Natrix™

HAMPTON RESEARCH
Solutions for Crystal Growth

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, Sodium cacodylate, HEPES sodium, and TRIS hydrochloride 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 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 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.

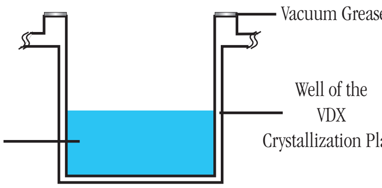
One may wish to include a polyamine such as spermine or spermidine at a concentration of 0.5 to 1.5 mM. The polyamine need not be added to the reservoir. Finally, the sample should be preheated to 60°C to 75°C for 10 minutes then cooled slowly to 22°C (room temperature). After cooling, centrifuge and mirco-filtrate the sample.

Performing The Screen

The following procedure describes the use of Natrix with the Hanging Drop Vapor Diffusion method. Natrix is also compatible with the Sitting Drop, Sandwich Drop, Microbatch, Microdialysis, and Free Interface Diffusion methods. A complete description of the Hanging, Sitting, Sandwich Drop, Dialysis and other crystallization methods are available from the Hampton Research Crystal Growth 101 Library.

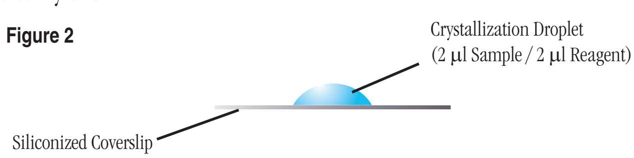
1. Prepare a VDX Plate (HR3-140) for Hanging Drop Vapor Diffusion by applying a thin bead of cover slide sealant to the upper edge of each of the 24 reservoirs. One may also use a Greased VDX Plate (HR3-170). Forty eight reservoirs are to be prepared for a complete Natrix. See Figure 1.

Figure 1
Cross section of a reservoir in the VDX plate.



2. Using a clean pipet tip, pipet 1 ml of Natrix reagent 1 into reservoir A1. Discard the pipet tip, add a new pipet tip and pipet 1 ml of Natrix reagent 2 into reservoir A2. Repeat the procedure for the remaining 46 Natrix reagents using a clean pipet tip for each reagent so as to avoid reagent contamination and carry over.

Figure 2

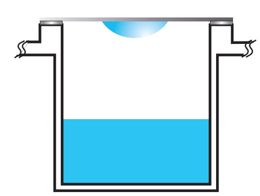


3. Pipet 2 µl of the sample to the center of a clean, siliconized 22 mm diameter circle or square cover slide. See Figure 2.

4. Pipet 2 µl of Natrix reagent 1 from reservoir A1 into the sample droplet and mix by aspirating and dispensing the droplet several times, keeping the tip in the drop during mixing to avoid foaming. See Figure 2.

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. See Figure 3.

Figure 3
Inverted siliconized coverslip placed over the reservoir.



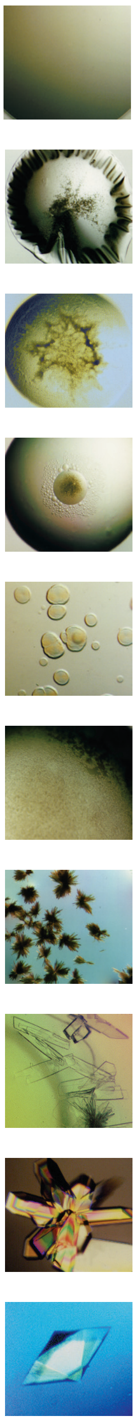
6. Repeat operations 3 through 5 for the remaining 47 Natrix reagents.

7. If the quantity of sample permits, perform Natrix in duplicate and incubate one set of plates at 4°C and the second set at room temperature. Incubate and store the crystallization plates in a stable temperature environment free of vibration.

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

Figure 4
Typical observations in a crystallization experiment



Clear Drop

Skin / Precipitate

Precipitate

Precipitate / Phase

Quasi Crystals

Microcrystals

Needle Cluster

Plates

Rod Cluster

Single Crystal

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 4 (on page 2) shows typical examples of what one might observe in a crystallization experiment.

