MembFac HT™

HAMPTON RESEARCH
Solutions for Crystal Growth

Applications
Preliminary screen for the crystallization of membrane proteins as well as soluble biological macromolecules.

Features
- Single 96 Deep Well block format
- Compatible with robotics and multi-channel pipets
- Primary screen variables : salt, pH, and precipitant (salts, polymers, volatile organics, and non-volatile organics)
- Samples pH 4.6 – 8.5
- Membrane protein sparse matrix screen reagent conditions based on MembFac™ and Crystal Screen Lite™
- Preformulated, ready to screen
- Formulated for use with detergents

General Description
MembFac HT™ is a highly effective sparse matrix specifically designed as a preliminary screen for the crystallization of membrane proteins as well as soluble biological macromolecules. MembFac HT is designed as a 96 reagent crystallization screen that combines the strategies of MembFac and Crystal Screen Lite 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.

MembFac 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 MembFac 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.

Within the 96 Deep Well block, rows A through D feature the 48 reagents of MembFac (HR2-114). These reagent variables consist of pH, buffer material salt, salt, and precipitant. Five different pH's: 4.6, 5.6, 6.5, 7.5, and 8.5 are utilized with Sodium acetate trihydrate, Sodium citrate tribasic dihydrate, ADA, HEPES sodium, and TRIS hydrochloride as the reagent buffers. The four categories of precipitating reagents consist of: volatile agents, non-volatile agents, salts, and a combination of these three. These reagents were selected specifically for use with detergents.

Rows E through H feature the first 48 reagents of Crystal Screen Lite (HR2-128). Based upon the original Jancarik and Kim screen also known as Crystal Screen and designed to provide a rapid screening method for the crystallization of biological macromolecules, this kit is also effective in determining the solubility of these macromolecules in a wide range of precipitants and pH.[3] The primary screens are salt, pH, and precipitant (salts, polymers, volatile organics, and non-volatile organics).[2] Crystal Screen Lite differs from the original Crystal Screen kit such that the primary precipitant reagents are one-half the concentration of that used in the original screen. The secondary salts, ion, and buffers remain at the original Crystal Screen concentration. Reducing the primary concentration of the primary precipitant results in a screen which is "more gentle" on the sample and typically produces much less precipitate conditions than the original Crystal Screen. Results comparing the Crystal Screen Lite formulation versus simply diluting the Crystal Screen formulation two-fold demonstrated more crystals using the Crystal Screen Lite protocol than the two-fold diluted Crystal Screen illustrating the importance of retaining the original salt, ion, and buffer concentration in Crystal Screen.[5] Results comparing simply diluting the sample versus using Crystal Screen Lite also demonstrated more crystals when using Crystal Screen Lite than when simply diluting the sample. Crystal Screen Lite should be used with samples which demonstrate limited solubility in traditional crystallization reagents.[1,4]

Refer to the enclosed MembFac HT reagent formulation for additional information on all 96 reagents.

Sample Preparation
The membrane protein of interest is isolated in the detergent which gives the highest stability/activity ratio. The final protein concentration should be 10 to 20 mg/ml and the detergent concentration should only be slightly above the CMC.

The sample should be as pure as is practically possible (>95%) and free of amorphous and particulate material. Remove amorphous material by centrifugation prior to use.

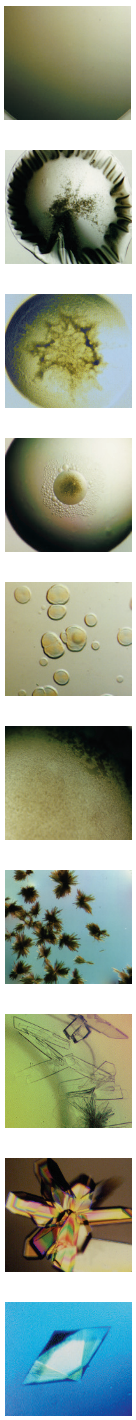
For additional sample preparation recommendation see Crystal Growth 101 - Preliminary Sample Preparation bulletin from Hampton Research.

Preparing the Deep Well Block for Use
It is recommended the Deep Well block be centrifuged 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 then pierced for reagent access.

Performing the Screen
<u>Manual Method - Sitting Drop Vapor Diffusion</u>

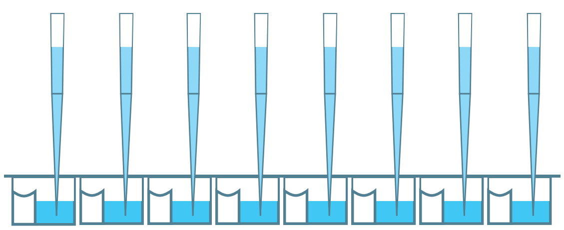
1. Using a 96 well sitting drop vapor diffusion plate, pipet the recommended

Figure 6
Typical observations in a crystallization experiment



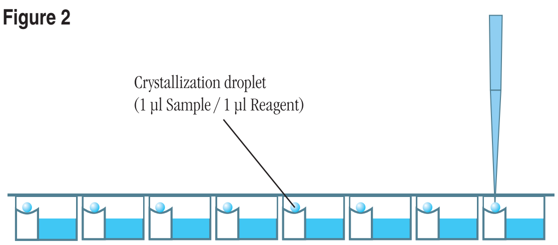
volume (typically 50 to 100 microliters) of crystallization reagent from the Deep Well block into the reservoirs of the crystallization plate. The Deep Well block is compatible with 8 and 12 channel pipets as well as many automated liquid handling systems. Use clean pipet tips for each reagent set transfer 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 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 multichannel 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 above.

