Crystallization Basic Kit for Proteins

ProductInformation

Product Code **82009**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

The Crystallization Basic Kit for Proteins is a rapid screening method to determine the best conditions for the crystallization of biomolecules. The conditions screened in this kit are based upon the original protocol of Jancarik and Kim.[1] Their sparse matrix sampling procedure is based on known crystallization conditions for proteins. Using a small volume of sample, the solutions in this kit provide for testing conditions over a wide range of pH with variations in buffer, salt, and precipitant.

Screening with the reagents in this kit will result in either crystal formation or solubility information of the target molecule. Information from crystallization will help optimize conditions to prepare crystals for analysis. Solubility information, obtained when crystals do not form, is useful for determining conditions for further screening.

The reagents and solutions in this kit are formulated using high purity reagents (primarily Microselect grade) and ultrapure water. Over the years Microselect grade chemicals have been successfully used for different crystallization methods.

All solutions have been filtered through 0.22 µm filters.

All solutions are available separately as 100 ml bottles and 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.

Sample Preparation Instructions

The protein sample should be as homogenous as possible with a purity of greater than 95%. Any amorphous material should be removed by centrifugation or filtration prior to use.[2,3,4]

A sample concentration of 5 to 25 mg/ml in water is recommended. For best determination of crystallization conditions, dialyze the sample against ultrapure water. However, during sample preparation, ligands, reducing agents, ions, or other additives may be required to aid solubility, stability, or activity of the target molecule. Note: Phosphate, borate, or carbonate buffers should be used at concentrations of 10 mM or less. These buffers can give false positives by the formation of inorganic crystals with reagents containing divalent cations such as magnesium, calcium, or zinc. It is also possible to exchange the phosphate, borate, or carbonate buffers with a more suitable buffer, which does not complex with divalent cations.

Storage/Stability

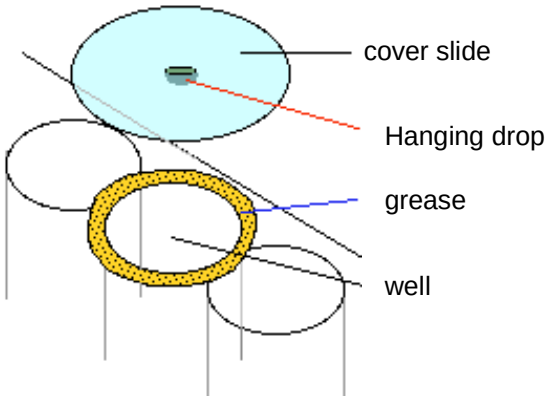
It is recommended that the reagents of this kit be stored at 2–8 °C. Storage at –20 °C will not adversely affect the kit reagents and the reagents as supplied are stable at room temperature for short-term storage.

Procedure

The hanging drop vapor diffusion method is the most frequently used procedure for crystallization. The use of the Crystallization Basic Kit for Proteins in this procedure is described in this section. Procedures for these other crystallization methods (sitting drop, sandwich drop, microbatch, and microdialysis) are available from Fluka Technical Service.

1. Prepare a 24 well plate for the hanging drop vapor diffusion method. It is recommended to put a thin film of grease on the upper edge of each of the 24 wells (See Figure 1). This prevents movement of the cover slide when it is placed on top of the well. Fifty wells should be prepared for the complete Crystallization Basic Kit for Proteins.

Figure 1.
Preparation of wells for the Hanging Drop Vapor Diffusion method



2. Add 800 µl of Reagent 1 from the Crystallization Basic Kit for Proteins into the first well using a clean pipette tip. Repeat this step for each of the remaining reagents. Avoid contamination by using a clean pipette tip for each reagent.

3. Pipette 2 µl of the protein sample onto the center of a clean, siliconized cover slide. Either a circular or square cover slide may be used (See Figure 1).

4. Pipette 2 µl of Reagent 1 from the first well into the sample droplet. Mix the droplet by pipetting up and down. Take care to avoid foaming by keeping the pipette tip in the drop during mixing.

