

User Guide

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Applications

Crystallization screen for nucleic acids and nucleic acid-protein complexes.

Features

The kit is designed to provide a biased sparse matrix of trial conditions selected from known and published crystallization conditions. The reagent parameter variables are:

- pH
- Buffer material
- Salt
- Precipitant

Seven different pH's 5.5, 5.6, 6.0, 6.5, 7.0, 7.5, and 8.5 are utilized with six buffers:

- MES monohydrate
- Sodium cacodylate trihydrate
- HEPES sodium
- MOPS
- PIPES
- TRIS hydrochloride

The four categories of precipitating agents utilized are:

- Volatile agents
- Non-volatile agents
- Salts
- A combination of volatile agents, non-volatile agents, and salts

Refer to the enclosed Natrix HT reagent formulation for additional information.

General Description

Natrix 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.

Sample Preparation

The sample should be as pure as practically possible (> 95%) and free of amorphous and particulate material. Remove amorphous material by centrifugation or micro-filtration when appropriate, prior to use.

Recommended stock concentration of the nucleic acid is 0.5 to 1.0 mM or 5 to 10 mg/ml depending upon the solubility and size of the sample. The nucleic acid should be solubilized in a water based system which promotes the stability and mono-dispersity of the nucleic acid. If a buffer is utilized for nucleic acid preparation, a concentration of 5 to 10 mM is recommended in order to allow the buffers in Natrix HT to alter the pH of the sample drop.

One may wish to include a polyamine such as spermine or spermidine at a concentration of 0.5 to 1.5 mM. The polyamine need not be added to the reservoir. Finally, when sample annealing is desired, the sample should be preheated to 50°C to 95°C for 10 minutes then cooled slowly to 25°C (room

temperature) in the presence of the samples buffer and 5-20 mM Magnesium chloride to produce native molecules.¹ After cooling, centrifuge and micro-filter the sample.

Preparing the Deep Well Block for Use

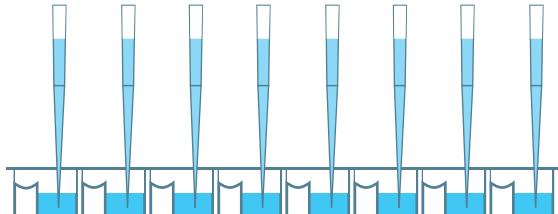
It is recommended the Deep Well block be centrifuged before removing the sealing film. Centrifugation at 500 rpm for five minutes will remove stray reagent from the sealing film. Removing the reagent from the film prevents stray reagent droplets from falling into neighboring wells during film removal. 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

Manual Method - Sitting Drop Vapor Diffusion

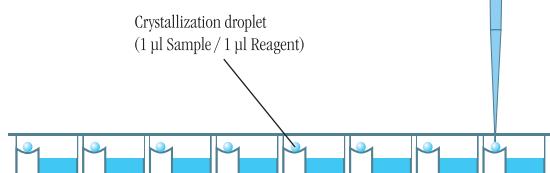
1. Using a 96 well sitting drop vapor diffusion plate, pipet the recommended volume (typically 50 to 100 microliters) of crystallization reagent from the Deep Well block into the reservoirs of the crystallization plate. The Deep Well block is compatible with 8 and 12 channel pipets as well as many automated liquid handling systems. Use clean pipet tips for each reagent set transfer and change pipet tips when changing reagents. For an 8 channel pipet, transfer reagents A1-H1 to reservoirs A1-H1 of the crystallization plate. Repeat this procedure for reagent columns 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



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Figure 6

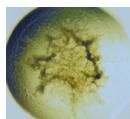
Typical observations in a crystallization experiment



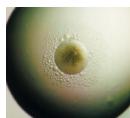
Clear Drop



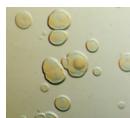
Skin /
Precipitate



Precipitate



Precipitate /
Phase



Quasi
Crystals



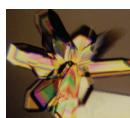
Microcrystals



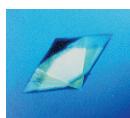
Needle
Cluster



Plates



Rod Cluster



Single
Crystal

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 on page 1.

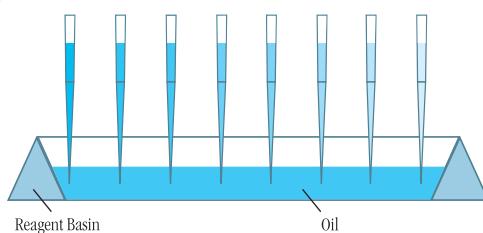
4. Seal the crystallization plate as per the manufacturer's recommendation. Most 96 well crystallization plates are sealed using a clear sealing tape or film. View and score the experiment as desired. See Hampton Research technical bulletin Crystal Growth 101 - Viewing Crystallization Experiments for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

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

Manual Method – Microbatch 96 well format

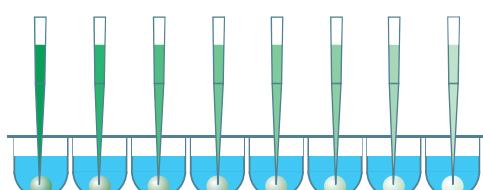
1. Using a 96 well clear polystyrene microplate (U-bottom recommended for best drop centering, flat-bottom recommended for best optics) pipet 50 to 150 microliters of microbatch compatible oil into each of the 96 reservoirs. This can be accomplished using an 8 or 12 channel pipet and pipetting the oil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

Figure 3



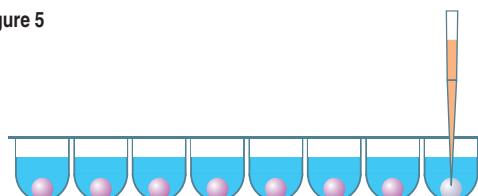
2. Once the plate is oiled, use an 8 or 12 channel pipet to aspirate reagent from the Deep Well block and dispense the reagent under the oil in the Microbatch plate. Change tips when changing reagent to prevent cross reagent contamination. To save time and pipet tips, set multiple plates at one time.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

Natrix 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 bipyramidal crystals, clear drop, 3+ needle shaped crystals in 1+ white precipitate.

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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 Natrix 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.

Natrix 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 OptimizeTM and StockOptionsTM stock solutions of salts, polymers and bu-

fers. 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 it is strongly recommended that crystallization reagents 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 Natrix 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.

