GRAS 5 SCREEN

HAMPTON RESEARCH
Solutions for Crystal Growth

Applications

GRAS reagent crystallization screen for proteins, including monoclonal antibodies, where low molecular weight Polyethylene glycol 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
- Polyethylene glycol 300, 400, MME 550, & 600
- Vapor diffusion, microbatch, free interface diffusion

Refer to the enclosed GRAS Screen 5 Reagent Formulation for more information.

General Description

GRAS Screen™ 5 was developed by Hampton Research for the crystallization of proteins, including monoclonal antibodies. Each of the chemicals in GRAS Screen 5 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 5 samples four low molecular weight Polyethylene glycols (300, 400, MME 550, and 600) at three concentrations versus eight unique buffers encompassing pH 4.5 to 10. GRAS Screen 5 is supplied in a 96 Deep Well block format and is compatible with robotic and multi-channel pipet liquid handling systems. GRAS Screen 5 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 5 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.

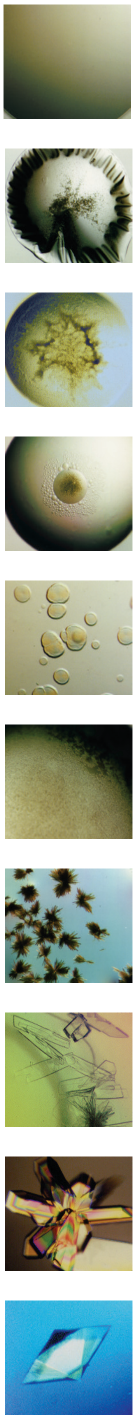
3. Using a clean pipet tip, pipet the same volume (typically 0.05 to 2 microliters) of sample to the reagent drop in the sitting drop well. Work carefully but quickly to minimize evaporation from the crystallization plate.

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 5

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 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 5 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 5 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 5 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 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™ 5