5. Invert the cover slide and droplet over the first well containing Reagent 1 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 the remaining 49 reagents contained in this kit.

7. Temperature is a parameter which may affect crystallization. Each screen in this kit can be performed in parallel at 4 °C and at room temperature, if the protein sample volume permits. The plates should be stored in a place with stable temperature and free of vibration.

8. Examination of Droplets
   Immediately after the screen is set up, examine the droplets under a stereomicroscope at 10 to 100x magnification. Scan the focal plane for small crystals and record observations for all droplets. For the first week, information should 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:
   (8= single crystal), small tetrahedral
   (5=Possetes of Sperulites), red/brown
   (1=clear drop), green
   (6= needle shaped crystals) in a fine yellow precipitate.
   It is useful to record the largest crystal size.

Results
A clear drop may be an indication that the drop has not yet reached complete equilibration. If the drop remains clear after 3 to 4 weeks, the relative sample and reagent supersaturation may be too low. One may repeat the screen using a protein sample at twice the initial concentration. If two thirds of the drops remain clear, consider repeating the entire screen using a 2-fold concentrated protein sample.

There are several reasons for precipitation in a drop. A precipitate can indicate that the relative sample and reagent supersaturation is too high. In this case reduce the protein sample concentration and repeat the screen. If two thirds of the drops contain a precipitate with no crystals present, consider a 2-fold dilution of the sample and repeating the entire screen.
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 a substrate or other ligand, a reducing agent, salts, glycerol, or other appropriate stabilizing reagents. 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. Do not discard or ignore a drop containing a precipitate as it is possible for crystals to form from a precipitate. If possible, 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 preliminary information 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 stabilizing additives.

Sample solubility is temperature dependent. Comparison of results of a screen at two different temperatures (4 °C and room temperature) helps determine the magnitude of temperature effects on sample solubility. Temperature may be an important variable in subsequent screens and optimization experiments.

Crystals can grow extremely fast (in 15 minutes) or may require much more time (up to 1 year). Store and record the plates until the drops are dried out.

References

1. Jancarik, J., and Kim, S.H., Sparse Matrix Sampling: a screening method for crystallization of proteins. J. Appl. Oryst., **24**, 409-411 (1991).
2. Crystallization of Nucleic Acids and Proteins: A Practical Approach, Ducruix, A., and Giege, R., eds., Oxford University Press (Oxford, UK: 1992).
3. McPherson, A., Current approaches to macromolecular crystallization. Eur. J. Biochem., **189**,1-23, (1990).
4. Protein and Nucleic Acid Crystallization Methods, A Companion to Methods in Enzymology, Academic Press, **1**, (1990).

