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Product Information

Crystallization Kit for Proteins for Automatic Screening
Product Number 56783
Store at 4°C

TECHNICAL BULLETIN

Application

Crystallization Kit for Proteins is an empirical screening method for the direct determination of suitable crystallization conditions for biological macromolecules like proteins, etc.

The Kit is based on our Crystallization Kit for Proteins and the Crystallization Extension Kit for Proteins, both developed according to the described methods of Jancarik and Kim (1). 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.

This kits comes in a deepwell block, to allow convenient handling by either automated liquid handling or multi channel pipets.

Quality of reagents – a key to success

All the reagents within this kit 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 experiences where the Biochemika ultra chemicals (former Microselect grade) have successfully been used for different crystallization methods. All solutions are sterile filtered using 0.22 micron filters.
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 4 °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 ultraviolet 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 at least >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 depending 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 direct into liquid nitrogen (5).

Sample concentration used in practice varies heavily, but concentrations of 5 or 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 macro-molecule. 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 bad method because after this step it is nearly impossible to remove the salt by dialyzing or with a desalting column.
Note:
Following Ions could be responsible for inorganic crystals building by the presence of bivalent cations: CO_3^{2-}, BO_3^{3-}, PO_4^{3-}
Example for bivalent cations: Zn^{2+}, Ca^{2+}, Mg^{2+}
Concentration of less than 10 mM are not a problem.

Handling and Procedures

Before use, centrifugation of the deepwell block with a swing-out rotor centrifuge is suggested, to remove solutions from the cover foil. Centrifugation prevents cross contamination while opening the plate. Otherwise drops under the cover foil may be shaken down. The film should be very gently peeled from the plate.

First open the strap and turn it down to the surface of the deep well plate (Fig.1). Then smoothly pull the strap in horizontal direction so that the aluminium foil moves parallel to the plate surface (Fig.2) to avoid contamination of the wells by drops hanging on the foil.

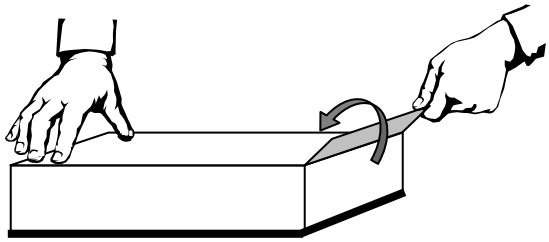

Fig. 1

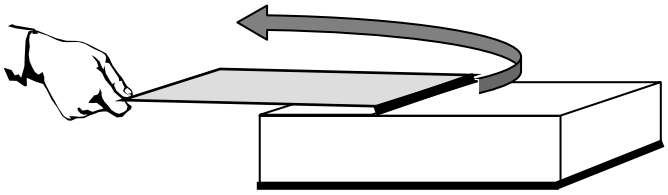

Fig. 2

Instead of peeling the film, it can also be left intact and pierced to take out reagent.

The following procedure describes the use of this kit with the Sitting Drop Vapor Diffusion method.

For manual handling, either a 8 or 12 channel pipet can be used to pipet the recommended volume (50-100 µl) into the reservoirs of a 96 well crystallization plate. Pipet tips should be changed for each set of conditions. But several crystallization plates may be prepared at once (row or column wise) to save tips.

Transfer 50 nl to 2 µl or crystallization reagent from reservoir to the sitting drop well (for each well). Again a 8 or 12 channel pipet may be used. Pipet tips should be changed for each well or set of wells.

Pipet same volume of sample (50 nl to 2 µl) into each sitting drop well. Mixing can be performed by gently aspirating and dispensing the drop several times.

Seal the crystallization plate as you usually do or according to the suppliers recommendations. Seal the deepwell block using foils or cover mats (for short term storage). You may use the enclosed aluminium foils. Simply pull the aluminium foil from the protective film and put the foil on the plate by pressing it onto the surface with evenly spread pressure. Take care that the foil is flush with one side of the plate and overhanging on the other side. To simplify the reopening of the kit put a piece of adhesive tape on the underside of the overhanging aluminium foil.

