GRAS 7 SCREEN

HAMPTON RESEARCH
Solutions for Crystal Growth

User Guide

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
- Ammonium — acetate, chloride, citrate, formate, phosphate, sulfate, tartrate, Potassium phosphate, & Sodium acetate
- 8 buffer controls without salt or PEG
- Vapor diffusion, microbatch, free interface diffusion

Refer to the enclosed GRAS Screen 7 Reagent Formulation for more information.

General Description
GRAS Screen™ 7 was developed by Hampton Research for the crystallization of proteins, including monoclonal antibodies. Each of the chemicals in GRAS Screen 7 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 7 samples 9 salts (Ammonium — acetate, chloride, citrate, formate, phosphate, sulfate, tartrate, Potassium phosphate, & Sodium acetate) at 4 concentrations, sampling 8 unique buffers (pH 4.5 to 10.0) as well as 8 buffer controls without salt. GRAS Screen 7 is supplied in a 96 Deep Well block format and is compatible with robotic and multi-channel pipet liquid handling systems. GRAS Screen 7 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 7 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.

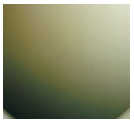
2. Using clean pipet tips, pipet the desired volume of crystallization reagent (typically 0.05 to 2 microliters) from the crystallization plate reservoir to the sitting drop well. Some 96 well crystallization plates allow this procedure to be performed using a multichannel pipet where other plates require the use of a single channel pipet. Change the pipet tip between reagents.

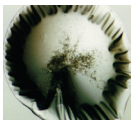
3. Using a clean pipet tip, pipet the same volume (typically 0.05 to 2 microliters) of sample to the reagent drop in the sitting drop well. Work carefully but quickly to minimize evaporation from the crystallization plate.

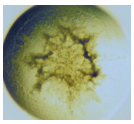
4. Seal the crystallization plate using an optically clear sealing film or tape. Seal the remaining reagent in the Deep Well block using AlumaSeal II sealing film.

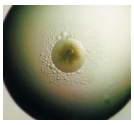
Examine the Drop
Carefully examine the drops under a stereo microscope (10 to 100x magnification) after setting the screen. Record all observations and be particularly careful to scan the focal plane for small crystals. Observe the drops once each day for the first week, then once a week there after 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

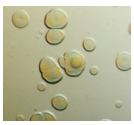
Figure 1
Typical observations in a crystallization experiment

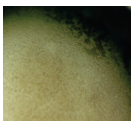

Clear Drop


Skin / Precipitate

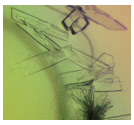

Precipitate


Precipitate / Phase

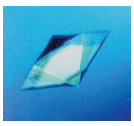

Quasi Crystals


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 7

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 7 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 7 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 7 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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HAMPTON RESEARCH
Solutions for Crystal Growth

