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Product Information

Crystallization Extension Kit for Proteins
Product Number **70437**
Store at 2-8 °C

TECHNICAL BULLETIN

Application

The Crystallization Extension Kit for Proteins is used in conjunction with the Crystallization Basic Kit for Proteins (82009). It provides additional screening conditions beyond those in the basic kit. The extension kit has 50 additional reagents and solutions with which to determine the best conditions for the crystallization of biological macromolecules such as proteins. This kit allows screening with precipitants and their combinations not available in the Crystallization Basic Kit for Proteins. The Crystallization Extension Kit for Proteins is also effective in determining the solubility of a protein with these additional precipitants and over the same pH range as the Crystallization Basic Kit for Proteins.

The Crystallization Extension Kit for Proteins may be used initially with the Crystallization Basic Kit for Proteins, when sample size permits, to provide as many potential screens for crystallization conditions as possible. It may be used if the basic kit did not yield, or is not expected to yield, any successful screens. The extension kit may be used to obtain additional information when the initial screens resulted in crystals of low quality for analysis (size, diffraction properties, homogeneity).
The solution and crystallization conditions are empirically derived based on known or published crystallization conditions of various proteins in the past, so as to sample as large a range of buffer, pH, additive and precipitant variables as possible, using small amounts of proteins.

Quality of reagents – the key to success

The Crystallization Extension Kit for Proteins reagents are formulated using high purity reagents. These reagents are specially purified and analyzed to ensure the absence of any significant traces of ions or other impurities. This enables the reliable and precise formulation of crystallization conditions as required for best results. There are many instances where the Biochemika ultra/MicroSelect chemicals have been used successfully for different crystallization methods. All solutions are sterile filtered using 0.22 micron filters.
The kit contains 10 ml of each component, but all solutions are available separately as 100 ml bottles. Larger quantities are available on request.

Precautions and Disclaimer

This product is for laboratory research use only. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

It is recommended that the reagents of this kit be stored at 2-8 °C. Storage at –20 °C will not adversely affect the kit reagents and the reagents as supplied are stable at room temperature for short-term storage. Kit reagents should not be set under ultravi-olet light to protect them from microorganisms.

Sample Preparation Instruction

The sample has to be as pure as possible and free of amorphous material or other particles. The purity should be >90% when stained with Coomassie on an SDS gel. Amorphous material can be removed by centrifugation or micro-filtration (2, 3, 4, 5). For the storage of protein it is recommended to have a high concentration, for example 5 mg/ml. The temperature depends on the protein, generally cells or bacteria tolerate freezing at –70 °C better than purified proteins. A method to do so is to pipet the sample directly into liquid nitrogen (5). Sample concentrations used in practice vary widely, but concentrations of 5 to 10 mg/ml have been used most frequently and may be useful as a starting point. If possible the sample should just contain water and the macromolecule. Unnecessary additives can falsify the result. In case disturbing ions, reducing agents, ligands or other additives are present, they may be removed by dialysis of sample against water. The purification or concentration by ammonium sulfate precipitation is a poor choice because after this step it is nearly impossible to remove the salt by dialyzing or with a desalting column.

Note:
The following anions could be responsible for inorganic crystals forming due to the presence of bivalent cations: CO_3^{2-}, BO_3^{3-}, PO_4^{3-}.
Example of bivalent cations: Zn^{2+}, Ca^{2+}, Mg^{2+}
Concentrations of less than 10 mM are not a problem.

Procedures

The application method described below is the most common method of crystallization: the Hanging Drop Vapor Diffusion method. Other methods like the Sitting Drop, Microdialysis, MicroBatch. and Sandwich Drop methods are also applicable for the Crystallization Extension Kit for Proteins. The sitting drop method is being used more and more. It will probably gain further importance since the hanging drop procedure is difficult to use with automated procedures. Directions for the Hanging, Sitting Drop and other crystallization methods are available from our Technical Service.