NP/MAM 2/02

Kit Reagents and Solutions

Reagent Number	Product Code	Product Name
1	89754	0.02 M calcium chloride, 0.1 M sodium acetate, pH 4.6, 30% 2-methyl-2,4-pentanediol
2	78016	0.4 M potassium/sodium tartrate
3	77104	0.4 M $NH_4H_2PO_4$
4	84653	0.1 M Tris-HCl, pH 8.5, 2.0 M ammonium sulfate
5	77103	0.2 M sodium citrate, 0.1 M HEPES sodium salt, pH 7.5, 30% 2-methyl-2,4-pentanediol
6	78760	0.2 M magnesium chloride, 0.1 M Tris-HCl, pH 8.5, 30% PEG 4000
7	70114	0.1 M sodium cacodylate, pH 6.5, 1.4 M sodium acetate
8	85887	0.2 M sodium citrate, 0.1 M sodium cacodylate, pH 6.5, 30% 2-propanol
9	73374	0.2 M ammonium acetate, 0.1 M sodium citrate, pH 5.6, 30% PEG 4000
10	76028	0.2 M ammonium acetate, 0.1 M sodium acetate, pH 4.6, 30% PEG 4000
11	78993	0.1 M sodium citrate, pH 5.6, 1.0 M $NH_4H_2PO_4$
12	73682	0.2 M magnesium chloride, 0.1 M HEPES sodium salt, pH 7.5, 30% 2-propanol
13	76018	0.2 M sodium citrate, 0.1 M Tris-HCl, pH 8.5, 30% PEG 400
14	79052	0.2 M calcium chloride, 0.1 M HEPES sodium salt, pH 7.5, 28% PEG 400
15	86686	0.2 M ammonium sulfate, 0.1 M sodium cacodylate, pH 6.5, 30% PEG 8000
16	86445	0.1 M HEPES sodium salt, pH 7.5, 1.5 M lithium sulfate
17	89786	0.2 M lithium sulfate, 0.1 M Tris-HCl, pH 8.5, 30% PEG 4000
18	88518	0.2 M magnesium acetate, 0.1 M sodium cacodylate, pH 6.5, 20% PEG 8000
19	88512	0.2 M ammonium acetate, 0.1 M Tris-HCl, pH 8.5, 30% 2-propanol
20	82406	0.2 M ammonium sulfate, 0.1 M sodium acetate, pH 4.6, 25% PEG 4000
21	87924	0.2 M magnesium acetate, 0.1 M sodium cacodylate, pH 6.5, 30% 2-methyl-2,4-pentanediol
22	88543	0.2 M sodium acetate, 0.1 M Tris-HCl, pH 8.5, 30% PEG 4000
23	73642	0.2 M magnesium chloride, 0.1 M HEPES sodium salt, pH 7.5, 30% PEG 400
24	92644	0.2 M calcium chloride, 0.1 M sodium acetate, pH 4.6, 20% 2-propanol
25	70377	0.1 M imidazole, pH 6.5, 1 M sodium acetate

Kit Reagents and Solutions (Continued)

Reagent Number	Product Code	Product Name
26	88530	0.2 M ammonium acetate, 0.1 M sodium citrate, pH 5.6, 30% 2-methyl-2,4-pentanediol
27	92465	0.2 M sodium citrate, 0.1 M HEPES sodium salt, pH 7.5, 20% 2-propanol
28	96345	0.2 M sodium acetate, 0.1 M sodium cacodylate, pH 6.5, 30% PEG 8000
29	93268	0.1 M HEPES sodium salt, pH 7.5, 0.8 M potassium/sodium tartrate
30	71907	0.2 M ammonium sulfate, 30% PEG 8000
31	80677	0.2 M ammonium sulfate, 30% PEG 4000
32	76399	2 M ammonium sulfate
33	73932	4 M sodium formate
34	76728	0.1 M sodium acetate, pH 4.6,2 M sodium formate
35	71835	0.1 M HEPES sodium salt, pH 7.5, 0.8 M KH_2PO_4, 0.8 M NaH_2PO_4
36	80526	0.1 M Tris-HCl, pH 8.5, 8% PEG 8000
37	77436	0.1 M sodium acetate, pH 4.6, 8% PEG 4000
38	73931	0.1 M HEPES sodium salt, pH 7.5, 1.4 M sodium citrate
39	80346	0.1 M HEPES sodium salt, pH 7.5, 2% PEG 400, 2.0 M ammonium sulfate
40	73933	0.1 M sodium citrate, pH 5.6, 20% 2-propanol, 20% PEG 4000
41	80565	0.1 M HEPES sodium salt, pH 7.5, 10% 2-propanol, 20% PEG 4000
42	80806	0.05 M KH_2PO_4, 20% PEG 8000
43	78225	30% PEG 1500
44	73934	0.2 M magnesium formate
45	82409	0.2 M zinc acetate, 0.1 M sodium cacodylate, pH 6.5, 18% PEG 8000
46	91113	0.2 M calcium acetate, 0.1 M sodium cacodylate, pH 6.5, 18% PEG 8000
47	91633	0.1 M sodium acetate, pH 4.6, 2.0 M ammonium sulfate
48	85934	0.1 M Tris-HCl, pH 8.5, 2.0 M $NH_4H_2PO_4$
49	91114	1.0 M lithium sulfate, 2% PEG 8000
50	88862	1.0 M lithium sulfate, 15% PEG 8000