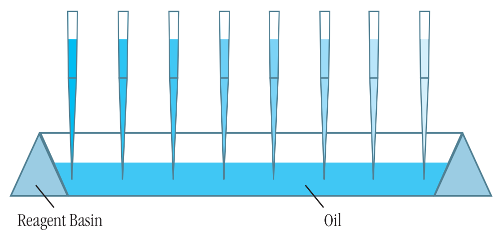
4. Seal the crystallization plate as per the manufacturer's recommendation. Most 96 well crystallization plates are sealed using a clear sealing tape or film. View and score the experiment as desired. See Hampton Research technical bulletin Crystal Growth 101 - Viewing Crystallization Experiments for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

5. Seal the remaining reagent in the Deep Well block using either clear sealing tape, film, or cap mat.

<u>Manual Method – Microbatch 96 well format</u>

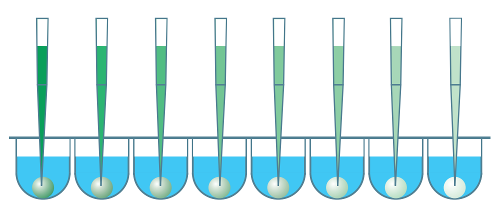
1. Using a 96 well clear polystyrene microplate (U-bottom recommended for best drop centering, flat-bottom recommended for best optics) pipet 50 to 150 microliters of microbatch compatible oil into each of the 96 reservoirs. This can be accomplished using an 8 or 12 channel pipet and pipetting the oil from a reagent basin. See Figure 3.

Figure 3



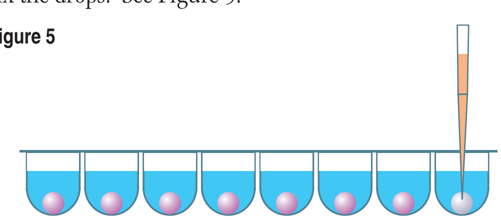
2. Once the plate is oiled, use an 8 or 12 channel pipet to aspirate reagent from the Deep Well block and dispense the reagent under the oil in the Microbatch plate. Change tips when changing reagent to prevent cross reagent contamination. To save time and pipet tips, set multiple plates at one time.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

MembFac 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 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 the left side of page 2 shows typical examples of what one might observe in a crystallization experiment.

Interpreting MembFac 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 super saturation 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.