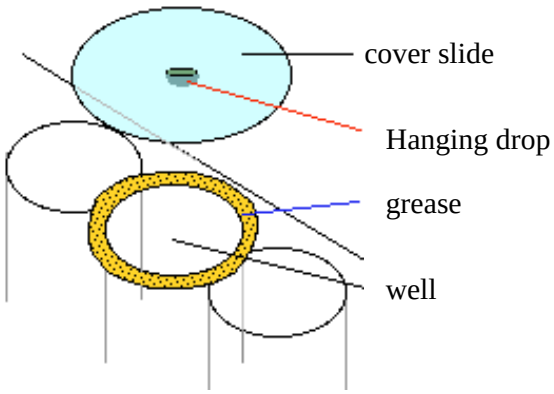

figure 1: well plate

1. Take three 24-Well plates (from Stratech, ICN Biomedicals, Costar, Falcon, Molecular Dimensions Ltd.). It is recommended that on each upper edge of the 24 wells should be put a thin film of grease. This ensures that the cover slide doesn't shift. Prepare fifty wells for one full screen of a protein. See figure 1.

2. Pipet 800 µl of each reagent into the fifty wells. *Use always a clean pipet tip for each reagent!*

3. 2 µul of sample are put into the middle of a clean, siliconized 22 mm (18 mm if you use Costar plate) diameter cover slide (Molecular Dimensions Ltd, Stratech). See figure 1. It is also possible to take a square cover slide.

4. Pipet 2 μl of the corresponding crystallization reagent from each well into the sample droplet. Mix with caution by dispensing and aspirating the droplet with the pipet. Avoid foaming by keeping the tip in the drop.

5. Invert the cover slide and droplet directly over the well containing the appropriate reagent and place the cover slide on top of the well. Make sure the grease seal is complete between the cover slide and the edge of the well.

6. Repeat steps 3. to 5. for all reagents.

7. A recommended practice is to perform the crystallization screen at 4 °C and at room temperature if there is enough sample. Incubate and store the plates in a place with stable temperature and free of vibration.

Sample solubility is also temperature dependent. Although most crystallizations have been achieved at room temperature, in many cases different temperatures have led to success. Comparison of results of screening at two different temperatures (4 °C and room temperature) helps determine the magnitude of temperature effects on sample solubility. Temperature may be an important parameter in the optimization procedure.

Observation

Drops are typically observed by a stereo microscope at 10 to 100X. Record all observations by scanning every droplet on the slides.

Scan the focal plane for small crystals and record observations for all droplets. Scan the first time shortly after the screen is set up. Then for the first 5-10 days, information may be recorded daily and, thereafter, on a weekly basis. Records should include the clarity of the droplet (clear, precipitate, or crystals), along with descriptive phrases and a numerical scale. The following are possible examples (see also observation sheet):
10(= crystal grown 1 D) shower of needles, yellow
6(= gelatinous protein precipitate) red/brown
1(= drop is clear), green
7 (= fully precipitated dark color) dark green
It can be useful to write down the largest crystal size!

Results and Interpretation

A clear drop may be an indication that the drop has not yet reached its final state. If the drop remains clear after 2 to 4 weeks, the relative sample and reagent supersaturation may be too low. If a majority of drops remain clear, consider repeating the entire screen using a protein sample at higher protein concentration.

There are several reasons for precipitation in a drop. A precipitate can indicate that the sample or precipitant concentration is too high (precipitation within 1 day) or it is not the preferred crystallization condition (within a few days). In the case of too high concentration repeat the screen with lower protein concentration. If more than a majority of drops contain a precipitate with no crystals present, consider also a dilution of the sample and repeating the entire screen. In the case of precipitation problems for several screens, it may be useful to dilute the precipitant in the reagent.
Precipitation may also be an indication that the target protein has denatured. It may be necessary to take steps to stabilize the target protein. These could include the addition of additives like salts, reducing agent, glycerol, ligands, non-detergent sulfobetaine
or other appropriate stabilizing reagents.
If you should have only denatured protein left, you may use the Renaturation Basic Kit for Proteins (96827) to find out appropriate renaturation conditions. Sample purity may also cause precipitation. Low sample purity, aggregation, or a heterogeneous preparation may be responsible for precipitation. In these cases, further sample purification is required. It is possible that a crystal may form out of a precipitate. Crystals can grow extremely fast, in few minutes, or may require much more time, up to a few months. This is the reason that crystallization plates should never be trashed, or a drop disregarded too early. Store and record the plates until the drops are dried out.

