GRAS 8 SCREEN

HAMPTON RESEARCH
Solutions for Crystal Growth

User Guide **HR2-458 (pg 1)**

Applications

GRAS reagent crystallization screen for proteins, including monoclonal antibodies, where salt is the primary reagent, sampling pH 4.5 to 10.

Features

- Generally Recognized As Safe reagent formulation
- Samples pH 4.5 to 10; 8 unique buffers
- Sodium — chloride, citrate, formate, phosphate & tartrate
- Vapor diffusion, microbatch, free interface diffusion

Refer to the enclosed GRAS Screen 8 Reagent Formulation for more information.

General Description

GRAS Screen™ 8 was developed by Hampton Research for the crystallization of proteins, including monoclonal antibodies. Each of the chemicals in GRAS Screen 8 has been used under one or more of the following categories. As (1) a Generally Recognized As Safe (GRAS) substance, (2) a pharmaceutical excipient, (3) a normal physiological constituent, (4) a metabolic byproduct, and/or (5) a Everything Added to Food in the United States (EAFUS) substance. GRAS Screen 8 samples five salts (Sodium — chloride, citrate, formate, phosphate & tartrate) at four concentrations versus up to eight unique buffers encompassing pH 4.5 to 10.0 GRAS Screen 8 is supplied in a 96 Deep Well block format and is compatible with robotic and multi-channel pipet liquid handling systems. GRAS Screen 8 is compatible with vapor diffusion, free interface diffusion, and microbatch crystallization methods. For research use only.

Sample Preparation

The protein sample should be homogenous, as pure as is practically possible (>95%), and free of amorphous material. Remove amorphous material by centrifugation or microfiltration prior to use. The recommended sample concentration is 5 to 25 mg/ml in 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 GRAS Screen 8 reagents. However, agents that promote and preserve sample solubility, stability, and homogeneity can and should be included in the sample buffer. For additional sample preparation recommendations see Hampton Research Crystal Growth 101 - Preliminary Sample Preparation.

Preparing the Deep Well Block for Use

Allow the Deep Well Block and reagents to stabilize at room temperature, then centrifuge at 500 rpm for 5 minutes to remove stray drops from the film before removing the sealing film. The film can be removed by grasping a corner of the film and gently peeling the film from the plate. Alternatively, the film can be left intact, pierced to access reagents, and resealed using AlumaSeal II Sealing Film.

Performing the Screen

Automated Method - Sitting Drop Vapor Diffusion

The Deep Well block is compatible with the SBS standard 96 well microplate format and is compatible with numerous automated liquid handling systems that accept 8 x 12, 96 well assay blocks. Follow the automation manufacturer's recommendation for handling Deep Well blocks.

1. Using a 96 well sitting drop vapor diffusion plate, dispense the recommended volume (typically 50 to 100 microliters) of crystallization reagent from the Deep Well block into the reagent reservoirs of the crystallization plate.

2. Dispense the desired volume of crystallization reagent (typically 50 to 200 nanoliters) from the crystallization plate reservoir to the sitting drop well.

3. Transfer the equivalent volume of sample to the reagent drop in the sitting drop well.

4. Seal the crystallization plate using a clear sealing tape or film. View and score the experiment. See Hampton Research Crystal Growth 101 - Viewing Crystallization Experiments for more information.

5. Seal the remaining reagent in the Deep Well block using AlumaSeal II Sealing Film.

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 reagent reservoirs of the crystallization plate. The Deep Well block is compatible with 8, 12, and 96 channel automated and manual pipettors. 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 2 through 12. 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 B through H.

2. Using clean pipet tips, pipet the desired volume of crystallization reagent (typically 0.05 to 2 microliters) 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.

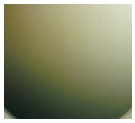
3. Using a clean pipet tip, pipet the same volume (typically 0.05 to 2 microliters) of sample to the reagent drop in the sitting drop well. Work carefully but quickly to minimize evaporation from the crystallization plate.

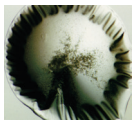
4. Seal the crystallization plate using an optically clear sealing film or tape. Seal the remaining reagent in the Deep Well block using AlumaSeal II sealing film.