MembFac 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

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Technical Support

Inquiries regarding MembFac 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)	0.1 M Sodium chloride	1. (A1)	0.1 M Sodium acetate trihydrate pH 4.6	1. (A1)	12% v/v (+/-)-2-Methyl-2,4-pentanediol
2. (A2)	0.1 M Zinc acetate dihydrate	2. (A2)	0.1 M Sodium acetate trihydrate pH 4.6	2. (A2)	12% w/v Polyethylene glycol 4,000
3. (A3)	0.2 M Ammonium sulfate	3. (A3)	0.1 M Sodium acetate trihydrate pH 4.6	3. (A3)	10% w/v Polyethylene glycol 4,000
4. (A4)	0.1 M Sodium chloride	4. (A4)	0.1 M Sodium acetate trihydrate pH 4.6	4. (A4)	12% v/v 2-Propanol
5. (A5)	None	5. (A5)	0.1 M Sodium acetate trihydrate pH 4.6	5. (A5)	12% w/v Polyethylene glycol 4,000
6. (A6)	None	6. (A6)	0.1 M Sodium acetate trihydrate pH 4.6	6. (A6)	1.0 M Ammonium sulfate
7. (A7)	None	7. (A7)	0.1 M Sodium acetate trihydrate pH 4.6	7. (A7)	1.0 M Magnesium sulfate heptahydrate
8. (A8)	0.1 M Magnesium chloride hexahydrate	8. (A8)	0.1 M Sodium acetate trihydrate pH 4.6	8. (A8)	18% v/v Polyethylene glycol 400
9. (A9)	0.1 M Lithium sulfate monohydrate	9. (A9)	0.1 M Sodium acetate trihydrate pH 4.6	9. (A9)	1.0 M Ammonium phosphate monobasic
10. (A10)	0.1 M Sodium chloride	10. (A10)	0.1 M Sodium acetate trihydrate pH 4.6	10. (A10)	12% w/v Polyethylene glycol 6,000
11. (A11)	0.1 M Magnesium chloride hexahydrate	11. (A11)	0.1 M Sodium acetate trihydrate pH 4.6	11. (A11)	12% w/v Polyethylene glycol 6,000
12. (A12)	0.1 M Sodium chloride	12. (A12)	0.1 M Sodium citrate tribasic dihydrate pH 5.6	12. (A12)	18% v/v Polyethylene glycol 400
13. (B1)	0.1 M Lithium sulfate monohydrate	13. (B1)	0.1 M Sodium citrate tribasic dihydrate pH 5.6	13. (B1)	12% w/v Polyethylene glycol 4,000
14. (B2)	0.1 M Sodium citrate tribasic dihydrate	14. (B2)	0.1 M Sodium citrate tribasic dihydrate pH 5.6	14. (B2)	10% v/v 2-Propanol
15. (B3)	0.1 M Sodium chloride	15. (B3)	0.1 M Sodium citrate tribasic dihydrate pH 5.6	15. (B3)	12% v/v (+/-)-2-Methyl-2,4-pentanediol
16. (B4)	None	16. (B4)	0.1 M Sodium citrate tribasic dihydrate pH 5.6	16. (B4)	1.0 M Magnesium sulfate heptahydrate
17. (B5)	0.1 M Sodium chloride	17. (B5)	0.1 M Sodium citrate tribasic dihydrate pH 5.6	17. (B5)	12% w/v Polyethylene glycol 4,000
18. (B6)	0.1 M Lithium sulfate monohydrate	18. (B6)	0.1 M Sodium citrate tribasic dihydrate pH 5.6	18. (B6)	12% w/v Polyethylene glycol 6,000
19. (B7)	0.1 M Magnesium chloride hexahydrate	19. (B7)	0.1 M Sodium citrate tribasic dihydrate pH 5.6	19. (B7)	4% v/v (+/-)-2-Methyl-2,4-pentanediol
20. (B8)	None	20. (B8)	0.1 M Sodium citrate tribasic dihydrate pH 5.6	20. (B8)	0.1 M Sodium chloride
21. (B9)	0.1 M Lithium sulfate monohydrate	21. (B9)	0.1 M Sodium citrate tribasic dihydrate pH 5.6	21. (B9)	4% v/v Polyethylene glycol 400
22. (B10)	None	22. (B10)	0.1 M ADA pH 6.5	22. (B10)	1.0 M Ammonium sulfate
