Wizard Screen HT

HAMPTON RESEARCH
Solutions for Crystal Growth

User Guide **HR2-463 (pg 1)**

Features

Wizard Screen HT is a high throughput reagent kit designed to provide a rapid screening method for the crystallization of biological macromolecules using a large range of precipitants, buffers, and salts, covering a broad range of crystallization space at pH levels from pH 4.5 to pH 10.5. 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.

Wizard Screen HT is comprised of Wizard Screen 1 and Wizard Screen 2, offering a sparse matrix of trial crystallization reagent conditions developed by Steve L. Sarfaty and Wim G. J. Hol. The primary screen variables are salt, pH, and precipitant (salts, polymers, volatile organics, and non-volatile organics).

General Description

Wizard 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 Wizard 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 Wizard Screen 1 and Wizard 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

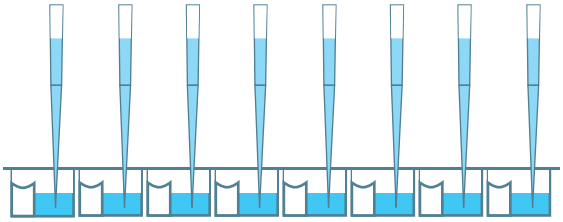
Allow the block to equilibrate to room temperature. To remove stray reagent from the sealing film, centrifuge the block at 500 rpm for 5 minutes. To remove film, grasp a corner of the film and gently peel film from the block. Alternatively, the film can be pierced to access reagents.

Performing the Screen

Manual Method - Sitting Drop Vapor Diffusion

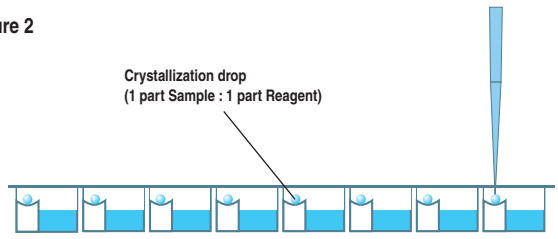
1. Using a 96 well sitting drop vapor diffusion plate, pipet the recommended volume (typically 100 microliters) of crystallization reagent from the Deep Well block into the reservoirs of the crystallization plate. The Deep Well block is compatible with 8 and 12 channel pipets as well as many automated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

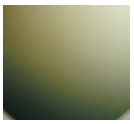
Figure 2

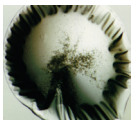


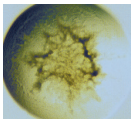
3. Using a clean pipet tip, pipet 0.05 to 2 microliters of sample to the reagent drop in the sitting drop well. One may choose to simply dispense the sample with no mixing or dispense with mixing by gently aspirating and dispensing the sample several times, keeping the tip in the drop during mixing to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

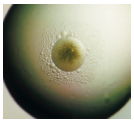
4. Seal the crystallization plate as per the manufacturer's recommendation. Most 96 well crystallization plates are sealed using a clear sealing tape, film, or cap mat. View and score the experiment as desired. See Hampton

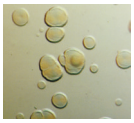
Figure 3
Typical observations in a crystallization experiment

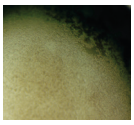

Clear Drop


Skin / Precipitate

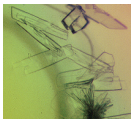

Precipitate


Precipitate / Phase

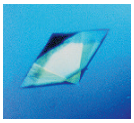

Quasi Crystals


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.

Wizard 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 Wizard 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.

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

Technical Support

Inquiries regarding Wizard 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 4:00 p.m. USA Pacific Standard Time.