HR2-455 Reagent Formulation

Well #	Buffer[1]	Titrant	Well #	PEG	Well #	pH[2]
1. (A1)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	1. (A1)	8% v/v Polyethylene glycol 300	1. (A1)	4.7
2. (A2)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	2. (A2)	26% v/v Polyethylene glycol 300	2. (A2)	5.1
3. (A3)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	3. (A3)	44% v/v Polyethylene glycol 300	3. (A3)	5.8
4. (A4)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	4. (A4)	8% v/v Polyethylene glycol 400	4. (A4)	4.7
5. (A5)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	5. (A5)	26% v/v Polyethylene glycol 400	5. (A5)	5.2
6. (A6)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	6. (A6)	44% v/v Polyethylene glycol 400	6. (A6)	5.8
7. (A7)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	7. (A7)	8% v/v Polyethylene glycol monomethyl ether 550	7. (A7)	4.7
8. (A8)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	8. (A8)	26% v/v Polyethylene glycol monomethyl ether 550	8. (A8)	5.2
9. (A9)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	9. (A9)	44% v/v Polyethylene glycol monomethyl ether 550	9. (A9)	5.9
10. (A10)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	10. (A10)	8% v/v Polyethylene glycol 600	10. (A10)	4.7
11. (A11)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	11. (A11)	26% v/v Polyethylene glycol 600	11. (A11)	5.2
12. (A12)	0.1 M Sodium acetate trihydrate pH 4.5	HCl	12. (A12)	44% v/v Polyethylene glycol 600	12. (A12)	5.8
13. (B1)	0.1 M Succinic acid pH 5.5	NaOH	13. (B1)	8% v/v Polyethylene glycol 300	13. (B1)	5.7
14. (B2)	0.1 M Succinic acid pH 5.5	NaOH	14. (B2)	26% v/v Polyethylene glycol 300	14. (B2)	6.2
15. (B3)	0.1 M Succinic acid pH 5.5	NaOH	15. (B3)	44% v/v Polyethylene glycol 300	15. (B3)	6.8
16. (B4)	0.1 M Succinic acid pH 5.5	NaOH	16. (B4)	8% v/v Polyethylene glycol 400	16. (B4)	5.7
17. (B5)	0.1 M Succinic acid pH 5.5	NaOH	17. (B5)	26% v/v Polyethylene glycol 400	17. (B5)	6.2
18. (B6)	0.1 M Succinic acid pH 5.5	NaOH	18. (B6)	44% v/v Polyethylene glycol 400	18. (B6)	6.9
19. (B7)	0.1 M Succinic acid pH 5.5	NaOH	19. (B7)	8% v/v Polyethylene glycol monomethyl ether 550	19. (B7)	5.7
20. (B8)	0.1 M Succinic acid pH 5.5	NaOH	20. (B8)	26% v/v Polyethylene glycol monomethyl ether 550	20. (B8)	6.2
21. (B9)	0.1 M Succinic acid pH 5.5	NaOH	21. (B9)	44% v/v Polyethylene glycol monomethyl ether 550	21. (B9)	6.9
22. (B10)	0.1 M Succinic acid pH 5.5	NaOH	22. (B10)	8% v/v Polyethylene glycol 600	22. (B10)	5.7
23. (B11)	0.1 M Succinic acid pH 5.5	NaOH	23. (B11)	26% v/v Polyethylene glycol 600	23. (B11)	6.2
