene glycol 8,000
37. (D1)	None	37. (D1)	0.1 M Sodium acetate trihydrate pH 4.6	37. (D1)	8% w/v Polyethylene glycol 4,000
38. (D2)	None	38. (D2)	0.1 M HEPES sodium pH 7.5	38. (D2)	1.4 M Sodium citrate tribasic dihydrate
39. (D3)	None	39. (D3)	0.1 M HEPES sodium pH 7.5	39. (D3)	2% v/v Polyethylene glycol 400, 2.0 M Ammonium sulfate
40. (D4)	None	40. (D4)	0.1 M Sodium citrate tribasic dihydrate pH 5.6	40. (D4)	20% v/v 2-Propanol, 20% w/v Polyethylene glycol 4,000
41. (D5)	None	41. (D5)	0.1 M HEPES sodium pH 7.5	41. (D5)	10% v/v 2-Propanol, 20% w/v Polyethylene glycol 4,000
42. (D6)	0.05 M Potassium phosphate monobasic	42. (D6)	None	42. (D6)	20% w/v Polyethylene glycol 8,000
43. (D7)	None	43. (D7)	None	43. (D7)	30% w/v Polyethylene glycol 1,500
44. (D8)	None	44. (D8)	None	44. (D8)	0.2 M Magnesium formate dihydrate
45. (D9)	0.2 M Zinc acetate dihydrate	45. (D9)	0.1 M Sodium cacodylate trihydrate pH 6.5	45. (D9)	18% w/v Polyethylene glycol 8,000
46. (D10)	0.2 M Calcium acetate hydrate	46. (D10)	0.1 M Sodium cacodylate trihydrate pH 6.5	46. (D10)	18% w/v Polyethylene glycol 8,000
47. (D11)	None	47. (D11)	0.1 M Sodium acetate trihydrate pH 4.6	47. (D11)	2.0 M Ammonium sulfate
48. (D12)	None	48. (D12)	0.1 M TRIS hydrochloride pH 8.5	48. (D12)	2.0 M Ammonium phosphate monobasic

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

Crystal Screen ™ (Deep Well Block) contains forty-eight unique reagents beginning at position A1. To determine the formulation of each reagent, simply read across the page.

