PEG/Ion 400™

HAMPTON RESEARCH
Solutions for Crystal Growth

Applications
Crystallization screen for membrane and soluble biological macromolecules.

Features
- Reagent formulation developed at Hampton Research
- Screens a profile of anions, cations, multivalent ions, and neutralized organic acids at varying pH levels in the presence of Polyethylene glycol 400
- pH range 5 - 9
- Buffer free formulation
- Compatible with lipidic cubic phase (LCP), vapor diffusion, microbatch, and free interface diffusion

General Description
PEG/Ion 400 is a crystallization reagent kit designed to provide a rapid screening method for the crystallization of membrane and soluble biological macromolecules. Most G protein-coupled receptors (GPCRs) have been crystallized in Polyethylene glycol (PEG) 400 based reagents.[1] PEG/Ion 400 is designed as a 96 reagent crystallization screen that is compatible with the Lipidic Cubic Phase (LCP).

PEG/Ion 400 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 400 is supplied with an adhesive sealing film which can be used to seal the block after removing the heat seal.

The screen combines a single concentration (30% v/v) of high purity Polyethylene glycol 400 and 48 different high purity salts, comprising both anions (malate, malonate, nitrate, phosphate, succinate, sulfate, tartrate, and thiocyanate) and cations (ammonium, cadmium, calcium, ethylammonium, lithium, magnesium, nickel, potassium, sodium, tetraethylammonium, and zinc) in two concentrations (0.1 and 0.4 M) which due to their unique pH characteristics also affords a reasonable pH screen (approximate pH range of 4 to 9.5). The primary screen variables are PEG, ion type, ionic strength, salt concentration, and pH.

Refer to the enclosed PEG/Ion 400 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. The recommended sample concentration is 5 to 80 mg/ml in sample buffer.

Preparing the Deep Well Block for Use
Allow the Deep Well Block and reagents to stabilize at room temperature. If reagents precipitate during cold storage, warm the sealed block at up to 50°C for up to 60 minutes, inverting the block several times to solubilize the reagents. Centrifuge the block 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 and pierced to access reagents. For storage, reseal using AlumaSeal II Sealing Film.

Performing The Screen
Lipidic Cubic Phase or in meso method
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 LCP Glass Sandwich Set, dispense the recommended volume of protein laden mesophase (typically 30-50 nl) onto position A1 of the glass base plate.

2. Dispense the desired volume of crystallization reagent (typically 600-800 nl) from the Deep Well block onto the protein laden mesophase drop in position A1 of the glass plate. A properly dispensed drop should appear as a fried egg on a pan, the mesophase appears as the yolk of the egg, the reagent appears as the white of the egg, and the perimeter of the well appears as the edge of the frying pan.

3. Repeat steps 1 and 2 for the remaining 95 wells, A2-H12.

4. Seal the crystallization plate using glass coverslip. 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.

Sitting Drop Vapor Diffusion method
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.

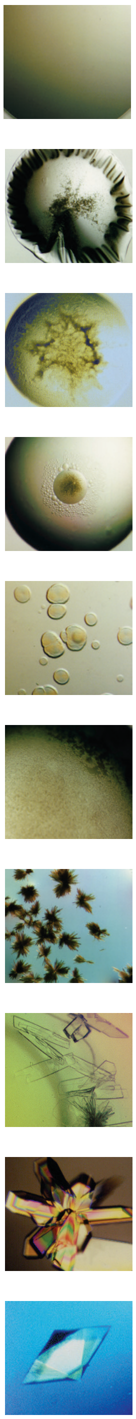
1. Using a 96 well sitting drop vapor diffusion plate, dispense the recommended volume (typically 50 to 100 microliters) of crystallization reagent from the Deep Well block into the reagent reservoirs of the crystallization plate.

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.

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

5. 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) 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 1 shows typical examples of what one might observe in a crystallization experiment.

