User Guide

IFTA AG
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DIN EN ISO 9001:2000-12
Reg. No. IC 03214 034

Jena Bioscience

JBS Crystallization Freshman Kit - Scholar

Get a head start crystallizing your protein

Cat. No.	Amount
CSK-102	1Kit

For *in vitro* use only
Quality guaranteed for 18 months
Store Screening reagents at 4°C
Plates and Cover Slides at RT

Application

The **JBS Crystallization Freshman Kit - Scholar** is addressed to newcomers in the field of protein crystallography who are interested in a rather extensive screening of initial crystallization conditions of proteins, peptides, nucleic acids and macromolecular complexes in order to grow single crystals suitable for X-ray diffraction analysis.

Kit Contents

The kit contains all the required material to crystallize one or more proteins using the 'Hanging Drop Method'.

(1) 40 Crystallization plates

The **pregreased SuperClear™** 24 well plates are designed for hanging drop vapor diffusion. They are made of crystal clear polystyrene. A bead of high vacuum grease is applied to the ring of the reservoir. This allows secure and leak-proof sealing of the reservoir.

(2) 1000 Cover Slides

The **siliconized 22 mm Thick Circular Cover Slides** allow secure sealing of the SuperClear™ crystallization plates. They are 0.5mm thick and hardly breakable. Due to the siliconized surface the protein drop does not flatten on the glass.

(3) 5 Classic Crystallization Kits, each containing 24 reagents at 10 ml

The reagents are selected from our successful **JBScreen Classic** series, covering of the most prominent buffers for protein crystallization. Their compositions result from data mining of several thousands of crystallized proteins. The reagents have been selected and indexed according to the main precipitant, i.e.:

- JBScreen Classic 1 - PEG 400 to PEG 3000
- JBScreen Classic 2 - PEG 4000
- JBScreen Classic 4 - PEG 5000 MME to 8000
- JBScreen Classic 6 - Ammonium Sulfate
- JBScreen Classic 7 - MPD

This allows easy extraction of all relevant information and is already a first step to a refinement: **Once you get a hit, you immediately see the effects of the neighbouring conditions.** Subsequent fine tuning of preliminary hits will be much more efficient.

Introduction

The first step in the determination of an X-ray crystal structure, which is often also the most difficult step, is the growth of sufficiently large protein crystals. A crystal is a three-dimensional periodic arrangement of building blocks. In our case, these building blocks are protein molecules.

How does one manage to grow crystals from such complicated molecules like proteins? The following protocol will help you screening for successful crystallization conditions.

A more detailed introduction to the theory and methods of protein crystallization can be found in the Background Information:
http://www.jenabioscience.com/images/baa1580ed1/Background_Information.pdf

Hanging-Drop Experimental Protocol

1. Sample preparation

The protein under investigation should be as pure as possible, i.e. 90 to 95% pure on a Coomassie stained SDS-PAGE.

In addition we recommend to centrifuge or micro-filtrate your sample before setting up trays.

The sample concentration should be in the range of 5 to 25 mg/ml.

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2. Setting up the tray

   Pipette 1ml of JBScreen Classic 1 reagent 1 into the reservoir A1 of your crystallization plate using a clean pipette tip.

   Use a new pipet tip to pipette 1ml of JBScreen Classic 1 reagent 2 into reservoir A2.

   Proceed in the same way until you pipetted the 24 solutions of JBScreen Classic 1 into the reservoir wells A1 to D6 using a new pipet tip for each reagent.

   Please note: Before pipetting, ensure that the crystallization plate is correctly oriented by checking that the row (A-D) and column (1-6) identifiers are rightside up.

3. Pipetting the crystallization drop

   Pipette 2 µl protein solution onto the center of a cover slide. Carefully add 2 µl of the reservoir solution A1 to the drop. Place the opening of the pipette tip carefully onto the surface of the protein drop and try to squeeze out the reservoir solution without forming bubbles in the drop.

   Subsequently, invert the cover slide and gently place it over reservoir A1 so that the hanging drop is positioned over the center of the reservoir. Now press down on each cover slide lightly with your fingers (or other soft object) so that the silicone grease forms a complete hermetic seal and isolates the interior reservoir chamber.

   Repeat the procedure for the remaining 23 wells. The pipette tip must be changed after each drop to avoid cross contamination of the drops.

4. After all reservoir wells are sealed with a cover slide, close the plate with the provided plate cover.

5. Repeat the operations 1.-3. for the remaining JBScreen Classic Kits.

Store the prepared crystallization plates in a suitable area with the temperature remaining as constant as possible (20-22°C).

Crystal formation can take place in a time space of a few minutes to more than a year.

Evaluation of the Experiment

Carefully remove the plate cover and examine the drops under a stereo microscope. Caution should be exercised when adjusting the microscope objective so as not to contaminate it with grease.

Inspect the drops directly after pipetting the plate, once a day during the first week and afterwards once a week. Record all your observations in the scoring sheet, i.e. if the drop is clear, contains precipitate or crystals. This will help you to interpret your results.

Clear drops indicate that supersaturation of the protein-precipitant mixture has not been reached or that drop and reservoir solution drop are not equilibrated yet. If more than 2/3 of the drops remain clear it might be helpful to repeat the screen with a higher protein concentration.

Drops with precipitate indicate that supersaturation of the protein-precipitant mixture is too high. If more than 2/3 of the drops contain precipitate it might be helpful to dilute the protein sample twofold and repeat screening.

Please Note: It is possible that crystals also grow from precipitate. So, do not discard plates containing drops with precipitate. Observing crystals under polarized light helps to differentiate between precipitate and micro-crystals.

If the drops contain crystals the concentration of protein and precipitant has been appropriately chosen. If you observe micro-crystals or needles the successful condition has to be optimized in order to improve crystal size and quality.

For help and further information please contact **xtals@jenabioscience.com**