

JBScreen Plus

Additive Screen

Application

Additive screening to manipulate sample-sample and sample-solvent interactions.

For *in vitro* use only

Quality guaranteed for 12 months

Store at 4°C

1. Contents of JBScreen Plus

The JBScreen Plus series (Cat.# CS-506) consists of five different kits of additives containing 24 different compounds each (120 in total):

(1) JBScreen Plus Kosmotropic

- Zwitterions
- Poly alcohols
- Cations
- Anions

(2) JBScreen Plus Chaotropic

- Non-ionic
- Cations
- Anions

(3) JBScreen Plus Salts

- Lithium salts
- Ammonium salts
- Sulfates
- Multivalent cations

(4) JBScreen Plus Additives

- Linker molecules and poly amines
- Organic hydrophilic polymers
- Sugars
- Other compounds

(5) JBScreen Plus Volatiles

- Small alcohols
- Non-ionic chaotropes
- Organic volatiles

The active ingredients are dissolved in purified water, pre-formulated at 10x the recommended final concentration and sterile filtered.

Please note: Some formulations of JBScreen Plus contain toxic compounds such as cadmium or beryllium, cyanates, or thiocyanates. Take care accordingly and follow appropriate safety procedures.

2. Introduction

The major bottle neck in a successful X-ray structure determination still is the growth of high quality crystals that diffract X-rays with a resolution better than 2.8 Å [1]. But although there are now sophisticated crystal screens on the market [2] that can be combined with robotics allowing increasing throughput for crystal set ups, many of the drops will not produce suitable crystals. Whereas those drops producing no precipitation of the protein to be crystallized at all are usually the toughest ones to deal with, there is quite some hope for those drops that produce amorphous precipitates, microcrystals, or – even better – larger crystals that do not show sufficient diffraction.

Due to the enormous number of possible parameters that may turn these initial findings into useful results however, the main challenge is to achieve the goal within the given time frame and according to the available resources (such as the available amount of protein to be crystallized)

The JBScreen Plus additive screen is therefore used before and during the optimization of preliminary crystallization conditions where special additives may assist in the crystallization of the sample and improve the quality of the crystal. The kit focuses mainly on small molecules which are known to interfere with sample-sample and sample-solvent interactions, and/or with the surface water molecules.

Numerous reports on the use of additives to improve the quality and size of macromolecular crystals have been evaluated to select the formulations of the JBScreen Plus solutions to allow the crystallographer to rapidly screen up to five sets of 24 unique additives with a minimum amount of sample.

JBScreen Plus

Additive Screen

3. Instructions

All **JBScreen Plus kits** are suitable for the common crystal set up techniques including *Sitting Drop Vapour Diffusion* (Figure 1), *Hanging Drop Vapour Diffusion*, or *Batch* techniques.

JBScreen Plus 1 - 4 (non-volatile additives) :

- (1) Pipet 1 ml of your crystallization buffer into the reservoir.
- (2) Pipet 5 μ l of protein solution onto the drop holding device.
- (3) Add 1 μ l of the **JBScreen Plus** additive solution into the protein drop.
- (4) Pipet 4 μ l of the reservoir solution into the drop. Mix gently to avoid bubbles and to minimize spreading of the drop. Some drop spreading may occur due to the nature of some of the additives.
- (5) Seal the reservoir.
- (6) Repeat for all other **JBScreen Plus** non-volatiles.

JBScreen Plus 5 (volatile additives):

Volatile compounds must be present in both the drop and the reservoir:

- (1) Pipet 900 μ l of your crystallization buffer into the reservoir.
- (2) Pipet 100 μ l of the volatile **JBScreen Plus** additive into the reservoir and mix thoroughly.
- (3) Pipet 5 μ l of protein solution onto the drop holding device.
- (4) Pipet 5 μ l of the reservoir solution (containing the additive) into the drop. Mix gently to avoid bubbles and to minimize spreading of the drop. Some drop spreading may occur due to the nature of some of the additives.
- (5) Seal the reservoir.
- (6) Repeat for all other **JBScreen Plus** volatiles.

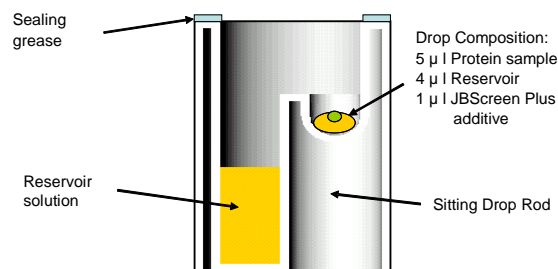


Figure 1: Setting of JBScreen Plus for the Sitting Drop Vapour Diffusion technique

4. References

- [1] Rayment (2002) Protein Structure. *Encyclopedia of Physical Science and Technology* **3rd Ed. (13)**:191.
- [2] Kundrot (2004) Which strategy for a protein crystallization project?. *Cell. Mol. Life Sci.* **61**:525.

5. JBScreen Plus Literature Citations

- Wolfova *et al.* (2005) Crystallization of the flavoprotein WrbA optimized by using additives and gels. *J. Crystal Growth* **284**:502.

6. Recommended Reading

- Herberhold *et al.* (2004) Effects of Chaotropic and Kosmotropic Cosolvents on the Pressure-Induced Unfolding and Denaturation of Proteins: An FT-IR Study on Staphylococcal Nuclease. *Biochemistry* **43**:3336.
- Batchelor *et al.* (2004) Impact of protein denaturants and stabilizers on water structure. *J. Am. Chem. Soc.* **126**:1958.
- Boström *et al.* (2003) Specific ion effects: Why the properties of lysozyme in salt solutions follow a Hofmeister series. *Biophys. J.* **85**:686.
- Uedaira *et al.* (2001) Role of hydration of polyhydroxy compounds in biological systems. *Cell. Mol. Biol.* **47**:823.
- <http://www.lsbu.ac.uk/water/kosmos.html>
- Cacace *et al.* (1997): The Hofmeister series: salt and solvent effects on interfacial phenomena. *Quarterly Reviews of Biophysics* **30**:241.
- Von Hippel *et al.* (1965) On the Conformational Stability of Globular Proteins: The Effects of Various Electrolytes and Non-electrolytes on the Thermal Ribonuclease Transition. *J. Biol. Chem.* **240**:3909.