HAM #HR2-116



# Natrix™

## nucleic acid sparse matrix

A complete reagent kit designed to provide a highly effective and rapid screening method for the crystallization of nucleic acids and protein - nucleic acid complexes. A variety of hammerhead ribozymes and other ribozymes, RNAs, DNAs, RNA-drug complexes, and RNA-protein complexes have been crystallized using the Natrix protocol. By using sparse matrix sampling technology Natrix allows one to quickly test wide ranges of pH, salts, and precipitants using less than 100 µl of sample. Natrix utilizes PEG 200, 400, 4000, 8000, as well as isopropanol, PEG MME, and 1,6 hexanediol as precipitants. Many of the polymeric and low molecular weight organic precipitants are combined with various monovalent salts as precipitating agents. This combination of salts, low molecular weight organics, and polyalcohols, as well as the utilization of varying chain length PEGs has proven to be a successful combination for producing nucleic acid and protein / nucleic acid complex crystals.

Each Natrix kit contains ten milliliters each of 48 unique reagents. Ready to use reagents are formulated using high purity salts, buffers, and precipitants with ultra pure water and are sterile filtered. Crystallization accessories are sold separately. Natrix does not include polyamines. It is recommended that a polyamine such as spermine be added to the sample prior to screening and that various polyamines be screened AFTER preliminary crystallization conditions have been determined.

**HR2-116 Natrix kit**

*Reference:*
*Scott, W. G., Finch, J.T., Grenfell, R., Fogg, J., Smith, T., Gait, M.J., Klug, A., Journal of Molecular Biology (1995) 250: 327-332.*

*Crystals above are those of a nucleic acid octamer by M.C. Wahl, Ohio State University.*

### Nucleic Acid Crystallization

Typical crystallization trials using Natrix will utilize the hanging or sitting drop methodology. Sample preparation is straightforward. The highly purified sample is heated to 75°C and annealed in the presence of the appropriate polyamine and buffer. After cooling to room temperature, the sample is mixed with an equal amount of Natrix solution and set against a reservior containing the same Natrix solution for vapor diffusion equilibration.