Please note: When kits are sealed manually we highly recommend to store the kits in upright position at 4°C in the dark.

Sample solubility is also temperature dependent. Allthough most crystallizations have been achieved at room temperature, in many cases different temperatures have lead 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.

Due to the standard format of deepwell blocks, they are suitable for any type of standard liquid handler (working with single tip or needle up to 96 parallel channels)

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 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 (preci-pitation within 1 day) or it is not the favorable crystallization condition (within a few days). In the case of too high concentration repeat the screen with lower protein concentration. If a majority of drops contain a precipitate with no crystals present, consider also a dilution of the sample and repeating the entire screen.

In case of precipitation problem for several times it may be useful to try out as a last essay 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 by some means just have denatured protein left, you may use the Renaturation Basic Kit for Proteins (96827) to find out sufficient 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 accrue out of a precipitate. Crystals can grow extremely fast in few minutes or may require much more time up to a few months. This are the reasons that crystallization plates should never been trashed or disregard a drop too early. Store and record the plates until the drops are dried out.

It is recommendable to use a high power microscope 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 cyrstallization. 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)

56783 Crystallization Kit for Proteins – List of Compounds

A1	89754	Ca-chloride 0.02M, Na-acetate (pH 4.6) 0.1M, 2-Methyl-2,4-pentanediol 30%
A2	73374	NH_4-acetate 0.2M, Na-citrate (pH 5.6) 0.1M, PEG 4000 30%
A3	89786	Li-sulfate 0.2M, TRIS-HCl (pH 8.5) 0.1M, PEG 4000 30%
A4	70377	Imidazole (pH 6.5) 0.1M, Na-acetate 1M
A5	76728	Na-acetate (pH 4.6) 0.1M,Na-formiate 2M
A6	80806	K-dihydrogenphosphate 0.05M, PEG 8000 20%
A7	88716	Na-chloride 2M, PEG 6000 10%
A8	93471	Na-chloride 2M, Na-acetate (pH 4.6) 0.1M
A9	94839	Na-citrate (pH 5.6) 0.1M, tert.-Butanol 35%
A10	72874	Co-chloride 0.01M, NH_4-sulfate 1.8M, MES (pH 6.5) 0.1M
A11	91476	NH_4-formate 2.0M, HEPES Na-salt (pH 7.5) 0.1M
A12	81935	Li-sulfate 1M, Ni(II)-chloride 0.01M, TRIS-HCl (pH 8.5) 0.1M
B1	78016	K-,Na-tartrate 0.4M
B2	76028	NH_4-acetate 0.2M, Na-acetate (pH 4.6) 0.1M, PEG 4000 30%