24. (B12)	0.1 M Succinic acid pH 5.5	NaOH	24. (B12)	44% v/v Polyethylene glycol 600	24. (B12)	6.9
25. (C1)	0.1 M BIS-TRIS pH 6.5	HCl	25. (C1)	8% v/v Polyethylene glycol 300	25. (C1)	6.4
26. (C2)	0.1 M BIS-TRIS pH 6.5	HCl	26. (C2)	26% v/v Polyethylene glycol 300	26. (C2)	6.4
27. (C3)	0.1 M BIS-TRIS pH 6.5	HCl	27. (C3)	44% v/v Polyethylene glycol 300	27. (C3)	6.5
28. (C4)	0.1 M BIS-TRIS pH 6.5	HCl	28. (C4)	8% v/v Polyethylene glycol 400	28. (C4)	6.4
29. (C5)	0.1 M BIS-TRIS pH 6.5	HCl	29. (C5)	26% v/v Polyethylene glycol 400	29. (C5)	6.4
30. (C6)	0.1 M BIS-TRIS pH 6.5	HCl	30. (C6)	44% v/v Polyethylene glycol 400	30. (C6)	6.5
31. (C7)	0.1 M BIS-TRIS pH 6.5	HCl	31. (C7)	8% v/v Polyethylene glycol monomethyl ether 550	31. (C7)	6.4
32. (C8)	0.1 M BIS-TRIS pH 6.5	HCl	32. (C8)	26% v/v Polyethylene glycol monomethyl ether 550	32. (C8)	6.4
33. (C9)	0.1 M BIS-TRIS pH 6.5	HCl	33. (C9)	44% v/v Polyethylene glycol monomethyl ether 550	33. (C9)	6.5
34. (C10)	0.1 M BIS-TRIS pH 6.5	HCl	34. (C10)	8% v/v Polyethylene glycol 600	34. (C10)	6.4
35. (C11)	0.1 M BIS-TRIS pH 6.5	HCl	35. (C11)	26% v/v Polyethylene glycol 600	35. (C11)	6.5
36. (C12)	0.1 M BIS-TRIS pH 6.5	HCl	36. (C12)	44% v/v Polyethylene glycol 600	36. (C12)	6.5
37. (D1)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	37. (D1)	8% v/v Polyethylene glycol 300	37. (D1)	7.3
38. (D2)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	38. (D2)	26% v/v Polyethylene glycol 300	38. (D2)	7.7
39. (D3)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	39. (D3)	44% v/v Polyethylene glycol 300	39. (D3)	8.2
40. (D4)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	40. (D4)	8% v/v Polyethylene glycol 400	40. (D4)	7.3
41. (D5)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	41. (D5)	26% v/v Polyethylene glycol 400	41. (D5)	7.7
42. (D6)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	42. (D6)	44% v/v Polyethylene glycol 400	42. (D6)	8.3
43. (D7)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	43. (D7)	8% v/v Polyethylene glycol monomethyl ether 550	43. (D7)	7.2
44. (D8)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	44. (D8)	26% v/v Polyethylene glycol monomethyl ether 550	44. (D8)	7.7
45. (D9)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	45. (D9)	44% v/v Polyethylene glycol monomethyl ether 550	45. (D9)	8.1
46. (D10)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	46. (D10)	8% v/v Polyethylene glycol 600	46. (D10)	7.3
47. (D11)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	47. (D11)	26% v/v Polyethylene glycol 600	47. (D11)	7.7
48. (D12)	0.1 M Sodium potassium phosphate pH 7.0[3]	None	48. (D12)	44% v/v Polyethylene glycol 600	48. (D12)	8.3