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Well #	Salt	Well #	Buffer ◊	Well #	Precipitant
1. (A1)	None	1. (A1)	0.1 M CHES pH 9.5	1. (A1)	20% w/v Polyethylene glycol 8,000
2. (A2)	0.2 M Sodium chloride	2. (A2)	0.1 M HEPES pH 7.5	2. (A2)	10% v/v 2-Propanol
3. (A3)	None	3. (A3)	0.1 M CHES pH 9.5	3. (A3)	15% v/v Ethanol
4. (A4)	0.2 M Magnesium chloride hexahydrate	4. (A4)	0.1 M Imidazole pH 8.0	4. (A4)	35% v/v (+/-)-2-Methyl-2,4-pentanediol
5. (A5)	None	5. (A5)	0.1 M CAPS pH 10.5	5. (A5)	30% v/v Polyethylene glycol 400
6. (A6)	None	6. (A6)	0.1 M Sodium citrate tribasic dihydrate pH 5.5	6. (A6)	20% w/v Polyethylene glycol 3,000
7. (A7)	0.2 M Zinc acetate dihydrate	7. (A7)	0.1 M MES monohydrate pH 6.0	7. (A7)	10% w/v Polyethylene glycol 8,000
8. (A8)	None	8. (A8)	0.1 M Sodium citrate tribasic dihydrate pH 5.5	8. (A8)	2.0 M Ammonium sulfate
9. (A9)	None	9. (A9)	0.1 M Sodium acetate trihydrate pH 4.5	9. (A9)	1.0 M Ammonium phosphate dibasic
10. (A10)	None	10. (A10)	0.1 M Tris pH 7.0	10. (A10)	20% w/v Polyethylene glycol monomethyl ether 2,000
11. (A11)	0.2 M Lithium sulfate monohydrate	11. (A11)	0.1 M MES monohydrate pH 6.0	11. (A11)	20% v/v 1,4-Butanediol
12. (A12)	0.2 M Calcium acetate hydrate	12. (A12)	0.1 M Imidazole pH 8.0	12. (A12)	20% w/v Polyethylene glycol 1,000
13. (B1)	None	13. (B1)	0.1 M Sodium cacodylate trihydrate pH 6.5	13. (B1)	1.26 M Ammonium sulfate
14. (B2)	None	14. (B2)	0.1 M Sodium cacodylate trihydrate pH 6.5	14. (B2)	1.0 M Sodium citrate tribasic dihydrate
15. (B3)	0.2 M Lithium sulfate monohydrate	15. (B3)	0.1 M Imidazole pH 8.0	15. (B3)	10% w/v Polyethylene glycol 3,000
16. (B4)	None	16. (B4)	0.068 M Sodium phosphate monobasic monohydrate, 0.032 M Potassium phosphate dibasic pH 6.2	16. (B4)	2.5 M Sodium chloride
17. (B5)	0.2 M Lithium sulfate monohydrate	17. (B5)	0.1 M Sodium acetate trihydrate pH 4.5	17. (B5)	30% w/v Polyethylene glycol 8,000
18. (B6)	0.2 M Sodium chloride	18. (B6)	0.1 M Imidazole pH 8.0	18. (B6)	1.0 M Potassium sodium tartrate tetrahydrate
19. (B7)	None	19. (B7)	0.1 M Tris pH 7.0	19. (B7)	20% w/v Polyethylene glycol 1,000
20. (B8)	0.2 M Sodium chloride	20. (B8)	0.1 M Imidazole pH 8.0	20. (B8)	1.6 M Potassium phosphate dibasic, 0.4 M Sodium phosphate monobasic monohydrate
21. (B9)	None	21. (B9)	0.1 M HEPES pH 7.5	21. (B9)	20% w/v Polyethylene glycol 8,000
22. (B10)	None	22. (B10)	0.1 M Tris pH 8.5	22. (B10)	10% v/v 2-Propanol
23. (B11)	0.2 M Magnesium chloride hexahydrate	23. (B11)	0.1 M Imidazole pH 8.0	23. (B11)	15% v/v Ethanol
24. (B12)	0.2 M Sodium chloride	24. (B12)	0.1 M Tris pH 7.0	24. (B12)	35% v/v (+/-)-2-Methyl-2,4-pentanediol