B3	88518	Mg-acetate 0.2M, Na-cacodylate pH (6.5) 0.1M, PEG 8000 20%
B4	88530	NH_4-acetate 0.2M, Na-citrate (pH 5.6) 0.1M, 2-Methyl-2,4-pentanediol 30%
B5	71835	HEPES Na-salt (pH 7.5) 0.1M, K-dihydrogenphosphate 0.8M, Na-dihydrogenphosphate 0.8M
B6	78225	PEG 1500 30%
B7	83935	Na-chloride 0.5M, Mg-chloride 0.01M, CTAB 0.01M
B8	93714	Na-chloride 0.2M, Na-acetate (pH 4.6) 0.1M, 2-Methyl-2,4-pentanediol 30%
B9	71228	Fe(III)-chloride 0.01M, Na-citrate (pH 5.6) 0.1M, Jeffamine M-600 10%
B10	79688	NH_4-sulfate 0.2M, MES (pH 6.5) 0.1M, PEG MME 5000 30%
B11	89718	Na-acetate 1.0M, Cd-sulfate 0.05M, HEPES Na-salt (pH 7.5) 0.1M
B12	82237	NH_4-sulfate 1.5M, TRIS-HCl (pH 8.5) 0.1M, Glycerol 12%
C1	77104	NH_4-dihydrogenphosphate 0.4M
C2	78993	Na-citrate (pH 5.6) 0.1M, NH_4-dihyrogenphosphate 1.0M
C3	88512	NH_4-acetate 0.2M, TRIS-HCl (pH 8.5) 0.1M, 2-Propanol 30%
C4	92465	Na-citrate 0.2M, HEPES Na-salt (pH 7.5) 0.1M, 2-Propanol 20%
C5	80526	TRIS-HCl (pH 8.5) 0.1M, PEG 8000 8%
C6	73934	Mg-formiate 0.2M
C7	72526	Ethylene glycol 25%
C8	96346	Co-chloride 0.01M, Na-acetate(pH 4.6) 0.1M, 1,6-Hexanediol 1M
C9	80557	Na-citrate (pH 5.6) 0.1M, 1,6-Hexanediol 2.5M
C10	74616	Zn-sulfate 0.01M, MES (pH 6.5) 0.1M, PEG MME 550 25%
C11	86953	HEPES Na-salt (pH 7.5) 0.1M, 2-Methyl-2,4-pentanediol 70%
C12	88438	TRIS-HCl (pH 8.5) 0.1M, Ethanol 20%
D1	84653	TRIS-HCl (pH 8.5) 0.1M, NH_4-sulfate 2.0M
D2	73682	Mg-chloride 0.2M, HEPES Na-salt (pH 7.5) 0.1M, 2-Propanol 30%
D3	82406	NH_4-sulfate 0.2M, Na-acetate (pH 4.6) 0.1M, PEG 4000 25%
D4	96345	Na-acetate 0.2M, Na-cacodylate pH (6.5) 0.1M, PEG 8000 30%
D5	77436	Na-acetate (pH 4.6) 0.1M, PEG 4000 8%
D6	82409	Zn-acetate 0.2M, Na-cacodylate (pH 6.5) 0.1M, PEG 8000 18%
D7	75691	Dioxane 35%
D8	95958	Cd-chloride 0.1M, Na-acetate (pH 4.6) 0.1M, PEG 400 30%
D9	80094	Mg-sulfate 1.6M, MES (pH 6.5) 0.1M
D10	73894	Na-citrate (pH 6.5) 1.6M
D11	82238	Na-chloride 4.3M, HEPES Na-salt (pH 7.5) 0.1M
D12	88439	Ni(II)-chloride 0.01M, TRIS-HCl (pH 8.5) 0.1M, PEG MME 2000 20%
E1	77103	Na-citrate 0.2M, HEPES Na-salt (pH 7.5) 0.1M, 2-Methyl-2,4-pentanediol 30%
E2	76018	Na-citrate 0.2M, TRIS-HCl (pH 8.5) 0.1M, PEG 400 30%
E3	87924	Mg-acetate 0.2M, Na-cacodylate (pH 6.5) 0.1M, 2-Methyl-2,4-pentanediol 30%
E4	93268	HEPES Na-salt (pH 7.5) 0.1M, K-,Na-tartrate 0.8M
E5	73931	HEPES Na-salt (pH 7.5) 0.1M, Na-citrate 1.4M
E6	91113	Ca-acetate 0.2M, Na-cacodylate (pH 6.5) 0.1M, PEG 8000 18%
E7	70293	NH_4-sulfate 2M, 2-Propanol 5%
E8	92913	NH_4-sulfate 0.2M, Na-acetate (pH 4.6) 0.1M, PEG MME 2000 30%
E9	77583	Na-chloride 2M, Ka-,Na- dihydrogenphosphate each 0.1M, MES (pH 6.5) 0.1M
E10	80386	NH_4-sulfate 0.5M, HEPES Na-salt (pH 7.5) 0.1M, 2-Methyl-2,4-pentanediol 30%
E11	90196	HEPES Na-salt (pH 7.5) 0.1M, Ethylene glycol 8%, PEG 8000 10%
E12	91229	Na-chloride 0.1M, Bicine (pH 9.0) 0.1M, PEG MME 550 20%
F1	78760	Mg-chloride 0.2M, TRIS-HCl (pH 8.5) 0.1M, PEG 4000 30%
F2	79052	Ca-chloride 0.2M, HEPES Na-salt (pH 7.5) 0.1M, PEG 400 28%
F3	88543	Na-acetate 0.2M, TRIS-HCl (pH 8.5) 0.1M, PEG 4000 30%
F4	71907	NH_4-sulfate 0.2M, PEG 8000 30%
F5	80346	HEPES Na-salt (pH 7.5) 0.1M, PEG 400 2%, NH_4-sulfate 2.0M
F6	91633	Na-acetate (pH 4.6) 0.1M, NH_4-sulfate 2.0M
F7	95514	Imidazole (pH 7.0) 1M
F8	96765	K-,Na-tartrate 0.2M, NH_4-sulfate 2M, Na-citrate (pH 5.6) 0.1M
F9	79975	MES (pH 6.5) 0.1M, PEG 20'000 12%
F10	80060	HEPES Na-salt (pH 7.5) 0.1M, 2-Methyl-2,4-pentanediol 5%, PEG 6000 10%
F11	82217	HEPES Na-salt (pH 7.5) 0.1M, PEG 10'000 20%