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 PEG and water (25°C)

[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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Well #	Buffer[1]	Titrant	Well #	PEG	Well #	pH[2]
49. (E1)	0.1 M HEPES pH 7.5	NaOH	49. (E1)	8% v/v Polyethylene glycol 300	49. (E1)	7.4
50. (E2)	0.1 M HEPES pH 7.5	NaOH	50. (E2)	26% v/v Polyethylene glycol 300	50. (E2)	7.4
51. (E3)	0.1 M HEPES pH 7.5	NaOH	51. (E3)	44% v/v Polyethylene glycol 300	51. (E3)	7.6
52. (E4)	0.1 M HEPES pH 7.5	NaOH	52. (E4)	8% v/v Polyethylene glycol 400	52. (E4)	7.4
53. (E5)	0.1 M HEPES pH 7.5	NaOH	53. (E5)	26% v/v Polyethylene glycol 400	53. (E5)	7.4
54. (E6)	0.1 M HEPES pH 7.5	NaOH	54. (E6)	44% v/v Polyethylene glycol 400	54. (E6)	7.6
55. (E7)	0.1 M HEPES pH 7.5	NaOH	55. (E7)	8% v/v Polyethylene glycol monomethyl ether 550	55. (E7)	7.3
56. (E8)	0.1 M HEPES pH 7.5	NaOH	56. (E8)	26% v/v Polyethylene glycol monomethyl ether 550	56. (E8)	7.4
57. (E9)	0.1 M HEPES pH 7.5	NaOH	57. (E9)	44% v/v Polyethylene glycol monomethyl ether 550	57. (E9)	7.6
58. (E10)	0.1 M HEPES pH 7.5	NaOH	58. (E10)	8% v/v Polyethylene glycol 600	58. (E10)	7.4
59. (E11)	0.1 M HEPES pH 7.5	NaOH	59. (E11)	26% v/v Polyethylene glycol 600	59. (E11)	7.5
60. (E12)	0.1 M HEPES pH 7.5	NaOH	60. (E12)	44% v/v Polyethylene glycol 600	60. (E12)	7.7
61. (F1)	0.1 M Tris pH 8.0	HCl	61. (F1)	8% v/v Polyethylene glycol 300	61. (F1)	8.0
62. (F2)	0.1 M Tris pH 8.0	HCl	62. (F2)	26% v/v Polyethylene glycol 300	62. (F2)	8.0
63. (F3)	0.1 M Tris pH 8.0	HCl	63. (F3)	44% v/v Polyethylene glycol 300	63. (F3)	8.1
64. (F4)	0.1 M Tris pH 8.0	HCl	64. (F4)	8% v/v Polyethylene glycol 400	64. (F4)	8.0
65. (F5)	0.1 M Tris pH 8.0	HCl	65. (F5)	26% v/v Polyethylene glycol 400	65. (F5)	8.1
66. (F6)	0.1 M Tris pH 8.0	HCl	66. (F6)	44% v/v Polyethylene glycol 400	66. (F6)	8.2
67. (F7)	0.1 M Tris pH 8.0	HCl	67. (F7)	8% v/v Polyethylene glycol monomethyl ether 550	67. (F7)	8.0
68. (F8)	0.1 M Tris pH 8.0	HCl	68. (F8)	26% v/v Polyethylene glycol monomethyl ether 550	68. (F8)	8.0
69. (F9)	0.1 M Tris pH 8.0	HCl	69. (F9)	44% v/v Polyethylene glycol monomethyl ether 550	69. (F9)	8.0
70. (F10)	0.1 M Tris pH 8.0	HCl	70. (F10)	8% v/v Polyethylene glycol 600	70. (F10)	8.0
71. (F11)	0.1 M Tris pH 8.0	HCl	71. (F11)	26% v/v Polyethylene glycol 600	71. (F11)	8.1
72. (F12)	0.1 M Tris pH 8.0	HCl	72. (F12)	44% v/v Polyethylene glycol 600	72. (F12)	8.2
