

Features

Crystal Screen HT™ is a high throughput reagent kit designed to provide a rapid screening method for the crystallization of biological macromolecules. The kit is straightforward, effective, and practical for the determination of preliminary crystallization conditions. The kit is also effective in determining the solubility of a macromolecule in a wide range of reagents and pH.

Crystal Screen HT is supplied in a sterile, polypropylene Deep Well block, each reservoir containing 1 ml of sterile filtered reagent. The block is heat sealed using a special polypropylene backed film.

Crystal Screen™ and Crystal Screen 2™ offer a sparse matrix of trial crystallization reagent conditions based upon the original Jancarik and Kim screen.³ The primary screen variables are salt, pH, and precipitant (salts, polymers, volatile organics, and non-volatile organics).

General Description

Crystal Screen HT is supplied in a sterile, polypropylene 96 Deep Well block, each reservoir containing 1 ml of sterile filtered reagent. The block is heat sealed using a special polypropylene backed film.

Each Crystal Screen HT kit is supplied with an adhesive sealing film which can be used to seal the block after removing the heat seal. Additional adhesive sealing films can be obtained from Hampton Research or laboratory supply companies which offer high throughput plates and seals.

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 microfiltration prior to use.^{1,2,4}

The recommended sample concentration is 5 to 25 mg/ml in sterile filtered, deionized water or 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 Crystal Screen and Crystal Screen 2 variables. However, agents that promote and preserve sample stability and homogeneity can and should be included in the sample. For additional sample preparation recommendation see Crystal Growth 101 - Preliminary Sample Preparation bulletin from Hampton Research.

Preparing the Deep Well Block for Use

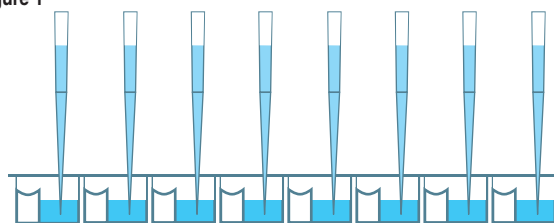
Allow the block to equilibrate to room temperature. To remove stray reagent from the sealing film, centrifuge the block at 500 rpm for 5 minutes. To remove film, grasp a corner of the film and gently peel film from the block. Alternatively, the film can be pierced to access reagents.

Performing the Screen

Manual Method - Sitting Drop Vapor Diffusion

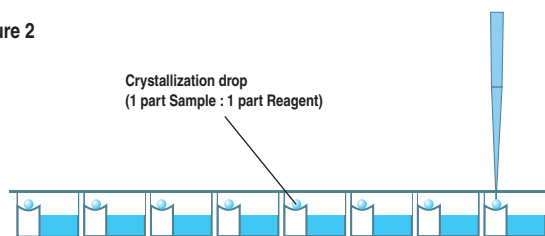
1. Using a 96 well sitting drop vapor diffusion plate, pipet the recommended volume (typically 100 microliters) of crystallization reagent from the Deep Well block into the reservoirs of the crystallization plate. The Deep Well block is compatible with 8 and 12 channel pipets as well as many automated 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 B through H. 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 1 through 12. See Figure 1. 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.

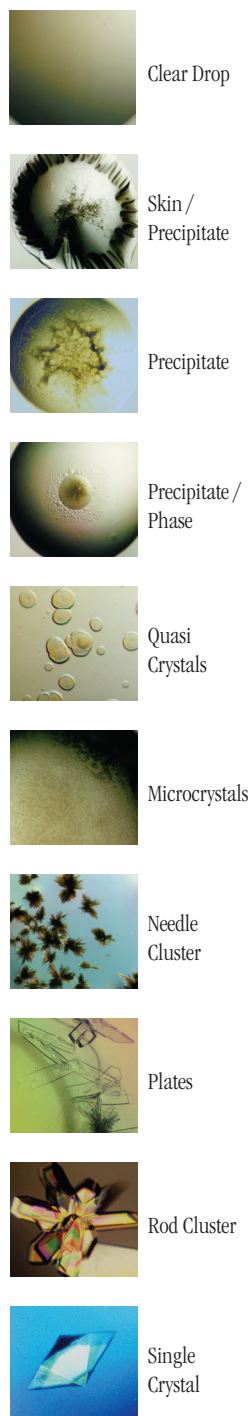
Figure 2



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 manufacturer's recommendation. Most 96 well crystallization plates are sealed using a clear sealing tape, film, or cap mat. View and score the experiment as desired. See Hampton

Figure 3
Typical observations in a crystallization experiment



Research technical bulletin Crystal Growth 101 - Viewing Crystallization Experiments for additional information on viewing drops.

5. Seal the remaining reagent in the Deep Well block using sealing film.

Crystal Screen 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 8 x 12 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 thereafter. 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 3, on the left side of page 2 shows typical examples of what one might observe in a crystallization experiment.

Interpreting Crystal Screen 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.

Crystal Screen 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).

Crystallization reagents are readily reproduced using Hampton Research Optimize™ and StockOptions™ stock solutions of salts, polymers and buffers. 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.

