



Crystallization PEG Grid Screening Kit

Product Number **36436**

Store at 2-8 °C

TECHNICAL BULLETIN

Application

The Crystallization PEG Grid Screening Kit for Proteins is a screening method for the determination of initial crystallization conditions for biological macromolecules like proteins.

The kit displays a range of conditions based on PEG 600 or PEG 6000 as precipitants and covers various buffers with pH values from pH 4.0 to pH 9.0. Whereas PEG is the most widely used type of precipitant, the size and concentration of PEG is critical for crystallization. On the other hand, pH is one of the major determinants for crystallization. The use of Crystallization PEG Grid Screening Kit enables determination of suitable conditions regarding these two crucial aspects. Further optimization may include the use of different types and concentrations of various salts.

Quality of reagents – the key to success

Crystallization Basic Kit for Proteins reagents are formulated using high purity reagents (mostly Biochemika ultra/MicroSelect from Fluka). 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 successfully been used 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 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 >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 interfering ions, reducing agents, ligands or other additives are present, they may be removed by dialysis against water. 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-} .
Examples 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 Basic 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 Fluka Technical Service.

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 below.

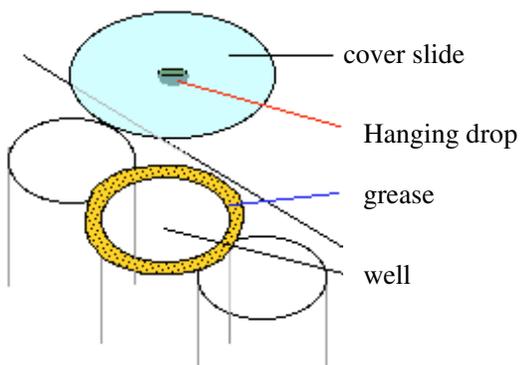


figure 1: well plate

2. Pipet 800 μl of each reagent into the fifty wells. *Use always a clean pipet tip for each reagent!*
3. 2 μl 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 is also 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 a majority of drops contain a precipitate with no crystals present, consider diluting 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 agents, 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 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. Cryst.*, 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)

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36436 Crystallization PEG Grid Screening Kit Observation Sheet

Sample description: _____ Date: _____
 concentration: _____ Incubation Temperature: _____
 buffer: _____ Reservoir Volume: _____
 1 Drop contains : Crystallization Reagent _____ ul Sample _____ ul Additive (name) _____ (volume) _____ ul

	precipitate without birefringent and edges	precipitates shows birefringent or has edges
1 drop is clear	3 mostly clear drop	7 spherulites 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

No.	Fluka No.	Reagent composition	Date:	Date:	Date:	Date:	Date:
1.	07196	0.1 M Citrate pH 4.0, 5% PEG 600					
2.	43830	0.1 M Citrate pH 4.0, 10% PEG 600					
3.	43288	0.1 M Citrate pH 4.0, 20% PEG 600					
4.	50546	0.1 M Citrate pH 4.0, 30% PEG 600					
5.	51255	0.1 M Citrate pH 5.0, 5% PEG 600					
6.	13195	0.1 M Citrate pH 5.0, 10% PEG 600					
7.	51422	0.1 M Citrate pH 5.0, 20% PEG 600					
8.	52218	0.1 M Citrate pH 5.0, 30% PEG 600					
9.	61798	0.1 M MES pH 6.0, 5% PEG 600					
10.	68591	0.1 M MES pH 6.0, 10% PEG 600					
11.	69045	0.1 M MES pH 6.0, 20% PEG 600					
12.	43627	0.1 M MES pH 6.0, 30% PEG 600					
13.	51768	0.1 M HEPES pH 7.0, 5% PEG 600					
14.	44232	0.1 M HEPES pH 7.0, 10% PEG 600					
15.	68128	0.1 M HEPES pH 7.0, 20% PEG 600					
16.	18268	0.1 M HEPES pH 7.0, 30% PEG 600					
17.	43529	0.1 M TRIS-HCl pH 8.0, 5% PEG 600					
18.	07066	0.1 M TRIS-HCl pH 8.0, 10% PEG 600					
19.	28531	0.1 M TRIS-HCl pH 8.0, 20% PEG 600					
20.	11075	0.1 M TRIS-HCl pH 8.0, 30% PEG 600					
21.	11084	0.1M BICINE pH 9.0, 5% PEG 600					
22.	00957	0.1M BICINE pH 9.0, 10% PEG 600					
23.	28603	0.1M BICINE pH 9.0, 20% PEG 600					
24.	14844	0.1M BICINE pH 9.0, 30% PEG 600					
25.	44389	0.1 M Citrate pH 4.0, 5% PEG 6000					
26.	44071	0.1 M Citrate pH 4.0, 10% PEG 6000					
27.	19053	0.1 M Citrate pH 4.0, 20% PEG 6000					
28.	39297	0.1 M Citrate pH 4.0, 30% PEG 6000					
29.	68609	0.1 M Citrate pH 5.0, 5% PEG 6000					
30.	05751	0.1 M Citrate pH 5.0, 10% PEG 6000					
31.	53803	0.1 M Citrate pH 5.0, 20% PEG 6000					
32.	52182	0.1 M Citrate pH 5.0, 30% PEG 6000					
33.	61969	0.1 M MES pH 6.0, 5% PEG 6000					
34.	52296	0.1 M MES pH 6.0, 10% PEG 6000					
35.	05162	0.1 M MES pH 6.0, 20% PEG 6000					
36.	07206	0.1 M MES pH 6.0, 30% PEG 6000					
37.	49339	0.1 M HEPES pH 7.0, 5% PEG 6000					
38.	49329	0.1 M HEPES pH 7.0, 10% PEG 6000					
39.	13169	0.1 M HEPES pH 7.0, 20% PEG 6000					
40.	41494	0.1 M HEPES pH 7.0, 30% PEG 6000					
41.	01879	0.1 M TRIS-HCl pH 8.0, 5% PEG 6000					
42.	61952	0.1 M TRIS-HCl pH 8.0, 10% PEG 6000					
43.	38731	0.1 M TRIS-HCl pH 8.0, 20% PEG 6000					
44.	53770	0.1 M TRIS-HCl pH 8.0, 30% PEG 6000					
45.	51815	0.1M BICINE pH 9.0, 5% PEG 6000					
46.	49395	0.1M BICINE pH 9.0, 10% PEG 6000					
47.	55434	0.1M BICINE pH 9.0, 20% PEG 6000					
48.	38864	0.1M BICINE pH 9.0, 30% PEG 6000					