25. (C1)	0.2 M Magnesium chloride hexahydrate	25. (C1)	0.1 M Tris pH 8.5	25. (C1)	30% v/v Polyethylene glycol 400
26. (C2)	None	26. (C2)	0.1 M CHES pH 9.5	26. (C2)	10% w/v Polyethylene glycol 3,000
27. (C3)	0.2 M Lithium sulfate monohydrate	27. (C3)	0.1 M CAPS pH 10.5	27. (C3)	0.8 M Potassium phosphate dibasic, 1.2 M Sodium phosphate monobasic monohydrate
28. (C4)	0.2 M Sodium chloride	28. (C4)	0.1 M HEPES pH 7.5	28. (C4)	20% w/v Polyethylene glycol 3,000
29. (C5)	0.2 M Sodium chloride	29. (C5)	0.1 M CHES pH 9.5	29. (C5)	10% w/v Polyethylene glycol 8,000
30. (C6)	0.2 M Sodium chloride	30. (C6)	0.1 M Sodium acetate trihydrate pH 4.5	30. (C6)	1.26 M Ammonium sulfate
31. (C7)	0.2 M Sodium chloride	31. (C7)	0.1 M Sodium citrate tribasic dihydrate pH 4.2	31. (C7)	20% w/v Polyethylene glycol 8,000
32. (C8)	None	32. (C8)	0.068 M Sodium phosphate monobasic monohydrate, 0.032 M Potassium phosphate dibasic pH 6.2	32. (C8)	10% w/v Polyethylene glycol 3,000
33. (C9)	0.2 M Lithium sulfate monohydrate	33. (C9)	0.1 M CAPS pH 10.5	33. (C9)	2.0 M Ammonium sulfate
34. (C10)	None	34. (C10)	0.1 M Imidazole pH 8.0	34. (C10)	1.0 M Ammonium phosphate dibasic
35. (C11)	None	35. (C11)	0.1 M Sodium acetate trihydrate pH 4.5	35. (C11)	20% v/v 1,4-Butanediol
36. (C12)	None	36. (C12)	0.1 M Imidazole pH 8.0	36. (C12)	1.0 M Sodium citrate tribasic dihydrate
37. (D1)	None	37. (D1)	0.1 M Imidazole pH 8.0	37. (D1)	2.5 M Sodium chloride
38. (D2)	0.2 M Lithium sulfate monohydrate	38. (D2)	0.1 M CHES pH 9.5	38. (D2)	1.0 M Potassium sodium tartrate tetrahydrate
39. (D3)	0.2 M Lithium sulfate monohydrate	39. (D3)	0.1 M Sodium citrate tribasic dihydrate pH 4.2	39. (D3)	20% w/v Polyethylene glycol 1,000
40. (D4)	0.2 M Calcium acetate hydrate	40. (D4)	0.1 M MES monohydrate pH 6.0	40. (D4)	10% v/v 2-Propanol
41. (D5)	None	41. (D5)	0.1 M CHES pH 9.5	41. (D5)	30% w/v Polyethylene glycol 3,000
42. (D6)	None	42. (D6)	0.1 M Tris pH 7.0	42. (D6)	15% v/v Ethanol
43. (D7)	None	43. (D7)	0.068 M Sodium phosphate monobasic monohydrate, 0.032 M Potassium phosphate dibasic pH 6.2	43. (D7)	35% v/v (+/-)-2-Methyl-2,4-pentanediol
44. (D8)	0.2 M Calcium acetate hydrate	44. (D8)	0.1 M Sodium acetate trihydrate pH 4.5	44. (D8)	30% v/v Polyethylene glycol 400
45. (D9)	None	45. (D9)	0.1 M Sodium acetate trihydrate pH 4.5	45. (D9)	20% w/v Polyethylene glycol 3,000
46. (D10)	0.2 M Calcium acetate hydrate	46. (D10)	0.1 M Imidazole pH 8.0	46. (D10)	10% w/v Polyethylene glycol 8,000
47. (D11)	0.2 M Lithium sulfate monohydrate	47. (D11)	0.1 M Tris pH 8.5	47. (D11)	1.26 M Ammonium sulfate
48. (D12)	0.2 M Zinc acetate dihydrate	48. (D12)	0.1 M Sodium acetate trihydrate pH 4.5	48. (D12)	20% w/v Polyethylene glycol 1,000

