PEG/pH HT™

HAMPTON RESEARCH
Solutions for Crystal Growth

User Guide **HR2-461 (pg 1)**

Applications

Crystallization screen for biological macromolecules, where Polyethylene glycol 4,000 is the primary, and buffer the secondary reagent, sampling a broad range of pH.

Features

- Samples pH 3.5 - 9.6 in 0.2 M buffer
- Polyethylene glycol 4,000 - primary reagent
- 20 unique buffers, 0.2 M - secondary reagent
- Vapor diffusion, microbatch, free interface diffusion

Refer to the enclosed PEG/pH HT Reagent Formulation for more information.

General Description

pH is an effective solubility, stability and crystallization variable because most proteins demonstrate pH dependent solubility minima and will solubilize, precipitate, or crystallize at particular pH values or in the presence of specific buffers. The solubility minima may correspond with the isoelectric point (pI) of the protein, but this is not always the case. The solubility minima and maxima is often complex and may depend on other chemical and physical variables in the crystallization experiment.

Using PEG/pH HT one isolates pH, buffer type and relative supersaturation from other chemical and physical variables to screen the effect that pH and buffer type have on the solubility, stability, homogeneity, monodispersity and crystallization of the sample. Varying the pH can alter the protonation state and charge of amino acid residues in the protein, generating different species of the protein for solubility and crystallization screening. The change in pH can have a dramatic effect on inter and intramolecular contacts in the protein and can manipulate how the protein interacts with itself, the surrounding solvent and chemicals in the drop. By screening buffer type and pH in a low ionic strength environment of Polyethylene glycol, PEG/pH HT simultaneously delivers as a solubility and a crystallization screen for proteins.

PEG/pH HT samples Polyethylene glycol 4,000 versus 20 different buffers, at 0.2 M, while sampling pH 3.5 - 9.6. PEG/pH HT is supplied in a 96 Deep Well block format and is compatible with robotic and multi-channel pipet liquid handling systems. PEG/pH HT 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 PEG/pH HT 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.

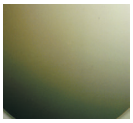
Manual Method - Sitting Drop Vapor Diffusion

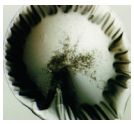
1. Using a 96 well sitting drop vapor diffusion plate, pipet the recommended volume (typically 50 to 100 microliters) of crystallization reagent from the Deep Well block into the reagent reservoirs of the crystallization plate. The Deep Well block is compatible with 8, 12, and 96 channel automated and 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.

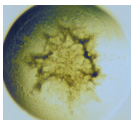
PEG/pH HT™

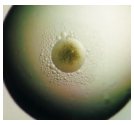
HAMPTON RESEARCH
Solutions for Crystal Growth

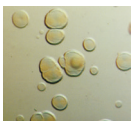
Figure 1
Typical observations in a crystallization experiment

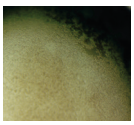

Clear Drop


Skin / Precipitate

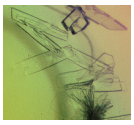

Precipitate


Precipitate / Phase

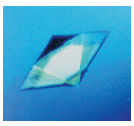

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

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 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 the Screen Results

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 the screen 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 the screen 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.

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™ 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. Protein Isoelectric Point as a Predictor for Increased Crystallization screening efficiency. Katherine A. Kantardjieff and Bernhard Rupp. Bioinformatics (2004) 20.
4. A protein crystallization strategy using automated grid searches on successively finer grid screens. Patricia C. Weber. Methods: A Companion to Methods in Enzymology. Vol. 1, No. 1, August, pp. 31-37, 1990.
5. Two approaches to the rapid screening of crystallization conditions. Alexander McPherson. Journal of Crystal Growth 122 (1992) 161-167.
6. Optimization of buffer solutions for protein crystallization. R. A. Gosavi, T. C. Mueser and C. A. Schall. Acta Cryst. (2008). D64, 506-514.
7. Buffer Solutions The Basics. R.J. Beynon and J.S. Easterby. 1996. IRL Press.