73. (G1)	0.1 M BIS-TRIS propane pH 9.0	HCl	73. (G1)	8% v/v Polyethylene glycol 300	73. (G1)	9.0
74. (G2)	0.1 M BIS-TRIS propane pH 9.0	HCl	74. (G2)	26% v/v Polyethylene glycol 300	74. (G2)	9.1
75. (G3)	0.1 M BIS-TRIS propane pH 9.0	HCl	75. (G3)	44% v/v Polyethylene glycol 300	75. (G3)	9.2
76. (G4)	0.1 M BIS-TRIS propane pH 9.0	HCl	76. (G4)	8% v/v Polyethylene glycol 400	76. (G4)	9.0
77. (G5)	0.1 M BIS-TRIS propane pH 9.0	HCl	77. (G5)	26% v/v Polyethylene glycol 400	77. (G5)	9.1
78. (G6)	0.1 M BIS-TRIS propane pH 9.0	HCl	78. (G6)	44% v/v Polyethylene glycol 400	78. (G6)	9.3
79. (G7)	0.1 M BIS-TRIS propane pH 9.0	HCl	79. (G7)	8% v/v Polyethylene glycol monomethyl ether 550	79. (G7)	9.0
80. (G8)	0.1 M BIS-TRIS propane pH 9.0	HCl	80. (G8)	26% v/v Polyethylene glycol monomethyl ether 550	80. (G8)	9.1
81. (G9)	0.1 M BIS-TRIS propane pH 9.0	HCl	81. (G9)	44% v/v Polyethylene glycol monomethyl ether 550	81. (G9)	9.2
82. (G10)	0.1 M BIS-TRIS propane pH 9.0	HCl	82. (G10)	8% v/v Polyethylene glycol 600	82. (G10)	9.0
83. (G11)	0.1 M BIS-TRIS propane pH 9.0	HCl	83. (G11)	26% v/v Polyethylene glycol 600	83. (G11)	9.1
84. (G12)	0.1 M BIS-TRIS propane pH 9.0	HCl	84. (G12)	44% v/v Polyethylene glycol 600	84. (G12)	9.3
85. (H1)	0.1 M CHES pH 10.0	NaOH	85. (H1)	8% v/v Polyethylene glycol 300	85. (H1)	10.0
86. (H2)	0.1 M CHES pH 10.0	NaOH	86. (H2)	26% v/v Polyethylene glycol 300	86. (H2)	10.0
87. (H3)	0.1 M CHES pH 10.0	NaOH	87. (H3)	44% v/v Polyethylene glycol 300	87. (H3)	10.0
88. (H4)	0.1 M CHES pH 10.0	NaOH	88. (H4)	8% v/v Polyethylene glycol 400	88. (H4)	10.0
89. (H5)	0.1 M CHES pH 10.0	NaOH	89. (H5)	26% v/v Polyethylene glycol 400	89. (H5)	10.0
90. (H6)	0.1 M CHES pH 10.0	NaOH	90. (H6)	44% v/v Polyethylene glycol 400	90. (H6)	10.1
91. (H7)	0.1 M CHES pH 10.0	NaOH	91. (H7)	8% v/v Polyethylene glycol monomethyl ether 550	91. (H7)	10.0
92. (H8)	0.1 M CHES pH 10.0	NaOH	92. (H8)	26% v/v Polyethylene glycol monomethyl ether 550	92. (H8)	9.9
93. (H9)	0.1 M CHES pH 10.0	NaOH	93. (H9)	44% v/v Polyethylene glycol monomethyl ether 550	93. (H9)	9.9
94. (H10)	0.1 M CHES pH 10.0	NaOH	94. (H10)	8% v/v Polyethylene glycol 600	94. (H10)	10.0
95. (H11)	0.1 M CHES pH 10.0	NaOH	95. (H11)	26% v/v Polyethylene glycol 600	95. (H11)	10.0
96. (H12)	0.1 M CHES pH 10.0	NaOH	96. (H12)	44% v/v Polyethylene glycol 600	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 PEG and water (25°C)