Crystallization reagents containing buffers are formulated by creating a 1.0 M stock buffer, titrated to the desired pH using

Hydrochloric acid or Sodium hydroxide. The buffer is then diluted with the other reagent components and water. No further pH adjustment is required.

Crystallization 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. Sparse Matrix Sampling: a screening method for crystallization of proteins. Jancarik, J. and Kim, S.H. J. Appl. Cryst., 24,409-411, 1991.
4. Protein and Nucleic Acid Crystallization. Methods, A Companion to Methods in Enzymology, Academic Press, Volume 1, Number 1, August 1990.

Technical Support

Inquiries regarding Crystal Screen 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 # | Buffer ◇ | Well # | Precipitant |
|-----------|---|-----------|--|-----------|--|
| 1. (A1) | 0.02 M Calcium chloride dihydrate | 1. (A1) | 0.1 M Sodium acetate trihydrate pH 4.6 | 1. (A1) | 30% v/v (+/-)-2-Methyl-2,4-pentanediol |
| 2. (A2) | None | 2. (A2) | None | 2. (A2) | 0.4 M Potassium sodium tartrate tetrahydrate |
| 3. (A3) | None | 3. (A3) | None | 3. (A3) | 0.4 M Ammonium phosphate monobasic |
| 4. (A4) | None | 4. (A4) | 0.1 M TRIS hydrochloride pH 8.5 | 4. (A4) | 2.0 M Ammonium sulfate |
| 5. (A5) | 0.2 M Sodium citrate tribasic dihydrate | 5. (A5) | 0.1 M HEPES sodium pH 7.5 | 5. (A5) | 30% v/v (+/-)-2-Methyl-2,4-pentanediol |
| 6. (A6) | 0.2 M Magnesium chloride hexahydrate | 6. (A6) | 0.1 M TRIS hydrochloride pH 8.5 | 6. (A6) | 30% w/v Polyethylene glycol 4,000 |
| 7. (A7) | None | 7. (A7) | 0.1 M Sodium cacodylate trihydrate pH 6.5 | 7. (A7) | 1.4 M Sodium acetate trihydrate |
| 8. (A8) | 0.2 M Sodium citrate tribasic dihydrate | 8. (A8) | 0.1 M Sodium cacodylate trihydrate pH 6.5 | 8. (A8) | 30% v/v 2-Propanol |
| 9. (A9) | 0.2 M Ammonium acetate | 9. (A9) | 0.1 M Sodium citrate tribasic dihydrate pH 5.6 | 9. (A9) | 30% w/v Polyethylene glycol 4,000 |
| 10. (A10) | 0.2 M Ammonium acetate | 10. (A10) | 0.1 M Sodium acetate trihydrate pH 4.6 | 10. (A10) | 30% w/v Polyethylene glycol 4,000 |
| 11. (A11) | None | 11. (A11) | 0.1 M Sodium citrate tribasic dihydrate pH 5.6 | 11. (A11) | 1.0 M Ammonium phosphate monobasic |
| 12. (A12) | 0.2 M Magnesium chloride hexahydrate | 12. (A12) | 0.1 M HEPES sodium pH 7.5 | 12. (A12) | 30% v/v 2-Propanol |
| 13. (B1) | 0.2 M Sodium citrate tribasic dihydrate | 13. (B1) | 0.1 M TRIS hydrochloride pH 8.5 | 13. (B1) | 30% v/v Polyethylene glycol 400 |
| 14. (B2) | 0.2 M Calcium chloride dihydrate | 14. (B2) | 0.1 M HEPES sodium pH 7.5 | 14. (B2) | 28% v/v Polyethylene glycol 400 |
| 15. (B3) | 0.2 M Ammonium sulfate | 15. (B3) | 0.1 M Sodium cacodylate trihydrate pH 6.5 | 15. (B3) | 30% w/v Polyethylene glycol 8,000 |
| 16. (B4) | None | 16. (B4) | 0.1 M HEPES sodium pH 7.5 | 16. (B4) | 1.5 M Lithium sulfate monohydrate |
| 17. (B5) | 0.2 M Lithium sulfate monohydrate | 17. (B5) | 0.1 M TRIS hydrochloride pH 8.5 | 17. (B5) | 30% w/v Polyethylene glycol 4,000 |
| 18. (B6) | 0.2 M Magnesium acetate tetrahydrate | 18. (B6) | 0.1 M Sodium cacodylate trihydrate pH 6.5 | 18. (B6) | 20% w/v Polyethylene glycol 8,000 |
| 19. (B7) | 0.2 M Ammonium acetate | 19. (B7) | 0.1 M TRIS hydrochloride pH 8.5 | 19. (B7) | 30% v/v 2-Propanol |
| 20. (B8) | 0.2 M Ammonium sulfate | 20. (B8) | 0.1 M Sodium acetate trihydrate pH 4.6 | 20. (B8) | 25% w/v Polyethylene glycol 4,000 |