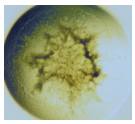
Examine the Drop

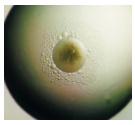
Carefully examine the drops under a stereo microscope (10 to 100x magnification) after setting the screen. Record all observations and be particularly careful to scan the focal plane for small crystals. Observe the drops once each day for the first week, then once a week there after for up to 60 days, or until the drop dries out. 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, 3+ needle shaped crystals in 1+ white precipitate. One may

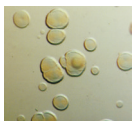
Figure 1
Typical observations in a crystallization experiment

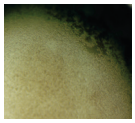

Clear Drop


Skin / Precipitate

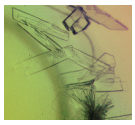

Precipitate


Precipitate / Phase

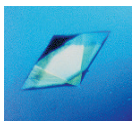

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

also employ a numerical scoring scheme (Clear = 0, Crystal = 1. Precipitate = 2). Figure 1 shows typical examples of what one might observe in a crystallization experiment.

Interpreting GRAS Screen 8

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 drops are clear, then 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 with sample buffer and repeat the screen condition. If more than 70 of the 96 drops contain precipitate and no crystals are present, then consider diluting the sample concentration in half by adding an equal volume of sample buffer to the sample and repeating the entire screen. If sample denaturation is suspect, take measures to stabilize the sample (add reducing agent, ligands, additives, 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 or UV optics to differentiate precipitate from microcrystals.

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 by varying salt concentration, screen pH, vary temperature between 4 and 30°C, screen additives, and evaluate other crystallization variables including sample construct, purity, stability, and homogeneity in order to achieve the desired crystal size and quality.

When sample quantity permits, set GRAS Screen 8 in duplicate (4°C and 25°C) or triplicate (10°C and 20°C and 30°C) to evaluate the effect of temperature on crystallization. Compare the observations between the different temperatures 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.

When sample quantity permits, set GRAS Screen 8 using multiple drops and drop ratios, such as 1:2, 1:1, and 2:1. See Hampton Research Crystal Growth 101: Drop Ratio for details.

GRAS Screen 8 Formulation

Crystallization reagents are formulated using the highest purity chemicals, ultrapure water (Formulated in Type 1+ ultrapure water: 18.2 megaohm-cm resistivity at 25°C, < 5 ppb Total Organic Carbon, bacteria free (<1 Bacteria (CFU/ml)), pyrogen free (<0.03 Endotoxin (EU/ml)), RNase-free (< 0.01 ng/mL) and DNase-free (< 4 pg/µL)) and are sterile filtered using 0.22 micron filters into sterile Deep Well blocks (no preservatives added). Store at -20°C. Best if used within 12 months of receipt.

Crystallization reagents can be reproduced using Hampton Research Optimize™ and StockOptions™ salts and buffers.

Recommended Reading

1. Introduction to protein crystallization. Alexander McPherson and Jose A. Gavira. Acta Crystallographica Section F Volume 70, Issue 1, pages 2-20, January 2014.

2. Optimization of crystallization conditions for biological macromolecules. Alexander McPherson and Bob Cudney. Acta Crystallographica Section F Volume 70, Issue 11, pages 1445–1467, November 2014.

3. Crystallization of intact monoclonal antibodies. Harris LJ, Skaletsky E, McPherson A. Proteins. 1995 Oct;23(2):285-9.

4. Crystalline monoclonal antibodies for subcutaneous delivery. Yang MX1, Shenoy B, Disttler M, Patel R, McGrath M, Pechenov S, Margolin AL. Proc Natl Acad Sci U S A. 2003 Jun 10;100(12):6934-9.

5. Fast and Scalable Purification of a Therapeutic Full-Length Antibody Based on Process Crystallization. Dariusch Hekmat et al, Biotechnology and Bioengineering, Vol. 110, No. 9, September, 2013.

6. Towards Protein Crystallization as a Process Step in Downstream Processing of Therapeutic Antibodies: Screening and Optimization at Microbatch Scale. Yuguo Zang et al, PLoS One. 2011; 6(9): e25282.

7. Crystallization and Liquid-Liquid Phase Separation of Monoclonal Antibodies and Fc-Fusion Proteins: Screening Results. Suresh Vunnum et al, Biotechnol Prog. 2011 Jul;27(4):1054-67.