GRAS Screen™ 7

HR2-457 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)	none	1. (A1)	4.6
2. (A2)	0.1 M Succinic acid pH 5.5	NaOH	2. (A2)	none	2. (A2)	5.5
3. (A3)	0.1 M BIS-TRIS pH 6.5	HCl	3. (A3)	none	3. (A3)	6.4
4. (A4)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	4. (A4)	none	4. (A4)	7.1
5. (A5)	0.1 M HEPES pH 7.5	NaOH	5. (A5)	none	5. (A5)	7.3
6. (A6)	0.1 M Tris pH 8.0	HCl	6. (A6)	none	6. (A6)	7.9
7. (A7)	0.1 M BIS-TRIS propane pH 9.0	HCl	7. (A7)	none	7. (A7)	8.8
8. (A8)	0.1 M CHES pH 10.0	NaOH	8. (A8)	none	8. (A8)	10.0
9. (A9)	0.1 M Tris pH 8.0	HCl	9. (A9)	0.2 M Ammonium acetate	9. (A9)	7.9
10. (A10)	0.1 M Tris pH 8.0	HCl	10. (A10)	1.5 M Ammonium acetate	10. (A10)	7.8
11. (A11)	0.1 M Tris pH 8.0	HCl	11. (A11)	2.7 M Ammonium acetate	11. (A11)	7.7
12. (A12)	0.1 M Tris pH 8.0	HCl	12. (A12)	4.0 M Ammonium acetate	12. (A12)	7.7
13. (B1)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	13. (B1)	0.2 M Ammonium chloride	13. (B1)	4.6
14. (B2)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	14. (B2)	1.3 M Ammonium chloride	14. (B2)	4.4
15. (B3)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	15. (B3)	2.4 M Ammonium chloride	15. (B3)	4.4
16. (B4)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	16. (B4)	3.5 M Ammonium chloride	16. (B4)	4.4
17. (B5)	0.1 M Succinic acid pH 5.5	NaOH	17. (B5)	0.2 M Ammonium chloride	17. (B5)	5.5
18. (B6)	0.1 M Succinic acid pH 5.5	NaOH	18. (B6)	1.3 M Ammonium chloride	18. (B6)	5.3
19. (B7)	0.1 M Succinic acid pH 5.5	NaOH	19. (B7)	2.4 M Ammonium chloride	19. (B7)	5.2
20. (B8)	0.1 M Succinic acid pH 5.5	NaOH	20. (B8)	3.5 M Ammonium chloride	20. (B8)	5.2
21. (B9)	0.1 M BIS-TRIS pH 6.5	HCl	21. (B9)	0.2 M Ammonium chloride	21. (B9)	6.5
22. (B10)	0.1 M BIS-TRIS pH 6.5	HCl	22. (B10)	1.3 M Ammonium chloride	22. (B10)	6.7
23. (B11)	0.1 M BIS-TRIS pH 6.5	HCl	23. (B11)	2.4 M Ammonium chloride	23. (B11)	6.7
24. (B12)	0.1 M BIS-TRIS pH 6.5	HCl	24. (B12)	3.5 M Ammonium chloride	24. (B12)	6.7
25. (C1)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	25. (C1)	0.2 M Ammonium citrate dibasic	25. (C1)	4.8
26. (C2)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	26. (C2)	0.7 M Ammonium citrate dibasic	26. (C2)	4.8