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PEG/pH HT™

HR2-461 Reagent Formulation

Well #	Buffer	pH ◊	Titrant	Precipitant
1. (A1)	0.2 M Citric acid	3.5	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
2. (A2)	0.2 M Citric acid	3.8	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
3. (A3)	0.2 M Citric acid	4.1	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
4. (A4)	0.2 M Citric acid	4.4	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
5. (A5)	0.2 M Sodium citrate tribasic dihydrate	3.6	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
6. (A6)	0.2 M Sodium citrate tribasic dihydrate	3.9	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
7. (A7)	0.2 M Sodium citrate tribasic dihydrate	4.2	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
8. (A8)	0.2 M Sodium citrate tribasic dihydrate	4.5	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
9. (A9)	0.2 M Sodium acetate trihydrate	3.7	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
10. (A10)	0.2 M Sodium acetate trihydrate	4.0	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
11. (A11)	0.2 M Sodium acetate trihydrate	4.3	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
12. (A12)	0.2 M Sodium acetate trihydrate	4.6	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
13. (B1)	0.2 M Sodium acetate trihydrate	4.9	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
14. (B2)	0.2 M DL-Malic acid	4.7	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
15. (B3)	0.2 M DL-Malic acid	5.0	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
16. (B4)	0.2 M DL-Malic acid	5.3	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
17. (B5)	0.2 M DL-Malic acid	5.6	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
18. (B6)	0.2 M DL-Malic acid	5.9	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
19. (B7)	0.2 M Succinic acid	4.8	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
20. (B8)	0.2 M Succinic acid	5.1	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
21. (B9)	0.2 M Succinic acid	5.4	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
22. (B10)	0.2 M Succinic acid	5.7	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
23. (B11)	0.2 M Succinic acid	6.0	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
24. (B12)	0.2 M Sodium cacodylate trihydrate	5.2	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
25. (C1)	0.2 M Sodium cacodylate trihydrate	5.5	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
26. (C2)	0.2 M Sodium cacodylate trihydrate	5.8	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
27. (C3)	0.2 M Sodium cacodylate trihydrate	6.1	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
28. (C4)	0.2 M Sodium cacodylate trihydrate	6.4	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
29. (C5)	0.2 M MES monohydrate	5.3	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
30. (C6)	0.2 M MES monohydrate	5.6	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
31. (C7)	0.2 M MES monohydrate	5.9	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
32. (C8)	0.2 M MES monohydrate	6.2	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
33. (C9)	0.2 M MES monohydrate	6.5	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
34. (C10)	0.2 M BIS-TRIS	5.7	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
35. (C11)	0.2 M BIS-TRIS	6.0	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
36. (C12)	0.2 M BIS-TRIS	6.3	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
37. (D1)	0.2 M BIS-TRIS	6.6	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
38. (D2)	0.2 M BIS-TRIS	6.9	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
39. (D3)	0.2 M ADA	5.8	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
40. (D4)	0.2 M ADA	6.1	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
41. (D5)	0.2 M ADA	6.4	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
42. (D6)	0.2 M ADA	6.7	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
43. (D7)	0.2 M ADA	7.0	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
44. (D8)	0.2 M Imidazole	6.2	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
45. (D9)	0.2 M Imidazole	6.5	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
46. (D10)	0.2 M Imidazole	6.8	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
47. (D11)	0.2 M Imidazole	7.1	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
48. (D12)	0.2 M Imidazole	7.4	Hydrochloric acid	20% w/v Polyethylene glycol 4,000