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

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Well #	Salt	Well #	Buffer ◊	Well #	Precipitant
49. (E1)	0.2 M Zinc acetate dihydrate	49. (E1)	0.1 M Sodium acetate trihydrate pH 4.5	49. (E1)	10% w/v Polyethylene glycol 3,000
50. (E2)	0.2 M Lithium sulfate monohydrate	50. (E2)	0.1 M MES monohydrate pH 6.0	50. (E2)	35% v/v (+/-)-2-Methyl-2,4-pentanediol
51. (E3)	0.2 M Magnesium chloride hexahydrate	51. (E3)	0.1 M Tris pH 8.5	51. (E3)	20% w/v Polyethylene glycol 8,000
52. (E4)	0.2 M Sodium chloride	52. (E4)	0.1 M Sodium cacodylate trihydrate pH 6.5	52. (E4)	2.0 M Ammonium sulfate
53. (E5)	0.2 M Sodium chloride	53. (E5)	0.1 M HEPES pH 7.5	53. (E5)	20% v/v 1,4-Butanediol
54. (E6)	0.2 M Lithium sulfate monohydrate	54. (E6)	0.1 M Sodium citrate tribasic dihydrate pH 4.2	54. (E6)	10% v/v 2-Propanol
55. (E7)	0.2 M Sodium chloride	55. (E7)	0.1 M Tris pH 7.0	55. (E7)	30% w/v Polyethylene glycol 3,000
56. (E8)	0.2 M Sodium chloride	56. (E8)	0.068 M Sodium phosphate monobasic monohydrate, 0.032 M Potassium phosphate dibasic pH 6.2	56. (E8)	10% w/v Polyethylene glycol 8,000
57. (E9)	None	57. (E9)	0.1 M Sodium citrate tribasic dihydrate pH 4.2	57. (E9)	2.0 M Ammonium sulfate
58. (E10)	None	58. (E10)	0.1 M Tris pH 8.5	58. (E10)	1.0 M Ammonium phosphate dibasic
59. (E11)	0.2 M Zinc acetate dihydrate	59. (E11)	0.1 M Sodium cacodylate trihydrate pH 6.5	59. (E11)	10% v/v 2-Propanol
60. (E12)	0.2 M Lithium sulfate monohydrate	60. (E12)	0.1 M Sodium cacodylate trihydrate pH 6.5	60. (E12)	30% v/v Polyethylene glycol 400
61. (F1)	0.2 M Lithium sulfate monohydrate	61. (F1)	0.1 M Sodium citrate tribasic dihydrate pH 5.5	61. (F1)	15% v/v Ethanol
62. (F2)	0.2 M Sodium chloride	62. (F2)	0.068 M Sodium phosphate monobasic monohydrate, 0.032 M Potassium phosphate dibasic pH 6.2	62. (F2)	20% w/v Polyethylene glycol 1,000
63. (F3)	None	63. (F3)	0.1 M HEPES pH 7.5	63. (F3)	1.26 M Ammonium sulfate
64. (F4)	None	64. (F4)	0.1 M CHES pH 9.5	64. (F4)	1.0 M Sodium citrate tribasic dihydrate
65. (F5)	0.2 M Magnesium chloride hexahydrate	65. (F5)	0.1 M Tris pH 7.0	65. (F5)	2.5 M Sodium chloride
66. (F6)	0.2 M Calcium acetate hydrate	66. (F6)	0.1 M Tris pH 7.0	66. (F6)	20% w/v Polyethylene glycol 3,000
67. (F7)	None	67. (F7)	0.1 M Sodium citrate tribasic dihydrate pH 4.2	67. (F7)	0.4 M Potassium phosphate dibasic, 1.6 M Sodium phosphate monobasic monohydrate
68. (F8)	0.2 M Zinc acetate dihydrate	68. (F8)	0.1 M MES monohydrate pH 6.0	68. (F8)	15% v/v Ethanol
69. (F9)	None	69. (F9)	0.1 M Sodium acetate trihydrate pH 4.5	69. (F9)	35% v/v (+/-)-2-Methyl-2,4-pentanediol
70. (F10)	None	70. (F10)	0.1 M Imidazole pH 8.0	70. (F10)	10% v/v 2-Propanol
71. (F11)	0.2 M Magnesium chloride hexahydrate	71. (F11)	0.1 M HEPES pH 7.5	71. (F11)	15% v/v Ethanol