27. (C3)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	27. (C3)	1.3 M Ammonium citrate dibasic	27. (C3)	4.8
28. (C4)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	28. (C4)	1.8 M Ammonium citrate dibasic	28. (C4)	4.8
29. (C5)	0.1 M HEPES pH 7.5	NaOH	29. (C5)	0.2 M Ammonium citrate tribasic	29. (C5)	7.4
30. (C6)	0.1 M HEPES pH 7.5	NaOH	30. (C6)	0.8 M Ammonium citrate tribasic	30. (C6)	7.5
31. (C7)	0.1 M HEPES pH 7.5	NaOH	31. (C7)	1.4 M Ammonium citrate tribasic	31. (C7)	7.7
32. (C8)	0.1 M HEPES pH 7.5	NaOH	32. (C8)	2.0 M Ammonium citrate tribasic	32. (C8)	7.8
33. (C9)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	33. (C9)	0.2 M Ammonium formate	33. (C9)	7.0
34. (C10)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	34. (C10)	1.1 M Ammonium formate	34. (C10)	6.7
35. (C11)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	35. (C11)	2.1 M Ammonium formate	35. (C11)	6.6
36. (C12)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	36. (C12)	3.0 M Ammonium formate	36. (C12)	6.5
37. (D1)	0.1 M Tris pH 8.0	HCl	37. (D1)	0.2 M Ammonium phosphate dibasic	37. (D1)	8.1
38. (D2)	0.1 M Tris pH 8.0	HCl	38. (D2)	1.0 M Ammonium phosphate dibasic	38. (D2)	8.2
39. (D3)	0.1 M Tris pH 8.0	HCl	39. (D3)	1.7 M Ammonium phosphate dibasic	39. (D3)	8.2
40. (D4)	0.1 M Tris pH 8.0	HCl	40. (D4)	2.5 M Ammonium phosphate dibasic	40. (D4)	8.2
41. (D5)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	41. (D5)	0.2 M Ammonium phosphate monobasic	41. (D5)	4.6
42. (D6)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	42. (D6)	0.8 M Ammonium phosphate monobasic	42. (D6)	4.3
43. (D7)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	43. (D7)	1.4 M Ammonium phosphate monobasic	43. (D7)	4.1
44. (D8)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	44. (D8)	2.0 M Ammonium phosphate monobasic	44. (D8)	4.0
45. (D9)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	45. (D9)	0.2 M Ammonium sulfate	45. (D9)	4.5
46. (D10)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	46. (D10)	0.8 M Ammonium sulfate	46. (D10)	4.5
47. (D11)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	47. (D11)	1.4 M Ammonium sulfate	47. (D11)	4.5
48. (D12)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	48. (D12)	2.0 M Ammonium sulfate	48. (D12)	4.6

