3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone (800) 325-5832 (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Crystallization Cryo Kit for Proteins

Product Code **75403**
Storage Temperature 2-8 °C

TECHNICAL BULLETIN

Product Description

The Crystallization Cryo Kit for Proteins is a rapid empirical screening method to determine the best crystallization conditions of biological macromolecules such as proteins in the presence of a cryoprotectant. The Crystallization Cryo Kit for Proteins also provides protein solubility information in a wide range of precipitants and over a wide range of pH.

The Crystallization Cryo Kit for Proteins allows for modification of the crystallization conditions based upon the original screening method of Jancarik and Kim.[1] The kit differs from the original method by having a cryoprotectant present in the screening reagents. Glycerol is most commonly used as a cryoprotectant. Low temperature minimizes radiation damage to the protein crystal during crystallographic data collection. The crystals are frozen in liquid nitrogen and maintained at –173 °C under a stream of nitrogen. To prevent ice crystals from forming during the freezing process, it is necessary to have a cryoprotectant present. One method of introducing the cryoprotectant is in the reagent used to crystallize the protein. This kit contains reagents and solutions formulated to form an amorphous glass upon cooling, the drop containing the protein crystal and mother liquor will remain clear and no “ice rings” appear in the diffraction pattern. The Crystallization Cryo Kit for Proteins offers as large a range of additive and precipitant variables, buffer, and pH as possible, while using small amounts of proteins.

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

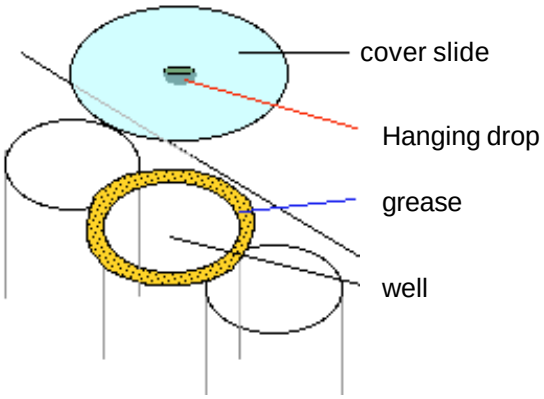
It is recommended that the reagents of this kit be stored at 2–8 °C. Storage at –20 °C will not adversely affect the kit reagents and the reagents as supplied are stable at room temperature for short-term storage.

Procedure

The hanging drop vapor diffusion method is the most frequently used procedure for crystallization. The use of the Crystallization Cryo 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 Cryo 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 Cryo 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.

The ideal cyroprotected crystallization reagent will form an amorphous glass at –173 °C, without ice damage to the crystal or observed "ice bands". If the crystal cracks or the drop has a milky appearance upon freezing, try a higher concentration of cryoprotectant in the drop. Alternatively, adjust the concentration of the screen reagent components.

For problematic crystallizations, one may have to evaluate different crystallization reagents and test other cryoprotectants. The following small molecules are also good cryoprotectants:
glycerol (Product Code 49769)
ethylene glycol (Product Code 03747)
erythriol (Product Code 45670)
glucose (Product Code 49140)
2R,3R-(-)-butane-2,3-diol (Product Code 18965)
PEG-400 (Product Code 81170)
2-methyl-2,4-pentandiol (MPD, Product Code 68338)
sucrose (Product Code 84099)
These cryoprotectants can be tested by placing in a cyrostream a frozen loop containing the final cryoprotectant solution alone and observing for the formation of ice crystals.

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).
5. Garman, E.F., and Mitchell, E.P., J. Appl. Cryst., **29**, 584-587 (1996).

NP/MAM 2/02

Kit Reagents and Solutions

Reagent Number	Product Code	Product Name
1	92599	0.02 M calcium chloride, 0.1 M sodium acetate, pH 4.6, 30% 2-methyl-2,4-pentanediol
2	96864	0.26 M potassium/sodium tartrate, 35% glycerol
3	96347	0.26 M $NH_4H_2PO_4$, 35% glycerol
4	94030	1.5 M ammonium sulfate, 0.1 M Tris-HCl, pH 8.5, 25% glycerol
5	93949	0.2 M sodium citrate, 0.1 M HEPES sodium salt, pH 7.5, 30% 2-methyl-2,4-pentanediol
6	93189	0.16 M magnesium chloride, 0.1 M Tris-HCl, pH 8.5, 20% glycerol, 24% PEG 4000
7	79973	0.98 M sodium acetate, 0.07 M sodium cacodylate, pH 6.5, 30% glycerol
8	74614	0.14 M sodium citrate, 0.07 M sodium cacodylate, pH 6.5, 21% 2-propanol, 30% glycerol
9	77437	0.17 M ammonium acetate, 0.085 M sodium citrate, pH 5.6, 15% glycerol, 25.5% PEG 4000
10	77582	0.17 M ammonium acetate, 0.085 M sodium acetate, pH 4.6, 15% glycerol, 25.5% PEG 4000

Kit Reagents and Solutions (Continued)