Interpreting Natrix

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 Natrix condition and doubling the sample concentration. If more than 33 of the 48 Natrix drops are clear consider doubling the sample concentration and repeating the entire screen.

Drops containing precipitate indicate that either the relative supersaturation of the sample and reagent is too high, the sample has denatured, or the sample is heterogeneous. To reduce the relative supersaturation, dilute the sample twofold and repeat the Natrix condition. If more than 33 of the 48 Natrix 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 good. 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 Formulation

Natrix 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 containers (no preservatives added).

Natrix reagents are readily reproduced using Hampton Research Optimize™ stock solutions of salts, polymers and buffers. Optimize stock reagents make reproducing Natrix reagents fast, convenient and easy. Dilutions can be performed directly into the crystallization plate using Optimize stock reagents.

Natrix 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.

Natrix 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 Natrix 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 Natrix 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 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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How to Reproduce Natrix Reagents

Natrix reagents and optimization conditions based on Natrix hits can be formulated using volumetric methods and carefully prepared reagent stocks (Table 1). Note the examples below.

Example 1. To prepare 1.0 milliliter of Natrix reagent 25 in a crystallization plate.

Solution Composition: 0.08 M Magnesium acetate tetrahydrate
0.05 M Sodium cacodylate trihydrate pH 6.5
30% w/v Polyethylene glycol 4,000

- 270 µl water[3]
- 80 µl 1.0 M Magnesium acetate tetrahydrate (CAS # 16674-78-5, Catalog # HR2-561)
- 50 µl 1.0 M Sodium cacodylate trihydrate pH 6.5 (CAS # 6131-99-3, Catalog # HR2-737)
- 600 µl 50% w/v Polyethylene glycol 4,000 (CAS # 25322-68-3, Catalog # HR2-529)

Make no pH adjustments. Mix well by aspirating and dispensing the solution multiple times.

Example 2. To prepare 1.0 milliliter of Natrix reagent 11.

Solution Composition: 0.01 M Magnesium chloride hexahydrate
0.05 M Sodium cacodylate trihydrate pH 6.0
1.0 M Lithium sulfate monohydrate

- 445 µl water[3]
- 50 µl 1.0 M Sodium cacodylate trihydrate pH 6.0 (CAS # 6131-99-3, Catalog # HR2-939-10)
- 5 µl 2.0 M Magnesium chloride hexahydrate (CAS # 7791-18-6, Catalog # HR2-559)
- 500 µl 2.0 M Lithium sulfate monohydrate (CAS # 10377-48-7, Catalog # HR2-545)

Make no pH adjustments. Mix well.

Example 3. To prepare 10 milliliters of Natrix reagent 48.

Solution Composition: 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

- 3,050 µl water[3]
- 50 µl 2.0 M Calcium chloride dihydrate (CAS # 10035-04-8, Catalog # HR2-557)
- 400 µl 5.0 M Ammonium chloride (CAS # 12125-02-9, Catalog # HR2-691)
- 500 µl 1.0 M TRIS hydrochloride pH 8.5 (CAS # 1185-53-1, Catalog # HR2-727)
- 6,000 µl 50% w/v Polyethylene glycol 4,000 (CAS # 25322-68-3, Catalog # HR2-529)

Make no pH adjustments. Mix well.

[3] ASTM Type I water.

Formulation Notes for Natrix Reagents

1. No additional pH adjustment is made to any reagent after formulation. Use the buffers in Table 1 to reproduce an Natrix reagent.
2. All Optimize solutions and screen reagents are sterile filtered using 0.22 µm filters into sterile containers.
3. Add water first as this will help maintain the solubility of subsequently added reagents.
4. When formulating reagents using a pipet, add the largest volume last (except water). Use this larger volume setting to aspirate and dispense the reagent until the solution is mixed.
5. When formulating reagents using a pipet, use a clean, sterile pipet tip for each reagent added to the solution.
6. Use the buffers in Table 2 to systematically vary the pH as a crystallization variable.

pH as a Crystallization Variable

The buffers listed in Table 2, can be used to vary the pH as a crystallization variable and are recommended when optimizing a crystal grown from an Natrix kit.