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™ 7

HR2-457 Reagent Formulation

Well #	Buffer[1]	Titrant	Well #	Salt	Well #	pH[2]
49. (E1)	0.1 M Succinic acid pH 5.5	NaOH	49. (E1)	0.2 M Ammonium sulfate	49. (E1)	5.5
50. (E2)	0.1 M Succinic acid pH 5.5	NaOH	50. (E2)	0.8 M Ammonium sulfate	50. (E2)	5.4
51. (E3)	0.1 M Succinic acid pH 5.5	NaOH	51. (E3)	1.4 M Ammonium sulfate	51. (E3)	5.3
52. (E4)	0.1 M Succinic acid pH 5.5	NaOH	52. (E4)	2.0 M Ammonium sulfate	52. (E4)	5.3
53. (E5)	0.1 M BIS-TRIS pH 6.5	HCl	53. (E5)	0.2 M Ammonium sulfate	53. (E5)	6.7
54. (E6)	0.1 M BIS-TRIS pH 6.5	HCl	54. (E6)	0.8 M Ammonium sulfate	54. (E6)	6.9
55. (E7)	0.1 M BIS-TRIS pH 6.5	HCl	55. (E7)	1.4 M Ammonium sulfate	55. (E7)	7.0
56. (E8)	0.1 M BIS-TRIS pH 6.5	HCl	56. (E8)	2.0 M Ammonium sulfate	56. (E8)	7.1
57. (E9)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	57. (E9)	0.2 M Ammonium sulfate	57. (E9)	6.8
58. (E10)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	58. (E10)	0.8 M Ammonium sulfate	58. (E10)	6.5
59. (E11)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	59. (E11)	1.4 M Ammonium sulfate	59. (E11)	6.4
60. (E12)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	60. (E12)	2.0 M Ammonium sulfate	60. (E12)	6.2
61. (F1)	0.1 M HEPES pH 7.5	NaOH	61. (F1)	0.2 M Ammonium sulfate	61. (F1)	7.4
62. (F2)	0.1 M HEPES pH 7.5	NaOH	62. (F2)	0.8 M Ammonium sulfate	62. (F2)	7.5
63. (F3)	0.1 M HEPES pH 7.5	NaOH	63. (F3)	1.4 M Ammonium sulfate	63. (F3)	7.5
64. (F4)	0.1 M HEPES pH 7.5	NaOH	64. (F4)	2.0 M Ammonium sulfate	64. (F4)	7.6
65. (F5)	0.1 M Succinic acid pH 5.5	NaOH	65. (F5)	0.2 M Ammonium tartrate dibasic	65. (F5)	5.6
66. (F6)	0.1 M Succinic acid pH 5.5	NaOH	66. (F6)	0.6 M Ammonium tartrate dibasic	66. (F6)	5.6
67. (F7)	0.1 M Succinic acid pH 5.5	NaOH	67. (F7)	1.1 M Ammonium tartrate dibasic	67. (F7)	5.8
68. (F8)	0.1 M Succinic acid pH 5.5	NaOH	68. (F8)	1.5 M Ammonium tartrate dibasic	68. (F8)	5.9
69. (F9)	0.1 M BIS-TRIS pH 6.5	HCl	69. (F9)	0.2 M Ammonium tartrate dibasic	69. (F9)	6.7
70. (F10)	0.1 M BIS-TRIS pH 6.5	HCl	70. (F10)	0.6 M Ammonium tartrate dibasic	70. (F10)	6.8
71. (F11)	0.1 M BIS-TRIS pH 6.5	HCl	71. (F11)	1.1 M Ammonium tartrate dibasic	71. (F11)	6.9
72. (F12)	0.1 M BIS-TRIS pH 6.5	HCl	72. (F12)	1.5 M Ammonium tartrate dibasic	72. (F12)	7.0
