

Additive Screen™

HAMPTON
RESEARCH

Solutions for Crystal Growth

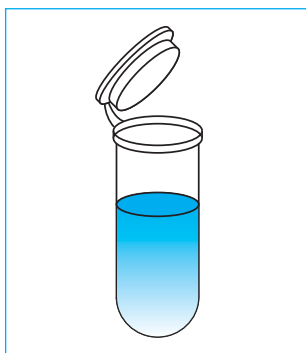
User Guide

HR2-428 (pg 1)

Additive Screen™ is a kit designed to allow the rapid and convenient evaluation of 96 unique additives and their ability to influence the crystallization of the sample. The screen is designed to be compatible with most popular crystallization reagents including all reagents utilized in all of the Hampton Research screens.

Each of the additives is preformulated in deionized water and sterile filtered using a 0.2 micron filter. Recommended long term storage for the Additive Screen kit is -20°C. Allow the kit to equilibrate to room temperature prior to use.

1



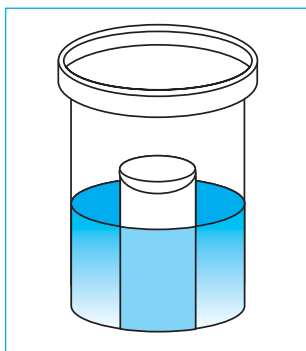
The Additive Screen™ kit is a complete reagent kit designed to provide a rapid screening method for the manipulation of sample-sample and sample solvent interactions to enhance or alter sample solubility.

The Additive Screen evaluates the manipulation factors of multivalent cations, salts, amino acid, dissociating agents, linkers, polyamines, chaotropes, co-factors, reducing agents, polymers, chelating agent, carbohydrates, polyols, non-detergents, amphiphiles, detergents, osmolyte, organic (non-volatile) and organic (volatile) reagents.

The Additive Screen kit is to be used before and during the optimization of preliminary crystallization conditions.

Each Additive Screen kit contains 1 milliliter of 96 unique additives formulated to allow one to rapidly screen with less than 100 microliters of sample.

2



This guide will describe the use of the Additive Screen kit using the Sitting Drop Vapor Diffusion method and a 1 milliliter reservoir volume. Other methods such as Hanging Drop Vapor Diffusion crystallization, and MicroBatch may also be utilized as well as smaller reservoir and drop volumes. A complete description of the Hanging, Sitting, Sandwich Drop, Dialysis and other crystallization methods are available from the Hampton Research Crystal Growth 101 Library.

Two separate methods of setup are to be used for volatile and non-volatile additives.

Reservoir setup for non-volatile Additives (A1 - G8):

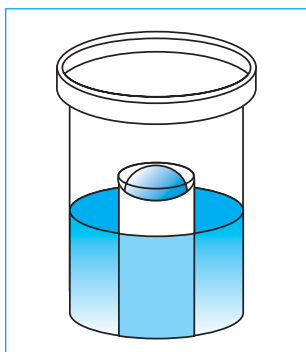
A. Pipet 1 milliliter of crystallization reagent into the reservoir only.

Reservoir setup for volatile Additives (G9 - H12):

A. Pipet 900 microliters of crystallization reagent into the reservoir.

B. Pipet and mix 100 microliters of the volatile additive into the reservoir.

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Drop setup for non-volatile Additives:

A. Pipet 5 microliters of sample onto a sitting drop post.

B. Pipet 1 microliter of additive into the sample drop.

C. Pipet 4 microliters of the crystallization reagent into the sample/additive drop.

D. Seal the reservoir with tape or grease and slides.

E. Repeat for remaining additives.

Drop setup for volatile Additives:

A. Pipet 5 microliters of sample onto a sitting drop post.

B. Pipet 5 microliters of the crystallization reagent/additive mixture from the reservoir into the sample drop.

C. Seal the reservoir with tape or grease and slides.

D. Repeat for the remaining additives.

Figure 1

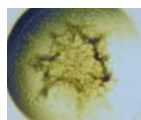
Typical observations in a crystallization experiment.



Clear Drop



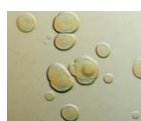
Skin/Precipitate



Precipitate



Precipitate/Phase



Quasi Crystals



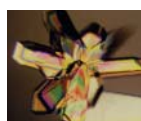
Microcrystals



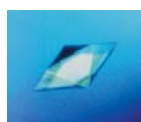
Needle Cluster



Plates



Rod Cluster



Single Crystal

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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 thereafter. 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 1 (left side of page 2) shows typical examples of what one might observe in a crystallization experiment.

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References and Readings

1. Crystallization of membrane proteins. Edited by Hartmut Michel, CRC Press, 1991.
2. Crystallization of nucleic acids and proteins, Edited by A. Ducruix and R. Giegé, The Practical Approach Series, Oxford Univ. Press, 1992 175-191.
3. Screening and optimization strategies for macromolecular crystal growth. Cudney, B. et al, Acta Cryst. (1994). D50, 414-423.
4. Use of glycerol, polyols and other protein structure stabilizing agents in protein crystallization. R. Sousa. Acta Cryst. (1995) D51, 271-277.
5. Influence of divalent cations on protein crystallization. Trakhanov, S. and Quiocho, EA. (1995) Protein Science 4(9): 1914-1919.
6. Non-detergent sulphobetaines: a new class of mild solubilizing agents for protein purification. L. Vuillard, C. Braun-Breton, T. Rabilloud, Biochem. J. (1995) 305, 337-343.
7. A new additive for protein crystallization. L. Vuillard, T. Rabilloud, R. Leberman, C. Berthet-Colominas, St. Cusack. FEBS Letters, 353 (1994) 294-296.

Technical Support

Inquiries regarding Additive 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 5:00 p.m. USA Pacific Standard Time.

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