PEG/Ion HT™

HAMPTON RESEARCH
Solutions for Crystal Growth

User Guide **HR2-139 (pg 1)**

Applications
Crystallization screen for soluble biological macromolecules.

Features
- Reagent formulation developed at Hampton Research
- Screens a profile of anions, cations, multivalent ions, titrated organic acids at varying pH levels in the presence of monodisperse Polyethylene glycol 3,350
- Tacsimate™ pH 4, 5, 6, 7, 8, and Polyethylene glycol
- pH range 3.4 - 9.2
- Novel CBTP buffer component
- Tryptone (peptide library)

General Description
PEG/Ion HT ™ is a crystallization reagent kit designed to provide a rapid screening method for the crystallization of biological macromolecules. PEG/Ion HT is designed as a 96 reagent crystallization screen that combines the strategies of PEG/Ion Screen™ (HR2-126) and PEG/Ion 2 Screen™ (HR2-098) into a highly effective and efficient format. This kit allows one to evaluate a large variety of potential crystallization conditions with the 96 unique reagents.

PEG/Ion HT is supplied in a sterile, polypropylene 96 Deep Well block, each reservoir containing 1 ml of sterile filtered reagent. The block is compatible with robotic and multi-channel pipet liquid handling systems and is heat sealed using a special polypropylene backed film. Each PEG/Ion HT kit is supplied with an adhesive sealing film which can be used to seal the block after removing the heat seal.

Within the 96 Deep Well block, rows A through D feature the 48 reagents of PEG/Ion Screen (HR2-126). The screen combines high purity Polyethylene glycol 3,350 and 48 different high purity salts, comprising both anions (sulfate, nitrate, tartrate, acetate, chloride, iodide, thiocyanate, formate, citrate, phosphate, and fluoride) and cations (sodium, potassium, ammonium, lithium, magnesium, and calcium) in a relatively low concentration (0.2 M) which due to their unique pH characteristics also affords a reasonable pH screen (approximate pH range of 4 to 9). The primary screen variables are PEG, ion type, ionic strength, and pH.

Rows E through H feature the 48 reagents of PEG/Ion 2 Screen (HR2-098). PEG/Ion 2 Screen is a crystallization reagent kit designed to provide a rapid screening method for the crystallization of biological macromolecules in the presence of Polyethylene glycol 3,350 and an array of neutralized and pH adjusted organic acids, multivalent ions, a novel Citrate BIS-TRIS propane buffer system (CBTP), Tryptone (peptide library), and pH. PEG/Ion 2 Screen utilizes a monodisperse (Mr 3,300-3,400), high purity, Polyethylene glycol 3,350.

Refer to the enclosed PEG/Ion HT reagent formulation for additional information on all 96 reagents.

Sample Preparation
The macromolecular sample should be homogenous, as pure as is practically possible (>95%) and free of amorphous and particulate material. Remove amorphous material by centrifugation or micro-filtration prior to use.[1-3]

The recommended sample concentration is 5 to 25 mg/ml in sample buffer. Initially, the sample should be free of any unnecessary additives in order to observe the effect of the PEG/Ion HT variables. Ideally, the initial screen should be performed with a sample which has been dialyzed against dilute buffer although ligands, ions, reducing agents, or other additives may be present as required by the sample for solubility, stability, or activity.

Preparing the Deep Well Block for Use
It is recommended the Deep Well block be centrifuged and at 25 degrees Celsius before removing the sealing film. Centrifugation at 500 rpm for five minutes will remove stray reagent from the sealing film. Removing the reagent from the film prevents stray reagent droplets from falling into neighboring wells during film removal. After centrifugation 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 and the pierced for reagent access.

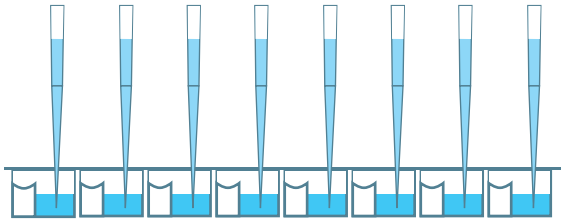
Performing The Screen
Since it is the most frequently reported method of crystallization, the following procedure describes the use of the PEG/Ion HT with the Sitting Drop Vapor Diffusion method. The PEG/Ion HT is also very compatible with the Hanging Drop, Sandwich Drop, Microbatch, and Microdialysis methods. A complete description of the Hanging, Sitting, Sandwich Drop, Dialysis and other crystallization methods are available from the Hampton Research Crystal Growth 101 Library.

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 reservoirs of the crystallization plate. The Deep Well block is compatible with 8 and 12 channel pipets as well as many automated liquid handling systems. 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

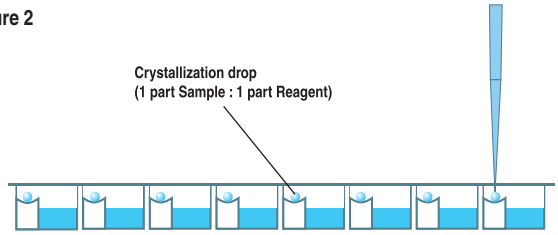
when moving between reagent columns. For a 12 channel pipet, transfer reagents A1-A12 to reservoirs A1-A12 of the crystallization plate. Repeat this procedure for reagent rows B through H. See Figure 1 below. Time and pipet tips can be conserved by batch pipetting multiple plates with the same (row or column) of reagent before changing reagent and pipet tips.