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Well #	Salt	Well #	Buffer ♦	Well #	Precipitant
1. (A1)	0.01 M Magnesium chloride hexahydrate	1. (A1)	0.05 M MES monohydrate pH 5.6	1. (A1)	1.8 M Lithium sulfate monohydrate
2. (A2)	0.01 M Magnesium acetate tetrahydrate	2. (A2)	0.05 M MES monohydrate pH 5.6	2. (A2)	2.5 M Ammonium sulfate
3. (A3)	0.1 M Magnesium acetate tetrahydrate	3. (A3)	0.05 M MES monohydrate pH 5.6	3. (A3)	20% v/v (+/-)-2-Methyl-2,4-pentanediol
4. (A4)	0.2 M Potassium chloride, 0.01 M Magnesium sulfate heptahydrate	4. (A4)	0.05 M MES monohydrate pH 5.6	4. (A4)	10% v/v Polyethylene glycol 400
5. (A5)	0.2 M Potassium chloride, 0.01 M Magnesium chloride hexahydrate	5. (A5)	0.05 M MES monohydrate pH 5.6	5. (A5)	5% w/v Polyethylene glycol 8,000
6. (A6)	0.1 M Ammonium sulfate, 0.01 M Magnesium chloride hexahydrate	6. (A6)	0.05 M MES monohydrate pH 5.6	6. (A6)	20% w/v Polyethylene glycol 8,000
7. (A7)	0.02 M Magnesium chloride hexahydrate	7. (A7)	0.05 M MES monohydrate pH 6.0	7. (A7)	15% v/v 2-Propanol
8. (A8)	0.1 M Ammonium acetate, 0.005 M Magnesium sulfate heptahydrate	8. (A8)	0.05 M MES monohydrate pH 6.0	8. (A8)	0.6 M Sodium chloride
9. (A9)	0.1 M Potassium chloride, 0.01 M Magnesium chloride hexahydrate	9. (A9)	0.05 M MES monohydrate pH 6.0	9. (A9)	10% v/v Polyethylene glycol 400
10. (A10)	0.005 M Magnesium sulfate heptahydrate	10. (A10)	0.05 M MES monohydrate pH 6.0	10. (A10)	5% w/v Polyethylene glycol 4,000
11. (A11)	0.01 M Magnesium chloride hexahydrate	11. (A11)	0.05 M Sodium cacodylate trihydrate pH 6.0	11. (A11)	1.0 M Lithium sulfate monohydrate
12. (A12)	0.01 M Magnesium sulfate heptahydrate	12. (A12)	0.05 M Sodium cacodylate trihydrate pH 6.0	12. (A12)	1.8 M Lithium sulfate monohydrate
13. (B1)	0.015 M Magnesium acetate tetrahydrate	13. (B1)	0.05 M Sodium cacodylate trihydrate pH 6.0	13. (B1)	1.7 M Ammonium sulfate
14. (B2)	0.1 M Potassium chloride, 0.025 M Magnesium chloride hexahydrate	14. (B2)	0.05 M Sodium cacodylate trihydrate pH 6.0	14. (B2)	15% v/v 2-Propanol
15. (B3)	0.04 M Magnesium chloride hexahydrate	15. (B3)	0.05 M Sodium cacodylate trihydrate pH 6.0	15. (B3)	5% v/v (+/-)-2-Methyl-2,4-pentanediol
16. (B4)	0.04 M Magnesium acetate tetrahydrate	16. (B4)	0.05 M Sodium cacodylate trihydrate pH 6.0	16. (B4)	30% v/v (+/-)-2-Methyl-2,4-pentanediol
17. (B5)	0.2 M Potassium chloride, 0.01 M Calcium chloride dihydrate	17. (B5)	0.05 M Sodium cacodylate trihydrate pH 6.0	17. (B5)	10% w/v Polyethylene glycol 4,000
18. (B6)	0.01 M Magnesium acetate tetrahydrate	18. (B6)	0.05 M Sodium cacodylate trihydrate pH 6.5	18. (B6)	1.3 M Lithium sulfate monohydrate
19. (B7)	0.01 M Magnesium sulfate heptahydrate	19. (B7)	0.05 M Sodium cacodylate trihydrate pH 6.5	19. (B7)	2.0 M Ammonium sulfate
20. (B8)	0.1 M Ammonium acetate, 0.015 M Magnesium acetate tetrahydrate	20. (B8)	0.05 M Sodium cacodylate trihydrate pH 6.5	20. (B8)	10% v/v 2-Propanol
21. (B9)	0.2 M Potassium chloride, 0.005 M Magnesium chloride hexahydrate	21. (B9)	0.05 M Sodium cacodylate trihydrate pH 6.5	21. (B9)	0.9 M 1,6-Hexanediol
22. (B10)	0.08 M Magnesium acetate tetrahydrate	22. (B10)	0.05 M Sodium cacodylate trihydrate pH 6.5	22. (B10)	15% v/v Polyethylene glycol 400
23. (B11)	0.2 M Potassium chloride, 0.01 M Magnesium chloride hexahydrate	23. (B11)	0.05 M Sodium cacodylate trihydrate pH 6.5	23. (B11)	10% w/v Polyethylene glycol 4,000
24. (B12)	0.2 M Ammonium acetate, 0.01 M Calcium chloride dihydrate	24. (B12)	0.05 M Sodium cacodylate trihydrate pH 6.5	24. (B12)	10% w/v Polyethylene glycol 4,000
