GRAS 3 SCREEN

HAMPTON RESEARCH
Solutions for Crystal Growth

User Guide **HR2-453 (pg 1)**

Applications

GRAS reagent crystallization screen for proteins, including monoclonal antibodies, where salt is the primary, and low molecular weight Polyethylene glycol the secondary reagent, sampling pH 4-9.

Features

- Generally Recognized As Safe reagent formulation
- Samples pH 4.0 - 9.0 without an added buffer
- 24 unique salts - primary reagent
- Polyethylene glycol 300, 400, MME 550, & 600 - secondary reagent
- Vapor diffusion, microbatch, free interface diffusion

Refer to the enclosed GRAS Screen 3 Reagent Formulation for more information.

General Description

GRAS Screen™ 3 was developed by Hampton Research for the crystallization of proteins, including monoclonal antibodies. Each of the chemicals in GRAS Screen 3 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 3 samples twenty four salts, encompassing pH 4-9 versus four low molecular weight Polyethylene glycols (300, 400, MME 550, & 600). GRAS Screen 3 is supplied in a 96 Deep Well block format and is compatible with robotic and multi-channel pipet liquid handling systems. GRAS Screen 3 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 3 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.

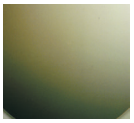
3. Using a clean pipet tip, pipet the same volume (typically 0.05 to 2 microliters) of sample to the reagent drop in the sitting drop well. Work carefully but quickly to minimize evaporation from the crystallization plate.

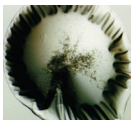
4. Seal the crystallization plate using an optically clear sealing film or tape. Seal the remaining reagent in the Deep Well block using AlumaSeal II sealing film.

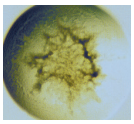
Examine the Drop

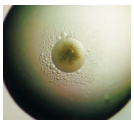
Carefully examine the drops under a stereo microscope (10 to 100x magnification) after setting the screen. Record all observations and be particularly careful to scan the focal plane for small crystals. Observe the drops once each day for the first week, then once a week there after for up to 60 days, 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

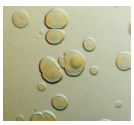
Figure 1
Typical observations in a crystallization experiment

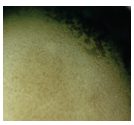

Clear Drop


Skin / Precipitate

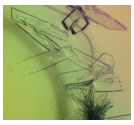

Precipitate


Precipitate / Phase

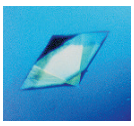

Quasi Crystals


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 3

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 and/or PEG 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 3 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 3 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 3 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™ polyethylene glycols and salts.

