

Additive Screen *HT*TM

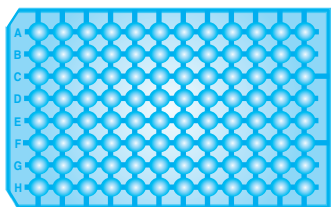
User Guide

HR2-138 (pg 1)

Additive Screen HTTM is a kit designed to allow rapid and convenient evaluation of 96 unique additives and their ability to influence the crystallization of the sample. The screen is designed to be compatible with most popular crystallization reagents including all reagents utilized in all of the Hampton Research screens.

Each of the additives is preformulated in deionized water and sterile filtered using a 0.2 micron filter. Recommended storage for the Additive Screen HT kit is -20 to 4°C. Allow the kit to equilibrate to room temperature prior to removing sealing film and using the reagents. If reagents precipitate during cold storage, warm the sealed block at 37°C for up to 60 minutes and invert block several times to solubilize the reagents.

1



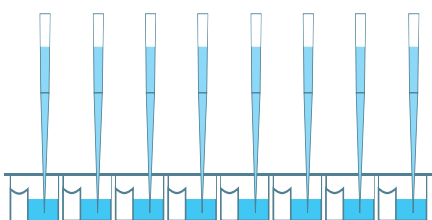
The Additive Screen HT kit is a complete reagent kit designed to provide a rapid screening method for the manipulation of sample-sample and sample-solvent interactions to enhance or alter sample solubility.

The Additive Screen HT is to be used before and during the optimization of preliminary crystallization conditions.

The Additive Screen HT evaluates the manipulation factors of multivalent cations, salts, amino acid, dissociating agents, linkers, polyamines, chaotropes, co-factors, reducing agents, polymers, chelating agent, carbohydrates, polyols, non-detergents, amphiphiles, detergents, osmolyte, organic (non-volatile) and organic (volatile) reagents.

The Additive Screen HT kit contains 1 milliliter of 96 unique additives formulated to allow one to rapidly screen with less than 100 microliters of sample.

2

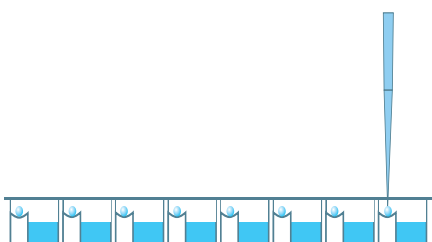


This guide will describe the use of the Additive Screen HT kit using the Sitting Drop Vapor Diffusion method and a 100 microliter reservoir volume. Other methods such as Hanging Drop Vapor Diffusion crystallization and MicroBatch may also be utilized as well as smaller reservoir and drop volumes. A complete description of the Hanging, Sitting, Sandwich Drop, Dialysis, and other crystallization methods are available from the Hampton Research Crystal Growth 101 Library.

Reservoir setup for Additives

- Pipet 90 microliters of crystallization reagent into the reservoir.
- Pipet and mix 10 microliters of the additive into the reservoir.