25. (C1)	0.08 M Magnesium acetate tetrahydrate	25. (C1)	0.05 M Sodium cacodylate trihydrate pH 6.5	25. (C1)	30% w/v Polyethylene glycol 4,000
26. (C2)	0.2 M Potassium chloride, 0.1 M Magnesium acetate tetrahydrate	26. (C2)	0.05 M Sodium cacodylate trihydrate pH 6.5	26. (C2)	10% w/v Polyethylene glycol 8,000
27. (C3)	0.2 M Ammonium acetate, 0.01 M Magnesium acetate tetrahydrate	27. (C3)	0.05 M Sodium cacodylate trihydrate pH 6.5	27. (C3)	30% w/v Polyethylene glycol 8,000
28. (C4)	0.05 M Magnesium sulfate hydrate	28. (C4)	0.05 M HEPES sodium pH 7.0	28. (C4)	1.6 M Lithium sulfate monohydrate
29. (C5)	0.01 M Magnesium chloride hexahydrate	29. (C5)	0.05 M HEPES sodium pH 7.0	29. (C5)	4.0 M Lithium chloride
30. (C6)	0.01 M Magnesium chloride hexahydrate	30. (C6)	0.05 M HEPES sodium pH 7.0	30. (C6)	1.6 M Ammonium sulfate
31. (C7)	0.005 M Magnesium chloride hexahydrate	31. (C7)	0.05 M HEPES sodium pH 7.0	31. (C7)	25% v/v Polyethylene glycol monomethyl ether 550
32. (C8)	0.2 M Potassium chloride, 0.01 M Magnesium chloride hexahydrate	32. (C8)	0.05 M HEPES sodium pH 7.0	32. (C8)	1.7 M 1,6-Hexanediol
33. (C9)	0.2 M Ammonium chloride, 0.01 M Magnesium chloride hexahydrate	33. (C9)	0.05 M HEPES sodium pH 7.0	33. (C9)	2.5 M 1,6-Hexanediol
34. (C10)	0.1 M Potassium chloride, 0.005 M Magnesium sulfate hydrate	34. (C10)	0.05 M HEPES sodium pH 7.0	34. (C10)	15% v/v (+/-)-2-Methyl-2,4-pentanediol
35. (C11)	0.1 M Potassium chloride, 0.01 M Magnesium chloride hexahydrate	35. (C11)	0.05 M HEPES sodium pH 7.0	35. (C11)	5% v/v Polyethylene glycol 400
36. (C12)	0.1 M Potassium chloride, 0.01 M Calcium chloride dihydrate	36. (C12)	0.05 M HEPES sodium pH 7.0	36. (C12)	10% v/v Polyethylene glycol 400
37. (D1)	0.2 M Potassium chloride, 0.025 M Magnesium sulfate hydrate	37. (D1)	0.05 M HEPES sodium pH 7.0	37. (D1)	20% v/v Polyethylene glycol 200
38. (D2)	0.2 M Ammonium acetate, 0.15 M Magnesium acetate tetrahydrate	38. (D2)	0.05 M HEPES sodium pH 7.0	38. (D2)	5% w/v Polyethylene glycol 4,000
39. (D3)	0.1 M Ammonium acetate, 0.02 M Magnesium chloride hexahydrate	39. (D3)	0.05 M HEPES sodium pH 7.0	39. (D3)	5% w/v Polyethylene glycol 8,000
40. (D4)	0.01 M Magnesium chloride hexahydrate	40. (D4)	0.05 M TRIS hydrochloride pH 7.5	40. (D4)	1.6 M Ammonium sulfate
41. (D5)	0.1 M Potassium chloride, 0.015 M Magnesium chloride hexahydrate	41. (D5)	0.05 M TRIS hydrochloride pH 7.5	41. (D5)	10% v/v Polyethylene glycol monomethyl ether 550
42. (D6)	0.01 M Magnesium chloride hexahydrate	42. (D6)	0.05 M TRIS hydrochloride pH 7.5	42. (D6)	5% v/v 2-Propanol
43. (D7)	0.05 M Ammonium acetate, 0.01 M Magnesium chloride hexahydrate	43. (D7)	0.05 M TRIS hydrochloride pH 7.5	43. (D7)	10% v/v (+/-)-2-Methyl-2,4-pentanediol
44. (D8)	0.2 M Potassium chloride, 0.05 M Magnesium chloride hexahydrate	44. (D8)	0.05 M TRIS hydrochloride pH 7.5	44. (D8)	10% w/v Polyethylene glycol 4,000
45. (D9)	0.025 M Magnesium sulfate hydrate	45. (D9)	0.05 M TRIS hydrochloride pH 8.5	45. (D9)	1.8 M Ammonium sulfate
46. (D10)	0.005 M Magnesium sulfate hydrate	46. (D10)	0.05 M TRIS hydrochloride pH 8.5	46. (D10)	2.9 M 1,6-Hexanediol
47. (D11)	0.1 M Potassium chloride, 0.01 M Magnesium chloride hexahydrate	47. (D11)	0.05 M TRIS hydrochloride pH 8.5	47. (D11)	30% v/v Polyethylene glycol 400
48. (D12)	0.2 M Ammonium chloride, 0.01 M Calcium chloride dihydrate	48. (D12)	0.05 M TRIS hydrochloride pH 8.5	48. (D12)	30% w/v Polyethylene glycol 4,000