Interpreting PEG/Ion 400

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 by varying PEG 400 concentration, salt concentration, pH, sample concentration, temperature, screening additives, as well as evaluating other crystallization variables in order to achieve crystals with the desired characteristics.

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 400 Formulation & Storage

Each of the reagents is 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 sterile filtered using a 0.2 micron filter. Recommended storage for PEG/Ion 400 is -20°C. Best if used within 12 months of receipt.

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. Successful Strategies to Determine High-Resolution Structures of GPCRs. Xiang J, Chun E, Liu C, Jing L, Al-Sahouri Z, Zhu L, Liu W. Trends Pharmacol Sci. 2016 Dec;37(12):1055-1069.
2. Development of an Automated High Throughput LCP-FRAP Assay to Guide Membrane Protein Crystallization in Lipid Mesophases. Fei Xu, Wei Liu, Michael A. Hanson, Raymond C. Stevens, and Vadim Cherezov. Cryst. Growth Des., 2011, 11 (4), pp 1193–1201.
3. A comprehensive review of the lipid cubic phase or in meso method for crystallizing membrane and soluble proteins and complexes. Martin Caffrey. Acta Crystallogr F Struct Biol Commun. 2015 Jan 1; 71(Pt 1): 3–18.

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Well #	Salt	Well #	Polymer	Well #	pH ◊
1. (A01)	0.1 M Ammonium acetate	1. (A01)	30% v/v Polyethylene glycol 400	1. (A01)	7.1
2. (A02)	0.4 M Ammonium acetate	2. (A02)	30% v/v Polyethylene glycol 400	2. (A02)	7.3
3. (A03)	0.1 M Calcium acetate hydrate	3. (A03)	30% v/v Polyethylene glycol 400	3. (A03)	7.2
4. (A04)	0.4 M Calcium acetate hydrate	4. (A04)	30% v/v Polyethylene glycol 400	4. (A04)	7.4
5. (A05)	0.1 M Lithium acetate dihydrate	5. (A05)	30% v/v Polyethylene glycol 400	5. (A05)	7.2
6. (A06)	0.4 M Lithium acetate dihydrate	6. (A06)	30% v/v Polyethylene glycol 400	6. (A06)	7.5
7. (A07)	0.1 M Magnesium acetate tetrahydrate	7. (A07)	30% v/v Polyethylene glycol 400	7. (A07)	7.2
8. (A08)	0.4 M Magnesium acetate tetrahydrate	8. (A08)	30% v/v Polyethylene glycol 400	8. (A08)	7.3
9. (A09)	0.1 M Potassium acetate	9. (A09)	30% v/v Polyethylene glycol 400	9. (A09)	7.3
10. (A10)	0.4 M Potassium acetate	10. (A10)	30% v/v Polyethylene glycol 400	10. (A10)	7.7
11. (A11)	0.1 M Sodium acetate trihydrate	11. (A11)	30% v/v Polyethylene glycol 400	11. (A11)	7.2
12. (A12)	0.4 M Sodium acetate trihydrate	12. (A12)	30% v/v Polyethylene glycol 400	12. (A12)	7.6
13. (B01)	0.1 M Zinc acetate dihydrate	13. (B01)	30% v/v Polyethylene glycol 400	13. (B01)	6.5
14. (B02)	0.4 M Zinc acetate dihydrate	14. (B02)	30% v/v Polyethylene glycol 400	14. (B02)	6.1
15. (B03)	0.1 M Tetraethylammonium bromide	15. (B03)	30% v/v Polyethylene glycol 400	15. (B03)	6.0
16. (B04)	0.4 M Tetraethylammonium bromide	16. (B04)	30% v/v Polyethylene glycol 400	16. (B04)	6.1
17. (B05)	0.1 M Ammonium chloride	17. (B05)	30% v/v Polyethylene glycol 400	17. (B05)	5.9