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GRAS Screen™ 8

HR2-458 Reagent Formulation

Well #	Buffer[1]	Titrant	Well #	Salt	Well #	pH[2]
1. (A1)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	1. (A1)	0.2 M Sodium chloride	1. (A1)	4.5
2. (A2)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	2. (A2)	1.1 M Sodium chloride	2. (A2)	4.4
3. (A3)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	3. (A3)	1.9 M Sodium chloride	3. (A3)	4.3
4. (A4)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	4. (A4)	2.8 M Sodium chloride	4. (A4)	4.3
5. (A5)	0.1 M Succinic acid pH 5.5	NaOH	5. (A5)	0.2 M Sodium chloride	5. (A5)	5.5
6. (A6)	0.1 M Succinic acid pH 5.5	NaOH	6. (A6)	1.1 M Sodium chloride	6. (A6)	5.3
7. (A7)	0.1 M Succinic acid pH 5.5	NaOH	7. (A7)	1.9 M Sodium chloride	7. (A7)	5.2
8. (A8)	0.1 M Succinic acid pH 5.5	NaOH	8. (A8)	2.8 M Sodium chloride	8. (A8)	5.1
9. (A9)	0.1 M BIS-TRIS pH 6.5	HCl	9. (A9)	0.2 M Sodium chloride	9. (A9)	6.5
10. (A10)	0.1 M BIS-TRIS pH 6.5	HCl	10. (A10)	1.1 M Sodium chloride	10. (A10)	6.7
11. (A11)	0.1 M BIS-TRIS pH 6.5	HCl	11. (A11)	1.9 M Sodium chloride	11. (A11)	6.7
12. (A12)	0.1 M BIS-TRIS pH 6.5	HCl	12. (A12)	2.8 M Sodium chloride	12. (A12)	6.8
13. (B1)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	13. (B1)	0.2 M Sodium chloride	13. (B1)	7.0
14. (B2)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	14. (B2)	1.1 M Sodium chloride	14. (B2)	6.6
15. (B3)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	15. (B3)	1.9 M Sodium chloride	15. (B3)	6.3
16. (B4)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	16. (B4)	2.8 M Sodium chloride	16. (B4)	6.1
17. (B5)	0.1 M HEPES pH 7.5	NaOH	17. (B5)	0.2 M Sodium chloride	17. (B5)	7.4
18. (B6)	0.1 M HEPES pH 7.5	NaOH	18. (B6)	1.1 M Sodium chloride	18. (B6)	7.5
19. (B7)	0.1 M HEPES pH 7.5	NaOH	19. (B7)	1.9 M Sodium chloride	19. (B7)	7.6
20. (B8)	0.1 M HEPES pH 7.5	NaOH	20. (B8)	2.8 M Sodium chloride	20. (B8)	7.7
21. (B9)	0.1 M Tris pH 8.0	HCl	21. (B9)	0.2 M Sodium chloride	21. (B9)	8.0
22. (B10)	0.1 M Tris pH 8.0	HCl	22. (B10)	1.1 M Sodium chloride	22. (B10)	8.1
23. (B11)	0.1 M Tris pH 8.0	HCl	23. (B11)	1.9 M Sodium chloride	23. (B11)	8.1