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

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

Well #	Salt	Well #	Buffer ♦	Well #	Precipitant	Well #	Additive
49.(E1)	0.04 M Lithium chloride, 0.02 M Magnesium chloride hexahydrate	49.(E1)	0.04 M Sodium cacodylate trihydrate pH 5.5	49.(E1)	30% v/v (+/-)-2-Methyl-2,4-pentanediol	49.(E1)	0.002 M Hexammine cobalt(III) chloride
50.(E2)	0.08 M Sodium chloride, 0.02 M Magnesium chloride hexahydrate	50.(E2)	0.04 M Sodium cacodylate trihydrate pH 5.5	50.(E2)	35% v/v (+/-)-2-Methyl-2,4-pentanediol	50.(E2)	0.002 M Hexammine cobalt(III) chloride
51.(E3)	0.012 M Sodium chloride, 0.08 M Potassium chloride	51.(E3)	0.04 M Sodium cacodylate trihydrate pH 5.5	51.(E3)	45% v/v (+/-)-2-Methyl-2,4-pentanediol	51.(E3)	0.002 M Hexammine cobalt(III) chloride
52.(E4)	0.02 M Magnesium chloride hexahydrate	52.(E4)	0.04 M Sodium cacodylate trihydrate pH 5.5	52.(E4)	40% v/v (+/-)-2-Methyl-2,4-pentanediol	52.(E4)	0.002 M Hexammine cobalt(III) chloride
53.(E5)	0.002 M Calcium chloride dihydrate	53.(E5)	0.05 M Sodium cacodylate trihydrate pH 6.0	53.(E5)	1.8 M Ammonium sulfate	53.(E5)	0.0005 M Spermine
54.(E6)		54.(E6)	0.05 M Sodium cacodylate trihydrate pH 6.0	54.(E6)	35% v/v Tacsimate™ pH 6.0	54.(E6)	0.001 M Spermine
55.(E7)	0.1 M Sodium chloride	55.(E7)	0.05 M Sodium cacodylate trihydrate pH 6.0	55.(E7)	10% w/w Polyethylene glycol 4,000	55.(E7)	0.0005 M Spermine
56.(E8)	0.05 M Potassium chloride	56.(E8)	0.05 M Sodium cacodylate trihydrate pH 6.0	56.(E8)	10% w/w Polyethylene glycol 8,000	56.(E8)	0.0005 M Spermine, 0.0005 M L-Aarginamide dihydrochloride
57.(E9)	0.1 M Potassium chloride	57.(E9)	0.05 M Sodium cacodylate trihydrate pH 6.0	57.(E9)	16% w/w Polyethylene glycol 1,000	57.(E9)	0.0005 M Spermine
58.(E10)	0.005 M Magnesium chloride hexahydrate, 0.002 M Calcium chloride dihydrate	58.(E10)	0.05 M Sodium cacodylate trihydrate pH 6.0	58.(E10)	15% v/v 2-Propanol	58.(E10)	0.001 M Spermine
59.(E11)	0.075 M Sodium chloride, 0.002 M Calcium chloride dihydrate	59.(E11)	0.05 M Sodium cacodylate trihydrate pH 6.0	59.(E11)	30% w/w 1,6-Hexanediol	59.(E11)	0.0005 M Spermine
60.(E12)	0.02 M Magnesium sulfate hydrate, 0.002 M Cobalt(II) chloride hexahydrate	60.(E12)	0.05 M Sodium cacodylate trihydrate pH 6.0	60.(E12)	25% v/v (+/-)-2-Methyl-2,4-pentanediol	60.(E12)	0.0005 M Spermine
61.(F1)		61.(F1)	0.05 M Sodium cacodylate trihydrate pH 6.0	61.(F1)	30% v/v (+/-)-2-Methyl-2,4-pentanediol	61.(F1)	
62.(F2)	0.08 M Sodium chloride, 0.012 M Potassium chloride, 0.02 M Magnesium chloride hexahydrate	62.(F2)	0.04 M Sodium cacodylate trihydrate pH 6.0	62.(F2)	30% v/v (+/-)-2-Methyl-2,4-pentanediol	62.(F2)	0.012 M Spermine tetrahydrochloride
63.(F3)	0.08 M Sodium chloride, 0.02 M Magnesium chloride hexahydrate	63.(F3)	0.04 M Sodium cacodylate trihydrate pH 6.0	63.(F3)	35% v/v (+/-)-2-Methyl-2,4-pentanediol	63.(F3)	0.012 M Spermine tetrahydrochloride
64.(F4)	0.08 M Strontium chloride hexahydrate	64.(F4)	0.04 M Sodium cacodylate trihydrate pH 6.0	64.(F4)	35% v/v (+/-)-2-Methyl-2,4-pentanediol	64.(F4)	0.012 M Spermine tetrahydrochloride
65.(F5)	0.08 M Potassium chloride, 0.02 M Barium chloride dihydrate	65.(F5)	0.04 M Sodium cacodylate trihydrate pH 6.0	65.(F5)	40% v/v (+/-)-2-Methyl-2,4-pentanediol	65.(F5)	0.012 M Spermine tetrahydrochloride
66.(F6)	0.08 M Potassium chloride, 0.02 M Magnesium chloride hexahydrate	66.(F6)	0.04 M Sodium cacodylate trihydrate pH 6.0	66.(F6)	45% v/v (+/-)-2-Methyl-2,4-pentanediol	66.(F6)	0.012 M Spermine tetrahydrochloride
67.(F7)	0.08 M Sodium chloride	67.(F7)	0.04 M Sodium cacodylate trihydrate pH 6.0	67.(F7)	45% v/v (+/-)-2-Methyl-2,4-pentanediol	67.(F7)	0.012 M Spermine tetrahydrochloride
68.(F8)	0.08 M Sodium chloride, 0.02 M Barium chloride dihydrate	68.(F8)	0.04 M Sodium cacodylate trihydrate pH 6.0	68.(F8)	45% v/v (+/-)-2-Methyl-2,4-pentanediol	68.(F8)	0.012 M Spermine tetrahydrochloride
69.(F9)	0.012 M Sodium chloride, 0.08 M Potassium chloride	69.(F9)	0.04 M Sodium cacodylate trihydrate pH 6.0	69.(F9)	50% v/v (+/-)-2-Methyl-2,4-pentanediol	69.(F9)	0.012 M Spermine tetrahydrochloride
70.(F10)	0.08 M Potassium chloride	70.(F10)	0.04 M Sodium cacodylate trihydrate pH 6.0	70.(F10)	55% v/v (+/-)-2-Methyl-2,4-pentanediol	70.(F10)	0.012 M Spermine tetrahydrochloride
71.(F11)	0.018 M Magnesium chloride hexahydrate	71.(F11)	0.05 M Sodium cacodylate trihydrate pH 6.5	71.(F11)	10% v/v 2-Propanol	71.(F11)	0.003 M Spermine