Reagents formulated in Type 1+ ultrapure grade water

◊ Measured pH at 25ºC after titration

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Well #	Buffer	pH ◊	Titrant	Precipitant
49. (E1)	0.2 M BIS-TRIS propane	6.4	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
50. (E2)	0.2 M BIS-TRIS propane	6.7	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
51. (E3)	0.2 M BIS-TRIS propane	7.0	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
52. (E4)	0.2 M BIS-TRIS propane	7.3	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
53. (E5)	0.2 M MOPS	6.5	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
54. (E6)	0.2 M MOPS	6.8	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
55. (E7)	0.2 M MOPS	7.1	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
56. (E8)	0.2 M MOPS	7.4	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
57. (E9)	0.2 M MOPS	7.7	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
58. (E10)	0.2 M HEPES sodium	6.6	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
59. (E11)	0.2 M HEPES sodium	6.9	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
60. (E12)	0.2 M HEPES sodium	7.2	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
61. (F1)	0.2 M HEPES sodium	7.5	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
62. (F2)	0.2 M HEPES	6.8	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
63. (F3)	0.2 M HEPES	7.1	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
64. (F4)	0.2 M HEPES	7.4	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
65. (F5)	0.2 M HEPES	7.7	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
66. (F6)	0.2 M TRIS hydrochloride	7.2	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
67. (F7)	0.2 M TRIS hydrochloride	7.5	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
68. (F8)	0.2 M TRIS hydrochloride	7.8	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
69. (F9)	0.2 M TRIS hydrochloride	8.1	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
70. (F10)	0.2 M Tris	7.3	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
71. (F11)	0.2 M Tris	7.6	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
72. (F12)	0.2 M Tris	7.9	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
73. (G1)	0.2 M Tris	8.2	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
74. (G2)	0.2 M Tris	8.5	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
75. (G3)	0.2 M Tricine	7.4	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
76. (G4)	0.2 M Tricine	7.7	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
77. (G5)	0.2 M Tricine	8.0	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
78. (G6)	0.2 M Tricine	8.3	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
79. (G7)	0.2 M Tricine	8.6	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
80. (G8)	0.2 M BICINE	7.5	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
81. (G9)	0.2 M BICINE	7.8	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
82. (G10)	0.2 M BICINE	8.1	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
83. (G11)	0.2 M BICINE	8.4	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
84. (G12)	0.2 M BICINE	8.7	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
85. (H1)	0.2 M BIS-TRIS propane	8.5	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
86. (H2)	0.2 M BIS-TRIS propane	8.8	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
87. (H3)	0.2 M BIS-TRIS propane	9.1	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
88. (H4)	0.2 M BIS-TRIS propane	9.4	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
89. (H5)	0.2 M Glycine	8.6	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
90. (H6)	0.2 M Glycine	8.9	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
91. (H7)	0.2 M Glycine	9.2	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
92. (H8)	0.2 M Glycine	9.5	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
93. (H9)	0.2 M AMPD	8.7	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
94. (H10)	0.2 M AMPD	9.0	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
95. (H11)	0.2 M AMPD	9.3	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
96. (H12)	0.2 M AMPD	9.6	Hydrochloric acid	20% w/v Polyethylene glycol 4,000

Reagents formulated in Type 1+ ultrapure grade water

◊ Measured pH at 25ºC after titration

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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)