| 21. (B9) | 0.2 M Magnesium acetate tetrahydrate | 21. (B9) | 0.1 M Sodium cacodylate trihydrate pH 6.5 | 21. (B9) | 30% v/v (+/-)-2-Methyl-2,4-pentanediol |
| 22. (B10) | 0.2 M Sodium acetate trihydrate | 22. (B10) | 0.1 M TRIS hydrochloride pH 8.5 | 22. (B10) | 30% w/v Polyethylene glycol 4,000 |
| 23. (B11) | 0.2 M Magnesium chloride hexahydrate | 23. (B11) | 0.1 M HEPES sodium pH 7.5 | 23. (B11) | 30% v/v Polyethylene glycol 400 |
| 24. (B12) | 0.2 M Calcium chloride dihydrate | 24. (B12) | 0.1 M Sodium acetate trihydrate pH 4.6 | 24. (B12) | 20% v/v 2-Propanol |
| 25. (C1) | None | 25. (C1) | 0.1 M Imidazole pH 6.5 | 25. (C1) | 1.0 M Sodium acetate trihydrate |
| 26. (C2) | 0.2 M Ammonium acetate | 26. (C2) | 0.1 M Sodium citrate tribasic dihydrate pH 5.6 | 26. (C2) | 30% v/v (+/-)-2-Methyl-2,4-pentanediol |
| 27. (C3) | 0.2 M Sodium citrate tribasic dihydrate | 27. (C3) | 0.1 M HEPES sodium pH 7.5 | 27. (C3) | 20% v/v 2-Propanol |
| 28. (C4) | 0.2 M Sodium acetate trihydrate | 28. (C4) | 0.1 M Sodium cacodylate trihydrate pH 6.5 | 28. (C4) | 30% w/v Polyethylene glycol 8,000 |
| 29. (C5) | None | 29. (C5) | 0.1 M HEPES sodium pH 7.5 | 29. (C5) | 0.8 M Potassium sodium tartrate tetrahydrate |
| 30. (C6) | 0.2 M Ammonium sulfate | 30. (C6) | None | 30. (C6) | 30% w/v Polyethylene glycol 8,000 |
| 31. (C7) | 0.2 M Ammonium sulfate | 31. (C7) | None | 31. (C7) | 30% w/v Polyethylene glycol 4,000 |
| 32. (C8) | None | 32. (C8) | None | 32. (C8) | 2.0 M Ammonium sulfate |
| 33. (C9) | None | 33. (C9) | None | 33. (C9) | 4.0 M Sodium formate |
| 34. (C10) | None | 34. (C10) | 0.1 M Sodium acetate trihydrate pH 4.6 | 34. (C10) | 2.0 M Sodium formate |
| 35. (C11) | None | 35. (C11) | 0.1 M HEPES sodium pH 7.5 | 35. (C11) | 0.8 M Sodium phosphate monobasic monohydrate, 0.8 M Potassium phosphate monobasic |
| 36. (C12) | None | 36. (C12) | 0.1 M TRIS hydrochloride pH 8.5 | 36. (C12) | 8% w/v Polyethylene glycol 8,000 |
| 37. (D1) | None | 37. (D1) | 0.1 M Sodium acetate trihydrate pH 4.6 | 37. (D1) | 8% w/v Polyethylene glycol 4,000 |
| 38. (D2) | None | 38. (D2) | 0.1 M HEPES sodium pH 7.5 | 38. (D2) | 1.4 M Sodium citrate tribasic dihydrate |
| 39. (D3) | None | 39. (D3) | 0.1 M HEPES sodium pH 7.5 | 39. (D3) | 2% v/v Polyethylene glycol 400, 2.0 M Ammonium sulfate |
| 40. (D4) | None | 40. (D4) | 0.1 M Sodium citrate tribasic dihydrate pH 5.6 | 40. (D4) | 20% v/v 2-Propanol, 20% w/v Polyethylene glycol 4,000 |
| 41. (D5) | None | 41. (D5) | 0.1 M HEPES sodium pH 7.5 | 41. (D5) | 10% v/v 2-Propanol, 20% w/v Polyethylene glycol 4,000 |
| 42. (D6) | 0.05 M Potassium phosphate monobasic | 42. (D6) | None | 42. (D6) | 20% w/v Polyethylene glycol 8,000 |
| 43. (D7) | None | 43. (D7) | None | 43. (D7) | 30% w/v Polyethylene glycol 1,500 |
| 44. (D8) | None | 44. (D8) | None | 44. (D8) | 0.2 M Magnesium formate dihydrate |
| 45. (D9) | 0.2 M Zinc acetate dihydrate | 45. (D9) | 0.1 M Sodium cacodylate trihydrate pH 6.5 | 45. (D9) | 18% w/v Polyethylene glycol 8,000 |
| 46. (D10) | 0.2 M Calcium acetate hydrate | 46. (D10) | 0.1 M Sodium cacodylate trihydrate pH 6.5 | 46. (D10) | 18% w/v Polyethylene glycol 8,000 |
| 47. (D11) | None | 47. (D11) | 0.1 M Sodium acetate trihydrate pH 4.6 | 47. (D11) | 2.0 M Ammonium sulfate |
| 48. (D12) | None | 48. (D12) | 0.1 M TRIS hydrochloride pH 8.5 | 48. (D12) | 2.0 M Ammonium phosphate monobasic |