72.(F12)	0.02 M Magnesium chloride hexahydrate	72.(F12)	0.05 M MOPS pH 7.0	72.(F12)	2.0 M Ammonium sulfate	72.(F12)	0.0005 M Spermine
73.(G1)		73.(G1)	0.05 M HEPES sodium pH 7.0	73.(G1)	40% v/v Tacsimate™ pH 7.0	73.(G1)	0.002 M Spermine, 0.002 M Hexammine cobalt(III) chloride
74.(G2)	0.02 M Magnesium chloride hexahydrate	74.(G2)	0.05 M MOPS pH 7.0	74.(G2)	55% v/v Tacsimate™ pH 7.0	74.(G2)	0.002 M Hexammine cobalt(III) chloride
75.(G3)	0.02 M Magnesium chloride hexahydrate	75.(G3)	0.05 M Sodium cacodylate trihydrate pH 7.0	75.(G3)	15% v/v 2-Propanol	75.(G3)	0.001 M Hexammine cobalt(III) chloride, 0.001 M Spermine
76.(G4)	0.005 M Magnesium chloride hexahydrate	76.(G4)	0.05 M MOPS pH 7.0	76.(G4)	25% v/v 1,4-Dioxane	76.(G4)	0.001 M Spermine
77.(G5)	0.01 M Magnesium chloride hexahydrate, 0.002 M Barium chloride dihydrate	77.(G5)	0.05 M MOPS pH 7.0	77.(G5)	30% v/v 1,4-Dioxane	77.(G5)	
78.(G6)	0.001 M Magnesium chloride hexahydrate, 0.002 M Calcium chloride dihydrate	78.(G6)	0.05 M MOPS pH 7.0	78.(G6)	15% v/v (+/-)-2-Methyl-2,4-pentanediol	78.(G6)	
79.(G7)	0.08 M Strontium chloride hexahydrate, 0.02 M Magnesium chloride hexahydrate	79.(G7)	0.04 M Sodium cacodylate trihydrate pH 7.0	79.(G7)	20% v/v (+/-)-2-Methyl-2,4-pentanediol	79.(G7)	0.012 M Spermine tetrahydrochloride
80.(G8)	0.08 M Sodium chloride	80.(G8)	0.04 M Sodium cacodylate trihydrate pH 7.0	80.(G8)	30% v/v (+/-)-2-Methyl-2,4-pentanediol	80.(G8)	0.012 M Spermine tetrahydrochloride
81.(G9)	0.04 M Lithium chloride, 0.08 M Strontium chloride hexahydrate	81.(G9)	0.04 M Sodium cacodylate trihydrate pH 7.0	81.(G9)	30% v/v (+/-)-2-Methyl-2,4-pentanediol	81.(G9)	0.012 M Spermine tetrahydrochloride
82.(G10)	0.04 M Lithium chloride, 0.08 M Strontium chloride hexahydrate, 0.02 M Magnesium chloride hexahydrate	82.(G10)	0.04 M Sodium cacodylate trihydrate pH 7.0	82.(G10)	30% v/v (+/-)-2-Methyl-2,4-pentanediol	82.(G10)	0.012 M Spermine tetrahydrochloride
83.(G11)	0.08 M Sodium chloride, 0.012 M Potassium chloride, 0.02 M Magnesium chloride hexahydrate	83.(G11)	0.04 M Sodium cacodylate trihydrate pH 7.0	83.(G11)	35% v/v (+/-)-2-Methyl-2,4-pentanediol	83.(G11)	0.012 M Spermine tetrahydrochloride
84.(G12)	0.012 M Sodium chloride, 0.08 M Potassium chloride	84.(G12)	0.04 M Sodium cacodylate trihydrate pH 7.0	84.(G12)	40% v/v (+/-)-2-Methyl-2,4-pentanediol	84.(G12)	0.012 M Spermine tetrahydrochloride
85.(H1)	0.08 M Sodium chloride, 0.02 M Barium chloride dihydrate	85.(H1)	0.04 M Sodium cacodylate trihydrate pH 7.0	85.(H1)	40% v/v (+/-)-2-Methyl-2,4-pentanediol	85.(H1)	0.012 M Spermine tetrahydrochloride
86.(H2)	0.08 M Sodium chloride, 0.02 M Magnesium chloride hexahydrate	86.(H2)	0.04 M Sodium cacodylate trihydrate pH 7.0	86.(H2)	40% v/v (+/-)-2-Methyl-2,4-pentanediol	86.(H2)	0.012 M Spermine tetrahydrochloride
87.(H3)	0.08 M Potassium chloride, 0.02 M Barium chloride dihydrate	87.(H3)	0.04 M Sodium cacodylate trihydrate pH 7.0	87.(H3)	40% v/v (+/-)-2-Methyl-2,4-pentanediol	87.(H3)	0.012 M Spermine tetrahydrochloride
88.(H4)	0.08 M Potassium chloride, 0.02 M Magnesium chloride hexahydrate	88.(H4)	0.04 M Sodium cacodylate trihydrate pH 7.0	88.(H4)	50% v/v (+/-)-2-Methyl-2,4-pentanediol	88.(H4)	0.012 M Spermine tetrahydrochloride
89.(H5)	0.08 M Potassium chloride	89.(H5)	0.04 M Sodium cacodylate trihydrate pH 7.0	89.(H5)	60% v/v (+/-)-2-Methyl-2,4-pentanediol	89.(H5)	0.012 M Spermine tetrahydrochloride
90.(H6)	0.02 M Magnesium chloride hexahydrate, 0.002 M Cobalt(II) chloride hexahydrate	90.(H6)	0.05 M HEPES sodium pH 7.5	90.(H6)	2.0 M Ammonium sulfate	90.(H6)	0.001 M Spermine
91.(H7)	0.02 M Magnesium chloride hexahydrate	91.(H7)	0.05 M PIPES pH 7.5	91.(H7)	4% w/w Polyethylene glycol 8,000	91.(H7)	0.001 M Spermine
92.(H8)	0.015 M Magnesium chloride hexahydrate, 0.002 M Barium chloride dihydrate	92.(H8)	0.05 M PIPES pH 7.5	92.(H8)	7% v/v 2-Propanol	92.(H8)	0.0005 M Spermine
93.(H9)	0.02 M Magnesium chloride hexahydrate	93.(H9)	0.05 M PIPES pH 7.5	93.(H9)	10% w/v 1,6-Hexanediol	93.(H9)	0.001 M Spermine
94.(H10)	0.01 M Magnesium chloride hexahydrate	94.(H10)	0.05 M HEPES sodium pH 7.5	94.(H10)	15% v/v (+/-)-2-Methyl-2,4-pentanediol	94.(H10)	0.0015 M Spermine
95.(H11)	0.2 M Calcium chloride dihydrate	95.(H11)	0.05 M HEPES sodium pH 7.5	95.(H11)	28% v/v Polyethylene glycol 400	95.(H11)	0.002 M Spermine
96.(H12)	0.002 M Copper(II) chloride dihydrate	96.(H12)	0.05 M TRIS hydrochloride pH 8.5	96.(H12)	1.8 M Lithium sulfate monohydrate	96.(H12)	0.0005 M Spermine

