Additive Screen Mini™

HAMPTON RESEARCH
Solutions for Crystal Growth

User Guide **HR2-464 (pg 1)**

Additive Screen Mini™ is a kit designed to allow the rapid and convenient evaluation of 24 unique additives and their ability to influence the crystallization of the sample by manipulation of sample-sample and sample solvent interactions to enhance or alter sample solubility. 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 storage for the Additive Screen kit is -20 to 4°C. Allow the kit to equilibrate to room temperature prior to removing the cap from the tube. If reagents precipitate during cold storage, warm the tube at 37°C for up to 60 minutes and invert several times to solubilize the reagents.

The Additive Screen evaluates the manipulation factors of multivalent cations, salts, dissociating agents, linkers, polyamines, chaotropes, co-factors, reducing agents, polymer, chelating agent, polyol, non-detergent, amphiphile, detergent, organic (non-volatile and 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 24 unique additives formulated to allow one to rapidly screen with less than 25 microliters of sample.

This guide will describe the use of the Additive Screen Mini kit using the Sitting Drop Vapor Diffusion method and a 1 milliliter reservoir volume. Other methods such as Hanging Drop Vapor Diffusion crystallization, and Micro-Batch 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.

Reservoir Setup Option 1

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

B. Pipet and mix 100 µl of the additive into the reservoir.

Drop Setup Option 1

A. Pipet 1 µl of sample into the sample well.

B. Pipet 1 µl of the crystallization reagent/additive mixture from the reservoir into the sample drop.

C. Repeat for the remaining additives.

E. Seal the plate.

Reservoir Setup Option 2

Reservoir setup for non-volatile Additives (1 - 23):

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

Reservoir setup for volatile Additives (24):

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

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

Drop Setup Option 2

Drop setup for non-volatile Additives:

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

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

C. Pipet 4 µl 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 µl of sample onto a sitting drop post.

B. Pipet 5 µl 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.

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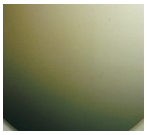
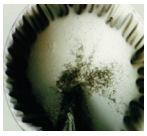
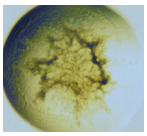
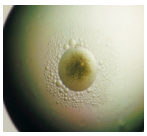
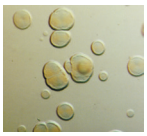
Figure 1
Typical observations in a crystallization experiment

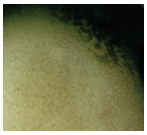

Clear Drop


Skin/Precipitate

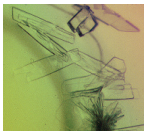

Precipitate


Precipitate/Phase

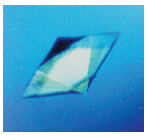

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

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

References and Readings

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2. Crystallization of nucleic acids and proteins, Edited by A. Ducruix and R. Giege, 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, F.A. (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.

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Tube #	Additive	Tube #	Classification	Tube #	Suggested Drop Concentration
1.	0.1 M Cadmium chloride hydrate	1.	Multivalent	1.	0.01 M (10 mM)
2.	0.1 M Calcium chloride dihydrate	2.	Multivalent	2.	0.01 M (10 mM)
3.	0.1 M Magnesium chloride hexahydrate	3.	Multivalent	3.	0.01 M (10 mM)
4.	0.1 M Zinc chloride	4.	Multivalent	4.	0.01 M (10 mM)
5.	0.1 M Praseodymium(III) acetate hydrate	5.	Multivalent	5.	0.01 M (10 mM)
6.	1.0 M Lithium chloride	6.	Salt	6.	0.1 M (100 mM)
7.	30% v/v Dimethyl sulfoxide	7.	Dissociating Agent	7.	3%
8.	0.1 M Sodium bromide	8.	Dissociating Agent	8.	0.01 M (10 mM)
9.	30% w/v 1,5-Diaminopentane dihydrochloride	9.	Linker	9.	3%
10.	1.0 M Glycine	10.	Linker	10.	0.1 M (100 mM)
11.	0.1 M Taurine	11.	Linker	11.	0.01 M (10 mM)
12.	0.1 M Spermidine	12.	Polyamine	12.	0.01 M (10 mM)
13.	0.1 M Spermine tetrahydrochloride	13.	Polyamine	13.	0.01 M (10 mM)
14.	0.1 M Hexammine cobalt(III) chloride	14.	Polyamine	14.	0.01 M (10 mM)
15.	0.1 M TCEP hydrochloride	15.	Reducing Agent	15.	0.01 M (10 mM)
16.	0.1 M Ethylenediaminetetraacetic acid disodium salt dihydrate	16.	Chelating Agent	16.	0.01 M (10 mM)
17.	30% w/v Dextran sulfate sodium salt	17.	Polymer	17.	3%
18.	30% v/v Glycerol	18.	Polyol	18.	3%
19.	3.0 M NDSB-195	19.	Non-detergent	19.	0.3 M (300 mM)
20.	20% w/v Benzamidine hydrochloride	20.	Amphiphile	20.	2%
21.	5% w/v n-Octyl-β-D-glucoside	21.	Detergent	21.	0.5%
22.	30% v/v (+/-)-2-Methyl-2,4-pentanediol	22.	Organic, Non-volatile	22.	3%
23.	40% v/v 2,5-Hexanediol	23.	Organic, Non-volatile	23.	4%
24.	30% v/v 2-Propanol	24.	Organic, Volatile	24.	3%

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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)

Additive Screen Mini™ - HR2-464 Scoring Sheet		Date:	Date:	Date:	Date:
1. 0.1 M Cadmium chloride hydrate	Multivalent				
2. 0.1 M Calcium chloride dihydrate	Multivalent				
3. 0.1 M Magnesium chloride hexahydrate	Multivalent				
4. 0.1 M Zinc chloride	Multivalent				
5. 0.1 M Praseodymium(III) acetate hydrate	Multivalent				
6. 1.0 M Lithium chloride	Salt				
7. 30% v/v Dimethyl sulfoxide	Dissociating Agent				
8. 0.1 M Sodium bromide	Dissociating Agent				
9. 30% w/v 1,5-Diaminopentane dihydrochloride	Linker				
10. 1.0 M Glycine	Linker				
11. 0.1 M Taurine	Linker				
12. 0.1 M Spermidine	Polyamine				
13. 0.1 M Spermine tetrahydrochloride	Polyamine				
14. 0.1 M Hexammine cobalt(III) chloride	Polyamine				
15. 0.1 M TCEP hydrochloride	Reducing Agent				
16. 0.1 M Ethylenediaminetetraacetic acid disodium salt dihydrate	Chelating Agent				
17. 30% w/v Dextran sulfate sodium salt (M_r 5,000)	Polymer				
18. 30% v/v Glycerol	Polyol				
19. 3.0 M NDSB-195	Non-detergent				
20. 20% w/v Benzamidine hydrochloride	Amphiphile				
21. 5% w/v n-Octyl-β-D-glucoside	Detergent				
22. 30% v/v (+/-)-2-Methyl-2,4-pentanediol	Organic, Non-volatile				
23. 40% v/v 2,5-Hexanediol	Organic, Non-volatile				
24. 30% v/v 2-Propanol	Organic, Volatile				

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