It is recommended that a high power microscope be used to examine the precipitate between crossed polarizing lenses. True amorphous precipitates do not glow. Birefringent microcrystalline precipitates can glow as a result of the plane of light polarization. It may be possible to use streak seeding to produce larger crystals from microcrystalline precipitates.

Screens, which produce crystals, provide the first clues regarding conditions for crystallization. It may be necessary to optimize these conditions to produce crystals with the proper size and quantity for analysis. The following parameters should be considered during optimization: pH, salt type and concentration, precipitant type and concentration, temperature, sample concentration, and other additives.

References

1. Sparse Matrix Sampling: a screening method for crystallization of proteins. Jancarik, J. and Kim, S.H. J. Appl. Oryst., 24, 409-411, 1991.
2. Crystallization of nucleic acids and proteins, A. Ducruix and R. Giege eds., The Practical Approach Series, Oxford Univ. Press, 1992.
3. Current approaches to macromolecular crystallization. McPherson, A. Eur. J. Biochem. 189,1.23, 1990.
4. Protein and Nucleic Acid Crystallization. Methods, A Companion to Methods in Enzymology, Academic Press, Volume 1, Number 1, August 1990.
5. Protein crystallization, Techniques, Strategies, and Tips, edited by Therese M. Bergfors, A laboratory Manual, International University Line (1999)

70437 Crystallization Extension Kit for Proteins Observation Sheet

Sample description: _____ Date: _____
concentration: _____ Incubation Temperature: _____
buffer: _____ Reservoir Volume: _____

1 Drop contains : Crystallization Reagent_____ul Sample_____ul Additive (name)_____(volume)_____ul

1 drop is clear
2 drop contains non-protein particles

precipitate without birefringent and edges
3 mostly clear drop
4 fully precipitated dark colour
5 gelatinous protein precipitate
6 phase separation

precipitates shows birefringent or has edges
7 sperulites or small structures maybe edges
8 crystal grown 1 D
9 crystal grown 2 D
10 crystal grown 3 D