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

*Crystal Screen™ (Deep Well Block) contains forty-eight unique reagents beginning at position A1.
To determine the formulation of each reagent, simply read across the page.*

| Well # | Salt | Well # | Buffer ◇ | Well # | Precipitant |
|-----------|--|-----------|--|-----------|---|
| 49. (E1) | 2.0 M Sodium chloride | 49. (E1) | None | 49. (E1) | 10% w/v Polyethylene glycol 6,000 |
| 50. (E2) | 0.5 M Sodium chloride, 0.01 M Magnesium chloride hexahydrate | 50. (E2) | None | 50. (E2) | 0.01 M Hexadecyltrimethylammonium bromide |
| 51. (E3) | None | 51. (E3) | None | 51. (E3) | 25% v/v Ethylene glycol |
| 52. (E4) | None | 52. (E4) | None | 52. (E4) | 35% v/v 1,4-Dioxane |
| 53. (E5) | 2.0 M Ammonium sulfate | 53. (E5) | None | 53. (E5) | 5% v/v 2-Propanol |
| 54. (E6) | None | 54. (E6) | None | 54. (E6) | 1.0 M Imidazole pH 7.0 |
| 55. (E7) | None | 55. (E7) | None | 55. (E7) | 10% w/v Polyethylene glycol 1,000, 10% w/v Polyethylene glycol 8,000 |
| 56. (E8) | 1.5 M Sodium chloride | 56. (E8) | None | 56. (E8) | 10% v/v Ethanol |
| 57. (E9) | None | 57. (E9) | 0.1 M Sodium acetate trihydrate pH 4.6 | 57. (E9) | 2.0 M Sodium chloride |
| 58. (E10) | 0.2 M Sodium chloride | 58. (E10) | 0.1 M Sodium acetate trihydrate pH 4.6 | 58. (E10) | 30% v/v (+/-)-2-Methyl-2,4-pentanediol |
| 59. (E11) | 0.01 M Cobalt(II) chloride hexahydrate | 59. (E11) | 0.1 M Sodium acetate trihydrate pH 4.6 | 59. (E11) | 1.0 M 1,6-Hexanediol |
| 60. (E12) | 0.1 M Cadmium chloride hydrate | 60. (E12) | 0.1 M Sodium acetate trihydrate pH 4.6 | 60. (E12) | 30% v/v Polyethylene glycol 400 |
| 61. (F1) | 0.2 M Ammonium sulfate | 61. (F1) | 0.1 M Sodium acetate trihydrate pH 4.6 | 61. (F1) | 30% w/v Polyethylene glycol monomethyl ether 2,000 |
| 62. (F2) | 0.2 M Potassium sodium tartrate tetrahydrate | 62. (F2) | 0.1 M Sodium citrate tribasic dihydrate pH 5.6 | 62. (F2) | 2.0 M Ammonium sulfate |
| 63. (F3) | 0.5 M Ammonium sulfate | 63. (F3) | 0.1 M Sodium citrate tribasic dihydrate pH 5.6 | 63. (F3) | 1.0 M Lithium sulfate monohydrate |
| 64. (F4) | 0.5 M Sodium chloride | 64. (F4) | 0.1 M Sodium citrate tribasic dihydrate pH 5.6 | 64. (F4) | 2% v/v Ethylene imine polymer |
| 65. (F5) | None | 65. (F5) | 0.1 M Sodium citrate tribasic dihydrate pH 5.6 | 65. (F5) | 35% v/v tert-Butanol |
| 66. (F6) | 0.01 M Iron(III) chloride hexahydrate | 66. (F6) | 0.1 M Sodium citrate tribasic dihydrate pH 5.6 | 66. (F6) | 10% v/v Jeffamine® M-600® |
| 67. (F7) | None | 67. (F7) | 0.1 M Sodium citrate tribasic dihydrate pH 5.6 | 67. (F7) | 2.5 M 1,6-Hexanediol |
| 68. (F8) | None | 68. (F8) | 0.1 M MES monohydrate pH 6.5 | 68. (F8) | 1.6 M Magnesium sulfate heptahydrate |
| 69. (F9) | 0.1 M Sodium phosphate monobasic monohydrate, 0.1 M Potassium phosphate monobasic | 69. (F9) | 0.1 M MES monohydrate pH 6.5 | 69. (F9) | 2.0 M Sodium chloride |
| 70. (F10) | None | 70. (F10) | 0.1 M MES monohydrate pH 6.5 | 70. (F10) | 12% w/v Polyethylene glycol 20,000 |
| 71. (F11) | 1.6 M Ammonium sulfate | 71. (F11) | 0.1 M MES monohydrate pH 6.5 | 71. (F11) | 10% v/v 1,4-Dioxane |