3

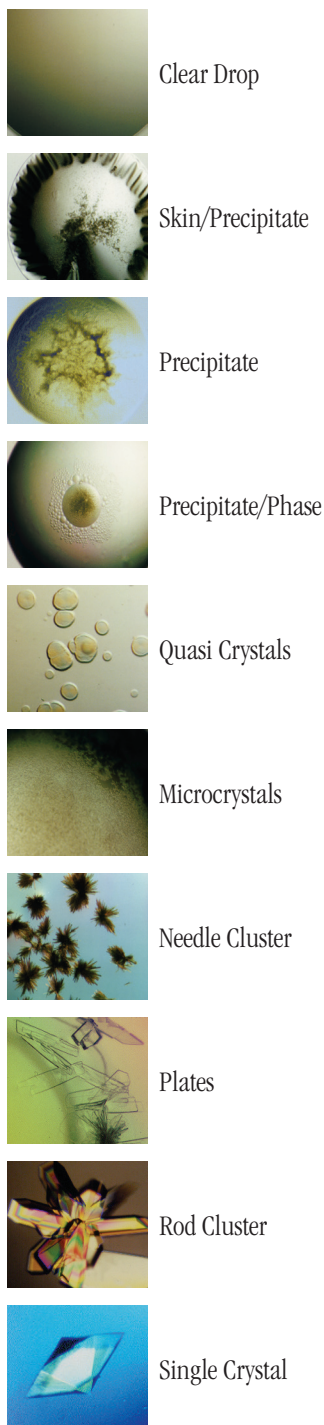


Drop setup for Additives

- Pipet 1 microliter of sample into the sample well.
- Pipet 1 microliter of the crystallization reagent/additive mixture from the reservoir into the sample drop.
- Repeat for the remaining additives.
- Seal the plate.

Figure 1

Typical observations in a crystallization experiment



4

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 1 (left side of page 2) shows typical examples of what one might observe in a crystallization experiment.

5

References and Readings

1. Crystallization of membrane proteins. Edited by Hartmut Michel, CRC Press, 1991.
2. Crystallization of nucleic acids and proteins, Edited by A. Ducruix and R. Giege, The Practical Approach Series, Oxford Univ. Press, 1992 175-191.
3. Screening and optimization strategies for macromolecular crystal growth. Cudney, B. et al, Acta Cryst. (1994). D50, 414-423.
4. Use of glycerol, polyols and other protein structure stabilizing agents in protein crystallization. R. Sousa. Acta Cryst. (1995) D51, 271-277.
5. Influence of divalent cations on protein crystallization. Trakhanov, S. and Quioco, F.A. (1995) Protein Science 4(9): 1914-1919.
6. Non-detergent sulphobetaines: a new class of mild solubilizing agents for protein purification. L. Vuillard, C. Braun-Breton, T. Rabilloud, Biochem. J. (1995) 305, 337-343.
7. A new additive for protein crystallization. L. Vuillard, T. Rabilloud, R. Leberman, C. Berthet-Colominas, St. Cusack. FEBS Letters, 353 (1994) 294-296.