18. (B06)	0.4 M Ammonium chloride	18. (B06)	30% v/v Polyethylene glycol 400	18. (B06)	5.8
19. (B07)	0.1 M Cadmium chloride hydrate	19. (B07)	30% v/v Polyethylene glycol 400	19. (B07)	5.4
20. (B08)	0.4 M Cadmium chloride hydrate	20. (B08)	30% v/v Polyethylene glycol 400	20. (B08)	4.9
21. (B09)	0.1 M Calcium chloride dihydrate	21. (B09)	30% v/v Polyethylene glycol 400	21. (B09)	5.7
22. (B10)	0.4 M Calcium chloride dihydrate	22. (B10)	30% v/v Polyethylene glycol 400	22. (B10)	5.3
23. (B11)	0.1 M Lithium chloride	23. (B11)	30% v/v Polyethylene glycol 400	23. (B11)	5.9
24. (B12)	0.4 M Lithium chloride	24. (B12)	30% v/v Polyethylene glycol 400	24. (B12)	5.8
25. (C01)	0.1 M Magnesium chloride hexahydrate	25. (C01)	30% v/v Polyethylene glycol 400	25. (C01)	5.7
26. (C02)	0.4 M Magnesium chloride hexahydrate	26. (C02)	30% v/v Polyethylene glycol 400	26. (C02)	5.3
27. (C03)	0.1 M Nickel(II) chloride hexahydrate	27. (C03)	30% v/v Polyethylene glycol 400	27. (C03)	5.5
28. (C04)	0.4 M Nickel(II) chloride hexahydrate	28. (C04)	30% v/v Polyethylene glycol 400	28. (C04)	5.0
29. (C05)	0.1 M Potassium chloride	29. (C05)	30% v/v Polyethylene glycol 400	29. (C05)	6.0
30. (C06)	0.4 M Potassium chloride	30. (C06)	30% v/v Polyethylene glycol 400	30. (C06)	5.9
31. (C07)	0.1 M Sodium chloride	31. (C07)	30% v/v Polyethylene glycol 400	31. (C07)	6.0
32. (C08)	0.4 M Sodium chloride	32. (C08)	30% v/v Polyethylene glycol 400	32. (C08)	5.8
33. (C09)	0.1 M Ammonium citrate dibasic	33. (C09)	30% v/v Polyethylene glycol 400	33. (C09)	5.6
34. (C10)	0.4 M Ammonium citrate dibasic	34. (C10)	30% v/v Polyethylene glycol 400	34. (C10)	5.3
35. (C11)	0.1 M Ammonium citrate tribasic pH 7.0	35. (C11)	30% v/v Polyethylene glycol 400	35. (C11)	7.3
36. (C12)	0.4 M Ammonium citrate tribasic pH 7.0	36. (C12)	30% v/v Polyethylene glycol 400	36. (C12)	7.2
37. (D01)	0.1 M Lithium citrate tribasic tetrahydrate	37. (D01)	30% v/v Polyethylene glycol 400	37. (D01)	7.6
38. (D02)	0.4 M Lithium citrate tribasic tetrahydrate	38. (D02)	30% v/v Polyethylene glycol 400	38. (D02)	7.7
39. (D03)	0.1 M Potassium citrate tribasic monohydrate	39. (D03)	30% v/v Polyethylene glycol 400	39. (D03)	7.9
40. (D04)	0.4 M Potassium citrate tribasic monohydrate	40. (D04)	30% v/v Polyethylene glycol 400	40. (D04)	8.2
41. (D05)	0.1 M Sodium citrate tribasic dihydrate	41. (D05)	30% v/v Polyethylene glycol 400	41. (D05)	7.7
42. (D06)	0.4 M Sodium citrate tribasic dihydrate	42. (D06)	30% v/v Polyethylene glycol 400	42. (D06)	7.8
43. (D07)	0.1 M Ammonium formate	43. (D07)	30% v/v Polyethylene glycol 400	43. (D07)	6.4
44. (D08)	0.4 M Ammonium formate	44. (D08)	30% v/v Polyethylene glycol 400	44. (D08)	6.6
45. (D09)	0.1 M Magnesium formate dihydrate	45. (D09)	30% v/v Polyethylene glycol 400	45. (D09)	6.4
46. (D10)	0.4 M Magnesium formate dihydrate	46. (D10)	30% v/v Polyethylene glycol 400	46. (D10)	6.5
47. (D11)	0.1 M Potassium formate	47. (D11)	30% v/v Polyethylene glycol 400	47. (D11)	6.4
48. (D12)	0.4 M Potassium formate	48. (D12)	30% v/v Polyethylene glycol 400	48. (D12)	6.7