Figure 1



2. Using clean pipet tips, pipet 0.05 to 2 microliters of crystallization reagent from the crystallization plate reservoir to the sitting drop well. Some 96 well crystallization plates allow this procedure to be performed using a multi-channel pipet where other plates require the use of a single channel pipet. Change the pipet tip between reagents. See Figure 2 below.

Figure 2



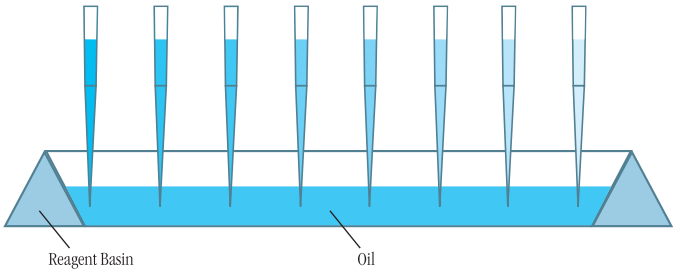
3. Using a clean pipet tip, pipet 0.05 to 2 microliters of sample to the reagent drop in the sitting drop well. One may choose to simply dispense the sample with no mixing or dispense with mixing by gently aspirating and dispensing the sample several times, keeping the tip in the drop during mixing to avoid foaming. Work carefully but quickly to minimize evaporation from the crystallization plate. See Figure 2.

4. Seal the crystallization plate as per the manufacturers recommendation. Most 96 well crystallization plates are sealed using a clear sealing tape or film. View and score the experiment as desired. See Hampton Research technical bulletin Crystal Growth 101 - Viewing Crystallization Experiments for additional information on viewing drops.

Manual Method – Microbatch 96 Well Format

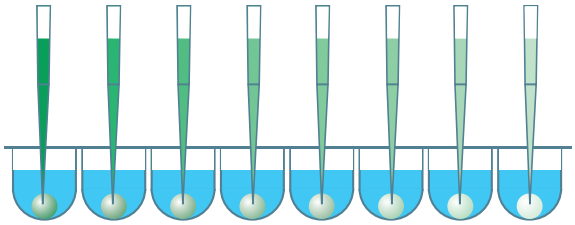
1. Using a 96 well clear polystyrene microplate (U-bottom recommended for best drop centering, flat-bottom recommended for best optics) pipet approximately 150 microliters of Microbatch compatible oil into each of the 96 reservoirs. This can be accomplished using an 8 or 12 channel pipet and pipetting the oil from a reagent basin. See Figure 3.

Figure 3



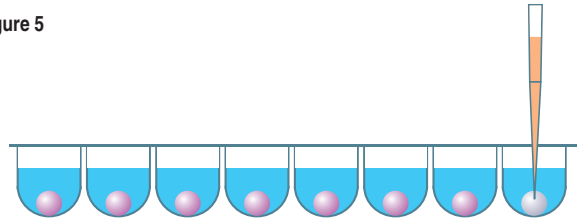
2. Once the plate is oiled, use an 8 or 12 channel pipet to aspirate reagent from the Deep Well block and dispense the reagent under the oil in the Microbatch plate. Change tips when changing reagent to prevent cross reagent contamination. To save time and pipet tips, set multiple plates at one time. See Figure 4.

Figure 4



3. Using a single channel pipet, aspirate the sample and dispense the sample under oil in the Microbatch plate. It is not necessary to dispense the sample drop into the reagent drop or mix the drops. See Figure 5.

Figure 5

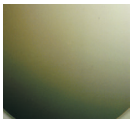


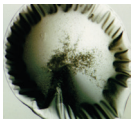
4. After all reagent and sample drops have been dispensed to the Microbatch plate, place the loose fitting clear cover on the Microbatch plate and centrifuge the plate for 10 minutes at 500 rpm. Centrifugation will cause the drops to coalesce into a single drop.

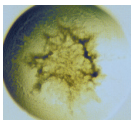
Note: If the drops appear flat or is fragmented into multiple drops, the centrifugation speed is too high and the centrifugation time is too long - adjust to obtain a spherical single drop in the center of the well.

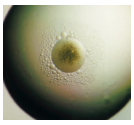
5. Store the plates with the loose fitting clear polystyrene cover and observe for crystals. See Hampton Research technical bulletin Crystal Growth 101 - Viewing Crystallization Experiments for additional information on viewing drops.

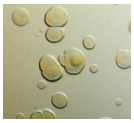
Figure 6
Typical observations in a crystallization experiment

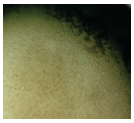

Clear Drop


Skin / Precipitate

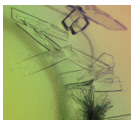

Precipitate


Precipitate / Phase

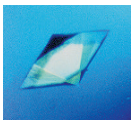

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

PEG/Ion HT Deep Well Block and Automated Liquid Handling Systems

The polypropylene Deep Well block is designed to be compatible with the SBS standard 96 microwell format and is therefore compatible with numerous automated liquid handling systems that accept 8x12 96 well assay blocks. Follow the manufacturer's recommendation for handling deep well microplates.

Examine the Drop

Carefully examine the drops under a stereo microscope (10 to 100x magnification) immediately after setting up 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. 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, 2+ small bipyramid crystals, clear drop, 3+ needle shaped crystals in 1+ white precipitate. One may also employ a standard numerical scoring scheme (Clear = 0, Precipitate = 1, Crystal = 10, etc). Figure 6 (on page 3) shows typical examples of what one might observe in a crystallization experiment.

Interpreting PEG/Ion HT

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 screen drops are clear 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 and repeat the screen condition. If more than 70 of the 96 screen drops contain precipitate and no crystals are present, consider diluting the sample concentration in half and repeating the entire screen. If sample denaturation is suspect, take measures to stabilize the sample (add reducing agent, ligands, glycerol, 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 optics to differentiate precipitate from microcrystalline material.