### Natrix formulation

1. 0.01 M Mg Chloride, 0.05 M MES pH 5.6, 2.0 M Lithium Sulfate
2. 0.01 M Mg Acetate, 0.05 M MES pH 5.6, 2.6 M Ammonium Sulfate
3. 0.1 M Mg Acetate, 0.05 M MES pH 5.6, 20% MPD
4. 0.2 M K Chloride, 0.01 M Mg Sulfate, 0.05 M MES pH 5.6, 10% PEG 400
5. 0.2 M K Chloride, 0.01 M Mg Chloride, 0.05 M MES pH 5.6, 5% PEG 8000
6. 0.1 M Ammonium Sulfate, 0.01 M Mg Chloride, 0.05 M MES pH 5.6, 20% PEG 8000
7. 0.02 M Mg Chloride, 0.05 M MES pH 6.0, 15% Iso-Propanol
8. 0.005 M Mg Sulfate, 0.1 M Ammonium Acetate, 0.05 M MES pH 6.0, 0.6 M Sodium Chloride
9. 0.1 M K Chloride, 0.01 M Mg Chloride, 0.05 M MES pH 6.0, 10% PEG 400
10. 0.005 M Mg Sulfate, 0.05 M MES pH 6.0, 5% PEG 4000
11. 0.01 M Mg Chloride, 0.05 M Na Cacodylate pH 6.0, 1.0 M Lithium Sulfate
12. 0.01 M Mg Sulfate, 0.05 M Na Cacodylate pH 6.0, 1.8 M Lithium Sulfate
13. 0.015 M Mg Acetate, 0.05 M Na Cacodylate pH 6.0, 1.7 M Ammonium Sulfate
14. 0.1 M K Chloride, 0.025 M Mg Chloride, 0.05 M Na Cacodylate pH 6.0, 15% Iso-Propanol
15. 0.04 M Mg Chloride, 0.05 M Na Cacodylate pH 6.0, 5% MPD
16. 0.04 M Mg Acetate, 0.05 M Na Cacodylate pH 6.0, 30% MPD
17. 0.2 M K Chloride, 0.01 M Ca Chloride, 0.05 M Na Cacodylate pH 6.0, 10% PEG 4000
18. 0.01 M Mg Acetate, 0.05 M Na Cacodylate pH 6.5, 1.3 M Lithium Sulfate
19. 0.01 M Mg Sulfate, 0.05 M Na Cacodylate pH 6.5, 2.0 M Ammonium sulfate
20. 0.1 M Ammonium Acetate, 0.015 M Mg Acetate, 0.05 M Na Cacodylate pH 6.5, 10% Iso-Propanol
21. 0.2 M K Chloride, 0.005 M Mg Chloride, 0.05 M Na Cacodylate pH 6.5, 10% 1, 6 hexanediol
22. 0.08 M Mg Acetate, 0.05 M Na Cacodylate pH 6.5, 15% PEG 400
23. 0.2 M K Chloride, 0.01 M Mg Chloride, 0.05 M Na Cacodylate pH 6.5, 10% PEG 4000
24. 0.2 M Ammonium Acetate, 0.01 M Ca Chloride, 0.05 M Na Cacodylate pH 6.5, 10% PEG 4000
25. 0.08 M Mg Acetate, 0.05 M Na Cacodylate pH 6.5, 30% PEG 4000
26. 0.2 M K Chloride, 0.1 M Mg Acetate, 0.05 M Na Cacodylate pH 6.5, 10% PEG 8000
27. 0.2 M Ammonium Acetate, 0.01 M Mg Acetate, 0.05 M Na Cacodylate pH 6.5, 30% PEG 8000
28. 0.05 M Mg Sulfate, 0.05 M Na Hepes pH 7.0, 1.6 M Lithium Sulfate
29. 0.01 M Mg Chloride, 0.05 M Na Hepes pH 7.0, 4.0 M Lithium Chloride
30. 0.01 M Mg Chloride, 0.05 M Na Hepes pH 7.0, 1.6 M Ammonium Sulfate
31. 0.005 M Mg Chloride, 0.05 M Na Hepes pH 7.0, 25% PEG Monomethyl Ether 550
32. 0.2 M KCl, 0.01M Mg Chloride, 0.05M Na Hepes pH 7.0, 20% 1,6 Hexanediol
33. 0.2 M Ammonium chloride, 0.01 M Mg Chloride, 0.05 M Na Hepes pH 7.0, 30% 1,6 Hexanediol
34. 0.1 M K Chloride, 0.005 M Mg Sulfate, 0.05 M Na Hepes pH 7.0, 15% MPD
35. 0.1 M K Chloride, 0.01 M Mg Chloride, 0.05 M Na Hepes pH 7.0, 5% PEG 400
36. 0.1 M K Chloride, 0.01 M Ca Chloride, 0.05 M Na Hepes pH 7.0, 10% PEG 400
37. 0.2 M K Chloride, 0.025 M Mg Sulfate, 0.05 M Na Hepes pH 7.0, 20% PEG 200
38. 0.2 M Ammonium Acetate, 0.15 M Mg Acetate, 0.05 M Na Hepes pH 7.0, 5% PEG 4000
39. 0.1 M Ammonium Acetate, 0.02 M Mg Chloride, 0.05 M Na Hepes pH 7.0, 5% PEG 8000
40. 0.01 M Mg Chloride, 0.05 M Tris HCl pH 7.5, 1.6 M Ammonium Sulfate
41. 0.1 M K Chloride, 0.015 M Mg Chloride, 0.05 M Tris HCl pH 7.5, 10% PEG Monomethyl Ether 550
42. 0.01 M Mg Chloride, 0.05 M Tris HCl pH 7.5, 5% Iso-Propanol
43. 0.01 M Mg Chloride, 0.05 M Ammonium Acetate, 0.05 M Tris HCl pH 7.5, 10% MPD
44. 0.2 M K Chloride, 0.05 M Mg Chloride, 0.05 M Tris HCl pH 7.5, 10% PEG 4000
45. 0.025 M Mg Sulfate, 0.05 M Tris HCl pH 8.5, 1.8 M Ammonium Sulfate
46. 0.005 M Mg Sulfate, 0.05 M Tris HCl pH 8.5, 35% 1,6 Hexanediol
47. 0.1 M K Chloride, 0.01 M Mg Chloride, 0.05 M Tris HCl pH 8.5, 30% PEG 400
48. 0.01 M Ca Chloride, 0.2 M Ammonium Chloride, 0.05 M Tris HCl pH 8.5, 30% PEG 4000