◊ Measured pH at 25 ° C

PEG/Ion 400 contains ninety-six unique reagents. To determine the formulation of each reagent, simply read across the page.

Well #	Salt	Well #	Polymer	Well #	pH ◊
49. (E01)	0.1 M Sodium formate	49. (E01)	30% v/v Polyethylene glycol 400	49. (E01)	6.5
50. (E02)	0.4 M Sodium formate	50. (E02)	30% v/v Polyethylene glycol 400	50. (E02)	6.8
51. (E03)	0.1 M DL-Malic acid pH 7.0	51. (E03)	30% v/v Polyethylene glycol 400	51. (E03)	7.1
52. (E04)	0.4 M DL-Malic acid pH 7.0	52. (E04)	30% v/v Polyethylene glycol 400	52. (E04)	7.1
53. (E05)	0.1 M Sodium malonate pH 7.0	53. (E05)	30% v/v Polyethylene glycol 400	53. (E05)	7.6
54. (E06)	0.4 M Sodium malonate pH 7.0	54. (E06)	30% v/v Polyethylene glycol 400	54. (E06)	7.5
55. (E07)	0.1 M Ammonium nitrate	55. (E07)	30% v/v Polyethylene glycol 400	55. (E07)	6.0
56. (E08)	0.1 M Ammonium nitrate	56. (E08)	30% v/v Polyethylene glycol 400	56. (E08)	5.8
57. (E09)	0.1 M Ethylammonium nitrate	57. (E09)	30% v/v Polyethylene glycol 400	57. (E09)	6.0
58. (E10)	0.4 M Ethylammonium nitrate	58. (E10)	30% v/v Polyethylene glycol 400	58. (E10)	5.8
59. (E11)	0.1 M Lithium nitrate	59. (E11)	30% v/v Polyethylene glycol 400	59. (E11)	5.9
60. (E12)	0.4 M Lithium nitrate	60. (E12)	30% v/v Polyethylene glycol 400	60. (E12)	5.7
61. (F01)	0.1 M Magnesium nitrate hexahydrate	61. (F01)	30% v/v Polyethylene glycol 400	61. (F01)	5.7
62. (F02)	0.4 M Magnesium nitrate hexahydrate	62. (F02)	30% v/v Polyethylene glycol 400	62. (F02)	5.3
63. (F03)	0.1 M Sodium nitrate	63. (F03)	30% v/v Polyethylene glycol 400	63. (F03)	6.0
64. (F04)	0.4 M Sodium nitrate	64. (F04)	30% v/v Polyethylene glycol 400	64. (F04)	5.8
65. (F05)	0.1 M Ammonium phosphate dibasic	65. (F05)	30% v/v Polyethylene glycol 400	65. (F05)	8.2
66. (F06)	0.4 M Ammonium phosphate dibasic	66. (F06)	30% v/v Polyethylene glycol 400	66. (F06)	8.2
67. (F07)	0.1 M Ammonium phosphate monobasic	67. (F07)	30% v/v Polyethylene glycol 400	67. (F07)	5.4
68. (F08)	0.4 M Ammonium phosphate monobasic	68. (F08)	30% v/v Polyethylene glycol 400	68. (F08)	4.8
69. (F09)	0.1 M Potassium phosphate dibasic	69. (F09)	30% v/v Polyethylene glycol 400	69. (F09)	9.0
70. (F10)	0.4 M Potassium phosphate dibasic	70. (F10)	30% v/v Polyethylene glycol 400	70. (F10)	9.3
71. (F11)	0.1 M Potassium phosphate monobasic	71. (F11)	30% v/v Polyethylene glycol 400	71. (F11)	5.5
72. (F12)	0.4 M Potassium phosphate monobasic	72. (F12)	30% v/v Polyethylene glycol 400	72. (F12)	5.0