73. (G1)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	73. (G1)	0.2 M Ammonium tartrate dibasic	73. (G1)	6.9
74. (G2)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	74. (G2)	0.6 M Ammonium tartrate dibasic	74. (G2)	6.7
75. (G3)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	75. (G3)	1.1 M Ammonium tartrate dibasic	75. (G3)	6.6
76. (G4)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	76. (G4)	1.5 M Ammonium tartrate dibasic	76. (G4)	6.5
77. (G5)	0.1 M HEPES pH 7.5	NaOH	77. (G5)	0.2 M Ammonium tartrate dibasic	77. (G5)	7.4
78. (G6)	0.1 M HEPES pH 7.5	NaOH	78. (G6)	0.6 M Ammonium tartrate dibasic	78. (G6)	7.5
79. (G7)	0.1 M HEPES pH 7.5	NaOH	79. (G7)	1.1 M Ammonium tartrate dibasic	79. (G7)	7.5
80. (G8)	0.1 M HEPES pH 7.5	NaOH	80. (G8)	1.5 M Ammonium tartrate dibasic	80. (G8)	7.6
81. (G9)	0.1 M CHES pH 10.0	NaOH	81. (G9)	0.2 M Potassium phosphate dibasic	81. (G9)	10.0
82. (G10)	0.1 M CHES pH 10.0	NaOH	82. (G10)	0.7 M Potassium phosphate dibasic	82. (G10)	9.8
83. (G11)	0.1 M CHES pH 10.0	NaOH	83. (G11)	1.3 M Potassium phosphate dibasic	83. (G11)	9.8
84. (G12)	0.1 M CHES pH 10.0	NaOH	84. (G12)	1.8 M Potassium phosphate dibasic	84. (G12)	9.8
85. (H1)	0.1 M Tris pH 8.0	HCl	85. (H1)	0.2 M Sodium acetate trihydrate	85. (H1)	8.1
86. (H2)	0.1 M Tris pH 8.0	HCl	86. (H2)	0.7 M Sodium acetate trihydrate	86. (H2)	8.2
87. (H3)	0.1 M Tris pH 8.0	HCl	87. (H3)	1.3 M Sodium acetate trihydrate	87. (H3)	8.3
88. (H4)	0.1 M Tris pH 8.0	HCl	88. (H4)	1.8 M Sodium acetate trihydrate	88. (H4)	8.4
89. (H5)	0.1 M BIS-TRIS propane pH 9.0	HCl	89. (H5)	0.2 M Sodium acetate trihydrate	89. (H5)	9.0
90. (H6)	0.1 M BIS-TRIS propane pH 9.0	HCl	90. (H6)	0.7 M Sodium acetate trihydrate	90. (H6)	9.1
91. (H7)	0.1 M BIS-TRIS propane pH 9.0	HCl	91. (H7)	1.3 M Sodium acetate trihydrate	91. (H7)	9.2
92. (H8)	0.1 M BIS-TRIS propane pH 9.0	HCl	92. (H8)	1.8 M Sodium acetate trihydrate	92. (H8)	9.3
93. (H9)	0.1 M CHES pH 10.0	NaOH	93. (H9)	0.2 M Sodium acetate trihydrate	93. (H9)	10.0
94. (H10)	0.1 M CHES pH 10.0	NaOH	94. (H10)	0.7 M Sodium acetate trihydrate	94. (H10)	10.1
95. (H11)	0.1 M CHES pH 10.0	NaOH	95. (H11)	1.3 M Sodium acetate trihydrate	95. (H11)	10.1
96. (H12)	0.1 M CHES pH 10.0	NaOH	96. (H12)	1.8 M Sodium acetate trihydrate	96. (H12)	10.1

