コスモ・バイオ株式会社

Grid Screen™

Sodium Malonate

HAMPTON RESEARCH

Solutions for Crystal Growth

User Guide **HR2-247**

Grid Screen™ Sodium Malonate is a preformulated reagent kit designed to provide a rapid screening method for the crystallization of biological macromolecules. The screen is simple and practical for finding initial crystallization conditions as well as determining the solubility of a macromolecule in Sodium Malonate between pH 4.0 and 7.0.

Sodium Malonate is an organic acid of relatively high charge density, which carries two negative charges at a neutral pH. The free acid of Sodium Malonate, Malonic Acid has a very high solubility in water and likely behaves as a cosmotrope in water (7) perhaps stabilizing protein structure as well as conform with water structure about the protein. Sodium Malonate has been demonstrated as a valuable crystallization reagent (8) along with other organic acids. In a study with 31 unique, previously crystallized proteins, 8 of the proteins failed to crystallize from any salt, however of the remaining 23 proteins, 19 were crystallized using Sodium Malonate as the primary crystallization reagent. Sodium Malonate proved twice as effective as the next most successful salt. High concentrations of Sodium Malonate freeze as a glass and can be a useful cryoprotectant (8) therefore crystals grown in Sodium Malonate may be successfully cryopreserved in high concentrations of Sodium Malonate.

Sample Preparation

The macromolecular sample should be homogenous, 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 (1, 2, 3).

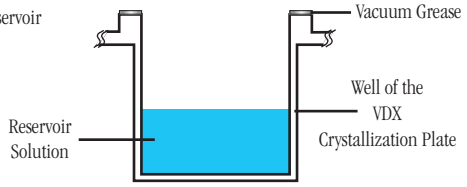
The recommended sample concentration is 5 to 25 mg/ml in water. Initially, the sample should be free of any unnecessary additives in order to observe the effect of the Grid Screen Sodium Malonate variables. Ideally, the initial screen should be performed with a sample which has been dialyzed against dilute (25 mM) buffer although ligands, ions, reducing agents, or other additives may be present as required by the sample for solubility, stability, or activity. Phosphate, borate and carbonate buffers are best avoided as these buffers tend to crystallize out of solution at high relative supersaturation as well as form crystalline material in the presence of divalent cations.

Performing the Screen

Since it is the most frequently reported method of crystallization, the following procedure describes the use of Grid Screen Sodium Malonate with the Hanging Drop Vapor Diffusion method. Grid Screen Sodium Malonate is also very compatible with the Sitting Drop, Sandwich Drop, MicroBatch, 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.

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). Twenty-four reservoirs are to be prepared for a complete Grid Screen Sodium Malonate. See Figure 1.

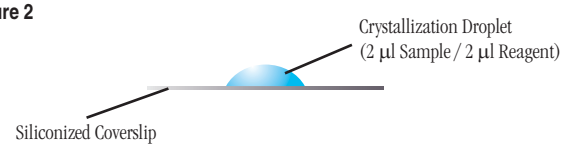
Figure 1
Cross section of a reservoir in the VDX plate.



2. Using a clean pipet tip, pipet 1 ml of Grid Screen Sodium Malonate reagent A1 into reservoir A1. Discard the pipet tip, add a new pipet tip (in order to avoid reagent cross contamination) and pipet 1 ml of Grid Screen Sodium Malonate reagent A2 into reservoir A2. Repeat the procedure for the remaining 22 Grid Screen Sodium Malonate reagents using a clean pipet tip for each reagent so as to avoid reagent contamination and carry over.

3. Pipet 0.5-2 µl of the sample to the center of a clean, siliconized 22 mm diameter circle or square cover slide. See Figure 2.

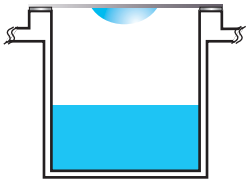
Figure 2



4. Pipet 0.5-2 µl of Grid Screen Sodium Malonate reagent A1 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 23 Grid Screen Sodium Malonate reagents.

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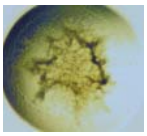
Figure 4
Typical observations in a crystallization experiment


Clear Drop


Skin/Precipitate


Precipitate


Precipitate/Phase

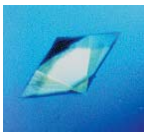

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

7. If the quantity of sample permits, perform Grid Screen Sodium Malonate in duplicate and incubate one set of plates between 4°C and 15°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. 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 Grid Screen Sodium Malonate

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 Grid Screen Sodium Malonate condition and doubling the sample concentration. If more than 70% Grid Screen Sodium Malonate drops are clear consider doubling the sample concentration and repeating the entire screen.

Drops containing precipitate indicate 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 Grid Screen Sodium Malonate condition. If more than 70% Grid Screen Sodium Malonate 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.

Grid Screen Sodium Malonate Formulation

Grid Screen Sodium Malonate 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).

Sodium Malonate is formulated by the titration of Malonic Acid with Sodium Hydroxide. The neutralization of Malonic Acid with Sodium Hydroxide can be a violent reaction and must be carried out slowly, in a vented hood, wearing safety gloves and eye protection and otherwise observing proper safety
procedures.

Hampton Research offers preformulated, sterile filtered stock solutions of 3.4 M Sodium Malonate titrated to pH 4.0, 5.0, 6.0, and 7.0 for reproduction and optimization of preliminary crystallization conditions determined using Grid Screen Sodium Malonate.

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Optimize Stock Solutions

HR2-747 3.4 M Sodium Malonate pH 4.0
HR2-749 3.4 M Sodium Malonate pH 5.0
HR2-751 3.4 M Sodium Malonate pH 6.0
HR2-707 3.4 M Sodium Malonate pH 7.0

References and Readings

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8. A comparison of salts for the crystallization of macromolecules. McPherson, A. Protein Science, 10:418-422, 2001.

Technical Support

Inquiries regarding Grid Screen Sodium Malonate 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 5:00 p.m. USA Pacific Standard Time.

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