| 72. (F12) | 0.05 M Cesium chloride | 72. (F12) | 0.1 M MES monohydrate pH 6.5 | 72. (F12) | 30% v/v Jeffamine® M-600® |
| 73. (G1) | 0.01 M Cobalt(II) chloride hexahydrate | 73. (G1) | 0.1 M MES monohydrate pH 6.5 | 73. (G1) | 1.8 M Ammonium sulfate |
| 74. (G2) | 0.2 M Ammonium sulfate | 74. (G2) | 0.1 M MES monohydrate pH 6.5 | 74. (G2) | 30% w/v Polyethylene glycol monomethyl ether 5,000 |
| 75. (G3) | 0.01 M Zinc sulfate heptahydrate | 75. (G3) | 0.1 M MES monohydrate pH 6.5 | 75. (G3) | 25% v/v Polyethylene glycol monomethyl ether 550 |
| 76. (G4) | None | 76. (G4) | None | 76. (G4) | 1.6 M Sodium citrate tribasic dihydrate pH 6.5 |
| 77. (G5) | 0.5 M Ammonium sulfate | 77. (G5) | 0.1 M HEPES pH 7.5 | 77. (G5) | 30% v/v (+/-)-2-Methyl-2,4-pentanediol |
| 78. (G6) | None | 78. (G6) | 0.1 M HEPES pH 7.5 | 78. (G6) | 10% w/v Polyethylene glycol 6,000, 5% v/v (+/-)-2-Methyl-2,4-pentanediol |
| 79. (G7) | None | 79. (G7) | 0.1 M HEPES pH 7.5 | 79. (G7) | 20% v/v Jeffamine® M-600® |
| 80. (G8) | 0.1 M Sodium chloride | 80. (G8) | 0.1 M HEPES pH 7.5 | 80. (G8) | 1.6 M Ammonium sulfate |
| 81. (G9) | None | 81. (G9) | 0.1 M HEPES pH 7.5 | 81. (G9) | 2.0 M Ammonium formate |
| 82. (G10) | 0.05 M Cadmium sulfate hydrate | 82. (G10) | 0.1 M HEPES pH 7.5 | 82. (G10) | 1.0 M Sodium acetate trihydrate |
| 83. (G11) | None | 83. (G11) | 0.1 M HEPES pH 7.5 | 83. (G11) | 70% v/v (+/-)-2-Methyl-2,4-pentanediol |
| 84. (G12) | None | 84. (G12) | 0.1 M HEPES pH 7.5 | 84. (G12) | 4.3 M Sodium chloride |
| 85. (H1) | None | 85. (H1) | 0.1 M HEPES pH 7.5 | 85. (H1) | 10% w/v Polyethylene glycol 8,000, 8% v/v Ethylene glycol |
| 86. (H2) | None | 86. (H2) | 0.1 M HEPES pH 7.5 | 86. (H2) | 20% w/v Polyethylene glycol 10,000 |
| 87. (H3) | 0.2 M Magnesium chloride hexahydrate | 87. (H3) | 0.1 M Tris pH 8.5 | 87. (H3) | 3.4 M 1,6-Hexanediol |
| 88. (H4) | None | 88. (H4) | 0.1 M Tris pH 8.5 | 88. (H4) | 25% v/v tert-Butanol |
| 89. (H5) | 0.01 M Nickel(II) chloride hexahydrate | 89. (H5) | 0.1 M Tris pH 8.5 | 89. (H5) | 1.0 M Lithium sulfate monohydrate |
| 90. (H6) | 1.5 M Ammonium sulfate | 90. (H6) | 0.1 M Tris pH 8.5 | 90. (H6) | 12% v/v Glycerol |
| 91. (H7) | 0.2 M Ammonium phosphate monobasic | 91. (H7) | 0.1 M Tris pH 8.5 | 91. (H7) | 50% v/v (+/-)-2-Methyl-2,4-pentanediol |
| 92. (H8) | None | 92. (H8) | 0.1 M Tris pH 8.5 | 92. (H8) | 20% v/v Ethanol |
| 93. (H9) | 0.01 M Nickel(II) chloride hexahydrate | 93. (H9) | 0.1 M Tris pH 8.5 | 93. (H9) | 20% w/v Polyethylene glycol monomethyl ether 2,000 |
| 94. (H10) | 0.1 M Sodium chloride | 94. (H10) | 0.1 M BICINE pH 9.0 | 94. (H10) | 20% v/v Polyethylene glycol monomethyl ether 550 |
| 95. (H11) | None | 95. (H11) | 0.1 M BICINE pH 9.0 | 95. (H11) | 2.0 M Magnesium chloride hexahydrate |
| 96. (H12) | None | 96. (H12) | 0.1 M BICINE pH 9.0 | 96. (H12) | 2% v/v 1,4-Dioxane, 10% w/v Polyethylene glycol 20,000 |

◇ Buffer pH is that of a 1.0 M (0.5 M for MES) stock prior to dilution with other reagent components:
pH with HCl or NaOH.