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

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

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)

Natrix HT™ - HR2-131 Scoring Sheet

Date: _____ Date: _____

1. (A1) 0.01 M Magnesium chloride hexahydrate, 0.05 M MES monohydrate pH 5.6, 1.8 M Lithium sulfate monohydrate	
2. (A2) 0.01 M Magnesium acetate tetrahydrate, 0.05 M MES monohydrate pH 5.6, 2.5 M Ammonium sulfate	
3. (A3) 0.1 M Magnesium acetate tetrahydrate, 0.05 M MES monohydrate pH 5.6, 20% v/v (+/-)-2-Methyl-2,4-pentanediol	
4. (A4) 0.2 M Potassium chloride, 0.01 M Magnesium sulfate heptahydrate, 0.05 M MES monohydrate pH 5.6, 10% v/v Polyethylene glycol 400	
5. (A5) 0.2 M Potassium chloride, 0.01 M Magnesium chloride hexahydrate, 0.05 M MES monohydrate pH 5.6, 5% w/v Polyethylene glycol 8,000	
6. (A6) 0.1 M Ammonium sulfate, 0.01 M Magnesium chloride hexahydrate, 0.05 M MES monohydrate pH 5.6, 20% w/v Polyethylene glycol 8,000	
7. (A7) 0.02 M Magnesium chloride hexahydrate, 0.05 M MES monohydrate pH 6.0, 15% v/v 2-Propanol	
8. (A8) 0.1 M Ammonium acetate, 0.005 M Magnesium sulfate heptahydrate, 0.05 M MES monohydrate pH 6.0, 0.6 M Sodium chloride	
9. (A9) 0.1 M Potassium chloride, 0.01 M Magnesium chloride hexahydrate, 0.05 M MES monohydrate pH 6.0, 10% v/v Polyethylene glycol 400	
10. (A10) 0.005 M Magnesium sulfate heptahydrate, 0.05 M MES monohydrate pH 6.0, 5% w/v Polyethylene glycol 4,000	
11. (A11) 0.01 M Magnesium chloride hexahydrate, 0.05 M Sodium cacodylate trihydrate pH 6.0, 1.0 M Lithium sulfate monohydrate	
12. (A12) 0.01 M Magnesium sulfate heptahydrate, 0.05 M Sodium cacodylate trihydrate pH 6.0, 1.8 M Lithium sulfate monohydrate	
13. (B1) 0.015 M Magnesium acetate tetrahydrate, 0.05 M Sodium cacodylate trihydrate pH 6.0, 1.7 M Ammonium sulfate	
14. (B2) 0.1 M Potassium chloride, 0.025 M Magnesium chloride hexahydrate, 0.05 M Sodium cacodylate trihydrate pH 6.0, 15% v/v 2-Propanol	
15. (B3) 0.04 M Magnesium chloride hexahydrate, 0.05 M Sodium cacodylate trihydrate pH 6.0, 5% v/v (+/-)-2-Methyl-2,4-pentanediol	
16. (B4) 0.04 M Magnesium acetate tetrahydrate, 0.05 M Sodium cacodylate trihydrate pH 6.0, 30% v/v (+/-)-2-Methyl-2,4-pentanediol	
17. (B5) 0.2 M Potassium chloride, 0.01 M Calcium chloride dihydrate, 0.05 M Sodium cacodylate trihydrate pH 6.0, 10% w/v Polyethylene glycol 4,000	
18. (B6) 0.01 M Magnesium acetate tetrahydrate, 0.05 M Sodium cacodylate trihydrate pH 6.5, 1.3 M Lithium sulfate monohydrate	
19. (B7) 0.01 M Magnesium sulfate heptahydrate, 0.05 M Sodium cacodylate trihydrate pH 6.5, 2.0 M Ammonium sulfate	
20. (B8) 0.1 M Ammonium acetate, 0.015 M Magnesium acetate tetrahydrate, 0.05 M Sodium cacodylate trihydrate pH 6.5, 10% v/v 2-Propanol	
21. (B9) 0.2 M Potassium chloride, 0.005 M Magnesium chloride hexahydrate, 0.05 M Sodium cacodylate trihydrate pH 6.5, 0.9 M 1,6-Hexanediol	
22. (B10) 0.08 M Magnesium acetate tetrahydrate, 0.05 M Sodium cacodylate trihydrate pH 6.5, 15% v/v Polyethylene glycol 400	
23. (B11) 0.2 M Potassium chloride, 0.01 M Magnesium chloride hexahydrate, 0.05 M Sodium cacodylate trihydrate pH 6.5, 10% w/v Polyethylene glycol 4,000	
24. (B12) 0.2 M Ammonium acetate, 0.01 M Calcium chloride dihydrate, 0.05 M Sodium cacodylate trihydrate pH 6.5, 10% w/v Polyethylene glycol 4,000	
25. (C1) 0.08 M Magnesium acetate tetrahydrate, 0.05 M Sodium cacodylate trihydrate pH 6.5, 30% w/v Polyethylene glycol 4,000	
26. (C2) 0.2 M Potassium chloride, 0.1 M Magnesium acetate tetrahydrate, 0.05 M Sodium cacodylate trihydrate pH 6.5, 10 % w/v Polyethylene glycol 8,000	
27. (C3) 0.2 M Ammonium acetate, 0.01 M Magnesium acetate tetrahydrate, 0.05 M Sodium cacodylate trihydrate pH 6.5, 30% w/v Polyethylene glycol 8,000	
28. (C4) 0.05 M Magnesium sulfate hydrate, 0.05 M HEPES sodium pH 7.0, 1.6 M Lithium sulfate monohydrate	
29. (C5) 0.01 M Magnesium chloride hexahydrate, 0.05 M HEPES sodium pH 7.0, 4.0 M Lithium chloride	
30. (C6) 0.01 M Magnesium chloride hexahydrate, 0.05 M HEPES sodium pH 7.0, 1.6 M Ammonium sulfate	
31. (C7) 0.005 M Magnesium chloride hexahydrate, 0.05 M HEPES sodium pH 7.0, 25% v/v Polyethylene glycol monomethyl ether 550	
32. (C8) 0.2 M Potassium chloride, 0.01 M Magnesium chloride hexahydrate, 0.05 M HEPES sodium pH 7.0, 1.7 M 1,6-Hexanediol	
33. (C9) 0.2 M Ammonium chloride, 0.01 M Magnesium chloride hexahydrate, 0.05 M HEPES sodium pH 7.0, 2.5 M 1,6-Hexanediol	
34. (C10) 0.1 M Potassium chloride, 0.005 M Magnesium sulfate hydrate, 0.05 M HEPES sodium pH 7.0, 15% v/v (+/-)-2-Methyl-2,4-pentanediol	
35. (C11) 0.1 M Potassium chloride, 0.01 M Magnesium chloride hexahydrate, 0.05 M HEPES sodium pH 7.0, 5% v/v Polyethylene glycol 400	
36. (C12) 0.1 M Potassium chloride, 0.01 M Calcium chloride dihydrate, 0.05 M HEPES sodium pH 7.0, 10% v/v Polyethylene glycol 400	
37. (D1) 0.2 M Potassium chloride, 0.025 M Magnesium sulfate hydrate, 0.05 M HEPES sodium pH 7.0, 20% v/v Polyethylene glycol 200	
38. (D2) 0.2 M Ammonium acetate, 0.15 M Magnesium acetate tetrahydrate, 0.05 M HEPES sodium pH 7.0, 5% w/v Polyethylene glycol 4,000	
39. (D3) 0.1 M Ammonium acetate, 0.02 M Magnesium chloride hexahydrate, 0.05 M HEPES sodium pH 7.0, 5% w/v Polyethylene glycol 8,000	
40. (D4) 0.01 M Magnesium chloride hexahydrate, 0.05 M TRIS hydrochloride pH 7.5, 1.6 M Ammonium sulfate	
41. (D5) 0.1 M Potassium chloride, 0.015 M Magnesium chloride hexahydrate, 0.05 M TRIS hydrochloride pH 7.5, 10% v/v Polyethylene glycol monomethyl ether 550	
42. (D6) 0.01 M Magnesium chloride hexahydrate, 0.05 M TRIS hydrochloride pH 7.5, 5% v/v 2-Propanol	
43. (D7) 0.05 M Ammonium acetate, 0.01 M Magnesium chloride hexahydrate, 0.05 M TRIS hydrochloride pH 7.5, 10% v/v (+/-)-2-Methyl-2,4-pentanediol	
44. (D8) 0.2 M Potassium chloride, 0.05 M Magnesium chloride hexahydrate, 0.05 M TRIS hydrochloride pH 7.5, 10% w/v Polyethylene glycol 4,000	
45. (D9) 0.025 M Magnesium sulfate hydrate, 0.05 M TRIS hydrochloride pH 8.5, 1.8 M Ammonium sulfate	
46. (D10) 0.005 M Magnesium sulfate hydrate, 0.05 M TRIS hydrochloride pH 8.5, 2.9 M 1,6-Hexanediol	
47. (D11) 0.1 M Potassium chloride, 0.01 M Magnesium chloride hexahydrate, 0.05 M TRIS hydrochloride pH 8.5, 30% v/v Polyethylene glycol 400	
48. (D12) 0.2 M Ammonium chloride, 0.01 M Calcium chloride dihydrate, 0.05 M TRIS hydrochloride pH 8.5, 30% w/v Polyethylene glycol 4,000	