Optimize™ buffer stocks are supplied as a 100 milliliters sterile filtered solution. Optimize buffers are available as an acid-base pair or titrated to a specific pH.

StockOptions™ buffer kits contain 10 milliliters each of ready to pipet buffers, titrated in 0.1 pH increments over the indicated pH range. The number of reagents offered in a StockOptions buffer kit depends upon the pH range of the buffer. The broader the pH range, the more buffers in the kit.

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Online Information

Visit www.hamptonresearch.com and enter one of the following:

- Reagent Catalog Number
- Kit Catalog Number
- CAS Number
- Reagent Name

To obtain reagent specifications, pH titration tables, user guides, certificates of analysis, material safety data sheets (MSDS), and any other additional information.

MakeTray™

MakeTray is a free, web based program at www.hamptonresearch.com which generates both a pipetting worksheet and a reagent formulation document for crystallization set ups. MakeTray allows one to enter general information about the sample and experiment, which is then printed on the pipet worksheet and the reagent formulation document. The plate size can be customized for any number of wells, so MakeTray works for: 24, 48, and 96 well plates. MakeTray is especially useful for the design and formulation of crystal optimization experiments.

Table 1. Recommended reagents for the formulation of Natrix and Optimization reagents.

Each of these reagents are available as an Optimize™ crystallization grade reagent from Hampton Research. Table 1 provides the common chemical name, the Hampton Research catalog number, supplied stock concentration, the supplied volume, and the CAS number for each reagent. For more information on a specific Optimize reagent, go to www.hamptonresearch.com. Using Search, enter either the catalog number, CAS number, or chemical name to obtain additional information for the Optimize reagent, including a Certificate of Analysis and MSDS (where applicable).

Salts	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
Ammonium acetate	HR2-565	1.0 M	100 ml	631-61-8
	HR2-799	8.0 M	200 ml	631-61-8
Ammonium chloride	HR2-691	5.0 M	200 ml	12125-02-9
Ammonium sulfate	HR2-541	3.5 M	200 ml	7783-20-2
Calcium chloride dihydrate	HR2-557	2.0 M	100 ml	10035-04-8
Lithium chloride	HR2-631	10.0 M	200 ml	7447-41-8
Lithium sulfate monohydrate	HR2-545	2.0 M	200 ml	10377-48-7
Magnesium acetate tetrahydrate	HR2-561	1.0 M	100 ml	16674-78-5
Magnesium chloride hexahydrate	HR2-559	2.0 M	100 ml	7791-18-6
	HR2-803	5.0 M	200 ml	7791-18-6
Magnesium sulfate heptahydrate	HR2-821	2.0 M	200 ml	10034-99-8
Magnesium sulfate hydrate	HR2-633	2.5 M	200 ml	22189-08-8
Potassium chloride	HR2-649	4.0 M	200 ml	7447-40-7
Sodium chloride	HR2-637	5.0 M	200 ml	7647-14-5
Polymers	**Hampton Research Catalog #**	**Supplied [Stock]**	**Supplied Volume**	**CAS #**
Polyethylene glycol 200	HR2-601	100	200 ml	25322-68-3
Polyethylene glycol 400	HR2-603	100%	200 ml	25322-68-3
Polyethylene glycol 4,000	HR2-529	50% w/v	200 ml	25322-68-3
Polyethylene glycol 8,000	HR2-535	50% w/v	200 ml	25322-68-3

(Continued on page 3)

Table 1 (Continued). Recommended reagents for the formulation of Natrix and Optimization reagents.