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 (pH, salt type, salt concentration, precipitant type, precipitant concentration, sample concentration, temperature, additives, and other crystallization variables) which produced the crystal in order to improve crystal size and quality.

Compare the observations between the 4°C and room temperature incubation 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.

Retain and observe plates until the drops are dried out. Crystal growth can occur within 15 minutes or one year.

PEG/Ion HT Formulation

Crystallization reagents are formulated using the highest purity chemicals, ultrapure water (18.2 Megohm-cm, 5 ppb TOC) and are sterile filtered using 0.22 micron filters into sterile Deep Well blocks (no preservatives added).

PEG/Ion HT reagents are readily reproduced using Hampton Research Optimize™ and StockOptions™ stock solutions of salts, polymers and buffers. Refer to PEG/Ion Screen and PEG/Ion 2 Screen Fundamentals for further information regarding reagent formulation. Optimize and StockOptions stock reagents make reproducing crystallization screen reagents accurate, precise, fast, convenient and easy. Dilutions can be performed directly into the crystallization plate using Optimize and StockOptions stock reagents.

PEG/Ion HT reagents are stable at room temperature and are best if used within 12 months of receipt. To enhance reagent stability the crystallization reagents can be stored at 4°C or -20°C. Avoid ultraviolet light to preserve reagent stability.

If the sample contains phosphate, borate, or carbonate buffers

it is possible to obtain inorganic crystals (false positives) when using crystallization reagents containing divalent cations such as magnesium, calcium, or zinc. To avoid false positives use phosphate, borate, or carbonate buffers at concentrations of 10 mM or less or exchange the phosphate, borate, or carbonate buffer with a more soluble buffer that does not complex with divalent cations.

References and Readings

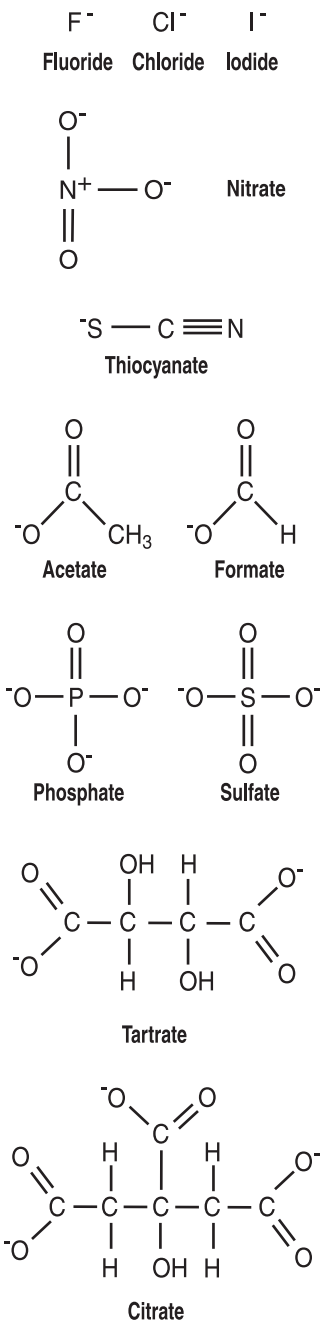
1. Crystallization of nucleic acids and proteins, Edited by A. Ducruix and R. Giege, The Practical Approach Series, Oxford Univ. Press, 1992.
2. Current approaches to macromolecular crystallization. McPherson, A. Eur. J. Biochem. 189, 1-23, 1990.
3. Protein and Nucleic Acid Crystallization. Methods, A Companion to Methods in Enzymology, Academic Press, Volume 1, Number 1, August 1990.

Technical Support

Inquiries regarding PEG/Ion HT reagent formulation, interpretation of screen results, optimization strategies and general inquiries regarding crystallization are welcome. Please e-mail, fax, or telephone your request to Hampton Research. Fax and e-mail Technical Support are available 24 hours a day. Telephone technical support is available 8:00 a.m. to 4:30 p.m. USA Pacific Standard Time.

Hampton Research
34 Journey
Aliso Viejo, CA 92656-3317 U.S.A.
Tel: (949) 425-1321 • Fax: (949) 425-1611
Technical Support e-mail: tech@hrmail.com
Website: www.hamptonresearch.com