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™ 3

HR2-453 Reagent Formulation

Well #	Salt	Well #	PEG	Well #	pH ◊
1. (A1)	3.0 M Ammonium acetate	1. (A1)	2% v/v Polyethylene glycol 300	1. (A1)	7.3
2. (A2)	3.0 M Ammonium acetate	2. (A2)	2% v/v Polyethylene glycol 400	2. (A2)	7.3
3. (A3)	3.0 M Ammonium acetate	3. (A3)	2% v/v Polyethylene glycol monomethyl ether 550	3. (A3)	7.3
4. (A4)	3.0 M Ammonium acetate	4. (A4)	2% v/v Polyethylene glycol 600	4. (A4)	7.3
5. (A5)	3.0 M Ammonium chloride	5. (A5)	2% v/v Polyethylene glycol 300	5. (A5)	4.7
6. (A6)	3.0 M Ammonium chloride	6. (A6)	2% v/v Polyethylene glycol 400	6. (A6)	4.8
7. (A7)	3.0 M Ammonium chloride	7. (A7)	2% v/v Polyethylene glycol monomethyl ether 550	7. (A7)	4.1
8. (A8)	3.0 M Ammonium chloride	8. (A8)	2% v/v Polyethylene glycol 600	8. (A8)	4.5
9. (A9)	1.5 M Ammonium citrate dibasic	9. (A9)	2% v/v Polyethylene glycol 300	9. (A9)	4.8
10. (A10)	1.5 M Ammonium citrate dibasic	10. (A10)	2% v/v Polyethylene glycol 400	10. (A10)	4.8
11. (A11)	1.5 M Ammonium citrate dibasic	11. (A11)	2% v/v Polyethylene glycol monomethyl ether 550	11. (A11)	4.8
12. (A12)	1.5 M Ammonium citrate dibasic	12. (A12)	2% v/v Polyethylene glycol 600	12. (A12)	4.8
13. (B1)	1.5 M Ammonium citrate tribasic	13. (B1)	2% v/v Polyethylene glycol 300	13. (B1)	7.5
14. (B2)	1.5 M Ammonium citrate tribasic	14. (B2)	2% v/v Polyethylene glycol 400	14. (B2)	7.4
15. (B3)	1.5 M Ammonium citrate tribasic	15. (B3)	2% v/v Polyethylene glycol monomethyl ether 550	15. (B3)	7.4
16. (B4)	1.5 M Ammonium citrate tribasic	16. (B4)	2% v/v Polyethylene glycol 600	16. (B4)	7.4
17. (B5)	6.0 M Ammonium formate	17. (B5)	2% v/v Polyethylene glycol 300	17. (B5)	7.0
18. (B6)	6.0 M Ammonium formate	18. (B6)	2% v/v Polyethylene glycol 400	18. (B6)	7.0
19. (B7)	6.0 M Ammonium formate	19. (B7)	2% v/v Polyethylene glycol monomethyl ether 550	19. (B7)	7.0
20. (B8)	6.0 M Ammonium formate	20. (B8)	2% v/v Polyethylene glycol 600	20. (B8)	7.0
21. (B9)	1.7 M Ammonium phosphate dibasic	21. (B9)	2% v/v Polyethylene glycol 300	21. (B9)	8.0
22. (B10)	1.7 M Ammonium phosphate dibasic	22. (B10)	2% v/v Polyethylene glycol 400	22. (B10)	8.0
23. (B11)	1.7 M Ammonium phosphate dibasic	23. (B11)	2% v/v Polyethylene glycol monomethyl ether 550	23. (B11)	8.0
24. (B12)	1.7 M Ammonium phosphate dibasic	24. (B12)	2% v/v Polyethylene glycol 600	24. (B12)	8.0
