

## The Additive Screen

## MD1-11

**The Additive Screen** is formulated for use before or during the optimization of proteins, peptides, nucleic acids, & water soluble small molecules.

This kit is designed to test the effect of 24 compounds on protein crystal growth; screen examples from different classes of additives typically used in crystal growth trials.

24 x 100  $\mu$ L conditions (500  $\mu$ L for volatile conditions).

### Features of The Additive Screen:

- Test the effect of 24 compounds on protein crystal growth.
- Samples amino acids, polyamines, salts, organics.
- 10  $\times$  concentration of additive solution to minimise drop dilution.
- Additives may improve the diffraction quality, morphology or size of a protein crystal.
- A simple and practical way to optimize crystallization conditions

### Introduction

The kit is designed to help test the effect of 24 compounds on protein crystal growth. Additives may affect hydration and intermolecular interactions between protein molecules or between protein molecule and solvent and even ligands. This kit is a screen and results may need to be interpreted with a view to designing further additive experiments using different compounds of the same type as the kit reagent that gave a promising result. Additives may improve the diffraction quality, morphology or size of a protein crystal.

There are 24 unique reagents supplied in the kit. The concentrations of the reagents have been designed to allow minimum dilution of the sample drop, and are sufficiently concentrated that vapour diffusion experiments can be carried out in as little as a 2.5 $\mu$ L drop. All additives have to be added to the sample protein. Non-volatile reagents can be added directly to a sitting or hanging drop. Volatile reagents must be added to the well solution also since they will equilibrate by vapour diffusion with the vapour (air space) above the well solution. Detailed instructions are given below so as to achieve the appropriate additive concentration in the drop.

### Formulation Notes

Additive Screen solutions are prepared using ultrapure water (>18.0 M $\Omega$ ) water and filtered through a 0.2 micron filter. No preservatives are added.

### Storage

**The kit can be stored at 4 degrees or at -20°C to -70°C . Allow the kit to equilibrate to room temperature before use.**

### Contact Us

Molecular Dimensions will be happy to discuss the precise formulation of individual reagents.

Individual reagents and stock solutions for optimisation are available from Molecular Dimensions.

Enquiries regarding The Additive Screen formulation, interpretation of results or optimization strategies are welcome.

Please e-mail, fax or phone your query to Molecular Dimensions.

Contact and product details can be found at [www.moleculardimensions.com](http://www.moleculardimensions.com)



## Instructions for Use:

### Additive Screen – Non-volatile reagents

The drop size may range from 2.5 $\mu$ l to 5.0  $\mu$ l.

Use half the drop volume for the sample and replace one fifth of the precipitant volume of the drop with additive. For example, for a 2.5 $\mu$ l drop use 1.25 $\mu$ l of sample; 1.0 $\mu$ l of precipitant (well solution) and 0.25 $\mu$ l of selected additive.

Drop Size ( $\mu$ l)	Sample ( $\mu$ l)	Additive ( $\mu$ l)	Precipitant ( $\mu$ l)
2.5	1.25	0.25	1.00
3.0	1.50	0.30	1.20
3.5	1.75	0.35	1.40
4.0	2.00	0.40	1.60
4.5	2.25	0.45	1.80
5.0	2.50	0.50	2.00

### Additive Screen – Volatile reagents

When using volatile additive reagents, the additive is placed in the reservoir to replace one tenth of the total well solution.

For example, to 450 $\mu$ l of well solution add 50 $\mu$ l of selected additive.

For a drop size of 2.5 $\mu$ l, mix 1.25 $\mu$ l of sample with 1.25 $\mu$ l of the well solution (pre-mixed with the additive). The drop size may range from 2.5 $\mu$ l to 5.0 $\mu$ l.

Drop Size ( $\mu$ l)	Sample ( $\mu$ l)	Reservoir Solution ( $\mu$ l) (pre-mixed with the additive)
2.5	1.25	1.25
3.0	1.50	1.50
3.5	1.75	1.75
4.0	2.00	2.00
4.5	2.25	2.25
5.0	2.50	2.50

- Place the drop on the coverslide or sitting-drop bridge and mix carefully to avoid bubbles and unnecessary spreading of the sample.
- Set-up a control experiment using water, in place of the additive.
- Using 4 – 20 $\times$  magnification observe the samples daily for 10 days and then weekly.
- Observe the sample for differences in solubility in comparison with the control experiment.
- When crystals are observed, initial crystallization conditions plus the concentration and type of additive included should be optimized.



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Tube #	Compound	Type	Molecular Weight, g/mol	Product concentration	Recommended drop concentration [M]
1	Glycine	Amino acid	75.07	0.5 M	0.05
2	L-Glutamine	Amino acid	146.15	0.1 M	0.05
3	Spermine tetrahydrochloride	Polyamine	348.19	0.1 M	0.01
4	Spermidine	Polyamine	145.25	0.1 M	0.01
5	EDTA	Chelating agent	372.24	0.05 M	0.005
6	DL-Dithiothreitol	Reducing agent	154.25	0.05 M	0.005
7	Sodium thiocyanate	Chaotropic salt	81.07	0.1 M	0.01
8	Taurine	Zwitterion	125.15	0.1 M	0.01
9	Betaine monohydrate	Linker	135.16	0.1 M	0.01
10	Dimethyl sulfoxide	Organic	78.13	100 %	5%*
11	Octyl glucoside	Detergent	292.38	5% w/v	0.5%
12	Xylitol	Sugar	152.10	0.2 M	0.02
13	Potassium iodide	Salt	166.01	1.0 M	0.1
14	Thymol	Organic/Anti-fungal	150.20	100 %	2-3 grains <sup>†</sup>
15	Sodium malonate dibasic monohydrate	Di sodium organic salt	148.00	0.5 M	0.05
16	Ethylenediamine	Amine	60.10	1.0 M	0.05*
17	Cadmium chloride hemi(pentahydrate)	Divalent cation	183.31	0.1 M	0.01
18	Cobalt(II) chloride hexahydrate	Divalent cation	237.93	0.1 M	0.01
19	Magnesium chloride hexahydrate	Divalent cation	203.31	0.1 M	0.01
20	Zinc chloride	Divalent cation	136.29	0.1 M	0.01
21	1,4-Dioxane	Organic	88.11	100 %	5%*
22	Phenol	Dissociating agent	94.11	0.2 M	0.01*
23	2-propanol	Organic	60.10	60 % v/v	3%*
24	Glycerol	Organic	92.09	20 % v/v	2%

\*These reagents are volatile and should be pre-mixed with the well solution as well as drop solution, as described in the instructions.

<sup>†</sup>2 –3 grains of thymol should be added to the well solution and then proceed as if it were a volatile reagent.

**Abbreviations: EDTA;** (ethylenediamine tetraacetic acid)

Manufacturer's safety data sheets are available upon request