**pH**

5.6, 6.0, 6.5, 7.0, 7.5, & 8.5

**Salts**

Ammonium Chloride
Calcium Chloride
Lithium Chloride
Magnesium Chloride
Potassium Chloride
Ammonium Sulfate
Lithium Sulfate
Magnesium Sulfate
Ammonium Acetate
Magnesium Acetate

**Polymers**

Polyethylene glycol 200
Polyethylene glycol 400
Polyethylene glycol 4000
Polyethylene glycol 8000
Polyethylene glycol MME 550

**Organic**

iso-Propanol

**Non-volatiles**

2-Methyl-2,4-pentanediol
1,6 Hexanediol

Utilized in Natrix

Salts, Polymers, Organic, and Non-volatiles

# FAQs About Natrix

The following are FAQs (frequently asked questions) about the use of Natrix™. We hope these answers will assist you in using Natrix.

***How do I accurately reproduce the Natrix reagents?***
First, think of Natrix as two sets of reagents; those with a buffer and those without. Reproducing the solutions without a buffer is simple. Use the package insert to determine the precise molarities and concentrations. No pH adjustments are required. For the solutions with buffers we suggest the following. First, make a 1.0 M stock of the buffer with the pH titrated to that indicated on the package insert or the side of the reagent tube. Use HCl or NaOH to adjust the pH (at room temperature!). Dilute this stock 1:10 (i.e. 1 ml to a final volume of 10 ml) with water and the other appropriate reagent components. Use ultra-pure water and sterile filter the solution with a 0.2 micron filter.

***How about an example?***
Okay, let's make Natrix reagent 15. If we were to make 100 ml of reagent 15 we would first add 5 ml of 1.0 M sodium cacodylate pH 6.0 buffer. We would then add 0.813 grams of magnesium chloride. Then add 5 milliliters of MPD and bring the final volume to 100 ml in a volumetric flask. Make no further pH adjustment. Adjust the final volume when the solution and flasks are at room temperature.

***This is a pain if I need to optimize! What can I do?***
First, pick two variables (such as pH and MPD concentration) of the screen to alter in a two dimensional (x,y) grid. See our Optimize™ line of stock solutions for accurate reproduction of reagents. Make an appropriate stock concentration of the precipitant and pH so that you can dilute the sample directly into the crystallization plate. Try a 1.0 M buffer and 100% MPD stock for the previous example. Dilute the solutions with water and mix well by aspirating and dispensing the reservoir solutions numerous times.

***What kind of preservative is used with Natrix?***
None, the solutions are sterile filtered using a 0.2 micron filter and filled into sterile vials.

***Can I get the Natrix reagents individually?***
No. Please see our Optimize™ line of stock solutions for accurate reproduction of reagents.

***I am getting salt crystals, what is going on?***
As you can see in the formulation insert, Natrix contains a number of cations such as magnesium which can complex with anions such as phosphate, carbonate, and borate to form beautiful inorganic salt crystals. We recommend you avoid using high concentrations (0.1 M or higher) of phosphate, carbonate, or borate buffers to avoid these effects. Remove by dialysis or replace with biological buffers such as HEPES, Tris, and Bicine.