25. (C1)	1.5 M Ammonium phosphate monobasic	25. (C1)	2% v/v Polyethylene glycol 300	25. (C1)	4.0
26. (C2)	1.5 M Ammonium phosphate monobasic	26. (C2)	2% v/v Polyethylene glycol 400	26. (C2)	4.0
27. (C3)	1.5 M Ammonium phosphate monobasic	27. (C3)	2% v/v Polyethylene glycol monomethyl ether 550	27. (C3)	4.0
28. (C4)	1.5 M Ammonium phosphate monobasic	28. (C4)	2% v/v Polyethylene glycol 600	28. (C4)	4.0
29. (C5)	1.5 M Ammonium sulfate	29. (C5)	2% v/v Polyethylene glycol 300	29. (C5)	5.1
30. (C6)	1.5 M Ammonium sulfate	30. (C6)	2% v/v Polyethylene glycol 400	30. (C6)	5.2
31. (C7)	1.5 M Ammonium sulfate	31. (C7)	2% v/v Polyethylene glycol monomethyl ether 550	31. (C7)	5.0
32. (C8)	1.5 M Ammonium sulfate	32. (C8)	2% v/v Polyethylene glycol 600	32. (C8)	5.1
33. (C9)	1.2 M Ammonium tartrate dibasic	33. (C9)	2% v/v Polyethylene glycol 300	33. (C9)	6.8
34. (C10)	1.2 M Ammonium tartrate dibasic	34. (C10)	2% v/v Polyethylene glycol 400	34. (C10)	6.8
35. (C11)	1.2 M Ammonium tartrate dibasic	35. (C11)	2% v/v Polyethylene glycol monomethyl ether 550	35. (C11)	6.7
36. (C12)	1.2 M Ammonium tartrate dibasic	36. (C12)	2% v/v Polyethylene glycol 600	36. (C12)	6.8
37. (D1)	1.8 M DL-Malic acid pH 7.0	37. (D1)	2% v/v Polyethylene glycol 300	37. (D1)	6.8
38. (D2)	1.8 M DL-Malic acid pH 7.0	38. (D2)	2% v/v Polyethylene glycol 400	38. (D2)	6.8
39. (D3)	1.8 M DL-Malic acid pH 7.0	39. (D3)	2% v/v Polyethylene glycol monomethyl ether 550	39. (D3)	6.8
40. (D4)	1.8 M DL-Malic acid pH 7.0	40. (D4)	2% v/v Polyethylene glycol 600	40. (D4)	6.9
41. (D5)	1.3 M Potassium phosphate dibasic	41. (D5)	2% v/v Polyethylene glycol 300	41. (D5)	9.3
42. (D6)	1.3 M Potassium phosphate dibasic	42. (D6)	2% v/v Polyethylene glycol 400	42. (D6)	9.3
43. (D7)	1.3 M Potassium phosphate dibasic	43. (D7)	2% v/v Polyethylene glycol monomethyl ether 550	43. (D7)	9.3
44. (D8)	1.3 M Potassium phosphate dibasic	44. (D8)	2% v/v Polyethylene glycol 600	44. (D8)	9.3
45. (D9)	0.9 M Potassium sodium tartrate tetrahydrate	45. (D9)	2% v/v Polyethylene glycol 300	45. (D9)	7.6
46. (D10)	0.9 M Potassium sodium tartrate tetrahydrate	46. (D10)	2% v/v Polyethylene glycol 400	46. (D10)	7.8
47. (D11)	0.9 M Potassium sodium tartrate tetrahydrate	47. (D11)	2% v/v Polyethylene glycol monomethyl ether 550	47. (D11)	7.3
48. (D12)	0.9 M Potassium sodium tartrate tetrahydrate	48. (D12)	2% v/v Polyethylene glycol 600	48. (D12)	8.0