23. (B11)	0.1 M Lithium sulfate monohydrate	23. (B11)	0.1 M ADA pH 6.5	23. (B11)	12% w/v Polyethylene glycol 4,000, 2% v/v 2-Propanol
24. (B12)	None	24. (B12)	0.1 M ADA pH 6.5	24. (B12)	1.0 M Ammonium phosphate dibasic
25. (C1)	0.1 M Magnesium chloride hexahydrate	25. (C1)	0.1 M ADA pH 6.5	25. (C1)	12% w/v Polyethylene glycol 6,000
26. (C2)	None	26. (C2)	0.1 M ADA pH 6.5	26. (C2)	12% v/v (+/-)-2-Methyl-2,4-pentanediol
27. (C3)	0.1 M Lithium sulfate monohydrate	27. (C3)	0.1 M ADA pH 6.5	27. (C3)	1.0 M Magnesium sulfate hydrate
28. (C4)	0.3 M Lithium sulfate monohydrate	28. (C4)	0.1 M ADA pH 6.5	28. (C4)	4% v/v Polyethylene glycol 400
29. (C5)	0.1 M Ammonium sulfate	29. (C5)	0.1 M HEPES sodium pH 7.5	29. (C5)	0.5 M Sodium phosphate dibasic dihydrate, 0.5 M Potassium phosphate dibasic
30. (C6)	0.1 M Sodium chloride	30. (C6)	0.1 M HEPES sodium pH 7.5	30. (C6)	10% w/v Polyethylene glycol 4,000
31. (C7)	0.1 M Magnesium chloride hexahydrate	31. (C7)	0.1 M HEPES sodium pH 7.5	31. (C7)	18% v/v Polyethylene glycol 400
32. (C8)	None	32. (C8)	0.1 M HEPES sodium pH 7.5	32. (C8)	1.0 M Potassium sodium tartrate tetrahydrate
33. (C9)	0.1 M Ammonium sulfate	33. (C9)	0.1 M HEPES sodium pH 7.5	33. (C9)	18% v/v Polyethylene glycol 400
34. (C10)	0.1 M Ammonium sulfate	34. (C10)	0.1 M HEPES sodium pH 7.5	34. (C10)	10% w/v Polyethylene glycol 4,000
35. (C11)	0.1 M Sodium citrate tribasic dihydrate	35. (C11)	0.1 M HEPES sodium pH 7.5	35. (C11)	12% v/v (+/-)-2-Methyl-2,4-pentanediol
36. (C12)	None	36. (C12)	0.1 M HEPES sodium pH 7.5	36. (C12)	1.0 M Sodium citrate tribasic dihydrate
37. (D1)	0.6 M Magnesium sulfate hydrate	37. (D1)	0.1 M HEPES sodium pH 7.5	37. (D1)	4% v/v Polyethylene glycol 400
38. (D2)	0.6 M Magnesium sulfate hydrate	38. (D2)	0.1 M HEPES sodium pH 7.5	38. (D2)	4% v/v (+/-)-2-Methyl-2,4-pentanediol
39. (D3)	0.1 M Lithium sulfate monohydrate	39. (D3)	0.1 M HEPES sodium pH 7.5	39. (D3)	0.1 M Potassium sodium tartrate tetrahydrate
40. (D4)	0.1 M Lithium sulfate monohydrate	40. (D4)	0.1 M TRIS hydrochloride pH 8.5	40. (D4)	12% v/v (+/-)-2-Methyl-2,4-pentanediol
41. (D5)	0.1 M Ammonium phosphate dibasic	41. (D5)	0.1 M TRIS hydrochloride pH 8.5	41. (D5)	0.5 M Sodium phosphate dibasic dihydrate, 0.5 M Potassium phosphate dibasic
42. (D6)	None	42. (D6)	0.1 M TRIS hydrochloride pH 8.5	42. (D6)	0.1 M Sodium acetate trihydrate
43. (D7)	None	43. (D7)	0.1 M TRIS hydrochloride pH 8.5	43. (D7)	0.1 M Sodium chloride
44. (D8)	0.1 M Ammonium phosphate dibasic	44. (D8)	0.1 M TRIS hydrochloride pH 8.5	44. (D8)	12% w/v Polyethylene glycol 6,000
45. (D9)	0.1 M Potassium sodium tartrate tetrahydrate	45. (D9)	0.1 M TRIS hydrochloride pH 8.5	45. (D9)	0.4 M Magnesium sulfate hydrate
46. (D10)	None	46. (D10)	0.1 M TRIS hydrochloride pH 8.5	46. (D10)	0.2 M Lithium sulfate monohydrate
47. (D11)	None	47. (D11)	0.1 M TRIS hydrochloride pH 8.5	47. (D11)	0.5 M Ammonium sulfate
48. (D12)	0.1 M Sodium citrate tribasic dihydrate	48. (D12)	0.1 M TRIS hydrochloride pH 8.5	48. (D12)	5% v/v Polyethylene glycol 400