Well #	Salt	Well #	Polymer	Well #	pH ◊
1. (A1)	0.2 M Sodium fluoride	1. (A1)	20% w/v Polyethylene glycol 3,350	1. (A1)	7.3
2. (A2)	0.2 M Potassium fluoride	2. (A2)	20% w/v Polyethylene glycol 3,350	2. (A2)	7.3
3. (A3)	0.2 M Ammonium fluoride	3. (A3)	20% w/v Polyethylene glycol 3,350	3. (A3)	6.2
4. (A4)	0.2 M Lithium chloride	4. (A4)	20% w/v Polyethylene glycol 3,350	4. (A4)	6.8
5. (A5)	0.2 M Magnesium chloride hexahydrate	5. (A5)	20% w/v Polyethylene glycol 3,350	5. (A5)	5.9
6. (A6)	0.2 M Sodium chloride	6. (A6)	20% w/v Polyethylene glycol 3,350	6. (A6)	6.9
7. (A7)	0.2 M Calcium chloride dihydrate	7. (A7)	20% w/v Polyethylene glycol 3,350	7. (A7)	5.1
8. (A8)	0.2 M Potassium chloride	8. (A8)	20% w/v Polyethylene glycol 3,350	8. (A8)	7.0
9. (A9)	0.2 M Ammonium chloride	9. (A9)	20% w/v Polyethylene glycol 3,350	9. (A9)	6.3
10. (A10)	0.2 M Sodium iodide	10. (A10)	20% w/v Polyethylene glycol 3,350	10. (A10)	7.0
11. (A11)	0.2 M Potassium iodide	11. (A11)	20% w/v Polyethylene glycol 3,350	11. (A11)	7.0
12. (A12)	0.2 M Ammonium iodide	12. (A12)	20% w/v Polyethylene glycol 3,350	12. (A12)	6.2
13. (B1)	0.2 M Sodium thiocyanate	13. (B1)	20% w/v Polyethylene glycol 3,350	13. (B1)	6.9
14. (B2)	0.2 M Potassium thiocyanate	14. (B2)	20% w/v Polyethylene glycol 3,350	14. (B2)	7.0
15. (B3)	0.2 M Lithium nitrate	15. (B3)	20% w/v Polyethylene glycol 3,350	15. (B3)	7.1
16. (B4)	0.2 M Magnesium nitrate hexahydrate	16. (B4)	20% w/v Polyethylene glycol 3,350	16. (B4)	5.9
17. (B5)	0.2 M Sodium nitrate	17. (B5)	20% w/v Polyethylene glycol 3,350	17. (B5)	6.8
18. (B6)	0.2 M Potassium nitrate	18. (B6)	20% w/v Polyethylene glycol 3,350	18. (B6)	6.8
19. (B7)	0.2 M Ammonium nitrate	19. (B7)	20% w/v Polyethylene glycol 3,350	19. (B7)	6.2
20. (B8)	0.2 M Magnesium formate dihydrate	20. (B8)	20% w/v Polyethylene glycol 3,350	20. (B8)	7.0
21. (B9)	0.2 M Sodium formate	21. (B9)	20% w/v Polyethylene glycol 3,350	21. (B9)	7.2
22. (B10)	0.2 M Potassium formate	22. (B10)	20% w/v Polyethylene glycol 3,350	22. (B10)	7.3
23. (B11)	0.2 M Ammonium formate	23. (B11)	20% w/v Polyethylene glycol 3,350	23. (B11)	6.6
24. (B12)	0.2 M Lithium acetate dihydrate	24. (B12)	20% w/v Polyethylene glycol 3,350	24. (B12)	7.9
25. (C1)	0.2 M Magnesium acetate tetrahydrate	25. (C1)	20% w/v Polyethylene glycol 3,350	25. (C1)	7.9
26. (C2)	0.2 M Zinc acetate dihydrate	26. (C2)	20% w/v Polyethylene glycol 3,350	26. (C2)	6.4
27. (C3)	0.2 M Sodium acetate trihydrate	27. (C3)	20% w/v Polyethylene glycol 3,350	27. (C3)	8.0
28. (C4)	0.2 M Calcium acetate hydrate	28. (C4)	20% w/v Polyethylene glycol 3,350	28. (C4)	7.5
29. (C5)	0.2 M Potassium acetate	29. (C5)	20% w/v Polyethylene glycol 3,350	29. (C5)	8.1
30. (C6)	0.2 M Ammonium acetate	30. (C6)	20% w/v Polyethylene glycol 3,350	30. (C6)	7.1
31. (C7)	0.2 M Lithium sulfate monohydrate	31. (C7)	20% w/v Polyethylene glycol 3,350	31. (C7)	6.0
32. (C8)	0.2 M Magnesium sulfate heptahydrate	32. (C8)	20% w/v Polyethylene glycol 3,350	32. (C8)	6.0
33. (C9)	0.2 M Sodium sulfate decahydrate	33. (C9)	20% w/v Polyethylene glycol 3,350	33. (C9)	6.7
34. (C10)	0.2 M Potassium sulfate	34. (C10)	20% w/v Polyethylene glycol 3,350	34. (C10)	6.8
35. (C11)	0.2 M Ammonium sulfate	35. (C11)	20% w/v Polyethylene glycol 3,350	35. (C11)	6.0
36. (C12)	0.2 M Sodium tartrate dibasic dihydrate	36. (C12)	20% w/v Polyethylene glycol 3,350	36. (C12)	7.3
37. (D1)	0.2 M Potassium sodium tartrate tetrahydrate	37. (D1)	20% w/v Polyethylene glycol 3,350	37. (D1)	7.4
38. (D2)	0.2 M Ammonium tartrate dibasic	38. (D2)	20% w/v Polyethylene glycol 3,350	38. (D2)	6.6
39. (D3)	0.2 M Sodium phosphate monobasic monohydrate	39. (D3)	20% w/v Polyethylene glycol 3,350	39. (D3)	4.7
40. (D4)	0.2 M Sodium phosphate dibasic dihydrate	40. (D4)	20% w/v Polyethylene glycol 3,350	40. (D4)	9.1
41. (D5)	0.2 M Potassium phosphate monobasic	41. (D5)	20% w/v Polyethylene glycol 3,350	41. (D5)	4.8
42. (D6)	0.2 M Potassium phosphate dibasic	42. (D6)	20% w/v Polyethylene glycol 3,350	42. (D6)	9.2
43. (D7)	0.2 M Ammonium phosphate monobasic	43. (D7)	20% w/v Polyethylene glycol 3,350	43. (D7)	4.6
44. (D8)	0.2 M Ammonium phosphate dibasic	44. (D8)	20% w/v Polyethylene glycol 3,350	44. (D8)	8.0
45. (D9)	0.2 M Lithium citrate tribasic tetrahydrate	45. (D9)	20% w/v Polyethylene glycol 3,350	45. (D9)	8.4
46. (D10)	0.2 M Sodium citrate tribasic dihydrate	46. (D10)	20% w/v Polyethylene glycol 3,350	46. (D10)	8.3
47. (D11)	0.2 M Potassium citrate tribasic monohydrate	47. (D11)	20% w/v Polyethylene glycol 3,350	47. (D11)	8.3
48. (D12)	0.2 M Ammonium citrate dibasic	48. (D12)	20% w/v Polyethylene glycol 3,350	48. (D12)	5.1



◊ Measured pH at 25 ° C

PEG/Ion HT contains ninety-six unique reagents. To determine the formulation of each reagent, simply read across the page.