Reagents formulated in Type 1+ ultrapure grade water

◊ Measured pH at 25°C, no pH adjustment made to reagent

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GRAS Screen™ 3

HAMPTON RESEARCH
Solutions for Crystal Growth

HR2-453 Reagent Formulation

Well #	Salt	Well #	PEG	Well #	pH ◊
49. (E1)	1.8 M Sodium acetate trihydrate	49. (E1)	2% v/v Polyethylene glycol 300	49. (E1)	8.7
50. (E2)	1.8 M Sodium acetate trihydrate	50. (E2)	2% v/v Polyethylene glycol 400	50. (E2)	8.9
51. (E3)	1.8 M Sodium acetate trihydrate	51. (E3)	2% v/v Polyethylene glycol monomethyl ether 550	51. (E3)	8.4
52. (E4)	1.8 M Sodium acetate trihydrate	52. (E4)	2% v/v Polyethylene glycol 600	52. (E4)	9.0
53. (E5)	3.0 M Sodium chloride	53. (E5)	2% v/v Polyethylene glycol 300	53. (E5)	5.2
54. (E6)	3.0 M Sodium chloride	54. (E6)	2% v/v Polyethylene glycol 400	54. (E6)	5.6
55. (E7)	3.0 M Sodium chloride	55. (E7)	2% v/v Polyethylene glycol monomethyl ether 550	55. (E7)	4.4
56. (E8)	3.0 M Sodium chloride	56. (E8)	2% v/v Polyethylene glycol 600	56. (E8)	5.4
57. (E9)	1.0 M Sodium citrate tribasic dihydrate	57. (E9)	2% v/v Polyethylene glycol 300	57. (E9)	8.2
58. (E10)	1.0 M Sodium citrate tribasic dihydrate	58. (E10)	2% v/v Polyethylene glycol 400	58. (E10)	8.2
59. (E11)	1.0 M Sodium citrate tribasic dihydrate	59. (E11)	2% v/v Polyethylene glycol monomethyl ether 550	59. (E11)	8.1
60. (E12)	1.0 M Sodium citrate tribasic dihydrate	60. (E12)	2% v/v Polyethylene glycol 600	60. (E12)	8.3
61. (F1)	3.0 M Sodium formate	61. (F1)	2% v/v Polyethylene glycol 300	61. (F1)	7.7
62. (F2)	3.0 M Sodium formate	62. (F2)	2% v/v Polyethylene glycol 400	62. (F2)	7.8
63. (F3)	3.0 M Sodium formate	63. (F3)	2% v/v Polyethylene glycol monomethyl ether 550	63. (F3)	7.5
64. (F4)	3.0 M Sodium formate	64. (F4)	2% v/v Polyethylene glycol 600	64. (F4)	7.9
65. (F5)	1.8 M Sodium malonate pH 7.0	65. (F5)	2% v/v Polyethylene glycol 300	65. (F5)	7.0
66. (F6)	1.8 M Sodium malonate pH 7.0	66. (F6)	2% v/v Polyethylene glycol 400	66. (F6)	7.0
67. (F7)	1.8 M Sodium malonate pH 7.0	67. (F7)	2% v/v Polyethylene glycol monomethyl ether 550	67. (F7)	7.0
68. (F8)	1.8 M Sodium malonate pH 7.0	68. (F8)	2% v/v Polyethylene glycol 600	68. (F8)	7.0
69. (F9)	0.6 M Sodium phosphate dibasic dihydrate	69. (F9)	2% v/v Polyethylene glycol 300	69. (F9)	8.9
70. (F10)	0.6 M Sodium phosphate dibasic dihydrate	70. (F10)	2% v/v Polyethylene glycol 400	70. (F10)	8.9
71. (F11)	0.6 M Sodium phosphate dibasic dihydrate	71. (F11)	2% v/v Polyethylene glycol monomethyl ether 550	71. (F11)	8.9