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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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™ 7 - HR2-457 Scoring Sheet	Date:	Date:	Date:	Date:
1. (A1) 0.1 M Sodium acetate trihydrate pH 4.5				
2. (A2) 0.1 M Succinic acid pH 5.5				
3. (A3) 0.1 M BIS-TRIS pH 6.5				
4. (A4) 0.1 M Sodium potassium phosphate pH 7.0				
5. (A5) 0.1 M HEPES pH 7.5				
6. (A6) 0.1 M Tris pH 8.0				
7. (A7) 0.1 M BIS-TRIS propane pH 9.0				
8. (A8) 0.1 M CHES pH 10.0				
9. (A9) 0.1 M Tris pH 8.0, 0.2 M Ammonium acetate				
10. (A10) 0.1 M Tris pH 8.0, 1.5 M Ammonium acetate				
11. (A11) 0.1 M Tris pH 8.0, 2.7 M Ammonium acetate				
12. (A12) 0.1 M Tris pH 8.0, 4.0 M Ammonium acetate				
13. (B1) 0.1 M Sodium acetate trihydrate pH 4.5, 0.2 M Ammonium chloride				
14. (B2) 0.1 M Sodium acetate trihydrate pH 4.5, 1.3 M Ammonium chloride				
15. (B3) 0.1 M Sodium acetate trihydrate pH 4.5, 2.4 M Ammonium chloride				
16. (B4) 0.1 M Sodium acetate trihydrate pH 4.5, 3.5 M Ammonium chloride				
17. (B5) 0.1 M Succinic acid pH 5.5, 0.2 M Ammonium chloride				
18. (B6) 0.1 M Succinic acid pH 5.5, 1.3 M Ammonium chloride				
19. (B7) 0.1 M Succinic acid pH 5.5, 2.4 M Ammonium chloride				
20. (B8) 0.1 M Succinic acid pH 5.5, 3.5 M Ammonium chloride				
21. (B9) 0.1 M BIS-TRIS pH 6.5, 0.2 M Ammonium chloride				
22. (B10) 0.1 M BIS-TRIS pH 6.5, 1.3 M Ammonium chloride				
23. (B11) 0.1 M BIS-TRIS pH 6.5, 2.4 M Ammonium chloride				
24. (B12) 0.1 M BIS-TRIS pH 6.5, 3.5 M Ammonium chloride				
25. (C1) 0.1 M Sodium acetate trihydrate pH 4.5, 0.2 M Ammonium citrate dibasic				
26. (C2) 0.1 M Sodium acetate trihydrate pH 4.5, 0.7 M Ammonium citrate dibasic				
27. (C3) 0.1 M Sodium acetate trihydrate pH 4.5, 1.3 M Ammonium citrate dibasic				
28. (C4) 0.1 M Sodium acetate trihydrate pH 4.5, 1.8 M Ammonium citrate dibasic				
29. (C5) 0.1 M HEPES pH 7.5, 0.2 M Ammonium citrate tribasic				
30. (C6) 0.1 M HEPES pH 7.5, 0.8 M Ammonium citrate tribasic				
31. (C7) 0.1 M HEPES pH 7.5, 1.4 M Ammonium citrate tribasic				
32. (C8) 0.1 M HEPES pH 7.5, 2.0 M Ammonium citrate tribasic				
33. (C9) 0.1 M Sodium potassium phosphate pH 7.0, 0.2 M Ammonium formate				
34. (C10) 0.1 M Sodium potassium phosphate pH 7.0, 1.1 M Ammonium formate				
35. (C11) 0.1 M Sodium potassium phosphate pH 7.0, 2.1 M Ammonium formate				
36. (C12) 0.1 M Sodium potassium phosphate pH 7.0, 3.0 M Ammonium formate				
37. (D1) 0.1 M Tris pH 8.0, 0.2 M Ammonium phosphate dibasic				
38. (D2) 0.1 M Tris pH 8.0, 1.0 M Ammonium phosphate dibasic				
39. (D3) 0.1 M Tris pH 8.0, 1.7 M Ammonium phosphate dibasic				
40. (D4) 0.1 M Tris pH 8.0, 2.5 M Ammonium phosphate dibasic				
41. (D5) 0.1 M Sodium acetate trihydrate pH 4.5, 0.2 M Ammonium phosphate monobasic				
42. (D6) 0.1 M Sodium acetate trihydrate pH 4.5, 0.8 M Ammonium phosphate monobasic				
43. (D7) 0.1 M Sodium acetate trihydrate pH 4.5, 1.4 M Ammonium phosphate monobasic				
44. (D8) 0.1 M Sodium acetate trihydrate pH 4.5, 2.0 M Ammonium phosphate monobasic				
45. (D9) 0.1 M Sodium acetate trihydrate pH 4.5, 0.2 M Ammonium sulfate				
46. (D10) 0.1 M Sodium acetate trihydrate pH 4.5, 0.8 M Ammonium sulfate				
47. (D11) 0.1 M Sodium acetate trihydrate pH 4.5, 1.4 M Ammonium sulfate				
48. (D12) 0.1 M Sodium acetate trihydrate pH 4.5, 2.0 M Ammonium sulfate				