HAMPTON RESEARCH
Solutions for Crystal Growth

Well #	Salt	Well #	Buffer ◊	Well #	Polymer
49. (E1)	0.1 M Sodium malonate pH 4.0	49. (E1)	None	49. (E1)	12% w/v Polyethylene glycol 3,350
50. (E2)	0.2 M Sodium malonate pH 4.0	50. (E2)	None	50. (E2)	20% w/v Polyethylene glycol 3,350
51. (E3)	0.1 M Sodium malonate pH 5.0	51. (E3)	None	51. (E3)	12% w/v Polyethylene glycol 3,350
52. (E4)	0.2 M Sodium malonate pH 5.0	52. (E4)	None	52. (E4)	20% w/v Polyethylene glycol 3,350
53. (E5)	0.1 M Sodium malonate pH 6.0	53. (E5)	None	53. (E5)	12% w/v Polyethylene glycol 3,350
54. (E6)	0.2 M Sodium malonate pH 6.0	54. (E6)	None	54. (E6)	20% w/v Polyethylene glycol 3,350
55. (E7)	0.1 M Sodium malonate pH 7.0	55. (E7)	None	55. (E7)	12% w/v Polyethylene glycol 3,350
56. (E8)	0.2 M Sodium malonate pH 7.0	56. (E8)	None	56. (E8)	20% w/v Polyethylene glycol 3,350
57. (E9)	4% v/v Tacsimate™ pH 4.0	57. (E9)	None	57. (E9)	12% w/v Polyethylene glycol 3,350
58. (E10)	8% v/v Tacsimate™ pH 4.0	58. (E10)	None	58. (E10)	20% w/v Polyethylene glycol 3,350
59. (E11)	4% v/v Tacsimate™ pH 5.0	59. (E11)	None	59. (E11)	12% w/v Polyethylene glycol 3,350
60. (E12)	8% v/v Tacsimate™ pH 5.0	60. (E12)	None	60. (E12)	20% w/v Polyethylene glycol 3,350
61. (F1)	4% v/v Tacsimate™ pH 6.0	61. (F1)	None	61. (F1)	12% w/v Polyethylene glycol 3,350
62. (F2)	8% v/v Tacsimate™ pH 6.0	62. (F2)	None	62. (F2)	20% w/v Polyethylene glycol 3,350
63. (F3)	4% v/v Tacsimate™ pH 7.0	63. (F3)	None	63. (F3)	12% w/v Polyethylene glycol 3,350
64. (F4)	8% v/v Tacsimate™ pH 7.0	64. (F4)	None	64. (F4)	20% w/v Polyethylene glycol 3,350
65. (F5)	4% v/v Tacsimate™ pH 8.0	65. (F5)	None	65. (F5)	12% w/v Polyethylene glycol 3,350
66. (F6)	8% v/v Tacsimate™ pH 8.0	66. (F6)	None	66. (F6)	20% w/v Polyethylene glycol 3,350
67. (F7)	0.1 M Succinic acid pH 7.0	67. (F7)	None	67. (F7)	12% w/v Polyethylene glycol 3,350
68. (F8)	0.2 M Succinic acid pH 7.0	68. (F8)	None	68. (F8)	20% w/v Polyethylene glycol 3,350
69. (F9)	0.1 M Ammonium citrate tribasic pH 7.0	69. (F9)	None	69. (F9)	12% w/v Polyethylene glycol 3,350
70. (F10)	0.2 M Ammonium citrate tribasic pH 7.0	70. (F10)	None	70. (F10)	20% w/v Polyethylene glycol 3,350
71. (F11)	0.1 M DL-Malic acid pH 7.0	71. (F11)	None	71. (F11)	12% w/v Polyethylene glycol 3,350
72. (F12)	0.2 M DL-Malic acid pH 7.0	72. (F12)	None	72. (F12)	20% w/v Polyethylene glycol 3,350
73. (G1)	0.1 M Sodium acetate trihydrate pH 7.0	73. (G1)	None	73. (G1)	12% w/v Polyethylene glycol 3,350
74. (G2)	0.2 M Sodium acetate trihydrate pH 7.0	74. (G2)	None	74. (G2)	20% w/v Polyethylene glycol 3,350