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Well #	Salt	Well #	Buffer ◊	Well #	Precipitant
49. (E1)	2.0 M Sodium chloride	49. (E1)	None	49. (E1)	10% w/v Polyethylene glycol 6,000
50. (E2)	0.5 M Sodium chloride, 0.01 M Magnesium chloride hexahydrate	50. (E2)	None	50. (E2)	0.01 M Hexadecyltrimethylammonium bromide
51. (E3)	None	51. (E3)	None	51. (E3)	25% v/v Ethylene glycol
52. (E4)	None	52. (E4)	None	52. (E4)	35% v/v 1,4-Dioxane
53. (E5)	2.0 M Ammonium sulfate	53. (E5)	None	53. (E5)	5% v/v 2-Propanol
54. (E6)	None	54. (E6)	None	54. (E6)	1.0 M Imidazole pH 7.0
55. (E7)	None	55. (E7)	None	55. (E7)	10% w/v Polyethylene glycol 1,000, 10% w/v Polyethylene glycol 8,000
56. (E8)	1.5 M Sodium chloride	56. (E8)	None	56. (E8)	10% v/v Ethanol
57. (E9)	None	57. (E9)	0.1 M Sodium acetate trihydrate pH 4.6	57. (E9)	2.0 M Sodium chloride
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
59. (E11)	0.01 M Cobalt(II) chloride hexahydrate	59. (E11)	0.1 M Sodium acetate trihydrate pH 4.6	59. (E11)	1.0 M 1,6-Hexanediol
60. (E12)	0.1 M Cadmium chloride hydrate	60. (E12)	0.1 M Sodium acetate trihydrate pH 4.6	60. (E12)	30% v/v Polyethylene glycol 400
61. (F1)	0.2 M Ammonium sulfate	61. (F1)	0.1 M Sodium acetate trihydrate pH 4.6	61. (F1)	30% w/v Polyethylene glycol monomethyl ether 2,000
62. (F2)	0.2 M Potassium sodium tartrate tetrahydrate	62. (F2)	0.1 M Sodium citrate tribasic dihydrate pH 5.6	62. (F2)	2.0 M Ammonium sulfate
63. (F3)	0.5 M Ammonium sulfate	63. (F3)	0.1 M Sodium citrate tribasic dihydrate pH 5.6	63. (F3)	1.0 M Lithium sulfate monohydrate
64. (F4)	0.5 M Sodium chloride	64. (F4)	0.1 M Sodium citrate tribasic dihydrate pH 5.6	64. (F4)	2% v/v Ethylene imine polymer
65. (F5)	None	65. (F5)	0.1 M Sodium citrate tribasic dihydrate pH 5.6	65. (F5)	35% v/v tert-Butanol
66. (F6)	0.01 M Iron(III) chloride hexahydrate	66. (F6)	0.1 M Sodium citrate tribasic dihydrate pH 5.6	66. (F6)	10% v/v Jeffamine® M-600®
67. (F7)	None	67. (F7)	0.1 M Sodium citrate tribasic dihydrate pH 5.6	67. (F7)	2.5 M 1,6-Hexanediol
68. (F8)	None	68. (F8)	0.1 M MES monohydrate pH 6.5	68. (F8)	1.6 M Magnesium sulfate heptahydrate
69. (F9)	0.1 M Sodium phosphate monobasic monohydrate, 0.1 M Potassium phosphate monobasic	69. (F9)	0.1 M MES monohydrate pH 6.5	69. (F9)	2.0 M Sodium chloride
70. (F10)	None	70. (F10)	0.1 M MES monohydrate pH 6.5	70. (F10)	12% w/v Polyethylene glycol 20,000
