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

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ProductInformation

Crystallization Low Ionic Kit for Proteins

Product Code **86684**
Storage Temperature 2-8 °C

TECHNICAL BULLETIN

Product Description

The crystallization conditions of the Crystallization Low Ionic Kit for Proteins are based upon the screening protocol of L. J. Harris, et al.[1] This protocol is an effective screen for determining the preliminary crystallization conditions of intact monoclonal antibodies. It is also possible to determine the crystallization conditions for monoclonal antibody fragments, as well as other soluble proteins. This low ionic strength crystallization screen for proteins may be effective in determining crystallization conditions as low ionic strength has not been routinely explored. The examples of salting-in reported in the literature concern protein solubility data near the isoelectric point (pI) of the protein.

In this kit, the variables for the crystallization conditions are the concentration of PEG 3350 (between 4 and 28%), a broad range of pH (from 3 to 10), and temperature (between 4 and 37 °C). At low ionic strength, pH and temperature have a greater effect on protein solubility. It is recommended to screen at several temperatures between 4 and 37 °C to determine the temperature effect on sample solubility at low ionic strength. Some advanced users of this kit may extend the pH range limits to 2 and 12. In addition, other concentrations of PEG 3350 are often tested. It is recommended to use Microselect grade products when preparing solutions for this additional testing.

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 highly purified and filtered through a 0.22 or 0.45 μm filter. A sample concentration of 3 to 5 mg/ml for an intact antibody or 10 to 20 mg/ml for other proteins in ultrapure 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

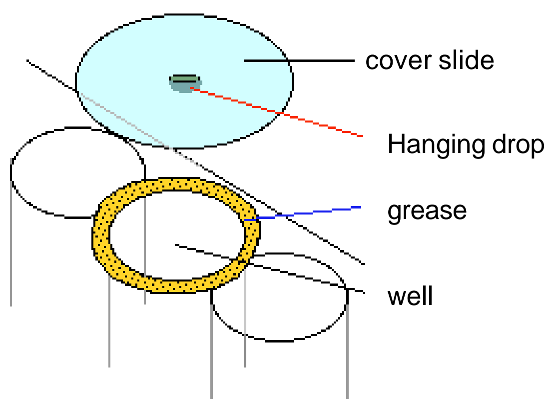
It is recommended that the reagents of this kit be stored at 2–8 °C. Storage at –20 °C will not adversely affect the kit reagents and the reagents as supplied are stable at room temperature for short-term storage.

Procedure

The hanging drop vapor diffusion method is the most frequently used procedure for crystallization. The use of the Crystallization Low Ionic 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 Low Ionic 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 Low Ionic 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 drops remain clear, leave the screen for a few weeks, but continue to observe the samples. Increasing the sample concentration may optimize the crystallization conditions. In the absence of visible crystals, inspect any drops with precipitate for microcrystallinity. 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 polarization. It may be possible to use streak seeding to produce larger crystals from microcrystalline precipitates. If a precipitate is present, consider repeating the screen with changes in the sample concentration, the precipitant concentration (up to 50%), or the temperature. If small crystals, not suitable for X-ray diffraction, are grown it may be possible to use seeding techniques to grow larger crystals.

Sample solubility is temperature dependent. Comparison of results of screens at different temperatures between 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. Harris, et al., Proteins: Structure, Function, and Genetics, **23**, 285-289 (1995).
2. Jancarik, J., and Kim, S.H., Sparse Matrix Sampling: a screening method for crystallization of proteins. J. Appl. Oryst., **24**, 409-411 (1991).
3. Crystallization of Nucleic Acids and Proteins: A Practical Approach, Ducruix, A., and Giege, R., eds., Oxford University Press (Oxford, UK: 1992).
4. McPherson, A., Current approaches to macromolecular crystallization. Eur. J. Biochem., **189**,1-23, (1990).
5. Protein and Nucleic Acid Crystallization Methods, A Companion to Methods in Enzymology, Academic Press, **1**, (1990).
6. Garman, E.F., and Mitchell, E.P., J. Appl. Cryst., **29**, 584-587 (1996).