75. (G3)	0.1 M Sodium formate pH 7.0	75. (G3)	None	75. (G3)	12% w/v Polyethylene glycol 3,350
76. (G4)	0.2 M Sodium formate pH 7.0	76. (G4)	None	76. (G4)	20% w/v Polyethylene glycol 3,350
77. (G5)	0.1 M Ammonium tartrate dibasic pH 7.0	77. (G5)	None	77. (G5)	12% w/v Polyethylene glycol 3,350
78. (G6)	0.2 M Ammonium tartrate dibasic pH 7.0	78. (G6)	None	78. (G6)	20% w/v Polyethylene glycol 3,350
79. (G7)	2% v/v Tacsimate™ pH 4.0	79. (G7)	0.1 M Sodium acetate trihydrate pH 4.6	79. (G7)	16% w/v Polyethylene glycol 3,350
80. (G8)	2% v/v Tacsimate™ pH 5.0	80. (G8)	0.1 M Sodium citrate tribasic dihydrate pH 5.6	80. (G8)	16% w/v Polyethylene glycol 3,350
81. (G9)	2% v/v Tacsimate™ pH 6.0	81. (G9)	0.1 M BIS-TRIS pH 6.5	81. (G9)	20% w/v Polyethylene glycol 3,350
82. (G10)	2% v/v Tacsimate™ pH 7.0	82. (G10)	0.1 M HEPES pH 7.5	82. (G10)	20% w/v Polyethylene glycol 3,350
83. (G11)	2% v/v Tacsimate™ pH 8.0	83. (G11)	0.1 M Tris pH 8.5	83. (G11)	16% w/v Polyethylene glycol 3,350
84. (G12)	None	84. (G12)	0.07 M Citric acid, 0.03 M BIS-TRIS propane / pH 3.4	84. (G12)	16% w/v Polyethylene glycol 3,350
85. (H1)	None	85. (H1)	0.06 M Citric acid, 0.04 M BIS-TRIS propane / pH 4.1	85. (H1)	16% w/v Polyethylene glycol 3,350
86. (H2)	None	86. (H2)	0.05 M Citric acid, 0.05 M BIS-TRIS propane / pH 5.0	86. (H2)	16% w/v Polyethylene glycol 3,350
87. (H3)	None	87. (H3)	0.04 M Citric acid, 0.06 M BIS-TRIS propane / pH 6.4	87. (H3)	20% w/v Polyethylene glycol 3,350
88. (H4)	None	88. (H4)	0.03 M Citric acid, 0.07 M BIS-TRIS propane / pH 7.6	88. (H4)	20% w/v Polyethylene glycol 3,350
89. (H5)	None	89. (H5)	0.02 M Citric acid, 0.08 M BIS-TRIS propane / pH 8.8	89. (H5)	16% w/v Polyethylene glycol 3,350
90. (H6)	0.02 M Calcium chloride dihydrate, 0.02 M Cadmium chloride hydrate, 0.02 M Cobalt(II) chloride hexahydrate	90. (H6)	None	90. (H6)	20% w/v Polyethylene glycol 3,350
91. (H7)	0.01 M Magnesium chloride hexahydrate 0.005 M Nickel(II) chloride hexahydrate	91. (H7)	0.1 M HEPES sodium pH 7.0	91. (H7)	15% w/v Polyethylene glycol 3,350
92. (H8)	0.02 M Zinc chloride	92. (H8)	None	92. (H8)	20% w/v Polyethylene glycol 3,350
93. (H9)	0.15 M Cesium chloride	93. (H9)	None	93. (H9)	15% w/v Polyethylene glycol 3,350
94. (H10)	0.2 M Sodium bromide	94. (H10)	None	94. (H10)	20% w/v Polyethylene glycol 3,350
95. (H11)	1% w/v Tryptone, 0.001 M Sodium azide	95. (H11)	0.05 M HEPES sodium pH 7.0	95. (H11)	12% w/v Polyethylene glycol 3,350
96. (H12)	1% w/v Tryptone, 0.001 M Sodium azide	96. (H12)	0.05 M HEPES sodium pH 7.0	96. (H12)	20% w/v Polyethylene glycol 3,350