72. (F12)	0.6 M Sodium phosphate dibasic dihydrate	72. (F12)	2% v/v Polyethylene glycol 600	72. (F12)	8.9
73. (G1)	3.0 M Sodium phosphate monobasic monohydrate	73. (G1)	2% v/v Polyethylene glycol 300	73. (G1)	3.7
74. (G2)	3.0 M Sodium phosphate monobasic monohydrate	74. (G2)	2% v/v Polyethylene glycol 400	74. (G2)	3.7
75. (G3)	3.0 M Sodium phosphate monobasic monohydrate	75. (G3)	2% v/v Polyethylene glycol monomethyl ether 550	75. (G3)	3.6
76. (G4)	3.0 M Sodium phosphate monobasic monohydrate	76. (G4)	2% v/v Polyethylene glycol 600	76. (G4)	3.7
77. (G5)	0.6 M Sodium sulfate decahydrate	77. (G5)	2% v/v Polyethylene glycol 300	77. (G5)	5.4
78. (G6)	0.6 M Sodium sulfate decahydrate	78. (G6)	2% v/v Polyethylene glycol 400	78. (G6)	5.7
79. (G7)	0.6 M Sodium sulfate decahydrate	79. (G7)	2% v/v Polyethylene glycol monomethyl ether 550	79. (G7)	5.3
80. (G8)	0.6 M Sodium sulfate decahydrate	80. (G8)	2% v/v Polyethylene glycol 600	80. (G8)	5.7
81. (G9)	0.9 M Sodium tartrate dibasic dihydrate	81. (G9)	2% v/v Polyethylene glycol 300	81. (G9)	7.5
82. (G10)	0.9 M Sodium tartrate dibasic dihydrate	82. (G10)	2% v/v Polyethylene glycol 400	82. (G10)	7.7
83. (G11)	0.9 M Sodium tartrate dibasic dihydrate	83. (G11)	2% v/v Polyethylene glycol monomethyl ether 550	83. (G11)	7.3
84. (G12)	0.9 M Sodium tartrate dibasic dihydrate	84. (G12)	2% v/v Polyethylene glycol 600	84. (G12)	8.0
85. (H1)	4.8 M Sodium thiocyanate	85. (H1)	2% v/v Polyethylene glycol 300	85. (H1)	6.8
86. (H2)	4.8 M Sodium thiocyanate	86. (H2)	2% v/v Polyethylene glycol 400	86. (H2)	7.0
87. (H3)	4.8 M Sodium thiocyanate	87. (H3)	2% v/v Polyethylene glycol monomethyl ether 550	87. (H3)	6.7
88. (H4)	4.8 M Sodium thiocyanate	88. (H4)	2% v/v Polyethylene glycol 600	88. (H4)	7.9
89. (H5)	0.7 M Succinic acid pH 7.0	89. (H5)	2% v/v Polyethylene glycol 300	89. (H5)	7.0
90. (H6)	0.7 M Succinic acid pH 7.0	90. (H6)	2% v/v Polyethylene glycol 400	90. (H6)	7.0
91. (H7)	0.7 M Succinic acid pH 7.0	91. (H7)	2% v/v Polyethylene glycol monomethyl ether 550	91. (H7)	7.0
92. (H8)	0.7 M Succinic acid pH 7.0	92. (H8)	2% v/v Polyethylene glycol 600	92. (H8)	7.0
93. (H9)	60% v/v Tacsimate™ pH 7.0	93. (H9)	2% v/v Polyethylene glycol 300	93. (H9)	7.0
94. (H10)	60% v/v Tacsimate™ pH 7.0	94. (H10)	2% v/v Polyethylene glycol 400	94. (H10)	7.0
95. (H11)	60% v/v Tacsimate™ pH 7.0	95. (H11)	2% v/v Polyethylene glycol monomethyl ether 550	95. (H11)	7.0
96. (H12)	60% v/v Tacsimate™ pH 7.0	96. (H12)	2% v/v Polyethylene glycol 600	96. (H12)	7.0