72. (F12)	0.2 M Sodium chloride	72. (F12)	0.1 M Imidazole pH 8.0	72. (F12)	30% w/v Polyethylene glycol 8,000
73. (G1)	0.2 M Sodium chloride	73. (G1)	0.1 M HEPES pH 7.5	73. (G1)	35% v/v (+/-)-2-Methyl-2,4-pentanediol
74. (G2)	None	74. (G2)	0.1 M CHES pH 9.5	74. (G2)	30% v/v Polyethylene glycol 400
75. (G3)	0.2 M Magnesium chloride hexahydrate	75. (G3)	0.1 M Sodium cacodylate trihydrate pH 6.5	75. (G3)	10% w/v Polyethylene glycol 3,000
76. (G4)	0.2 M Calcium acetate hydrate	76. (G4)	0.1 M MES monohydrate pH 6.0	76. (G4)	20% w/v Polyethylene glycol 8,000
77. (G5)	0.2 M Sodium chloride	77. (G5)	0.1 M CHES pH 9.5	77. (G5)	1.26 M Ammonium sulfate
78. (G6)	0.2 M Zinc acetate dihydrate	78. (G6)	0.1 M Imidazole pH 8.0	78. (G6)	20% v/v 1,4-Butanediol
79. (G7)	0.2 M Sodium chloride	79. (G7)	0.1 M Tris pH 7.0	79. (G7)	1.0 M Sodium citrate tribasic dihydrate
80. (G8)	None	80. (G8)	0.1 M Tris pH 8.5	80. (G8)	20% w/v Polyethylene glycol 1,000
81. (G9)	0.2 M Sodium chloride	81. (G9)	0.1 M Sodium citrate tribasic dihydrate pH 5.5	81. (G9)	1.0 M Ammonium phosphate dibasic
82. (G10)	None	82. (G10)	0.1 M Imidazole pH 8.0	82. (G10)	10% w/v Polyethylene glycol 8,000
83. (G11)	None	83. (G11)	0.1 M Sodium acetate trihydrate pH 4.5	83. (G11)	0.8 M Sodium phosphate monobasic monohydrate, 1.2 M Potassium phosphate dibasic
84. (G12)	0.2 M Sodium chloride	84. (G12)	0.1 M Sodium citrate tribasic dihydrate pH 4.2	84. (G12)	10% w/v Polyethylene glycol 3,000
85. (H1)	0.2 M Lithium sulfate monohydrate	85. (H1)	0.1 M Tris pH 7.0	85. (H1)	1.0 M Potassium sodium tartrate tetrahydrate
86. (H2)	0.2 M Lithium sulfate monohydrate	86. (H2)	0.1 M Sodium acetate trihydrate pH 4.5	86. (H2)	2.5 M Sodium chloride
87. (H3)	0.2 M Sodium chloride	87. (H3)	0.1 M CAPS pH 10.5	87. (H3)	20% w/v Polyethylene glycol 8,000
88. (H4)	0.2 M Zinc acetate dihydrate	88. (H4)	0.1 M Imidazole pH 8.0	88. (H4)	20% w/v Polyethylene glycol 3,000
89. (H5)	0.2 M Lithium sulfate monohydrate	89. (H5)	0.1 M Tris pH 7.0	89. (H5)	2.0 M Ammonium sulfate
90. (H6)	0.2 M Sodium chloride	90. (H6)	0.1 M HEPES pH 7.5	90. (H6)	30% v/v Polyethylene glycol 400
91. (H7)	0.2 M Magnesium chloride hexahydrate	91. (H7)	0.1 M Tris pH 7.0	91. (H7)	10% w/v Polyethylene glycol 8,000
92. (H8)	0.2 M Magnesium chloride hexahydrate	92. (H8)	0.1 M Sodium cacodylate trihydrate pH 6.5	92. (H8)	20% w/v Polyethylene glycol 1,000
93. (H9)	None	93. (H9)	0.1 M MES monohydrate pH 6.0	93. (H9)	1.26 M Ammonium sulfate
94. (H10)	0.2 M Sodium chloride	94. (H10)	0.1 M Imidazole pH 8.0	94. (H10)	1.0 M Ammonium phosphate dibasic
95. (H11)	0.2 M Zinc acetate dihydrate	95. (H11)	0.1 M Imidazole pH 8.0	95. (H11)	2.5 M Sodium chloride
96. (H12)	None	96. (H12)	0.1 M MES monohydrate pH 6.0	96. (H12)	1.0 M Potassium sodium tartrate tetrahydrate