73. (G01)	0.1 M Sodium potassium phosphate pH 7.0	73. (G01)	30% v/v Polyethylene glycol 400	73. (G01)	7.7
74. (G02)	0.4 M Sodium potassium phosphate pH 7.0	74. (G02)	30% v/v Polyethylene glycol 400	74. (G02)	7.4
75. (G03)	0.1 M Sodium phosphate monobasic monohydrate	75. (G03)	30% v/v Polyethylene glycol 400	75. (G03)	5.5
76. (G04)	0.4 M Sodium phosphate monobasic monohydrate	76. (G04)	30% v/v Polyethylene glycol 400	76. (G04)	4.9
77. (G05)	0.1 M Succinic acid pH 7.0	77. (G05)	30% v/v Polyethylene glycol 400	77. (G05)	7.4
78. (G06)	0.4 M Succinic acid pH 7.0	78. (G06)	30% v/v Polyethylene glycol 400	78. (G06)	7.4
79. (G07)	0.1 M Ammonium sulfate	79. (G07)	30% v/v Polyethylene glycol 400	79. (G07)	5.7
80. (G08)	0.4 M Ammonium sulfate	80. (G08)	30% v/v Polyethylene glycol 400	80. (G08)	5.6
81. (G09)	0.1 M Lithium sulfate monohydrate	81. (G09)	30% v/v Polyethylene glycol 400	81. (G09)	5.8
82. (G10)	0.4 M Lithium sulfate monohydrate	82. (G10)	30% v/v Polyethylene glycol 400	82. (G10)	5.5
83. (G11)	0.1 M Magnesium sulfate hydrate	83. (G11)	30% v/v Polyethylene glycol 400	83. (G11)	5.9
84. (G12)	0.4 M Magnesium sulfate hydrate	84. (G12)	30% v/v Polyethylene glycol 400	84. (G12)	5.6
85. (H01)	0.1 M Ammonium tartrate dibasic	85. (H01)	30% v/v Polyethylene glycol 400	85. (H01)	6.5
86. (H02)	0.4 M Ammonium tartrate dibasic	86. (H02)	30% v/v Polyethylene glycol 400	86. (H02)	6.6
87. (H03)	0.1 M Potassium sodium tartrate tetrahydrate	87. (H03)	30% v/v Polyethylene glycol 400	87. (H03)	6.7
88. (H04)	0.4 M Potassium sodium tartrate tetrahydrate	88. (H04)	30% v/v Polyethylene glycol 400	88. (H04)	7.0
89. (H05)	0.1 M Sodium tartrate dibasic dihydrate	89. (H05)	30% v/v Polyethylene glycol 400	89. (H05)	6.6
90. (H06)	0.4 M Sodium tartrate dibasic dihydrate	90. (H06)	30% v/v Polyethylene glycol 400	90. (H06)	6.9
91. (H07)	0.1 M Potassium thiocyanate	91. (H07)	30% v/v Polyethylene glycol 400	91. (H07)	6.1
92. (H08)	0.4 M Potassium thiocyanate	92. (H08)	30% v/v Polyethylene glycol 400	92. (H08)	6.0
93. (H09)	0.1 M Sodium thiocyanate	93. (H09)	30% v/v Polyethylene glycol 400	93. (H09)	6.1
94. (H10)	0.4 M Sodium thiocyanate	94. (H10)	30% v/v Polyethylene glycol 400	94. (H10)	6.1
95. (H11)	5% v/v Tacsimate™ pH 7.0	95. (H11)	30% v/v Polyethylene glycol 400	95. (H11)	7.4
96. (H12)	20% v/v Tacsimate™ pH 7.0	96. (H12)	30% v/v Polyethylene glycol 400	96. (H12)	7.4

◊ Measured pH at 25 ° C

PEG/Ion 400 contains ninety-six unique reagents.
To determine the formulation of each reagent, simply read across the page.