24. (B12)	0.1 M Tris pH 8.0	HCl	24. (B12)	2.8 M Sodium chloride	24. (B12)	8.1
25. (C1)	0.1 M BIS-TRIS propane pH 9.0	HCl	25. (C1)	0.2 M Sodium chloride	25. (C1)	9.0
26. (C2)	0.1 M BIS-TRIS propane pH 9.0	HCl	26. (C2)	1.1 M Sodium chloride	26. (C2)	9.1
27. (C3)	0.1 M BIS-TRIS propane pH 9.0	HCl	27. (C3)	1.9 M Sodium chloride	27. (C3)	9.1
28. (C4)	0.1 M BIS-TRIS propane pH 9.0	HCl	28. (C4)	2.8 M Sodium chloride	28. (C4)	9.1
29. (C5)	0.1 M CHES pH 10.0	NaOH	29. (C5)	0.2 M Sodium chloride	29. (C5)	10.0
30. (C6)	0.1 M CHES pH 10.0	NaOH	30. (C6)	1.1 M Sodium chloride	30. (C6)	10.0
31. (C7)	0.1 M CHES pH 10.0	NaOH	31. (C7)	1.9 M Sodium chloride	31. (C7)	10.1
32. (C8)	0.1 M CHES pH 10.0	NaOH	32. (C8)	2.8 M Sodium chloride	32. (C8)	10.1
33. (C9)	0.1 M Tris pH 8.0	HCl	33. (C9)	0.2 M Sodium citrate tribasic dihydrate	33. (C9)	8.3
34. (C10)	0.1 M Tris pH 8.0	HCl	34. (C10)	0.6 M Sodium citrate tribasic dihydrate	34. (C10)	8.5
35. (C11)	0.1 M Tris pH 8.0	HCl	35. (C11)	1.0 M Sodium citrate tribasic dihydrate	35. (C11)	8.6
36. (C12)	0.1 M Tris pH 8.0	HCl	36. (C12)	1.4 M Sodium citrate tribasic dihydrate	36. (C12)	8.7
37. (D1)	0.1 M BIS-TRIS propane pH 9.0	HCl	37. (D1)	0.2 M Sodium citrate tribasic dihydrate	37. (D1)	9.2
38. (D2)	0.1 M BIS-TRIS propane pH 9.0	HCl	38. (D2)	0.6 M Sodium citrate tribasic dihydrate	38. (D2)	9.3
39. (D3)	0.1 M BIS-TRIS propane pH 9.0	HCl	39. (D3)	1.0 M Sodium citrate tribasic dihydrate	39. (D3)	9.4
40. (D4)	0.1 M BIS-TRIS propane pH 9.0	HCl	40. (D4)	1.4 M Sodium citrate tribasic dihydrate	40. (D4)	9.5
41. (D5)	0.1 M CHES pH 10.0	NaOH	41. (D5)	0.2 M Sodium citrate tribasic dihydrate	41. (D5)	10.0
42. (D6)	0.1 M CHES pH 10.0	NaOH	42. (D6)	0.6 M Sodium citrate tribasic dihydrate	42. (D6)	10.1
43. (D7)	0.1 M CHES pH 10.0	NaOH	43. (D7)	1.0 M Sodium citrate tribasic dihydrate	43. (D7)	10.2
44. (D8)	0.1 M CHES pH 10.0	NaOH	44. (D8)	1.4 M Sodium citrate tribasic dihydrate	44. (D8)	10.2
45. (D9)	0.1 M Tris pH 8.0	HCl	45. (D9)	0.2 M Sodium formate	45. (D9)	8.1
46. (D10)	0.1 M Tris pH 8.0	HCl	46. (D10)	1.5 M Sodium formate	46. (D10)	8.3
47. (D11)	0.1 M Tris pH 8.0	HCl	47. (D11)	2.7 M Sodium formate	47. (D11)	8.4
48. (D12)	0.1 M Tris pH 8.0	HCl	48. (D12)	4.0 M Sodium formate	48. (D12)	8.5