Technical Support

Inquiries regarding Additive 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 #	Classification	Well #	Suggested Drop Concentration
1. (A1)	0.1 M Barium chloride dihydrate	1. (A1)	Multivalent	1. (A1)	0.01 M (10 mM)
2. (A2)	0.1 M Cadmium chloride hydrate	2. (A2)	Multivalent	2. (A2)	0.01 M (10 mM)
3. (A3)	0.1 M Calcium chloride dihydrate	3. (A3)	Multivalent	3. (A3)	0.01 M (10 mM)
4. (A4)	0.1 M Cobalt(II) chloride hexahydrate	4. (A4)	Multivalent	4. (A4)	0.01 M (10 mM)
5. (A5)	0.1 M Copper(II) chloride dihydrate	5. (A5)	Multivalent	5. (A5)	0.01 M (10 mM)
6. (A6)	0.1 M Magnesium chloride hexahydrate	6. (A6)	Multivalent	6. (A6)	0.01 M (10 mM)
7. (A7)	0.1 M Manganese(II) chloride tetrahydrate	7. (A7)	Multivalent	7. (A7)	0.01 M (10 mM)
8. (A8)	0.1 M Strontium chloride hexahydrate	8. (A8)	Multivalent	8. (A8)	0.01 M (10 mM)
9. (A9)	0.1 M Yttrium(III) chloride hexahydrate	9. (A9)	Multivalent	9. (A9)	0.01 M (10 mM)
10. (A10)	0.1 M Zinc chloride	10. (A10)	Multivalent	10. (A10)	0.01 M (10 mM)
11. (A11)	0.1 M Iron(III) chloride hexahydrate	11. (A11)	Multivalent	11. (A11)	0.01 M (10 mM)
12. (A12)	0.1 M Nickel(II) chloride hexahydrate	12. (A12)	Multivalent	12. (A12)	0.01 M (10 mM)
13. (B1)	0.1 M Chromium(III) chloride hexahydrate	13. (B1)	Multivalent	13. (B1)	0.01 M (10 mM)
14. (B2)	0.1 M Praseodymium(III) acetate hydrate	14. (B2)	Multivalent	14. (B2)	0.01 M (10 mM)
15. (B3)	1.0 M Ammonium sulfate	15. (B3)	Salt	15. (B3)	0.1 M (100 mM)
16. (B4)	1.0 M Potassium chloride	16. (B4)	Salt	16. (B4)	0.1 M (100 mM)
17. (B5)	1.0 M Lithium chloride	17. (B5)	Salt	17. (B5)	0.1 M (100 mM)
18. (B6)	2.0 M Sodium chloride	18. (B6)	Salt	18. (B6)	0.2 M (200 mM)
19. (B7)	0.5 M Sodium fluoride	19. (B7)	Salt	19. (B7)	0.05 M (50 mM)
20. (B8)	1.0 M Sodium iodide	20. (B8)	Salt	20. (B8)	0.1 M (100 mM)
21. (B9)	2.0 M Sodium thiocyanate	21. (B9)	Salt	21. (B9)	0.2 M (200 mM)
22. (B10)	1.0 M Potassium sodium tartrate tetrahydrate	22. (B10)	Salt	22. (B10)	0.1 M (100 mM)
23. (B11)	1.0 M Sodium citrate tribasic dihydrate	23. (B11)	Salt	23. (B11)	0.1 M (100 mM)
24. (B12)	1.0 M Cesium chloride	24. (B12)	Salt	24. (B12)	0.1 M (100 mM)
25. (C1)	1.0 M Sodium malonate pH 7.0	25. (C1)	Salt	25. (C1)	0.1 M (100 mM)
26. (C2)	0.1 M L-Proline	26. (C2)	Amino Acid	26. (C2)	0.01 M (10 mM)
27. (C3)	0.1 M Phenol	27. (C3)	Dissociating Agent	27. (C3)	0.01 M (10 mM)
28. (C4)	30% v/v Dimethyl sulfoxide	28. (C4)	Dissociating Agent	28. (C4)	3.0%
29. (C5)	0.1 M Sodium bromide	29. (C5)	Dissociating Agent	29. (C5)	0.01 M (10 mM)
30. (C6)	30% w/v 6-Aminohexanoic acid	30. (C6)	Linker	30. (C6)	3.0%
31. (C7)	30% w/v 1,5-Diaminopentane dihydrochloride	31. (C7)	Linker	31. (C7)	3.0%
32. (C8)	30% w/v 1,6-Diaminohexane	32. (C8)	Linker	32. (C8)	3.0%
33. (C9)	30% w/v 1,8-Diaminooctane	33. (C9)	Linker	33. (C9)	3.0%
34. (C10)	1.0 M Glycine	34. (C10)	Linker	34. (C10)	0.1 M (100 mM)
35. (C11)	0.3 M Glycyl-glycyl-glycine	35. (C11)	Linker	35. (C11)	0.03 M (30 mM)
36. (C12)	0.1 M Taurine	36. (C12)	Linker	36. (C12)	0.01 M (10 mM)
37. (D1)	0.1 M Betaine hydrochloride	37. (D1)	Linker	37. (D1)	0.01 M (10 mM)
38. (D2)	0.1 M Spermidine	38. (D2)	Polyamine	38. (D2)	0.01 M (10 mM)
39. (D3)	0.1 M Spermine tetrahydrochloride	39. (D3)	Polyamine	39. (D3)	0.01 M (10 mM)
40. (D4)	0.1 M Hexamine cobalt(III) chloride	40. (D4)	Polyamine	40. (D4)	0.01 M (10 mM)
41. (D5)	0.1 M Sarcosine	41. (D5)	Polyamine / Osmolyte	41. (D5)	0.01 M (10 mM)
42. (D6)	0.1 M Trimethylamine hydrochloride	42. (D6)	Chaotrope	42. (D6)	0.01 M (10 mM)
43. (D7)	1.0 M Guanidine hydrochloride	43. (D7)	Chaotrope	43. (D7)	0.1 M (100 mM)
44. (D8)	0.1 M Urea	44. (D8)	Chaotrope	44. (D8)	0.01 M (10 mM)
45. (D9)	0.1 M β -Nicotinamide adenine dinucleotide hydrate	45. (D9)	Co-factor	45. (D9)	0.01 M (10 mM)
46. (D10)	0.1 M Adenosine-5'-triphosphate disodium salt hydrate	46. (D10)	Co-factor	46. (D10)	0.01 M (10 mM)
47. (D11)	0.1 M TCEP hydrochloride	47. (D11)	Reducing Agent	47. (D11)	0.01 M (10 mM)
48. (D12)	0.01 M GSH (L-Glutathione reduced), 0.01 M GSSG (L-Glutathione oxidized)	48. (D12)	Reducing Agent	48. (D12)	0.001 M (1 mM)

