# Nucleic Acid Mini Screen™

HAMPTON RESEARCH
*Solutions for Crystal Growth*

**User Guide** **HR2-118 (pg 1)**

The Nucleic Acid Mini Screen™ is an efficient screen formulated to assist in the determination of preliminary crystallization conditions of nucleic acid fragments.

Using 1 to 4 mM oligonucleotide stock concentration the screen requires less than 25 microliters of sample.

The screen evaluates sample concentration, temperature, pH, monovalent cations, divalent cations, and polyamines. The screen is supplied as a kit comprised of twenty-four, 1 milliliter volumes of precipitant and a 250 milliliter bottle of 35% v/v (+/-)-2-Methyl-2,4-pentanediol as the dehydrant.

The composition of the nucleic acid mini screen allows one to apply the formulation to other nucleic acids such as deoxy- and ribozymes, pseudoknots and tRNAs.

## Formulation

All solutions are formulated using ultra-pure chemicals and deionized water and are sterile filtered.

## Storage

Recommended long term storage for the Nucleic Acid Mini Screen 24 unique reagents is -20°C. Allow the kit to equilibrate to room temperature prior to use.

Recommended storage for the 250 ml of dehydrant (35 % v/v (+/-)-2-Methyl-2,4-pentanediol) supplied with the kit is -20° to 25°C.

## Sample Preparation

Nucleic acid solutions should be highly purified and filtered using a 0.2 or 0.45 micron filter prior to crystallization screening. The nucleic acid sample should be suspended in deionized water or buffer of choice to a concentration of approximately 20 to 24 mM mononucleotide concentration. For example, a dodecamer, the single strand concentration should be approximately 2 mM.

No preincubation is recommended for this screen.

Centrifuge the sample to remove amorphous material prior to set up.

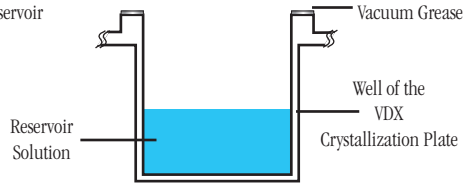
## Instructions

Since it is the most frequently reported method of crystallization, the following procedure describes the use of the Nucleic Acid Mini Screen with the Hanging Drop Vapor Diffusion method. The Nucleic Acid Mini Screen is also very compatible with the Sitting Drop, Sandwich Drop, Micro Batch, and Microdialysis 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.

Prior to use, remove the kit from -20°C storage and allow the reagents to equilibrate to 4°C or room temperature. The set up may be performed at room temperature and moved to a 4°C incubation or the set up may be performed in a 4°C cold room. To minimize condensation with 4°C incubation of room temperature set ups, place a sealed crystallization plate with water in each reservoir on the top and bottom of the plate stack.

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 VDX Plate with sealant (HR3-170). Twenty-four reservoirs are to be prepared for a complete screen. See Figure 1 below.

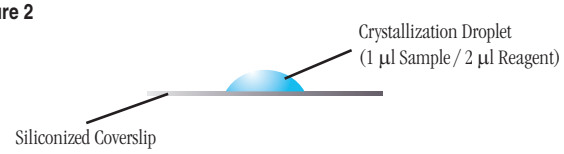
**Figure 1**
Cross section of a reservoir in the VDX plate.




2. Pipet 1 milliliter of 35 % v/v (+/-)-2-Methyl-2,4-pentanediol dehydrant into the reservoir of the crystallization plate.

3. Pipet 1 µl of sample (1 to 4 mM oligonucleotide stock) onto a clean, siliconized cover slide. Pipet 2 µl of Nucleic Acid Mini Screen reagent 1 to the 1 µl sample drop. It is recommended, but not required to pipet a second drop beside the initial drop. It is recommended the second drop contain 2 µl of sample and 2 µl of reagent. See Figure 2.

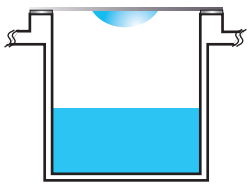
**Figure 2**




**Note:** Be sure to pipet the Nucleic Acid Mini Screen reagents/precipitants into sample drops and **NOT** the dehydrant!