71. (F11)	1.6 M Ammonium sulfate	71. (F11)	0.1 M MES monohydrate pH 6.5	71. (F11)	10% v/v 1,4-Dioxane
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®
73. (G1)	0.01 M Cobalt(II) chloride hexahydrate	73. (G1)	0.1 M MES monohydrate pH 6.5	73. (G1)	1.8 M Ammonium sulfate
74. (G2)	0.2 M Ammonium sulfate	74. (G2)	0.1 M MES monohydrate pH 6.5	74. (G2)	30% w/v Polyethylene glycol monomethyl ether 5,000
75. (G3)	0.01 M Zinc sulfate heptahydrate	75. (G3)	0.1 M MES monohydrate pH 6.5	75. (G3)	25% v/v Polyethylene glycol monomethyl ether 550
76. (G4)	None	76. (G4)	None	76. (G4)	1.6 M Sodium citrate tribasic dihydrate pH 6.5
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
78. (G6)	None	78. (G6)	0.1 M HEPES pH 7.5	78. (G6)	10% w/v Polyethylene glycol 6,000, 5% v/v (+/-)-2-Methyl-2,4-pentanediol
79. (G7)	None	79. (G7)	0.1 M HEPES pH 7.5	79. (G7)	20% v/v Jeffamine® M-600®
80. (G8)	0.1 M Sodium chloride	80. (G8)	0.1 M HEPES pH 7.5	80. (G8)	1.6 M Ammonium sulfate
81. (G9)	None	81. (G9)	0.1 M HEPES pH 7.5	81. (G9)	2.0 M Ammonium formate
82. (G10)	0.05 M Cadmium sulfate hydrate	82. (G10)	0.1 M HEPES pH 7.5	82. (G10)	1.0 M Sodium acetate trihydrate
83. (G11)	None	83. (G11)	0.1 M HEPES pH 7.5	83. (G11)	70% v/v (+/-)-2-Methyl-2,4-pentanediol
84. (G12)	None	84. (G12)	0.1 M HEPES pH 7.5	84. (G12)	4.3 M Sodium chloride
85. (H1)	None	85. (H1)	0.1 M HEPES pH 7.5	85. (H1)	10% w/v Polyethylene glycol 8,000, 8% v/v Ethylene glycol
86. (H2)	None	86. (H2)	0.1 M HEPES pH 7.5	86. (H2)	20% w/v Polyethylene glycol 10,000
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
88. (H4)	None	88. (H4)	0.1 M Tris pH 8.5	88. (H4)	25% v/v tert-Butanol
89. (H5)	0.01 M Nickel(II) chloride hexahydrate	89. (H5)	0.1 M Tris pH 8.5	89. (H5)	1.0 M Lithium sulfate monohydrate
90. (H6)	1.5 M Ammonium sulfate	90. (H6)	0.1 M Tris pH 8.5	90. (H6)	12% v/v Glycerol
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
92. (H8)	None	92. (H8)	0.1 M Tris pH 8.5	92. (H8)	20% v/v Ethanol
93. (H9)	0.01 M Nickel(II) chloride hexahydrate	93. (H9)	0.1 M Tris pH 8.5	93. (H9)	20% w/v Polyethylene glycol monomethyl ether 2,000
94. (H10)	0.1 M Sodium chloride	94. (H10)	0.1 M BICINE pH 9.0	94. (H10)	20% v/v Polyethylene glycol monomethyl ether 550
95. (H11)	None	95. (H11)	0.1 M BICINE pH 9.0	95. (H11)	2.0 M Magnesium chloride hexahydrate
96. (H12)	None	96. (H12)	0.1 M BICINE pH 9.0	96. (H12)	2% v/v 1,4-Dioxane, 10% w/v Polyethylene glycol 20,000