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

MembFac 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.

Well #	Salt	Well #	Buffer ◊	Well #	Precipitant
49. (E1)	0.02 M Calcium chloride dihydrate	49. (E1)	0.1 M Sodium acetate trihydrate pH 4.6	49. (E1)	15% v/v (+/-)-2-Methyl-2,4-pentanediol
50. (E2)	None	50. (E2)	None	50. (E2)	0.2 M Potassium sodium tartrate tetrahydrate
51. (E3)	None	51. (E3)	None	51. (E3)	0.2 M Ammonium phosphate monobasic
52. (E4)	None	52. (E4)	0.1 M TRIS hydrochloride pH 8.5	52. (E4)	1.0 M Ammonium sulfate
53. (E5)	0.2 M Sodium citrate tribasic dihydrate	53. (E5)	0.1 M HEPES sodium pH 7.5	53. (E5)	15% v/v (+/-)-2-Methyl-2,4-pentanediol
54. (E6)	0.2 M Magnesium chloride hexahydrate	54. (E6)	0.1 M TRIS hydrochloride pH 8.5	54. (E6)	15% w/v Polyethylene glycol 4,000
55. (E7)	None	55. (E7)	0.1 M Sodium cacodylate trihydrate pH 6.5	55. (E7)	0.7 M Sodium acetate trihydrate
56. (E8)	0.2 M Sodium citrate tribasic dihydrate	56. (E8)	0.1 M Sodium cacodylate trihydrate pH 6.5	56. (E8)	15% v/v 2-Propanol
57. (E9)	0.2 M Ammonium acetate	57. (E9)	0.1 M Sodium citrate tribasic dihydrate pH 5.6	57. (E9)	15% w/v Polyethylene glycol 4,000
58. (E10)	0.2 M Ammonium acetate	58. (E10)	0.1 M Sodium acetate trihydrate pH 4.6	58. (E10)	15% w/v Polyethylene glycol 4,000
59. (E11)	None	59. (E11)	0.1 M Sodium citrate tribasic dihydrate pH 5.6	59. (E11)	0.5 M Ammonium phosphate monobasic
60. (E12)	0.2 M Magnesium chloride hexahydrate	60. (E12)	0.1 M HEPES sodium pH 7.5	60. (E12)	15% v/v 2-Propanol
61. (F1)	0.2 M Sodium citrate tribasic dihydrate	61. (F1)	0.1 M TRIS hydrochloride pH 8.5	61. (F1)	15% v/v Polyethylene glycol 400
62. (F2)	0.2 M Calcium chloride dihydrate	62. (F2)	0.1 M HEPES sodium pH 7.5	62. (F2)	14% v/v Polyethylene glycol 400
63. (F3)	0.2 M Ammonium sulfate	63. (F3)	0.1 M Sodium cacodylate trihydrate pH 6.5	63. (F3)	15% w/v Polyethylene glycol 8,000
64. (F4)	None	64. (F4)	0.1 M HEPES sodium pH 7.5	64. (F4)	0.75 M Lithium sulfate monohydrate
65. (F5)	0.2 M Lithium sulfate monohydrate	65. (F5)	0.1 M TRIS hydrochloride pH 8.5	65. (F5)	15% w/v Polyethylene glycol 4,000
66. (F6)	0.2 M Magnesium acetate tetrahydrate	66. (F6)	0.1 M Sodium cacodylate trihydrate pH 6.5	66. (F6)	10% w/v Polyethylene glycol 8,000
67. (F7)	0.2 M Ammonium acetate	67. (F7)	0.1 M TRIS hydrochloride pH 8.5	67. (F7)	15% v/v 2-Propanol
68. (F8)	0.2 M Ammonium sulfate	68. (F8)	0.1 M Sodium acetate trihydrate pH 4.6	68. (F8)	12.5% w/v Polyethylene glycol 4,000
69. (F9)	0.2 M Magnesium acetate tetrahydrate	69. (F9)	0.1 M Sodium cacodylate trihydrate pH 6.5	69. (F9)	15% v/v (+/-)-2-Methyl-2,4-pentanediol
70. (F10)	0.2 M Sodium acetate trihydrate	70. (F10)	0.1 M TRIS hydrochloride pH 8.5	70. (F10)	15% w/v Polyethylene glycol 4,000