Polymers	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
Polyethylene glycol monomethyl ether 550	HR2-611	100%	200 ml	9004-74-4
Organics (non-volatile)	**Hampton Research Catalog #**	**Supplied [Stock]**	**Supplied Volume**	**CAS #**
1,6-Hexanediol	HR2-625	6.0 M	200 ml	629-11-8
(+/-)-2-Methyl-2,4-pentanediol	HR2-627	100%	200 ml	107-41-5
Organics (non-volatile)	**Hampton Research Catalog #**	**Supplied [Stock]**	**Supplied Volume**	**CAS #**
2-Propanol	HR2-619	100%	200 ml	67-63-0
Buffers	**Hampton Research Catalog #**	**Supplied [Stock]**	**Supplied Volume**	**CAS #**
MES monohydrate pH 5.6 [2]	HR2-943-05	1.0 M	185 ml	145224-94-8
MES monohydrate pH 6.0 [2]	HR2-943-09	1.0 M	185 ml	145224-94-8
HEPES sodium pH 7.0 [1]	HR2-931-03	1.0 M	185 ml	75277-39-3
Sodium cacodylate trihydrate pH 6.0 [1]	HR2-939-10	1.0 M	185 ml	6131-99-3
Sodium cacodylate trihydrate pH 6.5 [1]	HR2-737	1.0 M	100 ml	6131-99-3
TRIS hydrochloride pH 7.5 [2]	HR2-937-06	1.0 M	185 ml	1185-53-1
TRIS hydrochloride pH 8.5 [2]	HR2-727	1.0 M	100 ml	1185-53-1
[1] pH titrated using Hydrochloric acid (HR2-581) CAS # 7647-01-0				
[2] pH titrated using Sodium hydroxide (HR2-583) CAS # 1310-73-2				

Table 2. Recommended buffers for screening the pH of Natrix and Optimization reagents.

Buffer Solution or Kit	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #	pH range
MES monohydrate untitrated	HR2-587	0.5 M	100 ml	145224-94-8	5.2 - 7.1
Titrate with NaOH	HR2-583	1.0 M	100 ml	1310-73-2	—
StockOptions™ MES monohydrate kit [4]	HR2-243	1.0 M	10 ml each	145224-94-8	5.2 - 7.1
HEPES sodium untitrated	HR2-577	1.0 M	100 ml	75277-39-3	6.6 - 8.5
Titrate with HCl	HR2-581	1.0 M	100 ml	7647-01-0	—
StockOptions™ Sodium Hepes kit [4]	HR2-231	1.0 M	10 ml each	75277-39-3	6.8 - 8.2

(Continued on page 4)

Table 2. Recommended buffers for screening the pH of Natrix and Optimization reagents.

Buffer Solution or Kit	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #	pH range
Sodium cacodylate trihydrate untitrated	HR2-575	1.0 M	100 ml	6131-99-3	5.0 - 7.4
Titrate with HCl	HR2-581	1.0 M	100 ml	7647-01-0	—
StockOptions™ Sodium Cacodylate kit [4]	HR2-239	1.0 M	10 ml each	6131-99-3	5.1 - 7.4
Tris hydrochloride untitrated	HR2-579	1.0 M	100 ml	1185-53-1	7.0 - 9.0
Titrate with NaOH	HR2-583	1.0 M	100 ml	1310-73-2	—
StockOptions™ Tris Hydrochloride kit [4]	HR2-237	1.0 M	10 ml each	1185-53-1	7.0 - 9.0

[4] Individual StockOptions buffers titrated to any pH within the kit's pH range are available in 185 ml volumes from the Hampton Research Custom Shop