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Kit Reagents and Solutions

Reagent Number	Product Code	Product Name
1	83171	0.05 M sodium citrate, pH 3.0, 4% PEG 3350
2	95988	0.05 M sodium citrate, pH 3.0, 12% PEG 3350
3	96376	0.05 M sodium citrate, pH 3.0, 20% PEG 3350
4	95918	0.05 M sodium citrate, pH 3.0, 28% PEG 3350
5	94191	0.05 M sodium citrate, pH 4.0, 4% PEG 3350
6	96711	0.05 M sodium citrate, pH 4.0, 12% PEG 3350
7	75402	0.05 M sodium citrate, pH 4.0, 20% PEG 3350
8	78667	0.05 M sodium citrate, pH 4.0, 28% PEG 3350
9	80654	0.05 M sodium citrate, pH 4.5, 4% PEG 3350
10	71586	0.05 M sodium citrate, pH 4.5, 12% PEG 3350
11	80089	0.05 M sodium citrate, pH 4.5, 20% PEG 3350
12	80552	0.05 M sodium citrate, pH 4.5, 28% PEG 3350
13	73291	0.05 M sodium citrate, pH 5.0, 4% PEG 3350
14	78067	0.05 M sodium citrate, pH 5.0, 12% PEG 3350
15	80749	0.05 M sodium citrate, pH 5.0, 20% PEG 3350
16	77657	0.05 M sodium citrate, pH 5.0, 28% PEG 3350
17	77652	0.05 M sodium citrate, pH 5.5, 4% PEG 3350
18	80538	0.05 M sodium citrate, pH 5.5, 12% PEG 3350
19	87007	0.05 M sodium citrate, pH 5.5, 20% PEG 3350
20	91788	0.05 M sodium citrate, pH 5.5, 28% PEG 3350
21	86309	0.05 M MES sodium salt, pH 6.0, 4% PEG 3350
22	86287	0.05 M MES sodium salt, pH 6.0, 12% PEG 3350
23	89781	0.05 M MES sodium salt, pH 6.0, 20% PEG 3350
24	92179	0.05 M MES sodium salt, pH 6.0, 28% PEG 3350
25	91341	0.05 M Bis-tris, pH 6.5, 4% PEG 3350
26	89146	0.05 M Bis-tris, pH 6.5, 12% PEG 3350

Kit Reagents and Solutions (Continued)

27	89776	0.05 M Bis-tris, pH 6.5, 20% PEG 3350
28	87316	0.05 M Bis-tris, pH 6.5, 28% PEG 3350
29	86313	0.05 M imidazole-HCl, pH 7.0, 4% PEG 3350
30	95479	0.05 M imidazole-HCl, pH 7.0, 12% PEG 3350
31	74563	0.05 M imidazole-HCl, pH 7.0, 20% PEG 3350
32	94774	0.05 M imidazole-HCl, pH 7.0, 28% PEG 3350
33	92538	0.05 M HEPES sodium salt, pH 7.5, 4% PEG 3350
34	86461	0.05 M HEPES sodium salt, pH 7.5, 12% PEG 3350
35	92021	0.05 M HEPES sodium salt, pH 7.5, 20% PEG 3350
36	92146	0.05 M HEPES sodium salt, pH 7.5, 28% PEG 3350
37	92154	0.05 M Tris-HCl, pH 8.0, 4% PEG 3350
38	80866	0.05 M Tris-HCl, pH 8.0, 12% PEG 3350
39	89785	0.05 M Tris-HCl, pH 8.0, 20% PEG 3350
40	95129	0.05 M Tris-HCl, pH 8.0, 28% PEG 3350
41	92477	0.05 M Tris-HCl, pH 8.5, 4% PEG 3350
42	94611	0.05 M Tris-HCl, pH 8.5, 12% PEG 3350
43	94586	0.05 M Tris-HCl, pH 8.5, 20% PEG 3350
44	94845	0.05 M Tris-HCl, pH 8.5, 28% PEG 3350
45	93209	0.05 M glycine sodium salt, pH 9.0, 4% PEG 3350
46	92636	0.05 M glycine sodium salt, pH 9.0, 12% PEG 3350
47	96261	0.05 M glycine sodium salt, pH 9.0, 20% PEG 3350
48	75289	0.05 M glycine sodium salt, pH 9.0, 28% PEG 3350
49	77584	0.05 M glycine sodium salt, pH 10.0, 4% PEG 3350
50	88194	0.05 M glycine sodium salt, pH 10.0, 28% PEG 3350

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