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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)

PEG/Ion 400™ - HR2-460 Scoring Sheet		Date:	Date:	Date:	Date:
1. (A01)	0.1 M Ammonium acetate, 30% v/v Polyethylene glycol 400				
2. (A02)	0.4 M Ammonium acetate, 30% v/v Polyethylene glycol 400				
3. (A03)	0.1 M Calcium acetate hydrate, 30% v/v Polyethylene glycol 400				
4. (A04)	0.4 M Calcium acetate hydrate, 30% v/v Polyethylene glycol 400				
5. (A05)	0.1 M Lithium acetate dihydrate, 30% v/v Polyethylene glycol 400				
6. (A06)	0.4 M Lithium acetate dihydrate, 30% v/v Polyethylene glycol 400				
7. (A07)	0.1 M Magnesium acetate tetrahydrate, 30% v/v Polyethylene glycol 400				
8. (A08)	0.4 M Magnesium acetate tetrahydrate, 30% v/v Polyethylene glycol 400				
9. (A09)	0.1 M Potassium acetate, 30% v/v Polyethylene glycol 400				
10. (A10)	0.4 M Potassium acetate, 30% v/v Polyethylene glycol 400				
11. (A11)	0.1 M Sodium acetate trihydrate, 30% v/v Polyethylene glycol 400				
12. (A12)	0.4 M Sodium acetate trihydrate, 30% v/v Polyethylene glycol 400				
13. (B01)	0.1 M Zinc acetate dihydrate, 30% v/v Polyethylene glycol 400				
14. (B02)	0.4 M Zinc acetate dihydrate, 30% v/v Polyethylene glycol 400				
15. (B03)	0.1 M Tetraethylammonium bromide, 30% v/v Polyethylene glycol 400				
16. (B04)	0.4 M Tetraethylammonium bromide, 30% v/v Polyethylene glycol 400				
17. (B05)	0.1 M Ammonium chloride, 30% v/v Polyethylene glycol 400				
18. (B06)	0.4 M Ammonium chloride, 30% v/v Polyethylene glycol 400				
19. (B07)	0.1 M Cadmium chloride hydrate, 30% v/v Polyethylene glycol 400				
20. (B08)	0.4 M Cadmium chloride hydrate, 30% v/v Polyethylene glycol 400				
21. (B09)	0.1 M Calcium chloride dihydrate, 30% v/v Polyethylene glycol 400				
22. (B10)	0.4 M Calcium chloride dihydrate, 30% v/v Polyethylene glycol 400				
23. (B11)	0.1 M Lithium chloride, 30% v/v Polyethylene glycol 400				
24. (B12)	0.4 M Lithium chloride, 30% v/v Polyethylene glycol 400				
25. (C01)	0.1 M Magnesium chloride hexahydrate, 30% v/v Polyethylene glycol 400				
26. (C02)	0.4 M Magnesium chloride hexahydrate, 30% v/v Polyethylene glycol 400				
27. (C03)	0.1 M Nickel(II) chloride hexahydrate, 30% v/v Polyethylene glycol 400				
28. (C04)	0.4 M Nickel(II) chloride hexahydrate, 30% v/v Polyethylene glycol 400				
29. (C05)	0.1 M Potassium chloride, 30% v/v Polyethylene glycol 400				
30. (C06)	0.4 M Potassium chloride, 30% v/v Polyethylene glycol 400				
31. (C07)	0.1 M Sodium chloride, 30% v/v Polyethylene glycol 400				
32. (C08)	0.4 M Sodium chloride, 30% v/v Polyethylene glycol 400				
33. (C09)	0.1 M Ammonium citrate dibasic, 30% v/v Polyethylene glycol 400				
34. (C10)	0.4 M Ammonium citrate dibasic, 30% v/v Polyethylene glycol 400				
35. (C11)	0.1 M Ammonium citrate tribasic pH 7.0, 30% v/v Polyethylene glycol 400				
36. (C12)	0.4 M Ammonium citrate tribasic pH 7.0, 30% v/v Polyethylene glycol 400				
37. (D01)	0.1 M Lithium citrate tribasic tetrahydrate, 30% v/v Polyethylene glycol 400				
38. (D02)	0.4 M Lithium citrate tribasic tetrahydrate, 30% v/v Polyethylene glycol 400				
39. (D03)	0.1 M Potassium citrate tribasic monohydrate, 30% v/v Polyethylene glycol 400				
40. (D04)	0.4 M Potassium citrate tribasic monohydrate, 30% v/v Polyethylene glycol 400				
41. (D05)	0.1 M Sodium citrate tribasic dihydrate, 30% v/v Polyethylene glycol 400				
42. (D06)	0.4 M Sodium citrate tribasic dihydrate, 30% v/v Polyethylene glycol 400				
43. (D07)	0.1 M Ammonium formate, 30% v/v Polyethylene glycol 400				
44. (D08)	0.4 M Ammonium formate, 30% v/v Polyethylene glycol 400				
45. (D09)	0.1 M Magnesium formate dihydrate, 30% v/v Polyethylene glycol 400				
46. (D10)	0.4 M Magnesium formate dihydrate, 30% v/v Polyethylene glycol 400				
47. (D11)	0.1 M Potassium formate, 30% v/v Polyethylene glycol 400				
48. (D12)	0.4 M Potassium formate, 30% v/v Polyethylene glycol 400				