PEG/pH HT™ - HR2-461 Scoring Sheet		Date:	Date:	Date:	Date:
1. (A1)	0.2 M Citric acid pH 3.5, 20% w/v Polyethylene glycol 4,000				
2. (A2)	0.2 M Citric acid pH 3.8, 20% w/v Polyethylene glycol 4,000				
3. (A3)	0.2 M Citric acid pH 4.1, 20% w/v Polyethylene glycol 4,000				
4. (A4)	0.2 M Citric acid pH 4.4, 20% w/v Polyethylene glycol 4,000				
5. (A5)	0.2 M Sodium citrate tribasic dihydrate pH 3.6, 20% w/v Polyethylene glycol 4,000				
6. (A6)	0.2 M Sodium citrate tribasic dihydrate pH 3.9, 20% w/v Polyethylene glycol 4,000				
7. (A7)	0.2 M Sodium citrate tribasic dihydrate pH 4.2, 20% w/v Polyethylene glycol 4,000				
8. (A8)	0.2 M Sodium citrate tribasic dihydrate pH 4.5, 20% w/v Polyethylene glycol 4,000				
9. (A9)	0.2 M Sodium acetate trihydrate pH 3.7, 20% w/v Polyethylene glycol 4,000				
10. (A10)	0.2 M Sodium acetate trihydrate pH 4.0, 20% w/v Polyethylene glycol 4,000				
11. (A11)	0.2 M Sodium acetate trihydrate pH 4.3, 20% w/v Polyethylene glycol 4,000				
12. (A12)	0.2 M Sodium acetate trihydrate pH 4.6, 20% w/v Polyethylene glycol 4,000				
13. (B1)	0.2 M Sodium acetate trihydrate pH 4.9, 20% w/v Polyethylene glycol 4,000				
14. (B2)	0.2 M DL-Malic acid pH 4.7, 20% w/v Polyethylene glycol 4,000				
15. (B3)	0.2 M DL-Malic acid pH 5.0, 20% w/v Polyethylene glycol 4,000				
16. (B4)	0.2 M DL-Malic acid pH 5.3, 20% w/v Polyethylene glycol 4,000				
17. (B5)	0.2 M DL-Malic acid pH 5.6, 20% w/v Polyethylene glycol 4,000				
18. (B6)	0.2 M DL-Malic acid pH 5.9, 20% w/v Polyethylene glycol 4,000				
19. (B7)	0.2 M Succinic acid pH 4.8, 20% w/v Polyethylene glycol 4,000				
20. (B8)	0.2 M Succinic acid pH 5.1, 20% w/v Polyethylene glycol 4,000				
21. (B9)	0.2 M Succinic acid pH 5.4, 20% w/v Polyethylene glycol 4,000				
22. (B10)	0.2 M Succinic acid pH 5.7, 20% w/v Polyethylene glycol 4,000				
23. (B11)	0.2 M Succinic acid pH 6.0, 20% w/v Polyethylene glycol 4,000				
24. (B12)	0.2 M Sodium cacodylate trihydrate pH 5.2, 20% w/v Polyethylene glycol 4,000				
25. (C1)	0.2 M Sodium cacodylate trihydrate pH 5.5, 20% w/v Polyethylene glycol 4,000				
26. (C2)	0.2 M Sodium cacodylate trihydrate pH 5.8, 20% w/v Polyethylene glycol 4,000				
27. (C3)	0.2 M Sodium cacodylate trihydrate pH 6.1, 20% w/v Polyethylene glycol 4,000				
28. (C4)	0.2 M Sodium cacodylate trihydrate pH 6.4, 20% w/v Polyethylene glycol 4,000				
29. (C5)	0.2 M MES monohydrate pH 5.3, 20% w/v Polyethylene glycol 4,000				
30. (C6)	0.2 M MES monohydrate pH 5.6, 20% w/v Polyethylene glycol 4,000				
31. (C7)	0.2 M MES monohydrate pH 5.9, 20% w/v Polyethylene glycol 4,000				
32. (C8)	0.2 M MES monohydrate pH 6.2, 20% w/v Polyethylene glycol 4,000				
33. (C9)	0.2 M MES monohydrate pH 6.5, 20% w/v Polyethylene glycol 4,000				
34. (C10)	0.2 M BIS-TRIS pH 5.7, 20% w/v Polyethylene glycol 4,000				
35. (C11)	0.2 M BIS-TRIS pH 6.0, 20% w/v Polyethylene glycol 4,000				
36. (C12)	0.2 M BIS-TRIS pH 6.3, 20% w/v Polyethylene glycol 4,000				
37. (D1)	0.2 M BIS-TRIS pH 6.6, 20% w/v Polyethylene glycol 4,000				
38. (D2)	0.2 M BIS-TRIS pH 6.9, 20% w/v Polyethylene glycol 4,000				
39. (D3)	0.2 M ADA pH 5.8, 20% w/v Polyethylene glycol 4,000				
40. (D4)	0.2 M ADA pH 6.1, 20% w/v Polyethylene glycol 4,000				
41. (D5)	0.2 M ADA pH 6.4, 20% w/v Polyethylene glycol 4,000				
42. (D6)	0.2 M ADA pH 6.7, 20% w/v Polyethylene glycol 4,000				
43. (D7)	0.2 M ADA pH 7.0, 20% w/v Polyethylene glycol 4,000				
44. (D8)	0.2 M Imidazole pH 6.2, 20% w/v Polyethylene glycol 4,000				
45. (D9)	0.2 M Imidazole pH 6.5, 20% w/v Polyethylene glycol 4,000				
46. (D10)	0.2 M Imidazole pH 6.8, 20% w/v Polyethylene glycol 4,000				
47. (D11)	0.2 M Imidazole pH 7.1, 20% w/v Polyethylene glycol 4,000				
48. (D12)	0.2 M Imidazole pH 7.4, 20% w/v Polyethylene glycol 4,000				

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)