Technical Support

Inquiries regarding Natrix Fundamentals, 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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Tube #	Salt	Tube #	Buffer ◊	Tube #	Precipitant
1.	0.01 M Magnesium chloride hexahydrate	1.	0.05 M MES monohydrate pH 5.6	1.	1.8 M Lithium sulfate monohydrate
2.	0.01 M Magnesium acetate tetrahydrate	2.	0.05 M MES monohydrate pH 5.6	2.	2.5 M Ammonium sulfate
3.	0.1 M Magnesium acetate tetrahydrate	3.	0.05 M MES monohydrate pH 5.6	3.	20% v/v (+/-)-2-Methyl-2,4-pentanediol
4.	0.2 M Potassium chloride, 0.01 M Magnesium sulfate heptahydrate	4.	0.05 M MES monohydrate pH 5.6	4.	10% v/v Polyethylene glycol 400
5.	0.2 M Potassium chloride, 0.01 M Magnesium chloride hexahydrate	5.	0.05 M MES monohydrate pH 5.6	5.	5% w/v Polyethylene glycol 8,000
6.	0.1 M Ammonium sulfate, 0.01 M Magnesium chloride hexahydrate	6.	0.05 M MES monohydrate pH 5.6	6.	20% w/v Polyethylene glycol 8,000
7.	0.02 M Magnesium chloride hexahydrate	7.	0.05 M MES monohydrate pH 6.0	7.	15% v/v 2-Propanol
8.	0.1 M Ammonium acetate, 0.005 M Magnesium sulfate heptahydrate	8.	0.05 M MES monohydrate pH 6.0	8.	0.6 M Sodium chloride
9.	0.1 M Potassium chloride, 0.01 M Magnesium chloride hexahydrate	9.	0.05 M MES monohydrate pH 6.0	9.	10% v/v Polyethylene glycol 400
10.	0.005 M Magnesium sulfate heptahydrate	10.	0.05 M MES monohydrate pH 6.0	10.	5% w/v Polyethylene glycol 4,000
11.	0.01 M Magnesium chloride hexahydrate	11.	0.05 M Sodium cacodylate trihydrate pH 6.0	11.	1.0 M Lithium sulfate monohydrate
12.	0.01 M Magnesium sulfate heptahydrate	12.	0.05 M Sodium cacodylate trihydrate pH 6.0	12.	1.8 M Lithium sulfate monohydrate
13.	0.015 M Magnesium acetate tetrahydrate	13.	0.05 M Sodium cacodylate trihydrate pH 6.0	13.	1.7 M Ammonium sulfate
14.	0.1 M Potassium chloride, 0.025 M Magnesium chloride hexahydrate	14.	0.05 M Sodium cacodylate trihydrate pH 6.0	14.	15% v/v 2-Propanol
15.	0.04 M Magnesium chloride hexahydrate	15.	0.05 M Sodium cacodylate trihydrate pH 6.0	15.	5% v/v (+/-)-2-Methyl-2,4-pentanediol
16.	0.04 M Magnesium acetate tetrahydrate	16.	0.05 M Sodium cacodylate trihydrate pH 6.0	16.	30% v/v (+/-)-2-Methyl-2,4-pentanediol
17.	0.2 M Potassium chloride, 0.01 M Calcium chloride dihydrate	17.	0.05 M Sodium cacodylate trihydrate pH 6.0	17.	10% w/v Polyethylene glycol 4,000
18.	0.01 M Magnesium acetate tetrahydrate	18.	0.05 M Sodium cacodylate trihydrate pH 6.5	18.	1.3 M Lithium sulfate monohydrate
19.	0.01 M Magnesium sulfate heptahydrate	19.	0.05 M Sodium cacodylate trihydrate pH 6.5	19.	2.0 M Ammonium sulfate
20.	0.1 M Ammonium acetate, 0.015 M Magnesium acetate tetrahydrate	20.	0.05 M Sodium cacodylate trihydrate pH 6.5	20.	10% v/v 2-Propanol
21.	0.2 M Potassium chloride, 0.005 M Magnesium chloride hexahydrate	21.	0.05 M Sodium cacodylate trihydrate pH 6.5	21.	0.9 M 1,6-Hexanediol
22.	0.08 M Magnesium acetate tetrahydrate	22.	0.05 M Sodium cacodylate trihydrate pH 6.5	22.	15% v/v Polyethylene glycol 400
23.	0.2 M Potassium chloride, 0.01 Magnesium chloride hexahydrate	23.	0.05 M Sodium cacodylate trihydrate pH 6.5	23.	10% w/v Polyethylene glycol 4,000
24.	0.2 M Ammonium acetate, 0.01 M Calcium chloride dihydrate	24.	0.05 M Sodium cacodylate trihydrate pH 6.5	24.	10% w/v Polyethylene glycol 4,000