Additive Screen HT (Deep Well Block) contains ninety-six unique reagents beginning at position A1.
To determine the formulation of each reagent, simply read across the page.

Lot 2138** where ** ≥ 16

Well #	Salt	Well #	Classification	Well #	Suggested Drop Concentration
49. (E1)	0.1 M Ethylenediaminetetraacetic acid disodium salt dihydrate	49. (E1)	Chelating Agent	49. (E1)	0.01 M (10 mM)
50. (E2)	5% w/v Polyvinylpyrrolidone K15	50. (E2)	Polymer	50. (E2)	0.5%
51. (E3)	30% w/v Dextran sulfate sodium salt (M _r 5,000)	51. (E3)	Polymer	51. (E3)	3.0%
52. (E4)	40% v/v Pentaerythritol ethoxylate (3/4 EO/OH)	52. (E4)	Polymer	52. (E4)	4.0%
53. (E5)	10% w/v Polyethylene glycol 3,350	53. (E5)	Polymer	53. (E5)	1.0%
54. (E6)	30% w/v D-(+)-Glucose monohydrate	54. (E6)	Carbohydrate	54. (E6)	3.0%
55. (E7)	30% w/v Sucrose	55. (E7)	Carbohydrate	55. (E7)	3.0%
56. (E8)	30% w/v Xylitol	56. (E8)	Carbohydrate	56. (E8)	3.0%
57. (E9)	30% w/v D-Sorbitol	57. (E9)	Carbohydrate	57. (E9)	3.0%
58. (E10)	12% w/v myo-Inositol	58. (E10)	Carbohydrate	58. (E10)	1.2%
59. (E11)	30% w/v D-(+)-Trehalose dihydrate	59. (E11)	Carbohydrate	59. (E11)	3.0%
60. (E12)	30% w/v D-(+)-Galactose	60. (E12)	Carbohydrate	60. (E12)	3.0%
61. (F1)	30% v/v Ethylene glycol	61. (F1)	Polyol	61. (F1)	3.0%
62. (F2)	30% v/v Glycerol	62. (F2)	Polyol	62. (F2)	3.0%
63. (F3)	3.0 M NDSB-195	63. (F3)	Non-detergent	63. (F3)	0.3 M (300 mM)
64. (F4)	2.0 M NDSB-201	64. (F4)	Non-detergent	64. (F4)	0.2 M (200 mM)
65. (F5)	2.0 M NDSB-211	65. (F5)	Non-detergent	65. (F5)	0.2 M (200 mM)
66. (F6)	2.0 M NDSB-221	66. (F6)	Non-detergent	66. (F6)	0.2 M (200 mM)
67. (F7)	1.0 M NDSB-256	67. (F7)	Non-detergent	67. (F7)	0.1 M (200 mM)
68. (F8)	0.5% w/v 1,2,3-Heptanetriol	68. (F8)	Amphiphile	68. (F8)	0.05%
69. (F9)	20% w/v Benzamidine hydrochloride	69. (F9)	Amphiphile	69. (F9)	2.0%
70. (F10)	5% w/v n-dodecyl-N,N-dimethylamine-N-oxide, (LDAO, DDAO)	70. (F10)	Detergent	70. (F10)	0.5%
71. (F11)	5% w/v n-Octyl-β-D-glucoside	71. (F11)	Detergent	71. (F11)	0.5%
72. (F12)	5% w/v n-Dodecyl-β-D-maltoside	72. (F12)	Detergent	72. (F12)	0.5%
73. (G1)	30% w/v Trimethylamine N-oxide dihydrate	73. (G1)	Osmolyte	73. (G1)	3.0%
74. (G2)	30% w/v 1,6-Hexanediol	74. (G2)	Organic, Non-volatile	74. (G2)	3.0%