PEG/pH HT™ - HR2-461 Scoring Sheet	Date:	Date:	Date:	Date:
49. (E1) 0.2 M BIS-TRIS propane pH 6.4, 20% w/v Polyethylene glycol 4,000				
50. (E2) 0.2 M BIS-TRIS propane pH 6.7, 20% w/v Polyethylene glycol 4,000				
51. (E3) 0.2 M BIS-TRIS propane pH 7.0, 20% w/v Polyethylene glycol 4,000				
52. (E4) 0.2 M BIS-TRIS propane pH 7.3, 20% w/v Polyethylene glycol 4,000				
53. (E5) 0.2 M MOPS pH 6.5, 20% w/v Polyethylene glycol 4,000				
54. (E6) 0.2 M MOPS pH 6.8, 20% w/v Polyethylene glycol 4,000				
55. (E7) 0.2 M MOPS pH 7.1, 20% w/v Polyethylene glycol 4,000				
56. (E8) 0.2 M MOPS pH 7.4, 20% w/v Polyethylene glycol 4,000				
57. (E9) 0.2 M MOPS pH 7.7, 20% w/v Polyethylene glycol 4,000				
58. (E10) 0.2 M HEPES sodium pH 6.6, 20% w/v Polyethylene glycol 4,000				
59. (E11) 0.2 M HEPES sodium pH 6.9, 20% w/v Polyethylene glycol 4,000				
60. (E12) 0.2 M HEPES sodium pH 7.2, 20% w/v Polyethylene glycol 4,000				
61. (F1) 0.2 M HEPES sodium pH 7.5, 20% w/v Polyethylene glycol 4,000				
62. (F2) 0.2 M HEPES pH 6.8, 20% w/v Polyethylene glycol 4,000				
63. (F3) 0.2 M HEPES pH 7.1, 20% w/v Polyethylene glycol 4,000				
64. (F4) 0.2 M HEPES pH 7.4, 20% w/v Polyethylene glycol 4,000				
65. (F5) 0.2 M HEPES pH 7.7, 20% w/v Polyethylene glycol 4,000				
66. (F6) 0.2 M TRIS hydrochloride pH 7.2, 20% w/v Polyethylene glycol 4,000				
67. (F7) 0.2 M TRIS hydrochloride pH 7.5, 20% w/v Polyethylene glycol 4,000				
68. (F8) 0.2 M TRIS hydrochloride pH 7.8, 20% w/v Polyethylene glycol 4,000				
69. (F9) 0.2 M TRIS hydrochloride pH 8.1, 20% w/v Polyethylene glycol 4,000				
70. (F10) 0.2 M Tris pH 7.3, 20% w/v Polyethylene glycol 4,000				
71. (F11) 0.2 M Tris pH 7.6, 20% w/v Polyethylene glycol 4,000				
72. (F12) 0.2 M Tris pH 7.9, 20% w/v Polyethylene glycol 4,000				
73. (G1) 0.2 M Tris pH 8.2, 20% w/v Polyethylene glycol 4,000				
74. (G2) 0.2 M Tris pH 8.5, 20% w/v Polyethylene glycol 4,000				
75. (G3) 0.2 M Tricine pH 7.4, 20% w/v Polyethylene glycol 4,000				
76. (G4) 0.2 M Tricine pH 7.7, 20% w/v Polyethylene glycol 4,000				
77. (G5) 0.2 M Tricine pH 8.0, 20% w/v Polyethylene glycol 4,000				
78. (G6) 0.2 M Tricine pH 8.3, 20% w/v Polyethylene glycol 4,000				
79. (G7) 0.2 M Tricine pH 8.6, 20% w/v Polyethylene glycol 4,000				
80. (G8) 0.2 M BICINE pH 7.5, 20% w/v Polyethylene glycol 4,000				
81. (G9) 0.2 M BICINE pH 7.8, 20% w/v Polyethylene glycol 4,000				
82. (G10) 0.2 M BICINE pH 8.1, 20% w/v Polyethylene glycol 4,000				
83. (G11) 0.2 M BICINE pH 8.4, 20% w/v Polyethylene glycol 4,000				
84. (G12) 0.2 M BICINE pH 8.7, 20% w/v Polyethylene glycol 4,000				
85. (H1) 0.2 M BIS-TRIS propane pH 8.5, 20% w/v Polyethylene glycol 4,000				
86. (H2) 0.2 M BIS-TRIS propane pH 8.8, 20% w/v Polyethylene glycol 4,000				
87. (H3) 0.2 M BIS-TRIS propane pH 9.1, 20% w/v Polyethylene glycol 4,000				
88. (H4) 0.2 M BIS-TRIS propane pH 9.4, 20% w/v Polyethylene glycol 4,000				
89. (H5) 0.2 M Glycine pH 8.6, 20% w/v Polyethylene glycol 4,000				
90. (H6) 0.2 M Glycine pH 8.9, 20% w/v Polyethylene glycol 4,000				
91. (H7) 0.2 M Glycine pH 9.2, 20% w/v Polyethylene glycol 4,000				
92. (H8) 0.2 M Glycine pH 9.5, 20% w/v Polyethylene glycol 4,000				
93. (H9) 0.2 M AMPD pH 8.7, 20% w/v Polyethylene glycol 4,000				
94. (H10) 0.2 M AMPD pH 9.0, 20% w/v Polyethylene glycol 4,000				
95. (H11) 0.2 M AMPD pH 9.3, 20% w/v Polyethylene glycol 4,000				
96. (H12) 0.2 M AMPD pH 9.6, 20% w/v Polyethylene glycol 4,000				

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