◊ Buffer pH is that of a 1.0 M (0.5 M for MES) stock prior to dilution with other reagent components: pH with HCl or NaOH.

Crystal Screen 2™ (Deep Well Block) contains forty-eight unique reagents beginning at position E1. To determine the formulation of each reagent, simply read across the page.

34 Journey
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Tel: (949) 425-1321 • Fax: (949) 425-1611
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Website: www.hamptonresearch.com

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)

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Crystal Screen HT™ - HR2-130 Scoring Sheet		Date:	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)	0.4 M Potassium sodium tartrate tetrahydrate			
3. (A3)	0.4 M Ammonium phosphate monobasic			
4. (A4)	0.1 M TRIS hydrochloride pH 8.5, 2.0 M Ammonium sulfate			
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)	0.2 M Magnesium chloride hexahydrate, 0.1 M TRIS hydrochloride pH 8.5, 30% w/v Polyethylene glycol 4,000			
7. (A7)	0.1 M Sodium cacodylate trihydrate pH 6.5, 1.4 M Sodium acetate trihydrate			
8. (A8)	0.2 M Sodium citrate tribasic dihydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 30% v/v 2-Propanol			
9. (A9)	0.2 M Ammonium acetate, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 30% w/v Polyethylene glycol 4,000			
10. (A10)	0.2 M Ammonium acetate, 0.1 M Sodium acetate trihydrate pH 4.6, 30% w/v Polyethylene glycol 4,000			
11. (A11)	0.1 M Sodium citrate tribasic dihydrate pH 5.6, 1.0 M Ammonium phosphate monobasic			
12. (A12)	0.2 M Magnesium chloride hexahydrate, 0.1 M HEPES sodium pH 7.5, 30% v/v 2-Propanol			
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)	0.2 M Calcium chloride dihydrate, 0.1 M HEPES sodium pH 7.5, 28% v/v Polyethylene glycol 400			
15. (B3)	0.2 M Ammonium sulfate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 30% w/v Polyethylene glycol 8,000			
16. (B4)	0.1 M HEPES sodium pH 7.5, 1.5 M Lithium sulfate monohydrate			
17. (B5)	0.2 M Lithium sulfate monohydrate, 0.1 M TRIS hydrochloride pH 8.5, 30% w/v Polyethylene glycol 4,000			
18. (B6)	0.2 M Magnesium acetate tetrahydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 20% w/v Polyethylene glycol 8,000			
19. (B7)	0.2 M Ammonium acetate, 0.1 M TRIS hydrochloride pH 8.5, 30% v/v 2-Propanol			
20. (B8)	0.2 M Ammonium sulfate, 0.1 M Sodium acetate trihydrate pH 4.6, 25% w/v Polyethylene glycol 4,000			
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)	0.2 M Sodium acetate trihydrate, 0.1 M TRIS hydrochloride pH 8.5, 30% w/v Polyethylene glycol 4,000			
23. (B11)	0.2 M Magnesium chloride hexahydrate, 0.1 M HEPES sodium pH 7.5, 30% v/v Polyethylene glycol 400			
24. (B12)	0.2 M Calcium chloride dihydrate, 0.1 M Sodium acetate trihydrate pH 4.6, 20% v/v 2-Propanol			
25. (C1)	0.1 M Imidazole pH 6.5, 1.0 M Sodium acetate trihydrate			
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)	0.2 M Sodium citrate tribasic dihydrate, 0.1 M HEPES sodium pH 7.5, 20% v/v 2-Propanol			
28. (C4)	0.2 M Sodium acetate trihydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 30% w/v Polyethylene glycol 8,000			
29. (C5)	0.1 M HEPES sodium pH 7.5, 0.8 M Potassium sodium tartrate tetrahydrate			
30. (C6)	0.2 M Ammonium sulfate, 30% w/v Polyethylene glycol 8,000			
31. (C7)	0.2 M Ammonium sulfate, 30% w/v Polyethylene glycol 4,000			
32. (C8)	2.0 M Ammonium sulfate			
33. (C9)	4.0 M Sodium formate			
34. (C10)	0.1 M Sodium acetate trihydrate pH 4.6, 2.0 M Sodium formate			
35. (C11)	0.1 M HEPES sodium pH 7.5, 0.8 M Sodium phosphate monobasic monohydrate, 0.8 M Potassium phosphate monobasic			
36. (C12)	0.1 M TRIS hydrochloride pH 8.5, 8% w/v Polyethylene glycol 8,000			
37. (D1)	0.1 M Sodium acetate trihydrate pH 4.6, 8% w/v Polyethylene glycol 4,000			
38. (D2)	0.1 M HEPES sodium pH 7.5, 1.4 M Sodium citrate tribasic dihydrate			
39. (D3)	0.1 M HEPES sodium pH 7.5, 2% v/v Polyethylene glycol 400, 2.0 M Ammonium sulfate			
40. (D4)	0.1 M Sodium citrate tribasic dihydrate pH 5.6, 20% v/v 2-Propanol, 20% w/v Polyethylene glycol 4,000			
41. (D5)	0.1 M HEPES sodium pH 7.5, 10% v/v 2-Propanol, 20% w/v Polyethylene glycol 4,000			
42. (D6)	0.05 M Potassium phosphate monobasic, 20% w/v Polyethylene glycol 8,000			
43. (D7)	30% w/v Polyethylene glycol 1,500			
44. (D8)	0.2 M Magnesium formate dihydrate			
45. (D9)	0.2 M Zinc acetate dihydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 18% w/v Polyethylene glycol 8,000			
46. (D10)	0.2 M Calcium acetate hydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5 , 18% w/v Polyethylene glycol 8,000			
47. (D11)	0.1 M Sodium acetate trihydrate pH 4.6, 2.0 M Ammonium sulfate			
48. (D12)	0.1 M TRIS hydrochloride pH 8.5, 2.0 M Ammonium phosphate monobasic			