Reagents formulated in Type 1+ ultrapure grade water

◊ Measured pH at 25°C, no pH adjustment made to reagent

34 Journey
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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™ 3 - HR2-453 Scoring Sheet	Date:	Date:	Date:	Date:
1. (A1) 3.0 M Ammonium acetate, 2% v/v Polyethylene glycol 300				
2. (A2) 3.0 M Ammonium acetate, 2% v/v Polyethylene glycol 400				
3. (A3) 3.0 M Ammonium acetate, 2% v/v Polyethylene glycol monomethyl ether 550				
4. (A4) 3.0 M Ammonium acetate, 2% v/v Polyethylene glycol 600				
5. (A5) 3.0 M Ammonium chloride, 2% v/v Polyethylene glycol 300				
6. (A6) 3.0 M Ammonium chloride, 2% v/v Polyethylene glycol 400				
7. (A7) 3.0 M Ammonium chloride, 2% v/v Polyethylene glycol monomethyl ether 550				
8. (A8) 3.0 M Ammonium chloride, 2% v/v Polyethylene glycol 600				
9. (A9) 1.5 M Ammonium citrate dibasic, 2% v/v Polyethylene glycol 300				
10. (A10) 1.5 M Ammonium citrate dibasic, 2% v/v Polyethylene glycol 400				
11. (A11) 1.5 M Ammonium citrate dibasic, 2% v/v Polyethylene glycol monomethyl ether 550				
12. (A12) 1.5 M Ammonium citrate dibasic, 2% v/v Polyethylene glycol 600				
13. (B1) 1.5 M Ammonium citrate tribasic, 2% v/v Polyethylene glycol 300				
14. (B2) 1.5 M Ammonium citrate tribasic, 2% v/v Polyethylene glycol 400				
15. (B3) 1.5 M Ammonium citrate tribasic, 2% v/v Polyethylene glycol monomethyl ether 550				
16. (B4) 1.5 M Ammonium citrate tribasic, 2% v/v Polyethylene glycol 600				
17. (B5) 6.0 M Ammonium formate, 2% v/v Polyethylene glycol 300				
18. (B6) 6.0 M Ammonium formate, 2% v/v Polyethylene glycol 400				
19. (B7) 6.0 M Ammonium formate, 2% v/v Polyethylene glycol monomethyl ether 550				
20. (B8) 6.0 M Ammonium formate, 2% v/v Polyethylene glycol 600				
21. (B9) 1.7 M Ammonium phosphate dibasic, 2% v/v Polyethylene glycol 300				
22. (B10) 1.7 M Ammonium phosphate dibasic, 2% v/v Polyethylene glycol 400				
23. (B11) 1.7 M Ammonium phosphate dibasic, 2% v/v Polyethylene glycol monomethyl ether 550				
24. (B12) 1.7 M Ammonium phosphate dibasic, 2% v/v Polyethylene glycol 600				
25. (C1) 1.5 M Ammonium phosphate monobasic, 2% v/v Polyethylene glycol 300				
26. (C2) 1.5 M Ammonium phosphate monobasic, 2% v/v Polyethylene glycol 400				
27. (C3) 1.5 M Ammonium phosphate monobasic, 2% v/v Polyethylene glycol monomethyl ether 550				
28. (C4) 1.5 M Ammonium phosphate monobasic, 2% v/v Polyethylene glycol 600				
29. (C5) 1.5 M Ammonium sulfate, 2% v/v Polyethylene glycol 300				
30. (C6) 1.5 M Ammonium sulfate, 2% v/v Polyethylene glycol 400				
31. (C7) 1.5 M Ammonium sulfate, 2% v/v Polyethylene glycol monomethyl ether 550				
32. (C8) 1.5 M Ammonium sulfate, 2% v/v Polyethylene glycol 600				
33. (C9) 1.2 M Ammonium tartrate dibasic, 2% v/v Polyethylene glycol 300				
34. (C10) 1.2 M Ammonium tartrate dibasic, 2% v/v Polyethylene glycol 400				
35. (C11) 1.2 M Ammonium tartrate dibasic, 2% v/v Polyethylene glycol monomethyl ether 550				
36. (C12) 1.2 M Ammonium tartrate dibasic, 2% v/v Polyethylene glycol 600				
37. (D1) 1.8 M DL-Malic acid pH 7.0, 2% v/v Polyethylene glycol 300				
38. (D2) 1.8 M DL-Malic acid pH 7.0, 2% v/v Polyethylene glycol 400				
39. (D3) 1.8 M DL-Malic acid pH 7.0, 2% v/v Polyethylene glycol monomethyl ether 550				
40. (D4) 1.8 M DL-Malic acid pH 7.0, 2% v/v Polyethylene glycol 600				
41. (D5) 1.3 M Potassium phosphate dibasic, 2% v/v Polyethylene glycol 300				
42. (D6) 1.3 M Potassium phosphate dibasic, 2% v/v Polyethylene glycol 400				
43. (D7) 1.3 M Potassium phosphate dibasic, 2% v/v Polyethylene glycol monomethyl ether 550				
44. (D8) 1.3 M Potassium phosphate dibasic, 2% v/v Polyethylene glycol 600				
45. (D9) 0.9 M Potassium sodium tartrate tetrahydrate, 2% v/v Polyethylene glycol 300				
46. (D10) 0.9 M Potassium sodium tartrate tetrahydrate, 2% v/v Polyethylene glycol 400				
47. (D11) 0.9 M Potassium sodium tartrate tetrahydrate, 2% v/v Polyethylene glycol monomethyl ether 550				
48. (D12) 0.9 M Potassium sodium tartrate tetrahydrate, 2% v/v Polyethylene glycol 600				