F12	95633	Mg-chloride 2M, Bicine (pH 9.0) 0.1M
G1	70114	Na-cacodylate (pH 6.5) 0.1M, Na-acetate 1.4M
G2	86686	NH_4-sulfate 0.2M, Na-cacodylate (pH 6.5) 0.1M, PEG 8000 30%
G3	73642	Mg-chloride 0.2M, HEPES Na-salt (pH 7.5) 0.1M, PEG 400 30%
G4	80677	NH_4-sulfate 0.2M, PEG 4000 30%
G5	73933	Na-citrate (pH 5.6) 0.1M, 2-Propanol 20%, PEG 4000 20%
G6	91114	Li-sulfate 1.0M, PEG 8000 2%
G7	93593	PEG 1000 10%, PEG 8000 10%
G8	92347	Li-sulfate 1M, NH_4-sulfate 0.5M, Na-citrate (pH 5.6) 0.1M
G9	80095	NH_4-sulfate 1.6M, MES (pH 6.5) 0.1M, Dioxane 10%
G10	79976	HEPES Na-salt (pH 7.5) 0.1M, Jeffamine M-600 20%
G11	82208	Mg-chloride 0.2M, TRIS-HCl (pH 8.5) 0.1M, 1,6-Hexanediol 3.4M
G12	80106	Bicine (pH 9.0) 0.1M, Dioxane 2%, PEG 20'000 10%
H1	85887	Na-citrate 0.2M, Na-cacodylate (pH 6.5) 0.1M, 2-Propanol 30%
H2	86445	HEPES Na-salt (pH 7.5) 0.1M, Li-sulfate 1.5M
H3	92644	Ca-chloride 0.2M, Na-acetate (pH 4.6) 0.1M, 2-Propanol 20%
H4	76399	NH_4-sulfate 2M
H5	80565	HEPES Na-salt (pH 7.5) 0.1M, 2-Propanol 10%, PEG 4000 20%
H6	88862	Li-sulfate 0.5M, PEG 8000 15%
H7	92982	Na-chloride 1.5M, Ethanol 10%
H8	94832	Na-chloride 0.5M, Na-citrate (pH 5.6) 0.1M, Polyethylenimin 2%
H9	72873	Cs-chloride 0.05M, MES (pH 6.5) 0.1M, Jeffamine M-600 30%
H10	79687	NH_4-sulfate 1.6M, Na-chloride 0.1M, HEPES Na-salt (pH 7.5) 0.1M
H11	86261	TRIS-HCl (pH 8.5) 0.1M, tert.-Butanol 25%
H12	79924	Mg-chloride 0.1 M, TRIS-HCl (pH 8.5) 0.1M, PEG 20000 15%

56783 Crystallization Kit for Proteins – Observation Sheet

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

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

	precipitate without birefringent nd edges	**precipitates shows birefringent or has edges**
1 drop is clear	3 mostly clear drop	7 sperulites or small structures maybe edges
2 drop contains non-protein particles	4 fully precipitated dark colour	8 crystal grown 1 D
	5 gelatinous protein precipitate	9 crystal grown 2 D
	6 phase separation	10 crystal grown 3 D

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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.