71. (F11)	0.2 M Magnesium chloride hexahydrate	71. (F11)	0.1 M HEPES sodium pH 7.5	71. (F11)	15% v/v Polyethylene glycol 400
72. (F12)	0.2 M Calcium chloride dihydrate	72. (F12)	0.1 M Sodium acetate trihydrate pH 4.6	72. (F12)	10% v/v 2-Propanol
73. (G1)	None	73. (G1)	0.1 M Imidazole pH 6.5	73. (G1)	0.5 M Sodium acetate trihydrate
74. (G2)	0.2 M Ammonium acetate	74. (G2)	0.1 M Sodium citrate tribasic dihydrate pH 5.6	74. (G2)	15% v/v (+/-)-2-Methyl-2,4-pentanediol
75. (G3)	0.2 M Sodium citrate tribasic dihydrate	75. (G3)	0.1 M HEPES sodium pH 7.5	75. (G3)	10% v/v 2-Propanol
76. (G4)	0.2 M Sodium acetate trihydrate	76. (G4)	0.1 M Sodium cacodylate trihydrate pH 6.5	76. (G4)	15% w/v Polyethylene glycol 8,000
77. (G5)	None	77. (G5)	0.1 M HEPES sodium pH 7.5	77. (G5)	0.4 M Potassium sodium tartrate tetrahydrate
78. (G6)	0.2 M Ammonium sulfate	78. (G6)	None	78. (G6)	15% w/v Polyethylene glycol 8,000
79. (G7)	0.2 M Ammonium sulfate	79. (G7)	None	79. (G7)	15% w/v Polyethylene glycol 4,000
80. (G8)	None	80. (G8)	None	80. (G8)	1.0 M Ammonium sulfate
81. (G9)	None	81. (G9)	None	81. (G9)	2.0 M Sodium formate
82. (G10)	None	82. (G10)	0.1 M Sodium acetate trihydrate pH 4.6	82. (G10)	1.0 M Sodium formate
83. (G11)	None	83. (G11)	0.1 M HEPES sodium pH 7.5	83. (G11)	0.4 M Sodium phosphate monobasic monohydrate, 0.4 M Potassium phosphate monobasic
84. (G12)	None	84. (G12)	0.1 M TRIS hydrochloride pH 8.5	84. (G12)	4% w/v Polyethylene glycol 8,000
85. (H1)	None	85. (H1)	0.1 M Sodium acetate trihydrate pH 4.6	85. (H1)	4% w/v Polyethylene glycol 4,000
86. (H2)	None	86. (H2)	0.1 M HEPES sodium pH 7.5	86. (H2)	0.7 M Sodium citrate tribasic dihydrate
87. (H3)	None	87. (H3)	0.1 M HEPES sodium pH 7.5	87. (H3)	2% v/v Polyethylene glycol 400, 1.0 M Ammonium sulfate
88. (H4)	None	88. (H4)	0.1 M Sodium citrate tribasic dihydrate pH 5.6	88. (H4)	10% v/v 2-Propanol, 10% w/v Polyethylene glycol 4,000
89. (H5)	None	89. (H5)	0.1 M HEPES sodium pH 7.5	89. (H5)	5% v/v 2-Propanol, 10% w/v Polyethylene glycol 4,000
90. (H6)	0.05 M Potassium phosphate monobasic	90. (H6)	None	90. (H6)	10% w/v Polyethylene glycol 8,000
91. (H7)	None	91. (H7)	None	91. (H7)	15% w/v Polyethylene glycol 1,500
92. (H8)	None	92. (H8)	None	92. (H8)	0.1 M Magnesium formate dihydrate
93. (H9)	0.2 M Zinc acetate dihydrate	93. (H9)	0.1 M Sodium cacodylate trihydrate pH 6.5	93. (H9)	9% w/v Polyethylene glycol 8,000
94. (H10)	0.2 M Calcium acetate hydrate	94. (H10)	0.1 M Sodium cacodylate trihydrate pH 6.5	94. (H10)	9% w/v Polyethylene glycol 8,000
95. (H11)	None	95. (H11)	0.1 M Sodium acetate trihydrate pH 4.6	95. (H11)	1.0 M Ammonium sulfate
96. (H12)	None	96. (H12)	0.1 M TRIS hydrochloride pH 8.5	96. (H12)	1.0 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.