HAMPTON RESEARCH
Solutions for Crystal Growth

34 Journey
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Sample: ______ Sample Concentration: ______
Sample Buffer: ______ Date: ______
Reservoir Volume: ______ Temperature: ______
Drop Volume: Total ______ µl Sample ______ µl Reservoir ______ µl Additive ______ µl

1 Clear Drop
2 Phase Separation
3 Regular Granular Precipitate
4 Birefringent Precipitate or Microcrystals
5 Posettes or Spherulites
6 Needles (1D Growth)
7 Plates (2D Growth)
8 Single Crystals (3D Growth < 0.2 mm)
9 Single Crystals (3D Growth > 0.2 mm)

PEG/Ion 400™ - HR2-460 Scoring Sheet	Date:	Date:	Date:	Date:
49. (E01) 0.1 M Sodium formate, 30% v/v Polyethylene glycol 400				
50. (E02) 0.4 M Sodium formate, 30% v/v Polyethylene glycol 400				
51. (E03) 0.1 M DL-Malic acid pH 7.0, 30% v/v Polyethylene glycol 400				
52. (E04) 0.4 M DL-Malic acid pH 7.0, 30% v/v Polyethylene glycol 400				
53. (E05) 0.1 M Sodium malonate pH 7.0, 30% v/v Polyethylene glycol 400				
54. (E06) 0.4 M Sodium malonate pH 7.0, 30% v/v Polyethylene glycol 400				
55. (E07) 0.1 M Ammonium nitrate, 30% v/v Polyethylene glycol 400				
56. (E08) 0.4 M Ammonium nitrate, 30% v/v Polyethylene glycol 400				
57. (E09) 0.1 M Ethylammonium nitrate, 30% v/v Polyethylene glycol 400				
58. (E10) 0.4 M Ethylammonium nitrate, 30% v/v Polyethylene glycol 400				
59. (E11) 0.1 M Lithium nitrate, 30% v/v Polyethylene glycol 400				
60. (E12) 0.4 M Lithium nitrate, 30% v/v Polyethylene glycol 400				
61. (F01) 0.1 M Magnesium nitrate hexahydrate, 30% v/v Polyethylene glycol 400				
62. (F02) 0.4 M Magnesium nitrate hexahydrate, 30% v/v Polyethylene glycol 400				
63. (F03) 0.1 M Sodium nitrate, 30% v/v Polyethylene glycol 400				
64. (F04) 0.4 M Sodium nitrate, 30% v/v Polyethylene glycol 400				
65. (F05) 0.1 M Ammonium phosphate dibasic, 30% v/v Polyethylene glycol 400				
66. (F06) 0.4 M Ammonium phosphate dibasic, 30% v/v Polyethylene glycol 400				
67. (F07) 0.1 M Ammonium phosphate monobasic, 30% v/v Polyethylene glycol 400				
68. (F08) 0.4 M Ammonium phosphate monobasic, 30% v/v Polyethylene glycol 400				
69. (F09) 0.1 M Potassium phosphate dibasic, 30% v/v Polyethylene glycol 400				