***I am getting crystals but I am not sure they are protein.***
Look at the X-ray diffraction pattern. If this is not possible examine the crystals for birefringence. A destructive test is to touch the crystals with a needle. Macromolecular crystals are highly solvated and will be fragile, shattering when touched or manipulated. Inorganic salt crystals contain little water and will actually make a discernible crunch when broken with a probe. Finally, macromolecular crystals will readily absorb colored dyes where small molecule crystals will not. Upon standing (24 hours) the macromolecular crystals will absorb and concentrate the colored dye in the crystal where the salt crystal will remain clear and the surrounding solution will remain colored. Hampton Research offers a dye specially formulated for this application. The dye is called Izit and is catalog number HR4-710.

***How can I be certain to reproduce the crystals I obtain using Natrix?***
First, follow the formulation suggestions described earlier in this flyer. Then ask yourself these questions. Is the pH of the Natrix solution the same as your solution? Are you using fresh solutions or solutions which have been properly stored to avoid evaporation, pH change or degradation? Did the temperature remain the same? Is this sample from the same batch? Is the sample stable? Did you prepare the sample and perform the crystallization in exactly the same manner with the same volumes and technique? Finally, when making final dilutions into the crystallization plate, pipet carefully, especially with viscous compounds.

***How should Natrix reagents be stored?***
The reagents are stable at room temperature for at least 6 months. One should avoid exposure to UV light to prolong the stability of the PEGs. The solutions are stable at 4°C for longer periods and can even be frozen.

***I have more questions, what should I do?***
Give our technical support department a call at (714) 699-1040, fax your questions to (714) 586-1453, or send e-mail to the attention of Hampton Research Tech Support at the following address: "**xtalrox@aol.com**".

If you have any suggestions or comments regarding the use of Natrix please do not hesitate to contact us.

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# NATRIX REAGENT FORMULATION