No.	Product No.	Name	Date:	Date:	Date:	Date:	Date:
1.	88716	Na-chloride 2M, PEG 6000 10%					
2.	83935	Na-chloride 0.5M, Mg-chloride 0.01M, CTAB 0.01M					
3.	72526	Ethylene glycol 25%					
4.	75691	Dioxane 35%					
5.	70293	NH_4-sulfate 2M, 2-Propanol 5%					
6.	95514	Imidazole (pH 7.0) 1M					
7.	93593	PEG 1000 10%, PEG 8000 10%					
8.	92982	Na-chloride 1.5M, Ethanol 10%					
9.	93471	Na-chloride 2M, Na-acetate (pH 4.6) 0.1M					
10.	93714	Na-chloride 0.2M, Na-acetate (pH 4.6) 0.1M, 2-Methyl-2,4-pentanediol 30%					
11.	96346	Co-chloride 0.01M, Na-acetate (pH 4.6) 0.1M, 1,6-Hexanediol 1M					
12.	95958	Cd-chloride 0.1M, Na-acetate (pH 4.6) 0.1M, PEG 400 30%					
13.	92913	NH_4-sulfate 0.2M, Na-acetate (pH 4.6) 0.1M, PEG MME 2000 30%					
14.	96765	K-,Na-tartrate 0.2M, NH_4-sulfate 2M, Na-citrate (pH 5.6) 0.1M					
15.	92347	Li-sulfate 1M, NH_4-sulfate 0.5M, Na-citrate (pH 5.6) 0.1M					
16.	94832	Na-chloride 0.5M, Na-citrate (pH 5.6) 0.1M, Polyethylenimin 2%					
17.	94839	Na-citrate (pH 5.6) 0.1M, tert.-Butanol 35%					
18.	71228	Fe(III)-chloride 0.01M, Na-citrate (pH 5.6) 0.1M, Jeffamine M-600 10%					
19.	80557	Na-citrate (pH 5.6) 0.1M, 1,6-Hexanediol 2.5M					
20.	80094	Mg-sulfate 1.6M, MES (pH 6.5) 0.1M					
21.	77583	Na-chloride 2M, Ka-,Na- dihydrogenphosphate each 0.1M, MES (pH 6.5)					
22.	79975	MES (pH 6.5) 0.1M, PEG 20'000 12%					
23.	80095	NH_4-sulfate 1.6M, MES (pH 6.5) 0.1M, Dioxane 10%					
24.	72873	Cs-chloride 0.05M, MES (pH 6.5) 0.1M, Jeffamine M-600 30%					
25.	72874	Co-chloride 0.01M, NH_4-sulfate 1.8M, MES (pH 6.5) 0.1M					
26.	79688	NH_4-sulfate 0.2M, MES (pH 6.5) 0.1M, PEG MME 5000 30%					
27.	74616	Zn-sulfate 0.01M, MES (pH 6.5) 0.1M, PEG MME 550 25%					
28.	73894	Na-citrate (pH 6.5) 1.6M					
29.	80386	NH_4-sulfate 0.5M, HEPES Na-salt (pH 7.5) 0.1M, 2-Methyl-2,4-pentanediol					
30.	80060	HEPES Na-salt (pH 7.5) 0.1M, 2-Methyl-2,4-pentanediol 5%, PEG 6000 10%					
31.	79976	HEPES Na-salt (pH 7.5) 0.1M, Jeffamine M-600 20%					
32.	79687	NH_4-sulfate 1.6M, Na-chloride 0.1M, HEPES Na-salt (pH 7.5) 0.1M					
33.	91476	NH_4-formate 2.0M, HEPES Na-salt (pH 7.5) 0.1M					
34.	89718	Na-acetate 1.0M, Cd-sulfate 0.05M, HEPES Na-salt (pH 7.5) 0.1M					
35.	86953	HEPES Na-salt (pH 7.5) 0.1M, 2-Methyl-2,4-pentanediol 70%					
36.	82238	Na-chloride 4.3M, HEPES Na-salt (pH 7.5) 0.1M					
37.	90196	HEPES Na-salt (pH 7.5) 0.1M, Ethylene glycol 8%, PEG 8000 10%					
38.	82217	HEPES Na-salt (pH 7.5) 0.1M, PEG 10'000 20%					
39.	82208	Mg-chloride 0.2M, TRIS-HCl (pH 8.5) 0.1M, 1,6-Hexanediol 3.4M					
40.	86261	TRIS-HCl (pH 8.5) 0.1M, tert.-Butanol 25%					
41.	81935	Li-sulfate 1M, Ni(II)-chloride 0.01M, TRIS-HCl (pH 8.5) 0.1M					
42.	82237	NH_4-sulfate 1.5M, TRIS-HCl (pH 8.5) 0.1M, Glycerol 12%					
43.	80925	NH_4-phosphate 0.2M, TRIS-HCl (pH 8.5) 0.1M, 2-Methyl-2,4-pentanediol					
44.	88438	TRIS-HCl (pH 8.5) 0.1M, Ethanol 20%					
45.	88439	Ni(II)-chloride 0.01M, TRIS-HCl (pH 8.5) 0.1M, PEG MME 2000 20%					
46.	91229	Na-chloride 0.1M, Bicine (pH 9.0) 0.1M, PEG MME 550 20%					
47.	95633	Mg-chloride 2M, Bicine (pH 7.5) 0.1M					
48.	80106	Bicine (pH 9.0) 0.1M, Dioxane 2%, PEG 20'000 10%					
49.	79924	Mg-chloride 0.1 M, TRIS-HCl (pH 8.5) 0.1M, PEG 20000 15%					
50.	80105	PEG 20000 20 %					

Precautions and Disclaimer:
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.