Reagent Number	Product Code	Product Name
11	72871	0.7 M $NH_4H_2PO_4$, 0.07 M sodium citrate, pH 5.6, 30% glycerol
12	72872	0.18 M magnesium chloride, 0.09 M HEPES sodium salt, pH 7.5, 27% 2-propanol, 10% glycerol
13	80055	0.2 M sodium citrate, 0.1 M Tris-HCl, pH 8.5, 10% glycerol, 30% PEG 400
14	80556	0.19 M calcium chloride, 0.095 M HEPES sodium salt, pH 7.5, 5% glycerol, 26.6% PEG 400
15	80074	0.17 M ammonium sulfate, 0.085 M sodium cacodylate, pH 6.5, 15% glycerol, 25.5% PEG 8000
16	80084	1.125 M lithium sulfate, 0.075M HEPES sodium salt, pH 7.5, 25% glycerol
17	80091	0.17 M lithium sulfate, 0.085 M TrisHCl, pH 8.5, 15% glycerol, 25.5% PEG 4000
18	80093	0.16 M magnesium acetate, 0.08 M sodium cacodylate, pH 6.5, 20% glycerol, 16% PEG 8000
19	74613	0.16 M ammonium acetate, 0.08 M Tris-HCl, pH 8.5, 24% 2-propanol, 20% glycerol
20	80053	0.16 M ammonium sulfate, 0.08 M sodium acetate, pH 4.6, 20% glycerol, 20% PEG 4000
21	79974	0.2 M magnesium acetate, 0.1 M sodium cacodylate, pH 6.5, 30% 2-methyl-2,4-pentanediol
22	73803	0.17 M sodium acetate, 0.085 M Tris-HCl, pH 8.5, 15% glycerol, 25.5% PEG 4000
23	76525	0.2 M magnesium chloride, 0.1 M HEPES sodium salt, pH 7.5, 30% PEG 400
24	73806	0.14 M calcium chloride, 0.07 M sodium acetate, pH 4.6, 14% 2-propanol, 30% glycerol
25	71591	0.7 M sodium acetate, 0.07 M imidazole, pH 6.5, 30% glycerol
26	76658	0.2 M ammonium acetate, 0.1 M sodium citrate, pH 5.6, 30% 2-methyl-2,4-pentanediol
27	80384	0.14 M sodium citrate, 0.07 M HEPES sodium salt, pH 7.5, 14% 2-propanol, 30% glycerol
28	80387	0.17 M sodium acetate, 0.085 M sodium cacodylate, pH 6.5, 15% glycerol, 25.5% PEG 8000
29	76659	0.52 M potassium/sodium tartrate, 0.65 M HEPES sodium salt, pH 7.5, 35% glycerol
30	80803	0.17 M ammonium sulfate, 15% glycerol, 25.5% PEG 8000
31	87429	0.17 M ammonium sulfate, 15% glycerol, 25.5% PEG 4000
32	89252	1.5 M ammonium sulfate, 25% glycerol
33	81892	3.6 M sodium formate, 10% glycerol
34	81893	1.4 M sodium formate, 0.07 M sodium acetate, pH 4.6, 30% glycerol
35	80924	0.6 M NaH_2PO_4, 0.6 M KH_2PO_4, 0.075 M HEPES sodium salt, pH 7.5, 25% glycerol
36	89725	0.065 M Tris-HCl, pH 8.5, 35% glycerol, 5.2% PEG 8000
37	82216	0.07 M sodium acetate, pH 4.6, 35% glycerol, 5.6% PEG 4000
38	89734	1.26 M sodium citrate, 0.09 M HEPES sodium salt, pH 7.5, 10% glycerol
39	80864	1.7 M ammonium sulfate, 0.085 M HEPES sodium salt, pH 7.5, 15% glycerol, 1.7% PEG 400
40	91959	0.095 M sodium citrate, pH 5.6, 19% 2-propanol, 5% glycerol, 19% PEG 4000
41	82809	0.085 M HEPES sodium salt, pH 7.5, 8.5% 2-propanol, 15% glycerol, 17% PEG 4000
42	83617	0.04 M KH_2PO_4, 20% glycerol, 16% PEG 8000
43	90213	20% Glycerol, 24% PEG 1500
44	91638	0.1 M magnesium formate, 50% glycerol
45	83198	0.16 M zinc acetate, 0.08 M sodium cacodylate, pH 6.5, 20% glycerol, 14.4% PEG 8000
46	84461	0.16 M calcium acetate, 0.08 M sodium cacodylate, pH 6.5, 20% glycerol, 14.4% PEG 8000
47	83196	1.6 M ammonium sulfate, 0.08 M sodium acetate, pH 4.6, 20% glycerol
48	84796	1.6 M $NH_4H_2PO_4$, 0.08 M Tris-HCl, pH 8.5, 20% glycerol
49	81696	0.8 M lithium sulfate, 20% glycerol, 1.6% PEG 8000
50	81713	0.4 M lithium sulfate, 20% glycerol, 12% PEG 8000