70. (F10) 0.4 M Potassium phosphate dibasic, 30% v/v Polyethylene glycol 400				
71. (F11) 0.1 M Potassium phosphate monobasic, 30% v/v Polyethylene glycol 400				
72. (F12) 0.4 M Potassium phosphate monobasic, 30% v/v Polyethylene glycol 400				
73. (G01) 0.1 M Sodium potassium phosphate pH 7.0, 30% v/v Polyethylene glycol 400				
74. (G02) 0.4 M Sodium potassium phosphate pH 7.0, 30% v/v Polyethylene glycol 400				
75. (G03) 0.1 M Sodium phosphate monobasic monohydrate, 30% v/v Polyethylene glycol 400				
76. (G04) 0.4 M Sodium phosphate monobasic monohydrate, 30% v/v Polyethylene glycol 400				
77. (G05) 0.1 M Succinic acid pH 7.0, 30% v/v Polyethylene glycol 400				
78. (G06) 0.4 M Succinic acid pH 7.0, 30% v/v Polyethylene glycol 400				
79. (G07) 0.1 M Ammonium sulfate, 30% v/v Polyethylene glycol 400				
80. (G08) 0.4 M Ammonium sulfate, 30% v/v Polyethylene glycol 400				
81. (G09) 0.1 M Lithium sulfate monohydrate, 30% v/v Polyethylene glycol 400				
82. (G10) 0.4 M Lithium sulfate monohydrate, 30% v/v Polyethylene glycol 400				
83. (G11) 0.1 M Magnesium sulfate hydrate, 30% v/v Polyethylene glycol 400				
84. (G12) 0.4 M Magnesium sulfate hydrate, 30% v/v Polyethylene glycol 400				
85. (H01) 0.1 M Ammonium tartrate dibasic, 30% v/v Polyethylene glycol 400				
86. (H02) 0.4 M Ammonium tartrate dibasic, 30% v/v Polyethylene glycol 400				
87. (H03) 0.1 M Potassium sodium tartrate tetrahydrate, 30% v/v Polyethylene glycol 400				
88. (H04) 0.4 M Potassium sodium tartrate tetrahydrate, 30% v/v Polyethylene glycol 400				
89. (H05) 0.1 M Sodium tartrate dibasic dihydrate, 30% v/v Polyethylene glycol 400				
90. (H06) 0.4 M Sodium tartrate dibasic dihydrate, 30% v/v Polyethylene glycol 400				
91. (H07) 0.1 M Potassium thiocyanate, 30% v/v Polyethylene glycol 400				
92. (H08) 0.4 M Potassium thiocyanate, 30% v/v Polyethylene glycol 400				
93. (H09) 0.1 M Sodium thiocyanate, 30% v/v Polyethylene glycol 400				
94. (H10) 0.4 M Sodium thiocyanate, 30% v/v Polyethylene glycol 400				
95. (H11) 5% v/v Tacsimate™ pH 7.0, 30% v/v Polyethylene glycol 400				
96. (H12) 20% v/v Tacsimate™ pH 7.0, 30% v/v Polyethylene glycol 400				

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