| Tube Number | *Salt* | *Buffer* † | *Precipitant* |
|---|---|---|---|
| 1. | 0.01 M Magnesium chloride | 0.05 M MES pH 5.6 | 2.0 M Lithium sulfate |
| 2. | 0.01 M Magnesium acetate | 0.05 M MES pH 6.6 | 2.5 M Ammonium sulfate |
| 3. | 0.1 M Magnesium acetate | 0.05 M MES pH 5.6 | 20% v/v 2-methyl-2,4-pentanediol |
| 4. | 0.2 M Potassium chloride, 0.01 M Mg sulfate | 0.05 M MES pH 5.6 | 10% v/v PEG 400 |
| 5. | 0.2 M Potassium chloride, 0.01 M Mg chloride | 0.05 M MES pH 5.6 | 5% w/v PEG 8000 |
| 6. | 0.1 M Ammonium sulfate, 0.01 M Mg chloride | 0.05 M MES pH 5.6 | 20% w/v PEG 8000 |
| 7. | 0.02 M Magnesium chloride | 0.05 M MES pH 6.0 | 15% v/v Iso-Propanol |
| 8. | 0.1 M Ammonium acetate, 0.005 M Mg sulfate | 0.05 M MES pH 6.0 | 0.6 M Sodium chloride |
| 9. | 0.1 M Potassium chloride, 0.01 M Mg chloride | 0.05 M MES pH 6.0 | 10% v/v PEG 400 |
| 10. | 0.005 M Magnesium sulfate | 0.05 M MES pH 6.0 | 5% w/v PEG 4000 |
| 11. | 0.01 M Magnesium chloride | 0.05 M Na Cacodylate pH 6.0 | 1.0 M Lithium sulfate |
| 12. | 0.01 M Magnesium sulfate | 0.05 M Na Cacodylate pH 6.0 | 1.6 M Lithium sulfate |
| 13. | 0.015 M Magnesium acetate | 0.05 M Na Cacodylate pH 6.0 | 1.7 M Ammonium sulfate |
| 14. | 0.1 M Potassium chloride, 0.025 M Mg chloride | 0.05 M Na Cacodylate pH 6.0 | 15% v/v Iso-Propanol |
| 15. | 0.04 M Magnesium chloride | 0.05 M Na Cacodylate pH 6.0 | 5% v/v 2-methyl-2,4-pentanediol |
| 16. | 0.04 M Magnesium acetate | 0.05 M Na Cacodylate pH 6.0 | 30% v/v 2-methyl-2,4-pentanediol |
| 17. | 0.2 M Potassium chloride, 0.01 M Ca chloride | 0.05 M Na Cacodylate pH 6.0 | 10% w/v PEG 4000 |
| 18. | 0.01 M Magnesium acetate | 0.05 M Na Cacodylate pH 6.5 | 1.3 M Lithium sulfate |
| 19. | 0.01 M Magnesium sulfate | 0.05 M Na Cacodylate pH 6.5 | 2.0 M Ammonium sulfate |
| 20. | 0.1 M Ammonium acetate, 0.015 M Mg acetate | 0.05 M Na Cacodylate pH 6.5 | 10% v/v Iso-Propanol |
| 21. | 0.2 M Potassium chloride, 0.005 M Mg chloride | 0.05 M Na Cacodylate pH 6.5 | 10% w/v 1,6 Hexanediol |
| 22. | 0.08 M Magnesium acetate | 0.05 M Na Cacodylate pH 6.5 | 15% v/v PEG 400 |
| 23. | 0.2 M Potassium chloride, 0.01 M Mg chloride | 0.05 M Na Cacodylate pH 6.5 | 10% w/v PEG 4000 |
| 24. | 0.2 M Ammonium acetate, 0.01 M Ca chloride | 0.05 M Na Cacodylate pH 6.5 | 10% w/v PEG 4000 |
| 25. | 0.08 M Magnesium acetate | 0.05 M Na Cacodylate pH 6.5 | 30% w/v PEG 4000 |
| 26. | 0.2 M Potassium chloride, 0.1 M Mg acetate | 0.05 M Na Cacodylate pH 6.5 | 10% w/v PEG 8000 |
| 27. | 0.2 M Ammonium acetate, 0.01 M Mg acetate | 0.05 M Na Cacodylate pH 6.5 | 30% w/v PEG 8000 |
| 28. | 0.05 M Magnesium sulfate Aq. | 0.05 M Na HEPES pH 7.0 | 1.6 M Lithium sulfate |
| 29. | 0.01 M Magnesium chloride | 0.05 M Na HEPES pH 7.0 | 4.0 M Lithium chloride |
| 30. | 0.01 M Magnesium chloride | 0.05 M Na HEPES pH 7.0 | 1.6 M Ammonium sulfate |
| 31. | 0.005 M Magnesium chloride | 0.05 M Na HEPES pH 7.0 | 25% v/v PEG monomethyl ether 550 |
| 32. | 0.2 M Potassium chloride, 0.01 M Mg chloride | 0.05 M Na HEPES pH 7.0 | 20% w/v 1,6 Hexanediol |
| 33. | 0.2 M Ammonium chloride, 0.01 M Mg chloride | 0.05 M Na HEPES pH 7.0 | 30% w/v 1,6 Hexanediol |
| 34. | 0.1 M Potassium chloride, 0.005 M Mg sulfate Aq. | 0.05 M Na HEPES pH 7.0 | 15% v/v 2-methyl-2,4-pentanediol |
| 35. | 0.1 M Potassium chloride, 0.01 M Mg chloride | 0.05 M Na HEPES pH 7.0 | 5% v/v PEG 400 |
| 36. | 0.1 M Potassium chloride, 0.01 M Ca chloride | 0.05 M Na HEPES pH 7.0 | 10% v/v PEG 400 |
| 37. | 0.2 M Potassium chloride, 0.025 M Mg sulfate Aq. | 0.05 M Na HEPES pH 7.0 | 20% v/v PEG 200 |
| 38. | 0.2 M Ammonium acetate, 0.15 M Mg acetate | 0.05 M Na HEPES pH 7.0 | 5% w/v PEG 4000 |
| 39. | 0.1 M Ammonium acetate, 0.02 M Mg chloride | 0.05 M Na HEPES pH 7.0 | 5% w/v PEG 8000 |
| 40. | 0.01 M Magnesium chloride | 0.05 M Tris HCl pH 7.5 | 1.6 M Ammonium sulfate |
| 41. | 0.1 M Potassium chloride, 0.015 M Mg chloride | 0.05 M Tris HCl pH 7.5 | 10% v/v PEG monomethyl ether 550 |
| 42. | 0.01 M Magnesium chloride | 0.05 M Tris HCl pH 7.5 | 5% v/v Iso-Propanol |
| 43. | 0.05 M Ammonium acetate, 0.01 M Mg chloride | 0.05 M Tris HCl pH 7.5 | 10% v/v 2-methyl-2,4-pentanediol |
| 44. | 0.2 M Potassium Chloride, 0.05 M Mg chloride | 0.05 M Tris HCl pH 7.5 | 10% w/v PEG 4000 |
| 45. | 0.025 M Magnesium sulfate Aq. | 0.05 M Tris HCl pH 8.5 | 1.8 M Ammonium sulfate |
| 46. | 0.005 M Magnesium sulfate Aq. | 0.05 M Tris HCl pH 8.5 | 35% w/v 1,6 Hexanediol |
| 47. | 0.1 M Potassium chloride, 0.01 M Mg chloride | 0.05 M Tris HCl pH 8.5 | 30% v/v PEG 400 |
| 48. | 0.2 M Ammonium chloride, 0.01 M Ca chloride | 0.05 M Tris HCl pH 8.5 | 30% w/v PEG 4000 |