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™ 3 - HR2-453 Scoring Sheet	Date:	Date:	Date:	Date:
49. (E1) 1.8 M Sodium acetate trihydrate, 2% v/v Polyethylene glycol 300				
50. (E2) 1.8 M Sodium acetate trihydrate, 2% v/v Polyethylene glycol 400				
51. (E3) 1.8 M Sodium acetate trihydrate, 2% v/v Polyethylene glycol monomethyl ether 550				
52. (E4) 1.8 M Sodium acetate trihydrate, 2% v/v Polyethylene glycol 600				
53. (E5) 3.0 M Sodium chloride, 2% v/v Polyethylene glycol 300				
54. (E6) 3.0 M Sodium chloride, 2% v/v Polyethylene glycol 400				
55. (E7) 3.0 M Sodium chloride, 2% v/v Polyethylene glycol monomethyl ether 550				
56. (E8) 3.0 M Sodium chloride, 2% v/v Polyethylene glycol 600				
57. (E9) 1.0 M Sodium citrate tribasic dihydrate, 2% v/v Polyethylene glycol 300				
58. (E10) 1.0 M Sodium citrate tribasic dihydrate, 2% v/v Polyethylene glycol 400				
59. (E11) 1.0 M Sodium citrate tribasic dihydrate, 2% v/v Polyethylene glycol monomethyl ether 550				
60. (E12) 1.0 M Sodium citrate tribasic dihydrate, 2% v/v Polyethylene glycol 600				
61. (F1) 3.0 M Sodium formate, 2% v/v Polyethylene glycol 300				
62. (F2) 3.0 M Sodium formate, 2% v/v Polyethylene glycol 400				
63. (F3) 3.0 M Sodium formate, 2% v/v Polyethylene glycol monomethyl ether 550				
64. (F4) 3.0 M Sodium formate, 2% v/v Polyethylene glycol 600				
65. (F5) 1.8 M Sodium malonate pH 7.0, 2% v/v Polyethylene glycol 300				
66. (F6) 1.8 M Sodium malonate pH 7.0, 2% v/v Polyethylene glycol 400				
67. (F7) 1.8 M Sodium malonate pH 7.0, 2% v/v Polyethylene glycol monomethyl ether 550				
68. (F8) 1.8 M Sodium malonate pH 7.0, 2% v/v Polyethylene glycol 600				
69. (F9) 0.6 M Sodium phosphate dibasic dihydrate, 2% v/v Polyethylene glycol 300				
70. (F10) 0.6 M Sodium phosphate dibasic dihydrate, 2% v/v Polyethylene glycol 400				
71. (F11) 0.6 M Sodium phosphate dibasic dihydrate, 2% v/v Polyethylene glycol monomethyl ether 550				
72. (F12) 0.6 M Sodium phosphate dibasic dihydrate, 2% v/v Polyethylene glycol 600				
73. (G1) 3.0 M Sodium phosphate monobasic monohydrate, 2% v/v Polyethylene glycol 300				
74. (G2) 3.0 M Sodium phosphate monobasic monohydrate, 2% v/v Polyethylene glycol 400				
75. (G3) 3.0 M Sodium phosphate monobasic monohydrate, 2% v/v Polyethylene glycol monomethyl ether 550				
76. (G4) 3.0 M Sodium phosphate monobasic monohydrate, 2% v/v Polyethylene glycol 600				
77. (G5) 0.6 M Sodium sulfate decahydrate, 2% v/v Polyethylene glycol 300				
78. (G6) 0.6 M Sodium sulfate decahydrate, 2% v/v Polyethylene glycol 400				
79. (G7) 0.6 M Sodium sulfate decahydrate, 2% v/v Polyethylene glycol monomethyl ether 550				
80. (G8) 0.6 M Sodium sulfate decahydrate, 2% v/v Polyethylene glycol 600				
81. (G9) 0.9 M Sodium tartrate dibasic dihydrate, 2% v/v Polyethylene glycol 300				
82. (G10) 0.9 M Sodium tartrate dibasic dihydrate, 2% v/v Polyethylene glycol 400				
83. (G11) 0.9 M Sodium tartrate dibasic dihydrate, 2% v/v Polyethylene glycol monomethyl ether 550				
84. (G12) 0.9 M Sodium tartrate dibasic dihydrate, 2% v/v Polyethylene glycol 600				
85. (H1) 4.8 M Sodium thiocyanate, 2% v/v Polyethylene glycol 300				
86. (H2) 4.8 M Sodium thiocyanate, 2% v/v Polyethylene glycol 400				
87. (H3) 4.8 M Sodium thiocyanate, 2% v/v Polyethylene glycol monomethyl ether 550				
88. (H4) 4.8 M Sodium thiocyanate, 2% v/v Polyethylene glycol 600				
89. (H5) 0.7 M Succinic acid pH 7.0, 2% v/v Polyethylene glycol 300				
90. (H6) 0.7 M Succinic acid pH 7.0, 2% v/v Polyethylene glycol 400				
91. (H7) 0.7 M Succinic acid pH 7.0, 2% v/v Polyethylene glycol monomethyl ether 550				
92. (H8) 0.7 M Succinic acid pH 7.0, 2% v/v Polyethylene glycol 600				
93. (H9) 60% v/v Tacsimate™ pH 7.0, 2% v/v Polyethylene glycol 300				
94. (H10) 60% v/v Tacsimate™ pH 7.0, 2% v/v Polyethylene glycol 400				
95. (H11) 60% v/v Tacsimate™ pH 7.0, 2% v/v Polyethylene glycol monomethyl ether 550				
96. (H12) 60% v/v Tacsimate™ pH 7.0, 2% v/v Polyethylene glycol 600				

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