*Crystal Screen 2™ (Deep Well Block) contains forty-eight unique reagents beginning at position E1.
To determine the formulation of each reagent, simply read across the page.*

**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)

Crystal Screen HT™ - HR2-130 Scoring Sheet

Date: Date: Date:

| | | | | |
|-----------|---|--|--|--|
| 1. (A1) | 0.02 M Calcium chloride dihydrate, 0.1 M Sodium acetate trihydrate pH 4.6, 30% v/v (+/-)-2-Methyl-2,4-pentanediol | | | |
| 2. (A2) | 0.4 M Potassium sodium tartrate tetrahydrate | | | |
| 3. (A3) | 0.4 M Ammonium phosphate monobasic | | | |
| 4. (A4) | 0.1 M TRIS hydrochloride pH 8.5, 2.0 M Ammonium sulfate | | | |
| 5. (A5) | 0.2 M Sodium citrate tribasic dihydrate, 0.1 M HEPES sodium pH 7.5, 30% v/v (+/-)-2-Methyl-2,4-pentanediol | | | |
| 6. (A6) | 0.2 M Magnesium chloride hexahydrate, 0.1 M TRIS hydrochloride pH 8.5, 30% w/v Polyethylene glycol 4,000 | | | |
| 7. (A7) | 0.1 M Sodium cacodylate trihydrate pH 6.5, 1.4 M Sodium acetate trihydrate | | | |
| 8. (A8) | 0.2 M Sodium citrate tribasic dihydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 30% v/v 2-Propanol | | | |
| 9. (A9) | 0.2 M Ammonium acetate, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 30% w/v Polyethylene glycol 4,000 | | | |
| 10. (A10) | 0.2 M Ammonium acetate, 0.1 M Sodium acetate trihydrate pH 4.6, 30% w/v Polyethylene glycol 4,000 | | | |
| 11. (A11) | 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 1.0 M Ammonium phosphate monobasic | | | |
| 12. (A12) | 0.2 M Magnesium chloride hexahydrate, 0.1 M HEPES sodium pH 7.5, 30% v/v 2-Propanol | | | |
| 13. (B1) | 0.2 M Sodium citrate tribasic dihydrate, 0.1 M TRIS hydrochloride pH 8.5, 30% v/v Polyethylene glycol 400 | | | |
| 14. (B2) | 0.2 M Calcium chloride dihydrate, 0.1 M HEPES sodium pH 7.5, 28% v/v Polyethylene glycol 400 | | | |
| 15. (B3) | 0.2 M Ammonium sulfate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 30% w/v Polyethylene glycol 8,000 | | | |
| 16. (B4) | 0.1 M HEPES sodium pH 7.5, 1.5 M Lithium sulfate monohydrate | | | |
| 17. (B5) | 0.2 M Lithium sulfate monohydrate, 0.1 M TRIS hydrochloride pH 8.5, 30% w/v Polyethylene glycol 4,000 | | | |
| 18. (B6) | 0.2 M Magnesium acetate tetrahydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 20% w/v Polyethylene glycol 8,000 | | | |
| 19. (B7) | 0.2 M Ammonium acetate, 0.1 M TRIS hydrochloride pH 8.5, 30% v/v 2-Propanol | | | |
| 20. (B8) | 0.2 M Ammonium sulfate, 0.1 M Sodium acetate trihydrate pH 4.6, 25% w/v Polyethylene glycol 4,000 | | | |
| 21. (B9) | 0.2 M Magnesium acetate tetrahydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 30% v/v (+/-)-2-Methyl-2,4-pentanediol | | | |
| 22. (B10) | 0.2 M Sodium acetate trihydrate, 0.1 M TRIS hydrochloride pH 8.5, 30% w/v Polyethylene glycol 4,000 | | | |
| 23. (B11) | 0.2 M Magnesium chloride hexahydrate, 0.1 M HEPES sodium pH 7.5, 30% v/v Polyethylene glycol 400 | | | |
| 24. (B12) | 0.2 M Calcium chloride dihydrate, 0.1 M Sodium acetate trihydrate pH 4.6, 20% v/v 2-Propanol | | | |
| 25. (C1) | 0.1 M Imidazole pH 6.5, 1.0 M Sodium acetate trihydrate | | | |
| 26. (C2) | 0.2 M Ammonium acetate, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 30% v/v (+/-)-2-Methyl-2,4-pentanediol | | | |
| 27. (C3) | 0.2 M Sodium citrate tribasic dihydrate, 0.1 M HEPES sodium pH 7.5, 20% v/v 2-Propanol | | | |
| 28. (C4) | 0.2 M Sodium acetate trihydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 30% w/v Polyethylene glycol 8,000 | | | |
| 29. (C5) | 0.1 M HEPES sodium pH 7.5, 0.8 M Potassium sodium tartrate tetrahydrate | | | |
| 30. (C6) | 0.2 M Ammonium sulfate, 30% w/v Polyethylene glycol 8,000 | | | |
| 31. (C7) | 0.2 M Ammonium sulfate, 30% w/v Polyethylene glycol 4,000 | | | |
| 32. (C8) | 2.0 M Ammonium sulfate | | | |
| 33. (C9) | 4.0 M Sodium formate | | | |
| 34. (C10) | 0.1 M Sodium acetate trihydrate pH 4.6, 2.0 M Sodium formate | | | |
| 35. (C11) | 0.1 M HEPES sodium pH 7.5, 0.8 M Sodium phosphate monobasic monohydrate, 0.8 M Potassium phosphate monobasic | | | |
| 36. (C12) | 0.1 M TRIS hydrochloride pH 8.5, 8% w/v Polyethylene glycol 8,000 | | | |
| 37. (D1) | 0.1 M Sodium acetate trihydrate pH 4.6, 8% w/v Polyethylene glycol 4,000 | | | |
| 38. (D2) | 0.1 M HEPES sodium pH 7.5, 1.4 M Sodium citrate tribasic dihydrate | | | |
| 39. (D3) | 0.1 M HEPES sodium pH 7.5, 2% v/v Polyethylene glycol 400, 2.0 M Ammonium sulfate | | | |
| 40. (D4) | 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 20% v/v 2-Propanol, 20% w/v Polyethylene glycol 4,000 | | | |
| 41. (D5) | 0.1 M HEPES sodium pH 7.5, 10% v/v 2-Propanol, 20% w/v Polyethylene glycol 4,000 | | | |
| 42. (D6) | 0.05 M Potassium phosphate monobasic, 20% w/v Polyethylene glycol 8,000 | | | |
| 43. (D7) | 30% w/v Polyethylene glycol 1,500 | | | |
| 44. (D8) | 0.2 M Magnesium formate dihydrate | | | |
| 45. (D9) | 0.2 M Zinc acetate dihydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 18% w/v Polyethylene glycol 8,000 | | | |
| 46. (D10) | 0.2 M Calcium acetate hydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 18% w/v Polyethylene glycol 8,000 | | | |
| 47. (D11) | 0.1 M Sodium acetate trihydrate pH 4.6, 2.0 M Ammonium sulfate | | | |
| 48. (D12) | 0.1 M TRIS hydrochloride pH 8.5, 2.0 M Ammonium phosphate monobasic | | | |