† Buffer pH is that of a 1.0 M stock prior to dilution with other reagent components.

PEG = polyethylene glycol.

*Natrix contains forty-eight unique reagents. To determine the formulation of each reagent, simply read across the page.*

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5. If the droplets remains clear, continue to observe the screen for several weeks and consider increasing the sample's concentration, varying the polyamine and cation concentration, or screening an alternative polyamine or cation.

6. If small crystals are grown which are not suitalbe for x-ray diffraction analysis there are several options to pursue. One option is to use the small crystals as seeds to grow larger crystals. The second option is to set up additional screens to optimize crystal growth. Review all results in the set up to obtain information on what affects varying pH, salt, precipitant concentrations, polyamines, and cations have on crystal growth. Design subsequent screens to encompass pH, precipitant, polyamine, and cation ranges slightly above and below those conditions which produced small crystals.

7. If the results of the screen performed at 4°C do not appear different from that at room temperature pursue variations of precipitant type and concentration, pH, and additives as an optimization strategy. If temperature appears to influence solubility and crystal growth, implement temperature variations into the optimization protocol.

8. Change the nucleic acid sequence. See the first two references for additional information.

9. For additional information on suggestions for the optimization of crystallization conditions please refer to the techincal bulletin "Strategies for the Optimization of Crystallization Conditions for Biological Macromolecules." into the crystallization strategy.

***References & Recommended Reading:***


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◊ Doudna, J.A., Grosshans, C., Godding, A., and Kundrot, C.E., Proc. Natl. Acad. Sci. USA, Aug 1993, Vol. 90, 7829-7833.
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◊ Perona, J.J., Methods: A Companion to Methods in Enzymology, Vol. 1, 75-82, 1990.
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◊ Price, S.R., Ito, N., Oubridge, C., Avis, J.M., and Nagai, K., J. Mol. Bio., 249 398-408.
◊ Wincott, F., DiRenzo, A., Shaffer, C., Scaringe, S., and Usman, N., Nucleic Acid Res., Vol. 23, 2677-2687, 1995.



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# NATRIX™

## INSTRUCTIONS

## CRYSTALLIZATION REAGENT KIT FOR

## NUCLEIC ACIDS

## NUCLEIC ACID-PROTEIN COMPLEXES

## Description of Natrix

Natrix is a complete reagent kit designed to provide a rapid screening method for the crystallization of nucleic acids and nucleic acid-protein complexes. The screen is simple and practical for finding initial crystallization conditions as well as determining the solubility of nucleic acids in a wide range of precipitants and pH.