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)

Natrix HT™ - HR2-131 Scoring Sheet

Date: _____ Date: _____

49. (E1) 0.04 M Lithium chloride, 0.02 M Magnesium chloride hexahydrate, 0.04 M Sodium cacodylate trihydrate pH 5.5, 30% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.002 M Hexammine cobalt(III) chloride	
50. (E2) 0.08 M Sodium chloride, 0.02 M Magnesium chloride hexahydrate, 0.04 M Sodium cacodylate trihydrate pH 5.5, 35% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.002 M Hexammine cobalt(III) chloride	
51. (E3) 0.012 M Sodium chloride, 0.08 M Potassium chloride, 0.04 M Sodium cacodylate trihydrate pH 5.5, 45% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.002 M Hexammine cobalt(III) chloride	
52. (E4) 0.02 M Magnesium chloride hexahydrate, 0.04 M Sodium cacodylate trihydrate pH 5.5, 40% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.002 M Hexammine cobalt(III) chloride	
53. (E5) 0.002 M Calcium chloride dihydrate, 0.05 M Sodium cacodylate trihydrate pH 6.0, 1.8 M Ammonium sulfate, 0.0005 M Spermine	
54. (E6) 0.05 M Sodium cacodylate trihydrate pH 6.0, 35% v/v Tacsimate™ pH 6.0, 0.001 M Spermine	
55. (E7) 0.1 M Sodium chloride, 0.05 M Sodium cacodylate trihydrate pH 6.0, 10% w/v Polyethylene glycol 4,000, 0.0005 M Spermine	
56. (E8) 0.05 M Potassium chloride, 0.05 M Sodium cacodylate trihydrate pH 6.0, 10% w/v Polyethylene glycol 8,000, 0.0005 M Spermine, 0.0005 M L-Argininamide dihydrochloride	
57. (E9) 0.1 M Potassium chloride, 0.05 M Sodium cacodylate trihydrate pH 6.0, 16% w/v Polyethylene glycol 1,000, 0.0005 M Spermine	
58. (E10) 0.005 M Magnesium chloride hexahydrate, 0.002 M Calcium chloride dihydrate, 0.05 M Sodium cacodylate trihydrate pH 6.0, 15% v/v 2-Propanol, 0.001 M Spermine	
59. (E11) 0.075 M Sodium chloride, 0.002 M Calcium chloride dihydrate, 0.05 M Sodium cacodylate trihydrate pH 6.0, 30% w/v 1,6-Hexanediol, 0.0005 M Spermine	
60. (E12) 0.02 M Magnesium sulfate hydrate, 0.002 M Cobalt(II) chloride hexahydrate, 0.05 M Sodium cacodylate trihydrate pH 6.0, 25% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.0005 M Spermine	
61. (F1) 0.05 M Sodium cacodylate trihydrate pH 6.0, 30% v/v (+/-)-2-Methyl-2,4-pentanediol	
62. (F2) 0.08 M Sodium chloride, 0.012 M Potassium chloride, 0.02 M Magnesium chloride hexahydrate, 0.04 M Sodium cacodylate trihydrate pH 6.0, 30% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.012 M Spermine tetrahydrochloride	
63. (F3) 0.08 M Sodium chloride, 0.02 M Magnesium chloride hexahydrate, 0.04 M Sodium cacodylate trihydrate pH 6.0, 35% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.012 M Spermine tetrahydrochloride	
64. (F4) 0.08 M Strontium chloride hexahydrate, 0.04 M Sodium cacodylate trihydrate pH 6.0, 35% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.012 M Spermine tetrahydrochloride	
65. (F5) 0.08 M Potassium chloride, 0.02 M Barium chloride dihydrate, 0.04 M Sodium cacodylate trihydrate pH 6.0, 40% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.012 M Spermine tetrahydrochloride	
66. (F6) 0.08 M Potassium chloride, 0.02 M Magnesium chloride hexahydrate, 0.04 M Sodium cacodylate trihydrate pH 6.0, 45% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.012 M Spermine tetrahydrochloride	
67. (F7) 0.08 M Sodium chloride, 0.04 M Sodium cacodylate trihydrate pH 6.0, 45% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.012 M Spermine tetrahydrochloride	
68. (F8) 0.08 M Sodium chloride, 0.02 M Barium chloride dihydrate, 0.04 M Sodium cacodylate trihydrate pH 6.0, 45% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.012 M Spermine tetrahydrochloride	
69. (F9) 0.012 M Sodium chloride, 0.08 M Potassium chloride, 0.04 M Sodium cacodylate trihydrate pH 6.0, 50% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.012 M Spermine tetrahydrochloride	
70. (F10) 0.08 M Potassium chloride, 0.04 M Sodium cacodylate trihydrate pH 6.0, 55% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.012 M Spermine tetrahydrochloride	
71. (F11) 0.018 M Magnesium chloride hexahydrate, 0.05 M Sodium cacodylate trihydrate pH 6.5, 10% v/v 2-Propanol, 0.003 M Spermine	