Reagents formulated in Type 1+ ultrapure grade water

[1] pH of 1.0 M buffer titrated with HCl or NaOH [2] pH after buffer dilution with Salt and water (25ºC)

[3] 0.1 M Sodium potassium phosphate pH 7.0 = 0.0324 M Sodium phosphate monobasic monohydrate, 0.0676 M Potassium phosphate dibasic. No pH adjustment.

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GRAS Screen™ 8

HR2-458 Reagent Formulation

Well #	Buffer[1]	Titrant	Well #	Salt	Well #	pH[2]
49. (E1)	0.1 M BIS-TRIS propane pH 9.0	HCl	49. (E1)	0.2 M Sodium formate	49. (E1)	9.0
50. (E2)	0.1 M BIS-TRIS propane pH 9.0	HCl	50. (E2)	1.5 M Sodium formate	50. (E2)	9.2
51. (E3)	0.1 M BIS-TRIS propane pH 9.0	HCl	51. (E3)	2.7 M Sodium formate	51. (E3)	9.2
52. (E4)	0.1 M BIS-TRIS propane pH 9.0	HCl	52. (E4)	4.0 M Sodium formate	52. (E4)	9.4
53. (E5)	0.1 M CHES pH 10.0	NaOH	53. (E5)	0.2 M Sodium formate	53. (E5)	10.0
54. (E6)	0.1 M CHES pH 10.0	NaOH	54. (E6)	1.5 M Sodium formate	54. (E6)	10.0
55. (E7)	0.1 M CHES pH 10.0	NaOH	55. (E7)	2.7 M Sodium formate	55. (E7)	10.1
56. (E8)	0.1 M CHES pH 10.0	NaOH	56. (E8)	4.0 M Sodium formate	56. (E8)	10.2
57. (E9)	0.1 M Tris pH 8.0	HCl	57. (E9)	0.1 M Sodium phosphate dibasic dihydrate	57. (E9)	8.2
58. (E10)	0.1 M Tris pH 8.0	HCl	58. (E10)	0.2 M Sodium phosphate dibasic dihydrate	58. (E10)	8.4
59. (E11)	0.1 M Tris pH 8.0	HCl	59. (E11)	0.4 M Sodium phosphate dibasic dihydrate	59. (E11)	8.6
60. (E12)	0.1 M Tris pH 8.0	HCl	60. (E12)	0.6 M Sodium phosphate dibasic dihydrate	60. (E12)	8.7
61. (F1)	0.1 M BIS-TRIS propane pH 9.0	HCl	61. (F1)	0.1 M Sodium phosphate dibasic dihydrate	61. (F1)	9.1
62. (F2)	0.1 M BIS-TRIS propane pH 9.0	HCl	62. (F2)	0.2 M Sodium phosphate dibasic dihydrate	62. (F2)	9.2
63. (F3)	0.1 M BIS-TRIS propane pH 9.0	HCl	63. (F3)	0.4 M Sodium phosphate dibasic dihydrate	63. (F3)	9.3
64. (F4)	0.1 M BIS-TRIS propane pH 9.0	HCl	64. (F4)	0.6 M Sodium phosphate dibasic dihydrate	64. (F4)	9.3
65. (F5)	0.1 M CHES pH 10.0	NaOH	65. (F5)	0.1 M Sodium phosphate dibasic dihydrate	65. (F5)	9.9
66. (F6)	0.1 M CHES pH 10.0	NaOH	66. (F6)	0.2 M Sodium phosphate dibasic dihydrate	66. (F6)	9.8
67. (F7)	0.1 M CHES pH 10.0	NaOH	67. (F7)	0.4 M Sodium phosphate dibasic dihydrate	67. (F7)	9.7
68. (F8)	0.1 M CHES pH 10.0	NaOH	68. (F8)	0.6 M Sodium phosphate dibasic dihydrate	68. (F8)	9.6
69. (F9)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	69. (F9)	0.2 M Sodium phosphate monobasic monohydrate	69. (F9)	4.5
70. (F10)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	70. (F10)	0.8 M Sodium phosphate monobasic monohydrate	70. (F10)	4.3
71. (F11)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	71. (F11)	1.4 M Sodium phosphate monobasic monohydrate	71. (F11)	4.1