The kit is designed to provide a sparse matrix of trial conditions selected from known and published crystallization conditions. The reagent parameter variables are pH, buffer material, salt, and precipitant. Six different pH's: 5.6, 6.0, 6.5, 7.0, 7.5, and 8.5 are utilized with MES, Na Cacodylate, Na HEPES, and Tris HCl as the respective buffers. The four categories of precipitating agents utilized are volatile agents, non-volatile agents, salts, and a combination of these three. Refer to the enclosed Natrix reagent formulation for additional information.

## Sample Preparation

The sample should be as pure as is practically possible (>95%) and free of amorphous and particulate material. Remove amorphous material by centrifugation or micro-filtration 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 solublized in a water based system which promotes the stability and monodispersity of the nucleic acid. If a buffer is utilized for nucleic acid preparation, a concentration of 5 to 10 mM (pH 6.5) is recommended in order to allow the buffers in Natrix 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 reservior. Finally, the sample should be preheated to 60 to 70°C for 10 minutes then cooled slowly to 22°C (room temperature). After cooling, centrifuge and mirco-filtrate the sample.

## Hanging Drop Methodology

1. Prepare a standard crystallization plate (VDX Plate Catalog number: HR3-140) by applying a thin bead of sealant (vacuum grease or immersion oil) to the rim of each reservoir. This can be accomplished using a 10cc syringe filled with the sealant and appropriate tip (modified pipet or needle).

2. Fill reservoir A1 with 1 ml of Natrix reagent 1. Fill each of the remaining reservoirs with the appropriate Natrix reagents.

3. Apply 2 µl of sample to the center of a clean, siliconized cover slide. (Droplet size can vary from 1 to 10 µl).

4. Apply 2 µl (or a volume equal to the sample size) of Natrix reagent 1 from the reservior to the sample droplet and mix by aspirating and dispensing the droplet several times (avoid foaming).

5. Working quickly to minimize evaporation, invert the cover slide and droplet over reservoir A1 and seal the cover slide onto the edge of the reservoir.

6. Repeat this procedure for the remaining reagents.

7. If quantity of sample permits, perform the screen in duplicate and incubate one plate at 4°C and the second at 21°C (room temperature).

## Sitting Drop Methodology

1. Fill reservoir A1 with 1 ml of Natrix reagent 1. Fill each of the remaining reservoirs with the appropriate Natrix reagents.

2. Place 2 µl of sample onto the sitting drop post or MicroBridge (Catalog Number HR3-312) of reservoir A1.

3. Apply 2 µl (or a volume equal to the sample size) of Natrix reagent 1 from the reservior to the sample droplet and mix by aspirating and dispensing the droplet several times (avoid foaming).

4. Repeat this procedure for the remaining reagents.

5. Seal the individual reservoirs with cover slides and sealants as each sample is mixed, or seal the entire plate with Crystal Clear Sealing Tape (Catalog Number HR4-510) after all additions are completed.

6. If quantity of sample permits, perform the screen in duplicate and incubate one plate at 4°C and the second at 21°C (room temperature).

## Sandwich Drop Methodology

Using the Q Plate (Catalog Number: HR3-124), sitting, hanging, and sandwich drop methodologies can be performed using Natrix.

## Interpretation Of The Results

1. Examine the droplets under a stereo microscope (10 to 100x magnification) carefully after setting up the experiment. Record all observations and be particularly careful to scan the focal plane for small crystals.

2. Natrix is designed for the initial sampling of the sample's solubility. If crystals are obtained during initial screening, conditions may be optimized by varying the concentrations of the salt or precipitant, pH of the reagent, the experiment temperature, polyamine type and concentration, and cation type and concentration.

3. When crystals are not obtained in the initial screen, review droplets with precipitates for microcrystallinity. Examine the amorphous material under a high power microscope between crossed polarizing lenses to look for birefringence. True amorphous precipitates do not glow. Microcrystalline precipitates may glow as a result of the plane of polarization. Streak seeding may be used in these situations towards producing larger, single crystals.

4. If the amorphous material is precipitate, consider the following: a) screen an alternative polyamine or cation, b) increase or decrease the polyamine or cation concentration, c) vary the drop ratio, d) reduce the concentration of the nucleic acid or the precipitant and repeat the screen, and e) confirm the purity and monodispersity of the sample.