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

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HAMPTON RESEARCH
Solutions for Crystal Growth

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)

Wizard Screen HT - HR2-463 Scoring Sheet

	Date:	Date:	Date:
1. (A1) 0.1 M CHES pH 9.5, 20% w/v Polyethylene glycol 8,000			
2. (A2) 0.2 M Sodium chloride, 0.1 M HEPES pH 7.5, 10% v/v 2-Propanol			
3. (A3) 0.1 M CHES pH 9.5, 15% v/v Ethanol			
4. (A4) 0.2 M Magnesium chloride hexahydrate, 0.1 M Imidazole pH 8.0, 35% v/v (+/-)-2-Methyl-2,4-pentanediol			
5. (A5) 0.1 M CAPS pH 10.5, 30% v/v Polyethylene glycol 400			
6. (A6) 0.1 M Sodium citrate tribasic dihydrate pH 5.5, 20% w/v Polyethylene glycol 3,000			
7. (A7) 0.2 M Zinc acetate dihydrate, 0.1 M MES monohydrate pH 6.0, 10% w/v Polyethylene glycol 8,000			
8. (A8) 0.1 M Sodium citrate tribasic dihydrate pH 5.5, 2.0 M Ammonium sulfate			
9. (A9) 0.1 M Sodium acetate trihydrate pH 4.5, 1.0 M Ammonium phosphate dibasic			
10. (A10) 0.1 M Tris pH 7.0, 20% w/v Polyethylene glycol monomethyl ether 2,000			
11. (A11) 0.2 M Lithium sulfate monohydrate, 0.1 M MES monohydrate pH 6.0, 20% v/v 1,4-Butanediol			
12. (A12) 0.2 M Calcium acetate hydrate, 0.1 M Imidazole pH 8.0, 20% w/v Polyethylene glycol 1,000			
13. (B1) 0.1 M Sodium cacodylate trihydrate pH 6.5, 1.26 M Ammonium sulfate			
14. (B2) 0.1 M Sodium cacodylate trihydrate pH 6.5, 1.0 M Sodium citrate tribasic dihydrate			
15. (B3) 0.2 M Lithium sulfate monohydrate, 0.1 M Imidazole pH 8.0, 10% w/v Polyethylene glycol 3,000			
16. (B4) 0.1 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 6.2, 2.5 M Sodium chloride			
17. (B5) 0.2 M Lithium sulfate monohydrate, 0.1 M Sodium acetate trihydrate pH 4.5, 30% w/v Polyethylene glycol 8,000			
18. (B6) 0.2 M Sodium chloride, 0.1 M Imidazole pH 8.0, 1.0 M Potassium sodium tartrate tetrahydrate			
19. (B7) 0.1 M Tris pH 7.0, 20% w/v Polyethylene glycol 1,000			
20. (B8) 0.2 M Sodium chloride, 0.1 M Imidazole pH 8.0, 1.6 M Potassium phosphate dibasic, 0.4 M Na phosphate monobasic monohyd.			
21. (B9) 0.1 M HEPES pH 7.5, 20% w/v Polyethylene glycol 8,000			
22. (B10) 0.1 M Tris pH 8.5, 10% v/v 2-Propanol			
23. (B11) 0.2 M Magnesium chloride hexahydrate, 0.1 M Imidazole pH 8.0, 15% v/v Ethanol			
24. (B12) 0.2 M Sodium chloride, 0.1 M Tris pH 7.0, 35% v/v (+/-)-2-Methyl-2,4-pentanediol			
25. (C1) 0.2 M Magnesium chloride hexahydrate, 0.1 M Tris pH 8.5, 30% v/v Polyethylene glycol 400			
26. (C2) 0.1 M CHES pH 9.5, 10% w/v Polyethylene glycol 3,000			
27. (C3) 0.2 M Lithium sulfate monohydrate, 0.1 M CAPS pH 10.5, 0.8 M Potassium phosphate dibasic, 1.2 M Sodium phosphate monobasic monohydrate			
28. (C4) 0.2 M Sodium chloride, 0.1 M HEPES pH 7.5, 20% w/v Polyethylene glycol 3,000			
29. (C5) 0.2 M Sodium chloride, 0.1 M CHES pH 9.5, 10% w/v Polyethylene glycol 8,000			
30. (C6) 0.2 M Sodium chloride, 0.1 M Sodium acetate trihydrate pH 4.5, 1.26 M Ammonium sulfate			
31. (C7) 0.2 M Sodium chloride, 0.1 M Sodium citrate tribasic dihydrate pH 4.2, 20% w/v Polyethylene glycol 8,000			
32. (C8) 0.1 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 6.2, 10% w/v PEG 3,000			
33. (C9) 0.2 M Lithium sulfate monohydrate, 0.1 M CAPS pH 10.5, 2.0 M Ammonium sulfate			
34. (C10) 0.1 M Imidazole pH 8.0, 1.0 M Ammonium phosphate dibasic			
35. (C11) 0.1 M Sodium acetate trihydrate pH 4.5, 20% v/v 1,4-Butanediol			
36. (C12) 0.1 M Imidazole pH 8.0, 1.0 M Sodium citrate tribasic dihydrate			
37. (D1) 0.1 M Imidazole pH 8.0, 2.5 M Sodium chloride			
38. (D2) 0.2 M Lithium sulfate monohydrate, 0.1 M CHES pH 9.5, 1.0 M Potassium sodium tartrate tetrahydrate			
39. (D3) 0.2 M Lithium sulfate monohydrate, 0.1 M Sodium citrate tribasic dihydrate pH 4.2, 20% w/v Polyethylene glycol 1,000			
40. (D4) 0.2 M Calcium acetate hydrate, 0.1 M MES monohydrate pH 6.0, 10% v/v 2-Propanol			
41. (D5) 0.1 M CHES pH 9.5, 30% w/v Polyethylene glycol 3,000			
42. (D6) 0.1 M Tris pH 7.0, 15% v/v Ethanol			
43. (D7) 0.1 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 6.2, 35% v/v (+/-)-2-Methyl-2,4-pentanediol			
44. (D8) 0.2 M Calcium acetate hydrate, 0.1 M Sodium acetate trihydrate pH 4.5, 30% v/v Polyethylene glycol 400			
45. (D9) 0.1 M Sodium acetate trihydrate pH 4.5, 20% w/v Polyethylene glycol 3,000			
46. (D10) 0.1 M Imidazole pH 8.0, 0.2 M Calcium acetate hydrate, 10% w/v Polyethylene glycol 8,000			
47. (D11) 0.2 M Lithium sulfate monohydrate, 0.1 M Tris pH 8.5, 1.26 M Ammonium sulfate			
48. (D12) 0.2 M Zinc acetate dihydrate, 0.1 M Sodium acetate trihydrate pH 4.5, 20% w/v Polyethylene glycol 1,000			

