Natrix™ 2

HAMPTON RESEARCH
Solutions for Crystal Growth

User Guide **HR2-117 (pg 1)**

Natrix™ 2 is a 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 biased sparse matrix of trial conditions selected from known and published crystallization conditions. The reagent parameter variables are pH, buffer, salt, additive, and precipitant. Six different pH's: 5.5, 6.0, 6.5, 7.0, 7.5, and 8.5 are utilized with Sodium cacodylate, MOPS, HEPES sodium, PIPES, and TRIS hydrochloride as the 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 2 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 is recommended in order to allow the buffers in Natrix 2 to alter the pH of the sample drop.

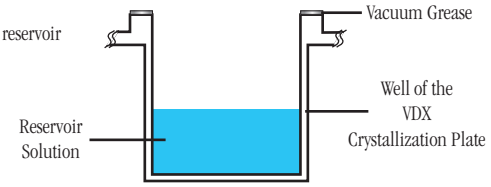
One may wish to include a polyamine such as spermine or spermidine at a concentration of 0.5 to 1.5 mM. The polyamine need not be added to the reservoir. Finally, when sample annealing is desired, the sample should be preheated to 50°C to 95°C for 10 minutes then cooled slowly to 25°C (room temperature), in the presence of the samples buffer and 5-20 mM Magnesium chloride to produce native molecules.[1] After cooling, centrifuge and mirco-filtrate the sample.

Performing The Screen

The following procedure describes the use of Natrix 2 with the Hanging Drop Vapor Diffusion method. Natrix 2 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.

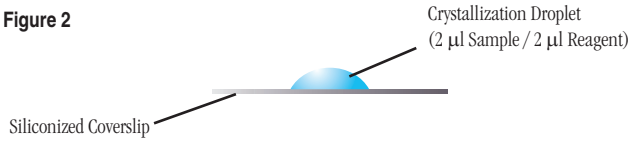
1. Prepare a VDX Plate (HR3-140) for Hanging Drop Vapor Diffusion by applying a thin bead of cover slide sealant to the upper edge of each of the 24 reservoirs. One may also use a Greased VDX Plate (HR3-170). Forty eight reservoirs are to be prepared for a complete Natrix 2. See Figure 1.

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



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

Figure 2

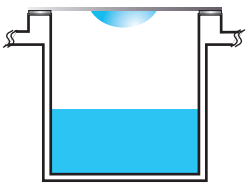


3. Pipet 2 µl of the sample to the center of a clean, siliconized 22 mm diameter circle or square cover slide. See Figure 2.

4. Pipet 2 µl of Natrix 2 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 2 reagents.

7. If the quantity of sample permits, perform Natrix 2 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.

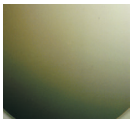
Examine The Drop

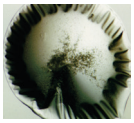
Carefully examine the drops under a stereo microscope (10 to 100x magnification) immediately after setting up the screen. Record all observations and be particularly careful to scan the focal plane for small crystals. Observe the drops once each day for the first week, then once a week there after. Records should indicate whether the drop is clear, contains precipitate, and or crystals. It is helpful to describe the drop contents using descriptive terms.

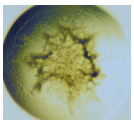
Natrix™ 2

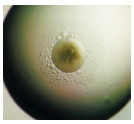
HAMPTON RESEARCH
Solutions for Crystal Growth

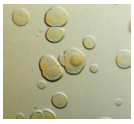
Figure 4
Typical observations in a crystallization experiment

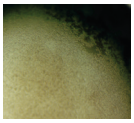

Clear Drop


Skin / Precipitate

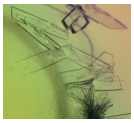

Precipitate


Precipitate / Phase

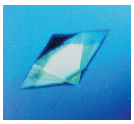

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

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 2

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 2 condition and doubling the sample concentration. If more than 33 of the 48 Natrix 2 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 2 condition. If more than 33 of the 48 Natrix 2 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 2 Formulation

Natrix 2 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 2 reagents are readily reproduced using Hampton Research Optimize™ stock solutions of salts, polymers and buffers. Optimize stock reagents make reproducing Natrix 2 reagents fast, convenient and easy. Dilutions can be performed directly into the crystallization plate using Optimize stock reagents.

Natrix 2 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 2 reagents are stable at room temperature and are best if used within 12 months of receipt. To enhance reagent stability it is recommended that Natrix 2 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 2 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

1. Crystallization of ribozymes and small RNA motifs by a sparse matrix approach. DOudna, J.A. et al. PNAS, USA, 90, 7829-7833, 1993.

2. Doudna, J.A., Grosshans, C., Godding, A., and Kundrot, C.E., Proc. Natl. Acad. Sci. USA, Aug 1993, Vol. 90, 7829-7833.

3. McPherson, A., Current Approaches to Macromolecular Crystallization, Eur. J. Biochem., 189, 1-23, 1990.

4. Protein and Nucleic Acid Crystallization, Methods: A Companion to Methods in Enzymology, Academic Press, Vol 1, No. 1, Aug 1990.

5. Crystallization of Nucleic Acids and Proteins - The Practical Approach Series, Edited by A. Ducruix and R. Giege, Oxford Univ. Press 1992.

6. Cudney, R., et al, Acta Cryst, D50, 414-423, 1994.

7. Schultz, S.C., Shields, G.C., and Steitz, T.A., J. of Mol. Bio., 213, 159-166, 1990.

8. Baeyens, K.J., Jancarik, J., and Holbrook, S.R., Acta Cryst, D50, 764-767, 1994.

9. Perona, J.J., Methods: A Companion to Methods in Enzymology, Vol. 1, 75-82, 1990.

10. Oubridge, C., Ito, N., Teo, C.-H., Fearnley, I., and Nagai, K., J. Mol. Bio., 249, 408-412, 1995.

11. Price, S.R., Ito, N., Oubridge, C., Avis, J.M., and Nagai, K., J. Mol. Bio., 249 398-408.

12. Wincott, F., DiRenzo, A., Shaffer, C., Scaringe, S., and Usman, N., Nucleic Acid Res., Vol. 23, 2677-2687, 1995.

Technical Support

Inquiries regarding Natrix 2 reagent formulation, interpretation of screen results, optimization strategies and general inquiries regarding crystallization are welcome. Please e-mail, fax, or telephone your request to Hampton Research. Fax and e-mail Technical Support are available 24 hours a day. Telephone technical support is available 8:00 a.m. to 4:30 p.m. USA Pacific Standard Time

Hampton Research
34 Journey
Aliso Viejo, CA 92656-3317 U.S.A.
Tel: (949) 425-1321 • Fax: (949) 425-1611
Technical Support e-mail: tech@hrmail.com
Website: www.hamptonresearch.com