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 Solutions for Crystal Growth

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 Also Viejo, CA 92656-5317 U.S.A.
 Tel: (949) 425-1321 • Fax: (949) 425-1611
 e-mail: tech@hampont.com
 Website: www.hampontresearch.com

Sample: _____ Sample Concentration: _____
 Sample Buffer: _____ Date: _____
 Reservoir Volume: _____ Temperature: _____
 Drop Volume: Total _____ µl Sample _____ µl Reservoir _____ µl Additive _____ µl

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

Crystal Screen HT™ - HR2-130 Scoring Sheet

Date: Date: Date:

| | | | | |
|-----------|--|--|--|--|
| 49. (E1) | 2.0 M Sodium chloride, 10% w/v Polyethylene glycol 6,000 | | | |
| 50. (E2) | 0.5 M Sodium chloride, 0.01 M Magnesium chloride hexahydrate, 0.01 M Hexadecyltrimethylammonium bromide | | | |
| 51. (E3) | 25% v/v Ethylene glycol | | | |
| 52. (E4) | 35% v/v 1,4-Dioxane | | | |
| 53. (E5) | 2.0 M Ammonium sulfate, 5% v/v 2-Propanol | | | |
| 54. (E6) | 1.0 M Imidazole pH 7.0 | | | |
| 55. (E7) | 10% w/v Polyethylene glycol 1,000, 10% w/v Polyethylene glycol 8,000 | | | |
| 56. (E8) | 1.5 M Sodium chloride, 10% v/v Ethanol | | | |
| 57. (E9) | 0.1 M Sodium acetate trihydrate pH 4.6, 2.0 M Sodium chloride | | | |
| 58. (E10) | 0.2 M Sodium chloride, 0.1 M Sodium acetate trihydrate pH 4.6, 30% v/v (+/-)-2-Methyl-2,4-pentanediol | | | |
| 59. (E11) | 0.01 M Cobalt(II) chloride hexahydrate, 0.1 M Sodium acetate trihydrate pH 4.6, 1.0 M 1,6-Hexanediol | | | |
| 60. (E12) | 0.1 M Cadmium chloride hydrate, 0.1 M Sodium acetate trihydrate pH 4.6, 30% v/v Polyethylene glycol 400 | | | |
| 61. (F1) | 0.2 M Ammonium sulfate, 0.1 M Sodium acetate trihydrate pH 4.6, 30% w/v Polyethylene glycol monomethyl ether 2,000 | | | |
| 62. (F2) | 0.2 M Potassium sodium tartrate tetrahydrate, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 2.0 M Ammonium sulfate | | | |
| 63. (F3) | 0.5 M Ammonium sulfate, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 1.0 M Lithium sulfate monohydrate | | | |
| 64. (F4) | 0.5 M Sodium chloride, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 2% v/v Ethylene imine polymer | | | |
| 65. (F5) | 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 35% v/v tert-Butanol | | | |
| 66. (F6) | 0.01 M Iron(III) chloride hexahydrate, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 10% v/v Jeffamine® M-600® | | | |
| 67. (F7) | 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 2.5 M 1,6-Hexanediol | | | |
| 68. (F8) | 0.1 M MES monohydrate pH 6.5, 1.6 M Magnesium sulfate heptahydrate | | | |
| 69. (F9) | 0.1 M Sodium phosphate monobasic monohydrate, 0.1 M Potassium phosphate monobasic, 0.1 M MES monohydrate pH 6.5, 2.0 M Sodium chloride | | | |