[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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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™ 5 - HR2-455 Scoring Sheet	Date:	Date:	Date:	Date:
1. (A1) 0.1 M Sodium acetate trihydrate pH 4.5, 8% v/v Polyethylene glycol 300				
2. (A2) 0.1 M Sodium acetate trihydrate pH 4.5, 26% v/v Polyethylene glycol 300				
3. (A3) 0.1 M Sodium acetate trihydrate pH 4.5, 44% v/v Polyethylene glycol 300				
4. (A4) 0.1 M Sodium acetate trihydrate pH 4.5, 8% v/v Polyethylene glycol 400				
5. (A5) 0.1 M Sodium acetate trihydrate pH 4.5, 26% v/v Polyethylene glycol 400				
6. (A6) 0.1 M Sodium acetate trihydrate pH 4.5, 44% v/v Polyethylene glycol 400				
7. (A7) 0.1 M Sodium acetate trihydrate pH 4.5, 8% v/v Polyethylene glycol monomethyl ether 550				
8. (A8) 0.1 M Sodium acetate trihydrate pH 4.5, 26% v/v Polyethylene glycol monomethyl ether 550				
9. (A9) 0.1 M Sodium acetate trihydrate pH 4.5, 44% v/v Polyethylene glycol monomethyl ether 550				
10. (A10) 0.1 M Sodium acetate trihydrate pH 4.5, 8% v/v Polyethylene glycol 600				
11. (A11) 0.1 M Sodium acetate trihydrate pH 4.5, 26% v/v Polyethylene glycol 600				
12. (A12) 0.1 M Sodium acetate trihydrate pH 4.5, 44% v/v Polyethylene glycol 600				
13. (B1) 0.1 M Succinic acid pH 5.5, 8% v/v Polyethylene glycol 300				
14. (B2) 0.1 M Succinic acid pH 5.5, 26% v/v Polyethylene glycol 300				
15. (B3) 0.1 M Succinic acid pH 5.5, 44% v/v Polyethylene glycol 300				
16. (B4) 0.1 M Succinic acid pH 5.5, 8% v/v Polyethylene glycol 400				
17. (B5) 0.1 M Succinic acid pH 5.5, 26% v/v Polyethylene glycol 400				
18. (B6) 0.1 M Succinic acid pH 5.5, 44% v/v Polyethylene glycol 400				
19. (B7) 0.1 M Succinic acid pH 5.5, 8% v/v Polyethylene glycol monomethyl ether 550				
20. (B8) 0.1 M Succinic acid pH 5.5, 26% v/v Polyethylene glycol monomethyl ether 550				
21. (B9) 0.1 M Succinic acid pH 5.5, 44% v/v Polyethylene glycol monomethyl ether 550				
22. (B10) 0.1 M Succinic acid pH 5.5, 8% v/v Polyethylene glycol 600				
23. (B11) 0.1 M Succinic acid pH 5.5, 26% v/v Polyethylene glycol 600				
24. (B12) 0.1 M Succinic acid pH 5.5, 44% v/v Polyethylene glycol 600				
25. (C1) 0.1 M BIS-TRIS pH 6.5, 8% v/v Polyethylene glycol 300				
26. (C2) 0.1 M BIS-TRIS pH 6.5, 26% v/v Polyethylene glycol 300				
27. (C3) 0.1 M BIS-TRIS pH 6.5, 44% v/v Polyethylene glycol 300				
28. (C4) 0.1 M BIS-TRIS pH 6.5, 8% v/v Polyethylene glycol 400				
29. (C5) 0.1 M BIS-TRIS pH 6.5, 26% v/v Polyethylene glycol 400				
30. (C6) 0.1 M BIS-TRIS pH 6.5, 44% v/v Polyethylene glycol 400				
31. (C7) 0.1 M BIS-TRIS pH 6.5, 8% v/v Polyethylene glycol monomethyl ether 550				
32. (C8) 0.1 M BIS-TRIS pH 6.5, 26% v/v Polyethylene glycol monomethyl ether 550				
33. (C9) 0.1 M BIS-TRIS pH 6.5, 44% v/v Polyethylene glycol monomethyl ether 550				
34. (C10) 0.1 M BIS-TRIS pH 6.5, 8% v/v Polyethylene glycol 600				
35. (C11) 0.1 M BIS-TRIS pH 6.5, 26% v/v Polyethylene glycol 600				
36. (C12) 0.1 M BIS-TRIS pH 6.5, 44% v/v Polyethylene glycol 600				
37. (D1) 0.1 M Sodium potassium phosphate pH 7.0, 8% v/v Polyethylene glycol 300				
38. (D2) 0.1 M Sodium potassium phosphate pH 7.0, 26% v/v Polyethylene glycol 300				
39. (D3) 0.1 M Sodium potassium phosphate pH 7.0, 44% v/v Polyethylene glycol 300				
40. (D4) 0.1 M Sodium potassium phosphate pH 7.0, 8% v/v Polyethylene glycol 400				
41. (D5) 0.1 M Sodium potassium phosphate pH 7.0, 26% v/v Polyethylene glycol 400				
42. (D6) 0.1 M Sodium potassium phosphate pH 7.0, 44% v/v Polyethylene glycol 400				
43. (D7) 0.1 M Sodium potassium phosphate pH 7.0, 8% v/v Polyethylene glycol monomethyl ether 550				
44. (D8) 0.1 M Sodium potassium phosphate pH 7.0, 26% v/v Polyethylene glycol monomethyl ether 550				
45. (D9) 0.1 M Sodium potassium phosphate pH 7.0, 44% v/v Polyethylene glycol monomethyl ether 550				
46. (D10) 0.1 M Sodium potassium phosphate pH 7.0, 8% v/v Polyethylene glycol 600				
47. (D11) 0.1 M Sodium potassium phosphate pH 7.0, 26% v/v Polyethylene glycol 600				
48. (D12) 0.1 M Sodium potassium phosphate pH 7.0, 44% v/v Polyethylene glycol 600				