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Website: hamptonresearch.com

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)

Wizard Screen HT - HR2-463 Scoring Sheet

	Date:	Date:	Date:
49. (E1) 0.2 M Zinc acetate dihydrate, 0.1 M Sodium acetate trihydrate pH 4.5, 10% w/v Polyethylene glycol 3,000			
50. (E2) 0.2 M Lithium sulfate monohydrate, 0.1 M MES monohydrate pH 6.0, 35% v/v (+/-)-2-Methyl-2,4-pentanediol			
51. (E3) 0.2 M Magnesium chloride hexahydrate, 0.1 M Tris pH 8.5, 20% w/v Polyethylene glycol 8,000			
52. (E4) 0.2 M Sodium chloride, 0.1 M Sodium cacodylate trihydrate pH 6.5, 2.0 M Ammonium sulfate			
53. (E5) 0.2 M Sodium chloride, 0.1 M HEPES pH 7.5, 20% v/v 1,4-Butanediol			
54. (E6) 0.2 M Lithium sulfate monohydrate, 0.1 M Sodium citrate tribasic dihydrate pH 4.2, 10% v/v 2-Propanol			
55. (E7) 0.2 M Sodium chloride, 0.1 M Tris pH 7.0, 30% w/v Polyethylene glycol 3,000			
56. (E8) 0.2 M NaCl, 0.1 M Na phosphate monobasic monohyd. / Potassium phosphate dibasic pH 6.2, 10% w/v PEG 8,000			
57. (E9) 0.1 M Sodium citrate tribasic dihydrate pH 4.2, 2.0 M Ammonium sulfate			
58. (E10) 0.1 M Tris pH 8.5, 1.0 M Ammonium phosphate dibasic			
59. (E11) 0.2 M Zinc acetate dihydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 10% v/v 2-Propanol			
60. (E12) 0.2 M Lithium sulfate monohydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 30% v/v Polyethylene glycol 400			
61. (F1) 0.2 M Lithium sulfate monohydrate, 0.1 M Sodium citrate tribasic dihydrate pH 5.5, 15% v/v Ethanol			
62. (F2) 0.2 M NaCl, 0.1 M Na phosphate monobasic monohyd. / Potassium phosphate dibasic pH 6.2, 20% w/v PEG 1,000			
63. (F3) 0.1 M HEPES pH 7.5, 1.26 M Ammonium sulfate			
64. (F4) 0.1 M CHES pH 9.5, 1.0 M Sodium citrate tribasic dihydrate			
65. (F5) 0.2 M Magnesium chloride hexahydrate, 0.1 M Tris pH 7.0, 2.5 M Sodium chloride			
66. (F6) 0.2 M Calcium acetate hydrate, 0.1 M Tris pH 7.0, 20% w/v Polyethylene glycol 3,000			
67. (F7) 0.1 M Sodium citrate tribasic dihydrate pH 4.2, 0.4 M Potassium phosphate dibasic,			
1.6 M Sodium phosphate monobasic monohydrate			
68. (F8) 0.2 M Zinc acetate dihydrate, 0.1 M MES monohydrate pH 6.0, 15% v/v Ethanol			
69. (F9) 0.1 M Sodium acetate trihydrate pH 4.5, 35% v/v (+/-)-2-Methyl-2,4-pentanediol			
70. (F10) 0.1 M Imidazole pH 8.0, 10% v/v 2-Propanol			
71. (F11) 0.2 M Magnesium chloride hexahydrate, 0.1 M HEPES pH 7.5, 15% v/v Ethanol			