25.	0.08 M Magnesium acetate tetrahydrate	25.	0.05 M Sodium cacodylate trihydrate pH 6.5	25.	30% w/v Polyethylene glycol 4,000
26.	0.2 M Potassium chloride, 0.1 M Magnesium acetate tetrahydrate	26.	0.05 M Sodium cacodylate trihydrate pH 6.5	26.	10% w/v Polyethylene glycol 8,000
27.	0.2 M Ammonium acetate, 0.01 M Magnesium acetate tetrahydrate	27.	0.05 M Sodium cacodylate trihydrate pH 6.5	27.	30% w/v Polyethylene glycol 8,000
28.	0.05 M Magnesium sulfate hydrate	28.	0.05 M HEPES sodium pH 7.0	28.	1.6 M Lithium sulfate monohydrate
29.	0.01 M Magnesium chloride hexahydrate	29.	0.05 M HEPES sodium pH 7.0	29.	4.0 M Lithium chloride
30.	0.01 M Magnesium chloride hexahydrate	30.	0.05 M HEPES sodium pH 7.0	30.	1.6 M Ammonium sulfate
31.	0.005 M Magnesium chloride hexahydrate	31.	0.05 M HEPES sodium pH 7.0	31.	25% v/v Polyethylene glycol monomethyl ether 550
32.	0.2 M Potassium chloride, 0.01 M Magnesium chloride hexahydrate	32.	0.05 M HEPES sodium pH 7.0	32.	1.7 M 1,6-Hexanediol
33.	0.2 M Ammonium chloride, 0.01 M Magnesium chloride hexahydrate	33.	0.05 M HEPES sodium pH 7.0	33.	2.5 M 1,6-Hexanediol
34.	0.1 M Potassium chloride, 0.005 M Magnesium sulfate hydrate	34.	0.05 M HEPES sodium pH 7.0	34.	15% v/v (+/-)-2-Methyl-2,4-pentanediol
35.	0.1 M Potassium chloride, 0.01 M Magnesium chloride hexahydrate	35.	0.05 M HEPES sodium pH 7.0	35.	5% v/v Polyethylene glycol 400
36.	0.1 M Potassium chloride, 0.01 M Calcium chloride dihydrate	36.	0.05 M HEPES sodium pH 7.0	36.	10% v/v Polyethylene glycol 400
37.	0.2 M Potassium chloride, 0.025 M Magnesium sulfate hydrate	37.	0.05 M HEPES sodium pH 7.0	37.	20% v/v Polyethylene glycol 200
38.	0.2 M Ammonium acetate, 0.15 M Magnesium acetate tetrahydrate	38.	0.05 M HEPES sodium pH 7.0	38.	5% w/v Polyethylene glycol 4,000
39.	0.1 M Ammonium acetate, 0.02 M Magnesium chloride hexahydrate	39.	0.05 M HEPES sodium pH 7.0	39.	5% w/v Polyethylene glycol 8,000
40.	0.01 M Magnesium chloride hexahydrate	40.	0.05 M TRIS hydrochloride pH 7.5	40.	1.6 M Ammonium sulfate
41.	0.1 M Potassium chloride, 0.015 M Magnesium chloride hexahydrate	41.	0.05 M TRIS hydrochloride pH 7.5	41.	10% v/v Polyethylene glycol monomethyl ether 550
42.	0.01 M Magnesium chloride hexahydrate	42.	0.05 M TRIS hydrochloride pH 7.5	42.	5% v/v 2-Propanol
43.	0.05 M Ammonium acetate, 0.01 M Magnesium chloride hexahydrate	43.	0.05 M TRIS hydrochloride pH 7.5	43.	10% v/v (+/-)-2-Methyl-2,4-pentanediol
44.	0.2 M Potassium chloride, 0.05 M Magnesium chloride hexahydrate	44.	0.05 M TRIS hydrochloride pH 7.5	44.	10% w/v Polyethylene glycol 4,000
45.	0.025 M Magnesium sulfate hydrate	45.	0.05 M TRIS hydrochloride pH 8.5	45.	1.8 M Ammonium sulfate
46.	0.005 M Magnesium sulfate hydrate	46.	0.05 M TRIS hydrochloride pH 8.5	46.	2.9 M 1,6-Hexanediol
47.	0.1 M Potassium chloride, 0.01 M Magnesium chloride hexahydrate	47.	0.05 M TRIS hydrochloride pH 8.5	47.	30% v/v Polyethylene glycol 400
48.	0.2 M Ammonium chloride, 0.01 M Calcium chloride dihydrate	48.	0.05 M TRIS hydrochloride pH 8.5	48.	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 contains forty-eight unique reagents. To determine the formulation of each reagent, simply read across the page.