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 HT™ - HR2-130 Scoring Sheet		Date:	Date:	Date:
49. (E1)	2.0 M Sodium chloride, 10% w/v Polyethylene glycol 6,000			
50. (E2)	0.5 M Sodium chloride, 0.01 M Magnesium chloride hexahydrate, 0.01 M Hexadecyltrimethylammonium bromide			
51. (E3)	25% v/v Ethylene glycol			
52. (E4)	35% v/v 1,4-Dioxane			
53. (E5)	2.0 M Ammonium sulfate, 5% v/v 2-Propanol			
54. (E6)	1.0 M Imidazole pH 7.0			
55. (E7)	10% w/v Polyethylene glycol 1,000, 10% w/v Polyethylene glycol 8,000			
56. (E8)	1.5 M Sodium chloride, 10% v/v Ethanol			
57. (E9)	0.1 M Sodium acetate trihydrate pH 4.6, 2.0 M Sodium chloride			
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)	0.01 M Cobalt(II) chloride hexahydrate, 0.1 M Sodium acetate trihydrate pH 4.6, 1.0 M 1,6-Hexanediol			
60. (E12)	0.1 M Cadmium chloride hydrate, 0.1 M Sodium acetate trihydrate pH 4.6, 30% v/v Polyethylene glycol 400			
61. (F1)	0.2 M Ammonium sulfate, 0.1 M Sodium acetate trihydrate pH 4.6, 30% w/v Polyethylene glycol monomethyl ether 2,000			
62. (F2)	0.2 M Potassium sodium tartrate tetrahydrate, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 2.0 M Ammonium sulfate			
63. (F3)	0.5 M Ammonium sulfate, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 1.0 M Lithium sulfate monohydrate			
64. (F4)	0.5 M Sodium chloride, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 2% v/v Ethylene imine polymer			
65. (F5)	0.1 M Sodium citrate tribasic dihydrate pH 5.6, 35% v/v tert-Butanol			
66. (F6)	0.01 M Iron(III) chloride hexahydrate, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 10% v/v Jeffamine® M-600®			
67. (F7)	0.1 M Sodium citrate tribasic dihydrate pH 5.6, 2.5 M 1,6-Hexanediol			
68. (F8)	0.1 M MES monohydrate pH 6.5, 1.6 M Magnesium sulfate heptahydrate			
69. (F9)	0.1 M Sodium phosphate monobasic monohydrate, 0.1 M Potassium phosphate monobasic, 0.1 M MES monohydrate pH 6.5,			
	2.0 M Sodium chloride			
70. (F10)	0.1 M MES monohydrate pH 6.5, 12% w/v Polyethylene glycol 20,000			
71. (F11)	1.6 M Ammonium sulfate, 0.1 M MES monohydrate pH 6.5, 10% v/v 1,4-Dioxane			
72. (F12)	0.05 M Cesium chloride, 0.1 M MES monohydrate pH 6.5, 30% v/v Jeffamine® M-600®			
73. (G1)	0.01 M Cobalt(II) chloride hexahydrate, 0.1 M MES monohydrate pH 6.5, 1.8 M Ammonium sulfate			
74. (G2)	0.2 M Ammonium sulfate, 0.1 M MES monohydrate pH 6.5, 30% w/v Polyethylene glycol monomethyl ether 5,000			
75. (G3)	0.01 M Zinc sulfate heptahydrate, 0.1 M MES monohydrate pH 6.5, 25% v/v Polyethylene glycol monomethyl ether 550			
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)	0.1 M HEPES pH 7.5, 10% w/v Polyethylene glycol 6,000, 5% v/v (+/-)-2-Methyl-2,4-pentanediol			
79. (G7)	0.1 M HEPES pH 7.5, 20% v/v Jeffamine® M-600®			
80. (G8)	0.1 M Sodium chloride, 0.1 M HEPES pH 7.5, 1.6 M Ammonium sulfate			
81. (G9)	0.1 M HEPES pH 7.5, 2.0 M Ammonium formate			
82. (G10)	0.05 M Cadmium sulfate hydrate, 0.1 M HEPES pH 7.5, 1.0 M Sodium acetate trihydrate			
83. (G11)	0.1 M HEPES pH 7.5, 70% v/v (+/-)-2-Methyl-2,4-pentanediol			
84. (G12)	0.1 M HEPES pH 7.5, 4.3 M Sodium chloride			
85. (H1)	0.1 M HEPES pH 7.5, 10% w/v Polyethylene glycol 8,000, 8% v/v Ethylene glycol			
86. (H2)	0.1 M HEPES pH 7.5, 20% w/v Polyethylene glycol 10,000			
87. (H3)	0.2 M Magnesium chloride hexahydrate, 0.1 M Tris pH 8.5, 3.4 M 1,6-Hexanediol			
88. (H4)	0.1 M Tris pH 8.5, 25% v/v tert-Butanol			
89. (H5)	0.01 M Nickel(II) chloride hexahydrate, 0.1 M Tris pH 8.5, 1.0 M Lithium sulfate monohydrate			
90. (H6)	1.5 M Ammonium sulfate, 0.1 M Tris pH 8.5, 12% 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)	0.1 M Tris pH 8.5, 20% v/v Ethanol			
93. (H9)	0.01 M Nickel(II) chloride hexahydrate, 0.1 M Tris pH 8.5, 20% w/v Polyethylene glycol monomethyl ether 2,000			
94. (H10)	0.1 M Sodium chloride, 0.1 M BICINE pH 9.0, 20% v/v Polyethylene glycol monomethyl ether 550			
95. (H11)	0.1 M BICINE pH 9.0, 2.0 M Magnesium chloride hexahydrate			
96. (H12)	0.1 M BICINE pH 9.0, 2% v/v 1,4-Dioxane, 10% w/v Polyethylene glycol 20,000			

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