◊ Buffer pH is that of a 1.0 M stock prior to dilution with other reagent components: pH with HCl or NaOH.

PEG/Ion HT contains ninety-six unique reagents. To determine the formulation of each reagent, simply read across the page.

34 Journey
Aliso Viejo, CA 92656-3317 U.S.A.
Tel: (949) 425-1321 • Fax: (949) 425-1611
E-mail: tech@hrmail.com
Website: www.hamptonresearch.com

HAMPTON RESEARCH

Solutions for Crystal Growth

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)

HAMPTON RESEARCH
Solutions for Crystal Growth

34 Journey
Aliso Viejo, CA 92656-3317 U.S.A.
Tel: (949) 425-1321 • Fax: (949) 425-1611
e-mail: tech@hrmail.com
Website: www.hamptonresearch.com

PEG/Ion HT™ - HR2-139 Scoring Sheet	Date:	Date:	Date:	Date:
1. (A1) 0.2 M Sodium fluoride, 20% w/v Polyethylene glycol 3,350				
2. (A2) 0.2 M Potassium fluoride, 20% w/v Polyethylene glycol 3,350				
3. (A3) 0.2 M Ammonium fluoride, 20% w/v Polyethylene glycol 3,350				
4. (A4) 0.2 M Lithium chloride, 20% w/v Polyethylene glycol 3,350				
5. (A5) 0.2 M Magnesium chloride hexahydrate, 20% w/v Polyethylene glycol 3,350				
6. (A6) 0.2 M Sodium chloride, 20% w/v Polyethylene glycol 3,350				
7. (A7) 0.2 M Calcium chloride dihydrate, 20% w/v Polyethylene glycol 3,350				
8. (A8) 0.2 M Potassium chloride, 20% w/v Polyethylene glycol 3,350				
9. (A9) 0.2 M Ammonium chloride, 20% w/v Polyethylene glycol 3,350				
10. (A10) 0.2 M Sodium iodide, 20% w/v Polyethylene glycol 3,350				
11. (A11) 0.2 M Potassium iodide, 20% w/v Polyethylene glycol 3,350				
12. (A12) 0.2 M Ammonium iodide, 20% w/v Polyethylene glycol 3,350				
13. (B1) 0.2 M Sodium thiocyanate, 20% w/v Polyethylene glycol 3,350				
14. (B2) 0.2 M Potassium thiocyanate, 20% w/v Polyethylene glycol 3,350				
15. (B3) 0.2 M Lithium nitrate, 20% w/v Polyethylene glycol 3,350				
16. (B4) 0.2 M Magnesium nitrate hexahydrate, 20% w/v Polyethylene glycol 3,350				
17. (B5) 0.2 M Sodium nitrate, 20% w/v Polyethylene glycol 3,350				
18. (B6) 0.2 M Potassium nitrate, 20% w/v Polyethylene glycol 3,350				
19. (B7) 0.2 M Ammonium nitrate, 20% w/v Polyethylene glycol 3,350				
20. (B8) 0.2 M Magnesium formate dihydrate, 20% w/v Polyethylene glycol 3,350				
21. (B9) 0.2 M Sodium formate, 20% w/v Polyethylene glycol 3,350				
22. (B10) 0.2 M Potassium formate, 20% w/v Polyethylene glycol 3,350				
23. (B11) 0.2 M Ammonium formate, 20% w/v Polyethylene glycol 3,350				
24. (B12) 0.2 M Lithium acetate dihydrate, 20% w/v Polyethylene glycol 3,350				
25. (C1) 0.2 M Magnesium acetate tetrahydrate, 20% w/v Polyethylene glycol 3,350				
26. (C2) 0.2 M Zinc acetate dihydrate, 20% w/v Polyethylene glycol 3,350				
27. (C3) 0.2 M Sodium acetate trihydrate, 20% w/v Polyethylene glycol 3,350				
28. (C4) 0.2 M Calcium acetate hydrate, 20% w/v Polyethylene glycol 3,350				
29. (C5) 0.2 M Potassium acetate, 20% w/v Polyethylene glycol 3,350				
30. (C6) 0.2 M Ammonium acetate, 20% w/v Polyethylene glycol 3,350				
31. (C7) 0.2 M Lithium sulfate monohydrate, 20% w/v Polyethylene glycol 3,350				
32. (C8) 0.2 M Magnesium sulfate heptahydrate, 20% w/v Polyethylene glycol 3,350				
33. (C9) 0.2 M Sodium sulfate decahydrate, 20% w/v Polyethylene glycol 3,350				
34. (C10) 0.2 M Potassium sulfate, 20% w/v Polyethylene glycol 3,350				
35. (C11) 0.2 M Ammonium sulfate, 20% w/v Polyethylene glycol 3,350				
36. (C12) 0.2 M Sodium tartrate dibasic dihydrate, 20% w/v Polyethylene glycol 3,350				
37. (D1) 0.2 M Potassium sodium tartrate tetrahydrate, 20% w/v Polyethylene glycol 3,350				
38. (D2) 0.2 M Ammonium tartrate dibasic, 20% w/v Polyethylene glycol 3,350				
39. (D3) 0.2 M Sodium phosphate monobasic monohydrate, 20% w/v Polyethylene glycol 3,350				
40. (D4) 0.2 M Sodium phosphate dibasic dihydrate, 20% w/v Polyethylene glycol 3,350				
41. (D5) 0.2 M Potassium phosphate monobasic, 20% w/v Polyethylene glycol 3,350				
42. (D6) 0.2 M Potassium phosphate dibasic, 20% w/v Polyethylene glycol 3,350				
43. (D7) 0.2 M Ammonium phosphate monobasic, 20% w/v Polyethylene glycol 3,350				
44. (D8) 0.2 M Ammonium phosphate dibasic, 20% w/v Polyethylene glycol 3,350				
45. (D9) 0.2 M Lithium citrate tribasic tetrahydrate, 20% w/v Polyethylene glycol 3,350				
46. (D10) 0.2 M Sodium citrate tribasic dihydrate, 20% w/v Polyethylene glycol 3,350				
47. (D11) 0.2 M Potassium citrate tribasic monohydrate, 20% w/v Polyethylene glycol 3,350				
48. (D12) 0.2 M Ammonium citrate dibasic, 20% w/v Polyethylene glycol 3,350				

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/Ion HT™ - HR2-139 Scoring Sheet