MembFac 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.

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

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Solutions for Crystal Growth

Sample: ______________________________
Sample Buffer: ______________________________
Reservoir Volume: __________
Drop Volume: Total ______ µl Sample ______ µl Reservoir ______ µl Additive ______ µl

Sample Concentration: __________
Date: ____________________
Temperature: ____________________

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)

MembFac HT™ - HR2-137 Scoring Sheet	Date:	Date:	Date:
1. (A1) 0.1 M Sodium chloride, 0.1 M Sodium acetate trihydrate pH 4.6, 12% v/v (+/-)-2-Methyl-2,4-pentanediol			
2. (A2) 0.1 M Zinc acetate dihydrate, 0.1 M Sodium acetate trihydrate pH 4.6, 12% w/v Polyethylene glycol 4,000			
3. (A3) 0.2 M Ammonium sulfate, 0.1 M Sodium acetate trihydrate pH 4.6, 10% w/v Polyethylene glycol 4,000			
4. (A4) 0.1 M Sodium chloride, 0.1 M Sodium acetate trihydrate pH 4.6, 12% v/v 2-Propanol			
5. (A5) 0.1 M Sodium acetate trihydrate pH 4.6, 12% w/v Polyethylene glycol 4,000			
6. (A6) 0.1 M Sodium acetate trihydrate pH 4.6, 1.0 M Ammonium sulfate			
7. (A7) 0.1 M Sodium acetate trihydrate pH 4.6, 1.0 M Magnesium sulfate heptahydrate			
8. (A8) 0.1 M Magnesium chloride hexahydrate, 0.1 M Sodium acetate trihydrate pH 4.6, 18% v/v Polyethylene glycol 400			
9. (A9) 0.1 M Lithium sulfate monohydrate, 0.1 M Sodium acetate trihydrate pH 4.6, 1.0 M Ammonium phosphate monobasic			
10. (A10) 0.1 M Sodium chloride, 0.1 M Sodium acetate trihydrate pH 4.6, 12% w/v Polyethylene glycol 6,000			
11. (A11) 0.1 M Magnesium chloride hexahydrate, 0.1 M Sodium acetate trihydrate pH 4.6, 12% w/v Polyethylene glycol 6,000			
12. (A12) 0.1 M Sodium chloride, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 18% v/v Polyethylene glycol 400			
13. (B1) 0.1 M Lithium sulfate monohydrate, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 12 % w/v Polyethylene glycol 4,000			
14. (B2) 0.1 M Sodium citrate tribasic dihydrate, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 10% v/v 2-Propanol			
15. (B3) 0.1 M Sodium chloride, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 12% v/v (+/-)-2-Methyl-2,4-pentanediol			
16. (B4) 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 1.0 M Magnesium sulfate heptahydrate			
17. (B5) 0.1 M Sodium chloride, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 12% w/v Polyethylene glycol 4,000			
18. (B6) 0.1 M Lithium sulfate monohydrate, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 12% w/v Polyethylene glycol 6,000			
19. (B7) 0.1 M Magnesium chloride hexahydrate, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 4% v/v (+/-)-2-Methyl-2,4-pentanediol			
20. (B8) 0.1 M Sodium citrate trihydrate dihydrate pH 5.6, 0.1 M Sodium chloride			
21. (B9) 0.1 M Lithium sulfate monohydrate, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 4% v/v Polyethylene glycol 400			
22. (B10) 0.1 M ADA pH 6.5, 1.0 M Ammonium sulfate			
23. (B11) 0.1 M Lithium sulfate monohydrate, 0.1 M ADA pH 6.5, 12% w/v Polyethylene glycol 4,000, 2% v/v 2-Propanol			
24. (B12) 0.1 M ADA pH 6.5, 1.0 M Ammonium phosphate dibasic			
25. (C1) 0.1 M Magnesium chloride hexahydrate, 0.1 M ADA pH 6.5, 12% w/v Polyethylene glycol 6,000			
26. (C2) 0.1 M ADA pH 6.5, 12% v/v (+/-)-2-Methyl-2,4-pentanediol			
27. (C3) 0.1 M Lithium sulfate monohydrate, 0.1 M ADA pH 6.5, 1.0 M Magnesium sulfate hydrate			
28. (C4) 0.3 M Lithium sulfate monohydrate, 0.1 M ADA pH 6.5, 4% v/v Polyethylene glycol 400			
29. (C5) 0.1 M Ammonium sulfate, 0.1 M HEPES sodium pH 7.5, 0.5 M Sodium phosphate dibasic dihydrate, 0.5 M Potassium phosphate dibasic			
30. (C6) 0.1 M Sodium chloride, 0.1 M HEPES sodium pH 7.5, 10% w/v Polyethylene glycol 4,000			
31. (C7) 0.1 M Magnesium chloride hexahydrate, 0.1 M HEPES sodium pH 7.5, 18% v/v Polyethylene glycol 400			
32. (C8) 0.1 M HEPES sodium pH 7.5, 1.0 M Potassium sodium tartrate tetrahydrate			
33. (C9) 0.1 M Ammonium sulfate, 0.1 M HEPES sodium pH 7.5, 18% v/v Polyethylene glycol 400			
34. (C10) 0.1 M Ammonium sulfate, 0.1 M HEPES sodium pH 7.5, 10% w/v Polyethylene glycol 4,000			
35. (C11) 0.1 M Sodium citrate tribasic dihydrate, 0.1 M HEPES sodium pH 7.5, 12% v/v (+/-)-2-Methyl-2,4-pentanediol			
36. (C12) 0.1 M HEPES sodium pH 7.5, 1.0 M Sodium citrate tribasic dihydrate			
37. (D1) 0.6 M Magnesium sulfate hydrate, 0.1 M HEPES sodium pH 7.5, 4% v/v Polyethylene glycol 400			
38. (D2) 0.6 M Magnesium sulfate hydrate, 0.1 M HEPES sodium pH 7.5, 4% v/v (+/-)-2-Methyl-2,4-pentanediol			
39. (D3) 0.1 M Lithium sulfate monohydrate, 0.1 M HEPES sodium pH 7.5, 0.1 M Potassium sodium tartrate tetrahydrate			
40. (D4) 0.1 M Lithium sulfate monohydrate, 0.1 M TRIS hydrochloride pH 8.5, 12% v/v (+/-)-2-Methyl-2,4-pentanediol			
41. (D5) 0.1 M Ammonium phosphate dibasic, 0.1 M TRIS hydrochloride pH 8.5, 0.5 M Na phosphate dibasic dihydrate, 0.5 M Potassium phosphate dibasic			
42. (D6) 0.1 M TRIS hydrochloride pH 8.5, 0.1 M Sodium acetate trihydrate			
43. (D7) 0.1 M TRIS hydrochloride pH 8.5, 0.1 M Sodium chloride			
44. (D8) 0.1 M Ammonium phosphate dibasic, 0.1 M TRIS hydrochloride pH 8.5, 12% w/v Polyethylene glycol 6,000			
45. (D9) 0.1 M Potassium sodium tartrate tetrahydrate, 0.1 M TRIS hydrochloride pH 8.5, 0.4 M Magnesium sulfate hydrate			
46. (D10) 0.1 M TRIS hydrochloride pH 8.5, 0.2 M Lithium sulfate monohydrate			
47. (D11) 0.1 M TRIS hydrochloride pH 8.5, 0.5 M Ammonium sulfate			
48. (D12) 0.1 M Sodium citrate tribasic dihydrate, 0.1 M TRIS hydrochloride pH 8.5, 5% v/v Polyethylene glycol 400			