HAMPTON RESEARCH
Solutions for Crystal Growth

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e-mail: tech@hrmail.com
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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™ 7 - HR2-457 Scoring Sheet	Date:	Date:	Date:	Date:
49. (E1) 0.1 M Succinic acid pH 5.5, 0.2 M Ammonium sulfate				
50. (E2) 0.1 M Succinic acid pH 5.5, 0.8 M Ammonium sulfate				
51. (E3) 0.1 M Succinic acid pH 5.5, 1.4 M Ammonium sulfate				
52. (E4) 0.1 M Succinic acid pH 5.5, 2.0 M Ammonium sulfate				
53. (E5) 0.1 M BIS-TRIS pH 6.5, 0.2 M Ammonium sulfate				
54. (E6) 0.1 M BIS-TRIS pH 6.5, 0.8 M Ammonium sulfate				
55. (E7) 0.1 M BIS-TRIS pH 6.5, 1.4 M Ammonium sulfate				
56. (E8) 0.1 M BIS-TRIS pH 6.5, 2.0 M Ammonium sulfate				
57. (E9) 0.1 M Sodium potassium phosphate pH 7.0, 0.2 M Ammonium sulfate				
58. (E10) 0.1 M Sodium potassium phosphate pH 7.0, 0.8 M Ammonium sulfate				
59. (E11) 0.1 M Sodium potassium phosphate pH 7.0, 1.4 M Ammonium sulfate				
60. (E12) 0.1 M Sodium potassium phosphate pH 7.0, 2.0 M Ammonium sulfate				
61. (F1) 0.1 M HEPES pH 7.5, 0.2 M Ammonium sulfate				
62. (F2) 0.1 M HEPES pH 7.5, 0.8 M Ammonium sulfate				
63. (F3) 0.1 M HEPES pH 7.5, 1.4 M Ammonium sulfate				
64. (F4) 0.1 M HEPES pH 7.5, 2.0 M Ammonium sulfate				
65. (F5) 0.1 M Succinic acid pH 5.5, 0.2 M Ammonium tartrate dibasic				
66. (F6) 0.1 M Succinic acid pH 5.5, 0.6 M Ammonium tartrate dibasic				
67. (F7) 0.1 M Succinic acid pH 5.5, 1.1 M Ammonium tartrate dibasic				
68. (F8) 0.1 M Succinic acid pH 5.5, 1.5 M Ammonium tartrate dibasic				
69. (F9) 0.1 M BIS-TRIS pH 6.5, 0.2 M Ammonium tartrate dibasic				
70. (F10) 0.1 M BIS-TRIS pH 6.5, 0.6 M Ammonium tartrate dibasic				
71. (F11) 0.1 M BIS-TRIS pH 6.5, 1.1 M Ammonium tartrate dibasic				
72. (F12) 0.1 M BIS-TRIS pH 6.5, 1.5 M Ammonium tartrate dibasic				
73. (G1) 0.1 M Sodium potassium phosphate pH 7.0, 0.2 M Ammonium tartrate dibasic				
74. (G2) 0.1 M Sodium potassium phosphate pH 7.0, 0.6 M Ammonium tartrate dibasic				
75. (G3) 0.1 M Sodium potassium phosphate pH 7.0, 1.1 M Ammonium tartrate dibasic				
76. (G4) 0.1 M Sodium potassium phosphate pH 7.0, 1.5 M Ammonium tartrate dibasic				
77. (G5) 0.1 M HEPES pH 7.5, 0.2 M Ammonium tartrate dibasic				
78. (G6) 0.1 M HEPES pH 7.5, 0.6 M Ammonium tartrate dibasic				
79. (G7) 0.1 M HEPES pH 7.5, 1.1 M Ammonium tartrate dibasic				
80. (G8) 0.1 M HEPES pH 7.5, 1.5 M Ammonium tartrate dibasic				
81. (G9) 0.1 M CHES pH 10.0, 0.2 M Potassium phosphate dibasic				
82. (G10) 0.1 M CHES pH 10.0, 0.7 M Potassium phosphate dibasic				
83. (G11) 0.1 M CHES pH 10.0, 1.3 M Potassium phosphate dibasic				
84. (G12) 0.1 M CHES pH 10.0, 1.8 M Potassium phosphate dibasic				
85. (H1) 0.1 M Tris pH 8.0, 0.2 M Sodium acetate trihydrate				
86. (H2) 0.1 M Tris pH 8.0, 0.7 M Sodium acetate trihydrate				
87. (H3) 0.1 M Tris pH 8.0, 1.3 M Sodium acetate trihydrate				
88. (H4) 0.1 M Tris pH 8.0, 1.8 M Sodium acetate trihydrate				
89. (H5) 0.1 M BIS-TRIS propane pH 9.0, 0.2 M Sodium acetate trihydrate				
90. (H6) 0.1 M BIS-TRIS propane pH 9.0, 0.7 M Sodium acetate trihydrate				
91. (H7) 0.1 M BIS-TRIS propane pH 9.0, 1.3 M Sodium acetate trihydrate				
92. (H8) 0.1 M BIS-TRIS propane pH 9.0, 1.8 M Sodium acetate trihydrate				
93. (H9) 0.1 M CHES pH 10.0, 0.2 M Sodium acetate trihydrate				
94. (H10) 0.1 M CHES pH 10.0, 0.7 M Sodium acetate trihydrate				
95. (H11) 0.1 M CHES pH 10.0, 1.3 M Sodium acetate trihydrate				
96. (H12) 0.1 M CHES pH 10.0, 1.8 M Sodium acetate trihydrate				

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