3. Seal the reservoir.

**Figure 3**
Inverted siliconized coverslip placed over the reservoir.



4. Repeat steps 2 through 3 for the remaining reagents 2 through 24.

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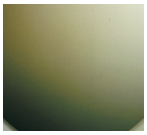
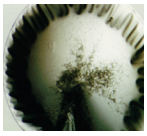
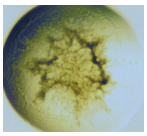
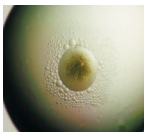
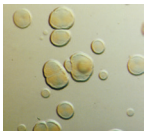
**Figure 4**
Typical observations in a crystallization experiment

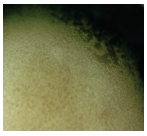

Clear Drop


Skin/Precipitate

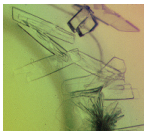

Precipitate


Precipitate/Phase

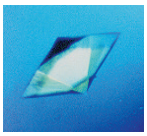

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

## Examine the Drop

Carefully examine the drops under a stereo microscope (10 to 100x magnification) immediately after setting up the screen. Record all observations and be particularly careful to scan the focal plane for small crystals. Observe the drops once each day for the first week, then once a week there after. Records should indicate whether the drop is clear, contains precipitate, and or crystals. It is helpful to describe the drop contents using descriptive terms. Adding magnitude is also helpful. Example: 4+ yellow/brown fine precipitate, 2+ small bipyramid crystals, clear drop, 3+ needle shaped crystals in 1+ white precipitate. One may also employ a standard numerical scoring scheme (Clear = 0, Precipitate = 1, Crystal = 10, etc). Figure 4 (on the left) shows typical examples of what one might observe in a crystallization experiment.

## Interpreting the Results

The sparse matrix screen used in Nucleic Acid Mini Screen is designed screen the samples solubility as well as determine preliminary crystallization conditions. When crystals are obtained during the initial screen, conditions may be optimized by varying the concentration of the precipitant (MPD) and/or salt, cation and polyamine, the pH, temperature, as well as other primary crystallization variables.

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 under polarization. If the amorphous material is precipitate, consider one of the following:

- Screen an alternate sample or precipitant concentration
- Alter the sample sequence
- Vary the drop ratio
- Change the temperature of the experiment

If the droplet remains clear, continue to observe the screen for several weeks and consider increasing sample concentration, increasing the concentration of the precipitant, varying the salt concentration, or screening an alternate sequence.

Using the unique dehydrant format also allows one simple increase the concentration of the dehydrant by adding concentrated dehydrant (100%) to the 35% v/v dehydrant to increase dehydrant concentration, or one can simply replace the 35% v/v dehydrant with a more concentrated dehydrant such as 50 or 65% v/v (+/-)-2-Methyl-2,4-pentanediol.

If small crystals are grown which are not suitable for X-ray diffraction analysis there are several options to pursue:

- Use the small crystals as seeds to grow larger crystals.
- Set optimization trials, varying the primary crystallization variables to optimize conditions for crystal growth. Review all of the results in the initial screen to obtain information on what affects pH, precipitant type and concentration, as well as the mixing of salts with precipitants have on crystal growth. Design subsequent trials to encompass these variables in a grid.
- Vary polyamine /cation type and concentration.

If the results of the screen performed at 4°C do not appear different from the room temperature screen, pursue varying pH, precipitant type and concentration, salt, cation and polyamine during optimization. If the presence or precipitate or crystals is dependent upon temperature, implement temperature variations into the crystallization strategy.

## References and Readings

1. Berger, et al, A Highly Effective 24 Condition Matrix for the Crystallization of Nucleic Acid Fragments. Acta Crystallographica Section D. Vol. D52 Part 3, 465-468, 1996.

## Technical Support

Inquiries regarding Nucleic Acid Mini Screen 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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