| 70. (F10) | 0.1 M MES monohydrate pH 6.5, 12% w/v Polyethylene glycol 20,000 | | | |
| 71. (F11) | 1.6 M Ammonium sulfate, 0.1 M MES monohydrate pH 6.5, 10% v/v 1,4-Dioxane | | | |
| 72. (F12) | 0.05 M Cesium chloride, 0.1 M MES monohydrate pH 6.5, 30% v/v Jeffamine® M-600® | | | |
| 73. (G1) | 0.01 M Cobalt(II) chloride hexahydrate, 0.1 M MES monohydrate pH 6.5, 1.8 M Ammonium sulfate | | | |
| 74. (G2) | 0.2 M Ammonium sulfate, 0.1 M MES monohydrate pH 6.5, 30% w/v Polyethylene glycol monomethyl ether 5,000 | | | |
| 75. (G3) | 0.01 M Zinc sulfate heptahydrate, 0.1 M MES monohydrate pH 6.5, 25% v/v Polyethylene glycol monomethyl ether 550 | | | |
| 76. (G4) | 1.6 M Sodium citrate tribasic dihydrate pH 6.5 | | | |
| 77. (G5) | 0.5 M Ammonium sulfate, 0.1 M HEPES pH 7.5, 30% v/v (+/-)-2-Methyl-2,4-pentanediol | | | |
| 78. (G6) | 0.1 M HEPES pH 7.5, 10% w/v Polyethylene glycol 6,000, 5% v/v (+/-)-2-Methyl-2,4-pentanediol | | | |
| 79. (G7) | 0.1 M HEPES pH 7.5, 20% v/v Jeffamine® M-600® | | | |
| 80. (G8) | 0.1 M Sodium chloride, 0.1 M HEPES pH 7.5, 1.6 M Ammonium sulfate | | | |
| 81. (G9) | 0.1 M HEPES pH 7.5, 2.0 M Ammonium formate | | | |
| 82. (G10) | 0.05 M Cadmium sulfate hydrate, 0.1 M HEPES pH 7.5, 1.0 M Sodium acetate trihydrate | | | |
| 83. (G11) | 0.1 M HEPES pH 7.5, 70% v/v (+/-)-2-Methyl-2,4-pentanediol | | | |
| 84. (G12) | 0.1 M HEPES pH 7.5, 4.3 M Sodium chloride | | | |
| 85. (H1) | 0.1 M HEPES pH 7.5, 10% w/v Polyethylene glycol 8,000, 8% v/v Ethylene glycol | | | |
| 86. (H2) | 0.1 M HEPES pH 7.5, 20% w/v Polyethylene glycol 10,000 | | | |
| 87. (H3) | 0.2 M Magnesium chloride hexahydrate, 0.1 M Tris pH 8.5, 3.4 M 1,6-Hexanediol | | | |
| 88. (H4) | 0.1 M Tris pH 8.5, 25% v/v tert-Butanol | | | |
| 89. (H5) | 0.01 M Nickel(II) chloride hexahydrate, 0.1 M Tris pH 8.5, 1.0 M Lithium sulfate monohydrate | | | |
| 90. (H6) | 1.5 M Ammonium sulfate, 0.1 M Tris pH 8.5, 12% v/v Glycerol | | | |
| 91. (H7) | 0.2 M Ammonium phosphate monobasic, 0.1 M Tris pH 8.5, 50% v/v (+/-)-2-Methyl-2,4-pentanediol | | | |
| 92. (H8) | 0.1 M Tris pH 8.5, 20% v/v Ethanol | | | |
| 93. (H9) | 0.01 M Nickel(II) chloride hexahydrate, 0.1 M Tris pH 8.5, 20% w/v Polyethylene glycol monomethyl ether 2,000 | | | |
| 94. (H10) | 0.1 M Sodium chloride, 0.1 M BICINE pH 9.0, 20% v/v Polyethylene glycol monomethyl ether 550 | | | |
| 95. (H11) | 0.1 M BICINE pH 9.0, 2.0 M Magnesium chloride hexahydrate | | | |
| 96. (H12) | 0.1 M BICINE pH 9.0, 2% v/v 1,4-Dioxane, 10% w/v Polyethylene glycol 20,000 | | | |



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@hrc.com
 Website: www.hamptonresearch.com

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