HAMPTON RESEARCH
Solutions for Crystal Growth

34 Journey
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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)

Natrix™ - HR2-116 Scoring Sheet	Date:	Date:	Date:
1. 0.01 M Magnesium chloride hexahydrate, 0.05 M MES monohydrate pH 5.6, 1.8 M Lithium sulfate monohydrate			
2. 0.01 M Magnesium acetate tetrahydrate, 0.05 M MES monohydrate pH 5.6, 2.5 M Ammonium sulfate			
3. 0.1 M Magnesium acetate tetrahydrate, 0.05 M MES monohydrate pH 5.6, 20% v/v (+/-)-2-Methyl-2,4-pentanediol			
4. 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. 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. 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. 0.02 M Magnesium chloride hexahydrate, 0.05 M MES monohydrate pH 6.0, 15% v/v 2-Propanol			
8. 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. 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. 0.005 M Magnesium sulfate heptahydrate, 0.05 M MES monohydrate pH 6.0, 5% w/v Polyethylene glycol 4,000			
11. 0.01 M Magnesium chloride hexahydrate, 0.05 M Sodium cacodylate trihydrate pH 6.0, 1.0 M Lithium sulfate monohydrate			
12. 0.01 M Magnesium sulfate heptahydrate, 0.05 M Sodium cacodylate trihydrate pH 6.0, 1.8 M Lithium sulfate monohydrate			
13. 0.015 M Magnesium acetate tetrahydrate, 0.05 M Sodium cacodylate trihydrate pH 6.0, 1.7 M Ammonium sulfate			
14. 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. 0.04 M Magnesium chloride hexahydrate, 0.05 M Sodium cacodylate trihydrate pH 6.0, 5% v/v (+/-)-2-Methyl-2,4-pentanediol			
16. 0.04 M Magnesium acetate tetrahydrate, 0.05 M Sodium cacodylate trihydrate pH 6.0, 30% v/v (+/-)-2-Methyl-2,4-pentanediol			
17. 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. 0.01 M Magnesium acetate tetrahydrate, 0.05 M Sodium cacodylate trihydrate pH 6.5, 1.3 M Lithium sulfate monohydrate			
19. 0.01 M Magnesium sulfate heptahydrate, 0.05 M Sodium cacodylate trihydrate pH 6.5, 2.0 M Ammonium sulfate			
20. 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. 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. 0.08 M Magnesium acetate tetrahydrate, 0.05 M Sodium cacodylate trihydrate pH 6.5, 15% v/v Polyethylene glycol 400			
23. 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. 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. 0.08 M Magnesium acetate tetrahydrate, 0.05 M Sodium cacodylate trihydrate pH 6.5, 30% w/v Polyethylene glycol 4,000			
26. 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. 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. 0.05 M Magnesium sulfate hydrate, 0.05 M HEPES sodium pH 7.0, 1.6 M Lithium sulfate monohydrate			
29. 0.01 M Magnesium chloride hexahydrate, 0.05 M HEPES sodium pH 7.0, 4.0 M Lithium chloride			
30. 0.01 M Magnesium chloride hexahydrate, 0.05 M HEPES sodium pH 7.0, 1.6 M Ammonium sulfate			
31. 0.005 M Magnesium chloride hexahydrate, 0.05 M HEPES sodium pH 7.0, 25% v/v Polyethylene glycol monomethyl ether 550			
32. 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. 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. 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. 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. 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. 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. 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. 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. 0.01 M Magnesium chloride hexahydrate, 0.05 M TRIS hydrochloride pH 7.5, 1.6 M Ammonium sulfate			
41. 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. 0.01 M Magnesium chloride hexahydrate, 0.05 M TRIS hydrochloride pH 7.5, 5% v/v 2-Propanol			
43. 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. 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. 0.025 M Magnesium sulfate hydrate, 0.05 M TRIS hydrochloride pH 8.5, 1.8 M Ammonium sulfate			
46. 0.005 M Magnesium sulfate hydrate, 0.05 M TRIS hydrochloride pH 8.5, 2.9 M 1,6-Hexanediol			
47. 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. 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			

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