72. (F12) 0.2 M Sodium chloride, 0.1 M Imidazole pH 8.0, 30% w/v Polyethylene glycol 8,000			
73. (G1) 0.2 M Sodium chloride, 0.1 M HEPES pH 7.5, 35% v/v (+/-)-2-Methyl-2,4-pentanediol			
74. (G2) 0.1 M CHES pH 9.5, 30% v/v Polyethylene glycol 400			
75. (G3) 0.2 M Magnesium chloride hexahydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5,			
10% w/v Polyethylene glycol 3,000			
76. (G4) 0.2 M Calcium acetate hydrate, 0.1 M MES monohydrate pH 6.0, 20% w/v Polyethylene glycol 8,000			
77. (G5) 0.2 M Sodium chloride, 0.1 M CHES pH 9.5, 1.26 M Ammonium sulfate			
78. (G6) 0.2 M Zinc acetate dihydrate, 0.1 M Imidazole pH 8.0, 20% v/v 1,4-Butanediol			
79. (G7) 0.2 M Sodium chloride, 0.1 M Tris pH 7.0, 1.0 M Sodium citrate tribasic dihydrate			
80. (G8) 0.1 M Tris pH 8.5, 20% w/v Polyethylene glycol 1,000			
81. (G9) 0.2 M Sodium chloride, 0.1 M Sodium citrate tribasic dihydrate pH 5.5, 1.0 M Ammonium phosphate dibasic			
82. (G10) 0.1 M Imidazole pH 8.0, 10% w/v Polyethylene glycol 8,000			
83. (G11) 0.1 M Sodium acetate trihydrate pH 4.5, 0.8 M Sodium phosphate monobasic monohyd., 1.2 M Potassium phosphate dibasic			
36. (G12) 0.2 M Sodium chloride, 0.1 M Sodium citrate tribasic dihydrate pH 4.2, 10% w/v Polyethylene glycol 3,000			
37. (H1) 0.2 M Lithium sulfate monohydrate, 0.1 M Tris pH 7.0, 1.0 M Potassium sodium tartrate tetrahydrate			
38. (H2) 0.2 M Lithium sulfate monohydrate, 0.1 M Sodium acetate trihydrate pH 4.5, 2.5 M Sodium chloride			
39. (H3) 0.2 M Sodium chloride, 0.1 M CAPS pH 10.5, 20% w/v Polyethylene glycol 8,000			
40. (H4) 0.2 M Zinc acetate dihydrate, 0.1 M Imidazole pH 8.0, 20% w/v Polyethylene glycol 3,000			
41. (H5) 0.2 M Lithium sulfate monohydrate, 0.1 M Tris pH 7.0, 2.0 M Ammonium sulfate			
42. (H6) 0.2 M Sodium chloride, 0.1 M HEPES pH 7.5, 30% v/v Polyethylene glycol 400			
43. (H7) 0.2 M Magnesium chloride hexahydrate, 0.1 M Tris pH 7.0, 10% w/v Polyethylene glycol 8,000			
44. (H8) 0.2 M Magnesium chloride hexahydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 20% w/v Polyethylene glycol 1,000			
45. (H9) 0.1 M MES monohydrate pH 6.0, 1.26 M Ammonium sulfate			
46. (H10) 0.2 M Sodium chloride, 0.1 M Imidazole pH 8.0, 1.0 M Ammonium phosphate dibasic			
47. (H11) 0.2 M Zinc acetate dihydrate, 0.1 M Imidazole pH 8.0, 2.5 M Sodium chloride			
48. (H12) 0.1 M MES monohydrate pH 6.0, 1.0 M Potassium sodium tartrate tetrahydrate			

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