72. (F12)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	72. (F12)	2.0 M Sodium phosphate monobasic monohydrate	72. (F12)	3.9
73. (G1)	0.1 M BIS-TRIS pH 6.5	HCl	73. (G1)	0.2 M Sodium tartrate dibasic dihydrate	73. (G1)	6.6
74. (G2)	0.1 M BIS-TRIS pH 6.5	HCl	74. (G2)	0.5 M Sodium tartrate dibasic dihydrate	74. (G2)	6.8
75. (G3)	0.1 M BIS-TRIS pH 6.5	HCl	75. (G3)	0.9 M Sodium tartrate dibasic dihydrate	75. (G3)	6.8
76. (G4)	0.1 M BIS-TRIS pH 6.5	HCl	76. (G4)	1.2 M Sodium tartrate dibasic dihydrate	76. (G4)	6.9
77. (G5)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	77. (G5)	0.2 M Sodium tartrate dibasic dihydrate	77. (G5)	6.9
78. (G6)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	78. (G6)	0.5 M Sodium tartrate dibasic dihydrate	78. (G6)	6.8
79. (G7)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	79. (G7)	0.9 M Sodium tartrate dibasic dihydrate	79. (G7)	6.7
80. (G8)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	80. (G8)	1.2 M Sodium tartrate dibasic dihydrate	80. (G8)	6.7
81. (G9)	0.1 M HEPES pH 7.5	NaOH	81. (G9)	0.2 M Sodium tartrate dibasic dihydrate	81. (G9)	7.4
82. (G10)	0.1 M HEPES pH 7.5	NaOH	82. (G10)	0.5 M Sodium tartrate dibasic dihydrate	82. (G10)	7.5
83. (G11)	0.1 M HEPES pH 7.5	NaOH	83. (G11)	0.9 M Sodium tartrate dibasic dihydrate	83. (G11)	7.6
84. (G12)	0.1 M HEPES pH 7.5	NaOH	84. (G12)	1.2 M Sodium tartrate dibasic dihydrate	84. (G12)	7.7
85. (H1)	0.1 M Tris pH 8.0	HCl	85. (H1)	0.2 M Sodium tartrate dibasic dihydrate	85. (H1)	8.2
86. (H2)	0.1 M Tris pH 8.0	HCl	86. (H2)	0.5 M Sodium tartrate dibasic dihydrate	86. (H2)	8.3
87. (H3)	0.1 M Tris pH 8.0	HCl	87. (H3)	0.9 M Sodium tartrate dibasic dihydrate	87. (H3)	8.4
88. (H4)	0.1 M Tris pH 8.0	HCl	88. (H4)	1.2 M Sodium tartrate dibasic dihydrate	88. (H4)	8.4
89. (H5)	0.1 M BIS-TRIS propane pH 9.0	HCl	89. (H5)	0.2 M Sodium tartrate dibasic dihydrate	89. (H5)	9.1
90. (H6)	0.1 M BIS-TRIS propane pH 9.0	HCl	90. (H6)	0.5 M Sodium tartrate dibasic dihydrate	90. (H6)	9.2
91. (H7)	0.1 M BIS-TRIS propane pH 9.0	HCl	91. (H7)	0.9 M Sodium tartrate dibasic dihydrate	91. (H7)	9.2
92. (H8)	0.1 M BIS-TRIS propane pH 9.0	HCl	92. (H8)	1.2 M Sodium tartrate dibasic dihydrate	92. (H8)	9.3
93. (H9)	0.1 M CHES pH 10.0	NaOH	93. (H9)	0.2 M Sodium tartrate dibasic dihydrate	93. (H9)	10.0
94. (H10)	0.1 M CHES pH 10.0	NaOH	94. (H10)	0.5 M Sodium tartrate dibasic dihydrate	94. (H10)	10.0
95. (H11)	0.1 M CHES pH 10.0	NaOH	95. (H11)	0.9 M Sodium tartrate dibasic dihydrate	95. (H11)	10.1
96. (H12)	0.1 M CHES pH 10.0	NaOH	96. (H12)	1.2 M Sodium tartrate dibasic dihydrate	96. (H12)	10.2