75. (G3)	30% v/v (+/-)-2-Methyl-2,4-pentanediol	75. (G3)	Organic, Non-volatile	75. (G3)	3.0%
76. (G4)	50% v/v Polyethylene glycol 400	76. (G4)	Organic, Non-volatile	76. (G4)	5.0%
77. (G5)	50% v/v Jeffamine® M-600® pH 7.0	77. (G5)	Organic, Non-volatile	77. (G5)	5.0%
78. (G6)	40% v/v 2,5-Hexanediol (mixture of isomers)	78. (G6)	Organic, Non-volatile	78. (G6)	4.0%
79. (G7)	40% v/v (±)-1,3-Butanediol	79. (G7)	Organic, Non-volatile	79. (G7)	4.0%
80. (G8)	40% v/v Polypropylene glycol P 400	80. (G8)	Organic, Non-volatile	80. (G8)	4.0%
81. (G9)	30% v/v 1,4-Dioxane	81. (G9)	Organic, Volatile	81. (G9)	3.0%
82. (G10)	30% v/v Ethanol	82. (G10)	Organic, Volatile	82. (G10)	3.0%
83. (G11)	30% v/v 2-Propanol	83. (G11)	Organic, Volatile	83. (G11)	3.0%
84. (G12)	30% v/v Methanol	84. (G12)	Organic, Volatile	84. (G12)	3.0%
85. (H1)	10% v/v 1,2-Butanediol	85. (H1)	Organic, Volatile	85. (H1)	1.0%
86. (H2)	40% v/v tert-Butanol	86. (H2)	Organic, Volatile	86. (H2)	4.0%
87. (H3)	40% v/v 1,3-Propanediol	87. (H3)	Organic, Volatile	87. (H3)	4.0%
88. (H4)	40% v/v Acetonitrile	88. (H4)	Organic, Volatile	88. (H4)	4.0%
89. (H5)	40% v/v Formamide	89. (H5)	Organic, Volatile	89. (H5)	4.0%
90. (H6)	40% v/v 1-Propanol	90. (H6)	Organic, Volatile	90. (H6)	4.0%
91. (H7)	5% v/v Ethyl acetate	91. (H7)	Organic, Volatile	91. (H7)	0.5%
92. (H8)	40% v/v Acetone	92. (H8)	Organic, Volatile	92. (H8)	4.0%
93. (H9)	0.25% v/v Dichloromethane	93. (H9)	Organic, Volatile	93. (H9)	0.025%
94. (H10)	7% v/v 1-Butanol	94. (H10)	Organic, Volatile	94. (H10)	0.7%
95. (H11)	40% v/v 2,2,2-Trifluoroethanol	95. (H11)	Organic, Volatile	95. (H11)	4.0%
96. (H12)	40% v/v 1,1,1,3,3,3-Hexafluoro-2-propanol	96. (H12)	Organic, Volatile	96. (H12)	4.0%

Additive Screen HT (Deep Well Block) contains ninety-six unique reagents beginning at position A1.
To determine the formulation of each reagent, simply read across the page.

Lot 2138** where ** ≥16

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



Solutions for Crystal Growth

© 1991 - 2018 Hampton Research Corp. all rights reserved
Printed in the United States of America. This guide or
parts thereof may not be reproduced in any form without
the written permission of the publishers.