	Date:	Date:	Date:	Date:
49. (E1) 0.1 M Sodium malonate pH 4.0, 12% w/v Polyethylene glycol 3,350				
50. (E2) 0.2 M Sodium malonate pH 4.0, 20% w/v Polyethylene glycol 3,350				
51. (E3) 0.1 M Sodium malonate pH 5.0, 12% w/v Polyethylene glycol 3,350				
52. (E4) 0.2 M Sodium malonate pH 5.0, 20% w/v Polyethylene glycol 3,350				
53. (E5) 0.1 M Sodium malonate pH 6.0, 12% w/v Polyethylene glycol 3,350				
54. (E6) 0.2 M Sodium malonate pH 6.0, 20% w/v Polyethylene glycol 3,350				
55. (E7) 0.1 M Sodium malonate pH 7.0, 12% w/v Polyethylene glycol 3,350				
56. (E8) 0.2 M Sodium malonate pH 7.0, 20% w/v Polyethylene glycol 3,350				
57. (E9) 4% v/v Tacsimate™ pH 4.0, 12% w/v Polyethylene glycol 3,350				
58. (E10) 8% v/v Tacsimate™ pH 4.0, 20% w/v Polyethylene glycol 3,350				
59. (E11) 4% v/v Tacsimate™ pH 5.0, 12% w/v Polyethylene glycol 3,350				
60. (E12) 8% v/v Tacsimate™ pH 5.0, 20% w/v Polyethylene glycol 3,350				
61. (F1) 4% v/v Tacsimate™ pH 6.0, 12% w/v Polyethylene glycol 3,350				
62. (F2) 8% v/v Tacsimate™ pH 6.0, 20% w/v Polyethylene glycol 3,350				
63. (F3) 4% v/v Tacsimate™ pH 7.0, 12% w/v Polyethylene glycol 3,350				
64. (F4) 8% v/v Tacsimate™ pH 7.0, 20% w/v Polyethylene glycol 3,350				
65. (F5) 4% v/v Tacsimate™ pH 8.0, 12% w/v Polyethylene glycol 3,350				
66. (F6) 8% v/v Tacsimate™ pH 8.0, 20% w/v Polyethylene glycol 3,350				
67. (F7) 0.1 M Succinic acid pH 7.0, 12% w/v Polyethylene glycol 3,350				
68. (F8) 0.2 M Succinic acid pH 7.0, 20% w/v Polyethylene glycol 3,350				
69. (F9) 0.1 M Ammonium citrate tribasic pH 7.0, 12% w/v Polyethylene glycol 3,350				
70. (F10) 0.2 M Ammonium citrate tribasic pH 7.0, 20% w/v Polyethylene glycol 3,350				
71. (F11) 0.1 M DL-Malic acid pH 7.0, 12% w/v Polyethylene glycol 3,350				
72. (F12) 0.2 M DL-Malic acid pH 7.0, 20% w/v Polyethylene glycol 3,350				
73. (G1) 0.1 M Sodium acetate trihydrate pH 7.0, 12% w/v Polyethylene glycol 3,350				
74. (G2) 0.2 M Sodium acetate trihydrate pH 7.0, 20% w/v Polyethylene glycol 3,350				
75. (G3) 0.1 M Sodium formate pH 7.0, 12% w/v Polyethylene glycol 3,350				
76. (G4) 0.2 M Sodium formate pH 7.0, 20% w/v Polyethylene glycol 3,350				
77. (G5) 0.1 M Ammonium tartrate dibasic pH 7.0, 12% w/v Polyethylene glycol 3,350				
78. (G6) 0.2 M Ammonium tartrate dibasic pH 7.0, 20% w/v Polyethylene glycol 3,350				
79. (G7) 2% v/v Tacsimate™ pH 4.0, 0.1 M Sodium acetate trihydrate pH 4.6, 16% w/v Polyethylene glycol 3,350				
80. (G8) 2% v/v Tacsimate™ pH 5.0, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 16% w/v Polyethylene glycol 3,350				
81. (G9) 2% v/v Tacsimate™ pH 6.0, 0.1 M BIS-TRIS pH 6.5, 20% w/v Polyethylene glycol 3,350				
82. (G10) 2% v/v Tacsimate™ pH 7.0, 0.1 M HEPES pH 7.5, 20% w/v Polyethylene glycol 3,350				
83. (G11) 2% v/v Tacsimate™ pH 8.0, 0.1 M Tris pH 8.5, 16% w/v Polyethylene glycol 3,350				
84. (G12) (0.07 M Citric acid, 0.03 M BIS-TRIS propane / pH 3.4), 16% w/v Polyethylene glycol 3,350				
85. (H1) (0.06 M Citric acid, 0.04 M BIS-TRIS propane / pH 4.1), 16% w/v Polyethylene glycol 3,350				
86. (H2) (0.05 M Citric acid, 0.05 M BIS-TRIS propane / pH 5.0), 16% w/v Polyethylene glycol 3,350				
87. (H3) (0.04 M Citric acid, 0.06 M BIS-TRIS propane / pH 6.4), 20% w/v Polyethylene glycol 3,350				
88. (H4) (0.03 M Citric acid, 0.07 M BIS-TRIS propane / pH 7.6), 20% w/v Polyethylene glycol 3,350				
89. (H5) (0.02 M Citric acid, 0.08 M BIS-TRIS propane / pH 8.8), 16% w/v Polyethylene glycol 3,350				
90. (H6) 0.02 M Calcium chloride dihydrate, 0.02 M Cadmium chloride hydrate,				
0.02 M Cobalt(II) chloride hexahydrate, 20% w/v Polyethylene glycol 3,350				
91. (H7) 0.01 M Magnesium chloride hexahydrate, 0.005 M Nickel(II) chloride hexahydrate				
0.1 M HEPES sodium pH 7.0, 15% w/v Polyethylene glycol 3,350				
92. (H8) 0.02 M Zinc chloride, 20% w/v Polyethylene glycol 3,350				
93. (H9) 0.15 M Cesium chloride, 15% w/v Polyethylene glycol 3,350				
94. (H10) 0.2 M Sodium bromide, 20% w/v Polyethylene glycol 3,350				
95. (H11) 1% w/v Tryptone, 0.001 M Sodium azide, 0.05 M HEPES sodium pH 7.0, 12% w/v Polyethylene glycol 3,350				
96. (H12) 1% w/v Tryptone, 0.001 M Sodium azide, 0.05 M HEPES sodium pH 7.0, 20% w/v Polyethylene glycol 3,350				

HAMPTON RESEARCH
Solutions for Crystal Growth
34 Journey
Aliso Viejo, CA 92656-3317 U.S.A.
Tel: (949) 425-1321 • Fax: (949) 425-1611
e-mail: tech@hrmail.com
Website: www.hamptonresearch.com