Reagents formulated in Type 1+ ultrapure grade water

[1] pH of 1.0 M buffer titrated with HCl or NaOH [2] pH after buffer dilution with Salt and water (25ºC)

[3] 0.1 M Sodium potassium phosphate pH 7.0 = 0.0324 M Sodium phosphate monobasic monohydrate, 0.0676 M Potassium phosphate dibasic. No pH adjustment.

34 Journey
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e-mail: tech@hrmail.com
Website: www.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)

GRAS Screen™ 8 - HR2-458 Scoring Sheet	Date:	Date:	Date:	Date:
1. (A1) 0.1 M Sodium acetate trihydrate pH 4.5, 0.2 M Sodium chloride				
2. (A2) 0.1 M Sodium acetate trihydrate pH 4.5, 1.1 M Sodium chloride				
3. (A3) 0.1 M Sodium acetate trihydrate pH 4.5, 1.9 M Sodium chloride				
4. (A4) 0.1 M Sodium acetate trihydrate pH 4.5, 2.8 M Sodium chloride				
5. (A5) 0.1 M Succinic acid pH 5.5, 0.2 M Sodium chloride				
6. (A6) 0.1 M Succinic acid pH 5.5, 1.1 M Sodium chloride				
7. (A7) 0.1 M Succinic acid pH 5.5, 1.9 M Sodium chloride				
8. (A8) 0.1 M Succinic acid pH 5.5, 2.8 M Sodium chloride				
9. (A9) 0.1 M BIS-TRIS pH 6.5, 0.2 M Sodium chloride				
10. (A10) 0.1 M BIS-TRIS pH 6.5, 1.1 M Sodium chloride				
11. (A11) 0.1 M BIS-TRIS pH 6.5, 1.9 M Sodium chloride				
12. (A12) 0.1 M BIS-TRIS pH 6.5, 2.8 M Sodium chloride				
13. (B1) 0.1 M Sodium potassium phosphate pH 7.0, 0.2 M Sodium chloride				
14. (B2) 0.1 M Sodium potassium phosphate pH 7.0, 1.1 M Sodium chloride				
15. (B3) 0.1 M Sodium potassium phosphate pH 7.0, 1.9 M Sodium chloride				
16. (B4) 0.1 M Sodium potassium phosphate pH 7.0, 2.8 M Sodium chloride				
17. (B5) 0.1 M HEPES pH 7.5, 0.2 M Sodium chloride				
18. (B6) 0.1 M HEPES pH 7.5, 1.1 M Sodium chloride				
19. (B7) 0.1 M HEPES pH 7.5, 1.9 M Sodium chloride				
20. (B8) 0.1 M HEPES pH 7.5, 2.8 M Sodium chloride				
21. (B9) 0.1 M Tris pH 8.0, 0.2 M Sodium chloride				
22. (B10) 0.1 M Tris pH 8.0, 1.1 M Sodium chloride				
23. (B11) 0.1 M Tris pH 8.0, 1.9 M Sodium chloride				
24. (B12) 0.1 M Tris pH 8.0, 2.8 M Sodium chloride				
25. (C1) 0.1 M BIS-TRIS propane pH 9.0, 0.2 M Sodium chloride				
26. (C2) 0.1 M BIS-TRIS propane pH 9.0, 1.1 M Sodium chloride				
27. (C3) 0.1 M BIS-TRIS propane pH 9.0, 1.9 M Sodium chloride				
28. (C4) 0.1 M BIS-TRIS propane pH 9.0, 2.8 M Sodium chloride				
29. (C5) 0.1 M CHES pH 10.0, 0.2 M Sodium chloride				
30. (C6) 0.1 M CHES pH 10.0, 1.1 M Sodium chloride				
31. (C7) 0.1 M CHES pH 10.0, 1.9 M Sodium chloride				
32. (C8) 0.1 M CHES pH 10.0, 2.8 M Sodium chloride				
33. (C9) 0.1 M Tris pH 8.0, 0.2 M Sodium citrate tribasic dihydrate				
34. (C10) 0.1 M Tris pH 8.0, 0.6 M Sodium citrate tribasic dihydrate				
35. (C11) 0.1 M Tris pH 8.0, 1.0 M Sodium citrate tribasic dihydrate				
36. (C12) 0.1 M Tris pH 8.0, 1.4 M Sodium citrate tribasic dihydrate				
37. (D1) 0.1 M BIS-TRIS propane pH 9.0, 0.2 M Sodium citrate tribasic dihydrate				
38. (D2) 0.1 M BIS-TRIS propane pH 9.0, 0.6 M Sodium citrate tribasic dihydrate				
39. (D3) 0.1 M BIS-TRIS propane pH 9.0, 1.0 M Sodium citrate tribasic dihydrate				
40. (D4) 0.1 M BIS-TRIS propane pH 9.0, 1.4 M Sodium citrate tribasic dihydrate				
41. (D5) 0.1 M CHES pH 10.0, 0.2 M Sodium citrate tribasic dihydrate				
42. (D6) 0.1 M CHES pH 10.0, 0.6 M Sodium citrate tribasic dihydrate				
43. (D7) 0.1 M CHES pH 10.0, 1.0 M Sodium citrate tribasic dihydrate				
44. (D8) 0.1 M CHES pH 10.0, 1.4 M Sodium citrate tribasic dihydrate				
45. (D9) 0.1 M Tris pH 8.0, 0.2 M Sodium formate				
46. (D10) 0.1 M Tris pH 8.0, 1.5 M Sodium formate				
47. (D11) 0.1 M Tris pH 8.0, 2.7 M Sodium formate				
48. (D12) 0.1 M Tris pH 8.0, 4.0 M Sodium formate				

HAMPTON RESEARCH
Solutions for Crystal Growth

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Sample: _____ Sample Concentration: _____