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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™ 5 - HR2-455 Scoring Sheet	Date:	Date:	Date:	Date:
49. (E1) 0.1 M HEPES pH 7.5, 8% v/v Polyethylene glycol 300				
50. (E2) 0.1 M HEPES pH 7.5, 26% v/v Polyethylene glycol 300				
51. (E3) 0.1 M HEPES pH 7.5, 44% v/v Polyethylene glycol 300				
52. (E4) 0.1 M HEPES pH 7.5, 8% v/v Polyethylene glycol 400				
53. (E5) 0.1 M HEPES pH 7.5, 26% v/v Polyethylene glycol 400				
54. (E6) 0.1 M HEPES pH 7.5, 44% v/v Polyethylene glycol 400				
55. (E7) 0.1 M HEPES pH 7.5, 8% v/v Polyethylene glycol monomethyl ether 550				
56. (E8) 0.1 M HEPES pH 7.5, 26% v/v Polyethylene glycol monomethyl ether 550				
57. (E9) 0.1 M HEPES pH 7.5, 44% v/v Polyethylene glycol monomethyl ether 550				
58. (E10) 0.1 M HEPES pH 7.5, 8% v/v Polyethylene glycol 600				
59. (E11) 0.1 M HEPES pH 7.5, 26% v/v Polyethylene glycol 600				
60. (E12) 0.1 M HEPES pH 7.5, 44% v/v Polyethylene glycol 600				
61. (F1) 0.1 M Tris pH 8.0, 8% v/v Polyethylene glycol 300				
62. (F2) 0.1 M Tris pH 8.0, 26% v/v Polyethylene glycol 300				
63. (F3) 0.1 M Tris pH 8.0, 44% v/v Polyethylene glycol 300				
64. (F4) 0.1 M Tris pH 8.0, 8% v/v Polyethylene glycol 400				
65. (F5) 0.1 M Tris pH 8.0, 26% v/v Polyethylene glycol 400				
66. (F6) 0.1 M Tris pH 8.0, 44% v/v Polyethylene glycol 400				
67. (F7) 0.1 M Tris pH 8.0, 8% v/v Polyethylene glycol monomethyl ether 550				
68. (F8) 0.1 M Tris pH 8.0, 26% v/v Polyethylene glycol monomethyl ether 550				
69. (F9) 0.1 M Tris pH 8.0, 44% v/v Polyethylene glycol monomethyl ether 550				
70. (F10) 0.1 M Tris pH 8.0, 8% v/v Polyethylene glycol 600				
71. (F11) 0.1 M Tris pH 8.0, 26% v/v Polyethylene glycol 600				
72. (F12) 0.1 M Tris pH 8.0, 44% v/v Polyethylene glycol 600				
73. (G1) 0.1 M BIS-TRIS propane pH 9.0, 8% v/v Polyethylene glycol 300				
74. (G2) 0.1 M BIS-TRIS propane pH 9.0, 26% v/v Polyethylene glycol 300				
75. (G3) 0.1 M BIS-TRIS propane pH 9.0, 44% v/v Polyethylene glycol 300				
76. (G4) 0.1 M BIS-TRIS propane pH 9.0, 8% v/v Polyethylene glycol 400				
77. (G5) 0.1 M BIS-TRIS propane pH 9.0, 26% v/v Polyethylene glycol 400				
78. (G6) 0.1 M BIS-TRIS propane pH 9.0, 44% v/v Polyethylene glycol 400				
79. (G7) 0.1 M BIS-TRIS propane pH 9.0, 8% v/v Polyethylene glycol monomethyl ether 550				
80. (G8) 0.1 M BIS-TRIS propane pH 9.0, 26% v/v Polyethylene glycol monomethyl ether 550				
81. (G9) 0.1 M BIS-TRIS propane pH 9.0, 44% v/v Polyethylene glycol monomethyl ether 550				
82. (G10) 0.1 M BIS-TRIS propane pH 9.0, 8% v/v Polyethylene glycol 600				
83. (G11) 0.1 M BIS-TRIS propane pH 9.0, 26% v/v Polyethylene glycol 600				
84. (G12) 0.1 M BIS-TRIS propane pH 9.0, 44% v/v Polyethylene glycol 600				
85. (H1) 0.1 M CHES pH 10.0, 8% v/v Polyethylene glycol 300				
86. (H2) 0.1 M CHES pH 10.0, 26% v/v Polyethylene glycol 300				
87. (H3) 0.1 M CHES pH 10.0, 44% v/v Polyethylene glycol 300				
88. (H4) 0.1 M CHES pH 10.0, 8% v/v Polyethylene glycol 400				
89. (H5) 0.1 M CHES pH 10.0, 26% v/v Polyethylene glycol 400				
90. (H6) 0.1 M CHES pH 10.0, 44% v/v Polyethylene glycol 400				
91. (H7) 0.1 M CHES pH 10.0, 8% v/v Polyethylene glycol monomethyl ether 550				
92. (H8) 0.1 M CHES pH 10.0, 26% v/v Polyethylene glycol monomethyl ether 550				
93. (H9) 0.1 M CHES pH 10.0, 44% v/v Polyethylene glycol monomethyl ether 550				
94. (H10) 0.1 M CHES pH 10.0, 8% v/v Polyethylene glycol 600				
95. (H11) 0.1 M CHES pH 10.0, 26% v/v Polyethylene glycol 600				
96. (H12) 0.1 M CHES pH 10.0, 44% v/v Polyethylene glycol 600				

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