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

- 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)

Additive Screen HT™ - HR2-138 Scoring Sheet

		Date:	Date:	Date:	Date:
49. (E1)	0.1 M Ethylenediaminetetraacetic acid disodium salt dihydrate	Chelating Agent			
50. (E2)	5% w/v Polyvinylpyrrolidone K15	Polymer			
51. (E3)	30% w/v Dextran sulfate sodium salt (Mr 5,000)	Polymer			
52. (E4)	40% v/v Pentaerythritol ethoxylate (3/4 EO/OH)	Polymer			
53. (E5)	10% w/v Polyethylene glycol 3,350	Polymer			
54. (E6)	30% w/v D-(+)-Glucose monohydrate	Carbohydrate			
55. (E7)	30% w/v Sucrose	Carbohydrate			
56. (E8)	30% w/v Xylitol	Carbohydrate			
57. (E9)	30% w/v D-Sorbitol	Carbohydrate			
58. (E10)	12% w/v myo-Inositol	Carbohydrate			
59. (E11)	30% w/v D-(+)-Trehalose dihydrate	Carbohydrate			
60. (E12)	30% w/v D-(+)-Galactose	Carbohydrate			
61. (F1)	30% v/v Ethylene glycol	Polyol			
62. (F2)	30% v/v Glycerol	Polyol			
63. (F3)	3.0 M NDSB-195	Non-detergent			
64. (F4)	2.0 M NDSB-201	Non-detergent			
65. (F5)	2.0 M NDSB-211	Non-detergent			
66. (F6)	2.0 M NDSB-221	Non-detergent			
67. (F7)	1.0 M NDSB-256	Non-detergent			
68. (F8)	0.5% w/v 1,2,3-Heptanetriol	Amphiphile			
69. (F9)	20% w/v Benzamidine hydrochloride	Amphiphile			
70. (F10)	5% w/v n-dodecyl-N,N-dimethylamine-N-oxide, (LDAO, DDAO)	Detergent			
71. (F11)	5% w/v n-Octyl- β -D-glucoside	Detergent			
72. (F12)	5% w/v n-Dodecyl- β -D-maltoside	Detergent			
73. (G1)	30% w/v Trimethylamine N-oxide dihydrate	Osmolyte			
74. (G2)	30% w/v 1,6-Hexanediol	Organic, Non-volatile			
75. (G3)	30% v/v (+/-)-2-Methyl-2,4-pentanediol	Organic, Non-volatile			
76. (G4)	50% v/v Polyethylene glycol 400	Organic, Non-volatile			
77. (G5)	50% v/v Jeffamine® M-600® pH 7.0	Organic, Non-volatile			
78. (G6)	40% v/v 2,5-Hexanediol	Organic, Non-volatile			
79. (G7)	40% v/v (\pm)-1,3-Butanediol	Organic, Non-volatile			
80. (G8)	40% v/v Polypropylene glycol P 400	Organic, Non-volatile			
81. (G9)	30% v/v 1,4-Dioxane	Organic, Volatile			
82. (G10)	30% v/v Ethanol	Organic, Volatile			
83. (G11)	30% v/v 2-Propanol	Organic, Volatile			
84. (G12)	30% v/v Methanol	Organic, Volatile			
85. (H1)	10% v/v 1,2-Butanediol	Organic, Volatile			
86. (H2)	40% v/v tert-Butanol	Organic, Volatile			
87. (H3)	40% v/v 1,3-Propanediol	Organic, Volatile			
88. (H4)	40% v/v Acetonitrile	Organic, Volatile			
89. (H5)	40% v/v Formamide	Organic, Volatile			
90. (H6)	40% v/v 1-Propanol	Organic, Volatile			
91. (H7)	5% v/v Ethyl acetate	Organic, Volatile			
92. (H8)	40% v/v Acetone	Organic, Volatile			
93. (H9)	0.25% v/v Dichloromethane	Organic, Volatile			
94. (H10)	7% v/v 1-Butanol	Organic, Volatile			
95. (H11)	40% v/v 2,2,2-Trifluoroethanol	Organic, Volatile			
96. (H12)	40% v/v 1,1,1,3,3,3-Hexafluoro-2-propanol	Organic, Volatile			



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@hrcr.com
 Website: www.hamptonresearch.com

© 1991 - 2018 Hampton Research Corp. all rights reserved
 Printed in the United States of America. This guide or parts thereof may not
 be reproduced in any form without the written permission of the publishers.