Sample Buffer: _____ Date: _____

Reservoir Volume: _____ Temperature: _____

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

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

GRAS Screen™ 8 - HR2-458 Scoring Sheet	Date:	Date:	Date:	Date:
49. (E1) 0.1 M BIS-TRIS propane pH 9.0, 0.2 M Sodium formate				
50. (E2) 0.1 M BIS-TRIS propane pH 9.0, 1.5 M Sodium formate				
51. (E3) 0.1 M BIS-TRIS propane pH 9.0, 2.7 M Sodium formate				
52. (E4) 0.1 M BIS-TRIS propane pH 9.0, 4.0 M Sodium formate				
53. (E5) 0.1 M CHES pH 10.0, 0.2 M Sodium formate				
54. (E6) 0.1 M CHES pH 10.0, 1.5 M Sodium formate				
55. (E7) 0.1 M CHES pH 10.0, 2.7 M Sodium formate				
56. (E8) 0.1 M CHES pH 10.0, 4.0 M Sodium formate				
57. (E9) 0.1 M Tris pH 8.0, 0.1 M Sodium phosphate dibasic dihydrate				
58. (E10) 0.1 M Tris pH 8.0, 0.2 M Sodium phosphate dibasic dihydrate				
59. (E11) 0.1 M Tris pH 8.0, 0.4 M Sodium phosphate dibasic dihydrate				
60. (E12) 0.1 M Tris pH 8.0, 0.6 M Sodium phosphate dibasic dihydrate				
61. (F1) 0.1 M BIS-TRIS propane pH 9.0, 0.1 M Sodium phosphate dibasic dihydrate				
62. (F2) 0.1 M BIS-TRIS propane pH 9.0, 0.2 M Sodium phosphate dibasic dihydrate				
63. (F3) 0.1 M BIS-TRIS propane pH 9.0, 0.4 M Sodium phosphate dibasic dihydrate				
64. (F4) 0.1 M BIS-TRIS propane pH 9.0, 0.6 M Sodium phosphate dibasic dihydrate				
65. (F5) 0.1 M CHES pH 10.0, 0.1 M Sodium phosphate dibasic dihydrate				
66. (F6) 0.1 M CHES pH 10.0, 0.2 M Sodium phosphate dibasic dihydrate				
67. (F7) 0.1 M CHES pH 10.0, 0.4 M Sodium phosphate dibasic dihydrate				
68. (F8) 0.1 M CHES pH 10.0, 0.6 M Sodium phosphate dibasic dihydrate				
69. (F9) 0.1 M Sodium acetate trihydrate pH 4.5, 0.2 M Sodium phosphate monobasic monohydrate				
70. (F10) 0.1 M Sodium acetate trihydrate pH 4.5, 0.8 M Sodium phosphate monobasic monohydrate				
71. (F11) 0.1 M Sodium acetate trihydrate pH 4.5, 1.4 M Sodium phosphate monobasic monohydrate				
72. (F12) 0.1 M Sodium acetate trihydrate pH 4.5, 2.0 M Sodium phosphate monobasic monohydrate				
73. (G1) 0.1 M BIS-TRIS pH 6.5, 0.2 M Sodium tartrate dibasic dihydrate				
74. (G2) 0.1 M BIS-TRIS pH 6.5, 0.5 M Sodium tartrate dibasic dihydrate				
75. (G3) 0.1 M BIS-TRIS pH 6.5, 0.9 M Sodium tartrate dibasic dihydrate				
76. (G4) 0.1 M BIS-TRIS pH 6.5, 1.2 M Sodium tartrate dibasic dihydrate				
77. (G5) 0.1 M Sodium potassium phosphate pH 7.0, 0.2 M Sodium tartrate dibasic dihydrate				
78. (G6) 0.1 M Sodium potassium phosphate pH 7.0, 0.5 M Sodium tartrate dibasic dihydrate				
79. (G7) 0.1 M Sodium potassium phosphate pH 7.0, 0.9 M Sodium tartrate dibasic dihydrate				
80. (G8) 0.1 M Sodium potassium phosphate pH 7.0, 1.2 M Sodium tartrate dibasic dihydrate				
81. (G9) 0.1 M HEPES pH 7.5, 0.2 M Sodium tartrate dibasic dihydrate				
82. (G10) 0.1 M HEPES pH 7.5, 0.5 M Sodium tartrate dibasic dihydrate				
83. (G11) 0.1 M HEPES pH 7.5, 0.9 M Sodium tartrate dibasic dihydrate				
84. (G12) 0.1 M HEPES pH 7.5, 1.2 M Sodium tartrate dibasic dihydrate				
85. (H1) 0.1 M Tris pH 8.0, 0.2 M Sodium tartrate dibasic dihydrate				
86. (H2) 0.1 M Tris pH 8.0, 0.5 M Sodium tartrate dibasic dihydrate				
87. (H3) 0.1 M Tris pH 8.0, 0.9 M Sodium tartrate dibasic dihydrate				
88. (H4) 0.1 M Tris pH 8.0, 1.2 M Sodium tartrate dibasic dihydrate				
89. (H5) 0.1 M BIS-TRIS propane pH 9.0, 0.2 M Sodium tartrate dibasic dihydrate				
90. (H6) 0.1 M BIS-TRIS propane pH 9.0, 0.5 M Sodium tartrate dibasic dihydrate				
91. (H7) 0.1 M BIS-TRIS propane pH 9.0, 0.9 M Sodium tartrate dibasic dihydrate				
92. (H8) 0.1 M BIS-TRIS propane pH 9.0, 1.2 M Sodium tartrate dibasic dihydrate				
93. (H9) 0.1 M CHES pH 10.0, 0.2 M Sodium tartrate dibasic dihydrate				
94. (H10) 0.1 M CHES pH 10.0, 0.5 M Sodium tartrate dibasic dihydrate				
95. (H11) 0.1 M CHES pH 10.0, 0.9 M Sodium tartrate dibasic dihydrate				
96. (H12) 0.1 M CHES pH 10.0, 1.2 M Sodium tartrate dibasic dihydrate				

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