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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

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)

MembFac HT™ - HR2-137 Scoring Sheet		Date:	Date:	Date:
49. (E1)	0.02 M Calcium chloride dihydrate, 0.1 M Sodium acetate trihydrate pH 4.6, 15% v/v (+/-)-2-Methyl-2,4-pentanediol			
50. (E2)	0.2 M Potassium sodium tartrate tetrahydrate			
51. (E3)	0.2 M Ammonium phosphate monobasic			
52. (E4)	0.1 M TRIS hydrochloride pH 8.5, 1.0 M Ammonium sulfate			
53. (E5)	0.2 M Sodium citrate tribasic dihydrate, 0.1 M HEPES sodium pH 7.5, 15% v/v (+/-)-2-Methyl-2,4-pentanediol			
54. (E6)	0.2 M Magnesium chloride hexahydrate, 0.1 M TRIS hydrochloride pH 8.5, 15% w/v Polyethylene glycol 4,000			
55. (E7)	0.1 M Sodium cacodylate trihydrate pH 6.5, 0.7 M Sodium acetate trihydrate			
56. (E8)	0.2 M Sodium citrate tribasic dihydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 15% v/v 2-Propanol			
57. (E9)	0.2 M Ammonium acetate, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 15% w/v Polyethylene glycol 4,000			
58. (E10)	0.2 M Ammonium acetate, 0.1 M Sodium acetate trihydrate pH 4.6, 15% w/v Polyethylene glycol 4,000			
59. (E11)	0.1 M Sodium citrate tribasic dihydrate pH 5.6, 0.5 M Ammonium phosphate monobasic			
60. (E12)	0.2 M Magnesium chloride hexahydrate, 0.1 M HEPES sodium pH 7.5, 15% v/v 2-Propanol			
61. (F1)	0.2 M Sodium citrate tribasic dihydrate, 0.1 M TRIS hydrochloride pH 8.5, 15% v/v Polyethylene glycol 400			
62. (F2)	0.2 M Calcium chloride dihydrate, 0.1 M HEPES sodium pH 7.5, 14% v/v Polyethylene glycol 400			
63. (F3)	0.2 M Ammonium sulfate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 15% w/v Polyethylene glycol 8,000			
64. (F4)	0.1 M HEPES sodium pH 7.5, 0.75 M Lithium sulfate monohydrate			
65. (F5)	0.2 M Lithium sulfate monohydrate, 0.1 M TRIS hydrochloride pH 8.5, 15% w/v Polyethylene glycol 4,000			
66. (F6)	0.2 M Magnesium acetate tetrahydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 10% w/v Polyethylene glycol 8,000			
67. (F7)	0.2 M Ammonium acetate, 0.1 M TRIS hydrochloride pH 8.5, 15% v/v 2-Propanol			
68. (F8)	0.2 M Ammonium sulfate, 0.1 M Sodium acetate trihydrate pH 4.6, 12.5% w/v Polyethylene glycol 4,000			
69. (F9)	0.2 M Magnesium acetate tetrahydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 15% v/v (+/-)-2-Methyl-2,4-pentanediol			
70. (F10)	0.2 M Sodium acetate trihydrate, 0.1 M TRIS hydrochloride pH 8.5, 15% w/v Polyethylene glycol 4,000			
71. (F11)	0.2 M Magnesium chloride hexahydrate, 0.1 M HEPES sodium pH 7.5, 15% v/v Polyethylene glycol 400			
72. (F12)	0.2 M Calcium chloride dihydrate, 0.1 M Sodium acetate trihydrate pH 4.6, 10% v/v 2-Propanol			
73. (G1)	0.1 M Imidazole pH 6.5, 0.5 M Sodium acetate trihydrate			
74. (G2)	0.2 M Ammonium acetate, 0.1 M Sodium citrate tribasic dihydrate pH 5.6, 15% v/v (+/-)-2-Methyl-2,4-pentanediol			
75. (G3)	0.2 M Sodium citrate tribasic dihydrate, 0.1 M HEPES sodium pH 7.5, 10% v/v 2-Propanol			
76. (G4)	0.2 M Sodium acetate trihydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 15% w/v Polyethylene glycol 8,000			
77. (G5)	0.1 M HEPES sodium pH 7.5, 0.4 M Potassium sodium tartrate tetrahydrate			
78. (G6)	0.2 M Ammonium sulfate, 15% w/v Polyethylene glycol 8,000			
79. (G7)	0.2 M Ammonium sulfate, 15% w/v Polyethylene glycol 4,000			
80. (G8)	1.0 M Ammonium sulfate			
81. (G9)	2.0 M Sodium formate			
82. (G10)	0.1 M Sodium acetate trihydrate pH 4.6, 1.0 M Sodium formate			
83. (G11)	0.1 M HEPES sodium pH 7.5, 0.4 M Sodium phosphate monobasic monohydrate, 0.4 M Potassium phosphate monobasic			
84. (G12)	0.1 M TRIS hydrochloride pH 8.5, 4% w/v Polyethylene glycol 8,000			
85. (H1)	0.1 M Sodium acetate trihydrate pH 4.6, 4% w/v Polyethylene glycol 4,000			
86. (H2)	0.1 M HEPES sodium pH 7.5, 0.7 M Sodium citrate tribasic dihydrate			
87. (H3)	0.1 M HEPES sodium pH 7.5, 2% v/v Polyethylene glycol 400, 1.0 M Ammonium sulfate			
88. (H4)	0.1 M Sodium citrate tribasic dihydrate pH 5.6, 10% v/v 2-Propanol, 10% w/v Polyethylene glycol 4,000			
89. (H5)	0.1 M HEPES sodium pH 7.5, 5% v/v 2-Propanol, 10% w/v Polyethylene glycol 4,000			
90. (H6)	0.05 M Potassium phosphate monobasic, 10% w/v Polyethylene glycol 8,000			
91. (H7)	15% w/v Polyethylene glycol 1,500			
92. (H8)	0.1 M Magnesium formate dihydrate			
93. (H9)	0.2 M Zinc acetate dihydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 9% w/v Polyethylene glycol 8,000			
94. (H10)	0.2 M Calcium acetate hydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 9% w/v Polyethylene glycol 8,000			
95. (H11)	0.1 M Sodium acetate trihydrate pH 4.6, 1.0 M Ammonium sulfate			
96. (H12)	0.1 M TRIS hydrochloride pH 8.5, 1.0 M Ammonium phosphate monobasic			

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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