72. (F12) 0.02 M Magnesium chloride hexahydrate, 0.05 M MOPS pH 7.0, 2.0 M Ammonium sulfate, 0.0005 M Spermine	
73. (G1) 0.05 M HEPES sodium pH 7.0, 40% v/v Tacsimate™ pH 7.0, 0.002 M Spermine, 0.002 M Hexammine cobalt(III) chloride	
74. (G2) 0.02 M Magnesium chloride hexahydrate, 0.05 M MOPS pH 7.0, 55% v/v Tacsimate™ pH 7.0, 0.002 M Hexammine cobalt(III) chloride	
75. (G3) 0.02 M Magnesium chloride hexahydrate, 0.05 M Sodium cacodylate trihydrate pH 7.0, 15% v/v 2-Propanol, 0.001 M Hexammine cobalt(III) chloride, 0.001 M Spermine	
76. (G4) 0.005 M Magnesium chloride hexahydrate, 0.05 M MOPS pH 7.0, 25% v/v 1,4-Dioxane, 0.001 M Spermine	
77. (G5) 0.01 M Magnesium chloride hexahydrate, 0.002 M Barium chloride dihydrate, 0.05 M MOPS pH 7.0, 30% v/v 1,4-Dioxane	
78. (G6) 0.001 M Magnesium chloride hexahydrate, 0.002 M Calcium chloride dihydrate, 0.05 M MOPS pH 7.0, 15% v/v (+/-)-2-Methyl-2,4-pentanediol	
79. (G7) 0.08 M Strontium chloride hexahydrate, 0.02 M Magnesium chloride hexahydrate, 0.04 M Sodium cacodylate trihydrate pH 7.0, 20% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.012 M Spermine tetrahydrochloride	
80. (G8) 0.08 M Sodium chloride, 0.04 M Sodium cacodylate trihydrate pH 7.0, 30% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.012 M Spermine tetrahydrochloride	
81. (G9) 0.04 M Lithium chloride, 0.08 M Strontium chloride hexahydrate, 0.04 M Sodium cacodylate trihydrate pH 7.0, 30% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.012 M Spermine tetrahydrochloride	
82. (G10) 0.04 M Lithium chloride, 0.08 M Strontium chloride hexahydrate, 0.02 M Magnesium chloride hexahydrate, 0.04 M Sodium cacodylate trihydrate pH 7.0, 30% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.012 M Spermine tetrahydrochloride	
83. (G11) 0.08 M Sodium chloride, 0.012 M Potassium chloride, 0.02 M Magnesium chloride hexahydrate, 0.04 M Sodium cacodylate trihydrate pH 7.0, 35% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.012 M Spermine tetrahydrochloride	
84. (G12) 0.012 M Sodium chloride, 0.08 M Potassium chloride, 0.04 M Sodium cacodylate trihydrate pH 7.0, 40% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.012 M Spermine tetrahydrochloride	
85. (H1) 0.08 M Sodium chloride, 0.02 M Barium chloride dihydrate, 0.04 M Sodium cacodylate trihydrate pH 7.0, 40% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.012 M Spermine tetrahydrochloride	
86. (H2) 0.08 M Sodium chloride, 0.02 M Magnesium chloride hexahydrate, 0.04 M Sodium cacodylate trihydrate pH 7.0, 40% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.012 M Spermine tetrahydrochloride	
87. (H3) 0.08 M Potassium chloride, 0.02 M Barium chloride dihydrate, 0.04 M Sodium cacodylate trihydrate pH 7.0, 40% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.012 M Spermine tetrahydrochloride	
88. (H4) 0.08 M Potassium chloride, 0.02 M Magnesium chloride hexahydrate, 0.04 M Sodium cacodylate trihydrate pH 7.0, 50% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.012 M Spermine tetrahydrochloride	
89. (H5) 0.08 M Potassium chloride, 0.04 M Sodium cacodylate trihydrate pH 7.0, 60% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.012 M Spermine tetrahydrochloride	
90. (H6) 0.02 M Magnesium chloride hexahydrate, 0.002 M Cobalt(II) chloride hexahydrate, 0.05 M HEPES sodium pH 7.5, 2.0 M Ammonium sulfate, 0.001 M Spermine	
91. (H7) 0.02 M Magnesium chloride hexahydrate, 0.05 M PIPES pH 7.5, 4% w/v Polyethylene glycol 8,000, 0.001 M Spermine	
92. (H8) 0.015 M Magnesium chloride hexahydrate, 0.002 M Barium chloride dihydrate, 0.05 M PIPES pH 7.5, 7% v/v 2-Propanol, 0.0005 M Spermine	
93. (H9) 0.02 M Magnesium chloride hexahydrate, 0.05 M PIPES pH 7.5, 10% w/v 1,6-Hexanediol, 0.001 M Spermine	
94. (H10) 0.01 M Magnesium chloride hexahydrate, 0.05 M HEPES sodium pH 7.5, 15% v/v (+/-)-2-Methyl-2,4-pentanediol, 0.0015 M Spermine	
95. (H11) 0.2 M Calcium chloride dihydrate, 0.05 M HEPES sodium pH 7.5, 28% v/v Polyethylene glycol 400, 0.002 M Spermine	
96. (H12) 0.002 M Copper(II) chloride dihydrate, 0.05 M TRIS hydrochloride pH 8.5, 1.8 M Lithium sulfate monohydrate, 0.0005 M Spermine	