**Crystallization Low Ionic Strength Kit for Proteins**
Product Number **86684**
Store at 2-8 °C

# TECHNICAL BULLETIN

## Application

With this Crystallization Low Ionic Strength Kit for Proteins it is possible to determine the crystallization conditions for numerous monoclonal antibody fragments, as well as other soluble proteins. The screen can be used for proteins where this strategy could be effective in determining preliminary crystallization screens.
The low ionic strength region has not been explored very much. Moreover, the examples of salting-in reported in the literature concern protein solubility data near the isoelectric point (pI) of the protein.
The method is based upon a screening protocol in the publication "Crystallization of intact monoclonal antibodies", Proteins: Structure, Function, and Genetics 23:285-289 (1995) by L.J. Harris, et al. In this publication, a screen is described for determining the preliminary crystallization conditions of intact monoclonal antibodies.

The important variable parameters, which give the conditions for the crystallization, are the concentration of PEG 3350 (between 4 and 28%), a broad range of the pH (from 3 to 10) and several temperatures (between 4 and 37°C). Some advanced users of this crystallisation kit screen a range of pH between 2 and 12. In addition they often test some other concentration of PEG 3350. For producing the solutions, it is recommended to use the Microselect products of Fluka.

## Quality of reagents – the key to success

Crystallization Low Ionic Strength 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 been used successfully 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

Sample concentrations used in practice vary widely, but concentrations of 3 to 20 mg/ml of intact antibody or protein (7,8,6,9,10,11) in ultrapure water have been used most frequently and may be useful as a starting point. Samples from, e.g., cell culture, should be filtered through a sterile micro-filter. The sample should be free of any unnecessary additives to detect the best crystallization conditions. Although in the real sample, ligands, ions, reducing agents, or other additives may be present for solubility, stability or activity, it is the best to dialyze the sample against ultrapure water.

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*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: $Zn^{2+}$, $Ca^{2+}$, $Mg^{2+}$
Concentration of less than 10 mM are not a problem. Or, one can exchange the phosphate, borate, or carbonate buffers with a more soluble buffer that does not complex with divalent cations.

**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 Low Ionic Strength  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.

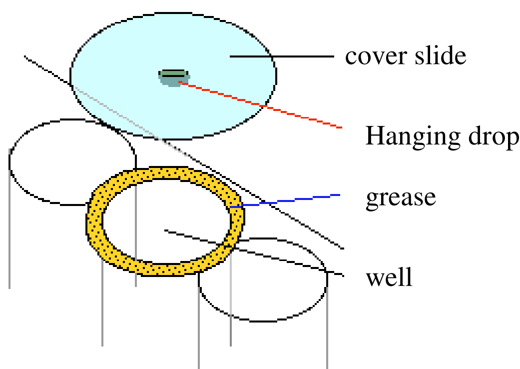


figure 1: well plate

*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.

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## 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 complete equilibration. In the absence of crystals, inspect any droplets with precipitate for microcrystallinity. Use a high power microscope to examine amorphous material 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 there is precipitate from amorphous material, consider repeating the screen while changing the sample concentration, the precipitant concentration (up to 50%) or the temperature. If the droplets remain clear, leave the screen for a few weeks but continue to observe the samples.
Increasing the sample concentration may optimize the conditions.

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


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# 86684 Crystallization Low Ionic Kit for Proteins Observation Sheet

Sample description: ________________________________ Date: ______________________________
concentration: ________________________________ Incubation Temperature: _______________
buffer: ________________________________ Reservoir Volume: ___________________
1 Drop contains : Crystallization Reagent_______ul Sample_______ul Additive (name)_______________(volume)_____ul

1 drop is clear
2 drop contains non-protein particles

**precipitate without birefringent and edges**
3 mostly clear drop
4 fully precipitated dark colour
5 gelatinous protein precipitate
6 phase separation

**precipitates shows birefringent or has edges**
7 sperulites or small structures maybe edges
8 crystal grown 1 D
9 crystal grown 2 D
10 crystal grown 3 D

| No. | Fluka No. | Name | Date: | Date: | Date: | Date: | Date: |
|---|---|---|---|---|---|---|---|
| 1. | 83171 | Na-citrate (pH 3.0) 0.05M, PEG 3350 4% | | | | | |
| 2. | 95988 | Na-citrate (pH 3.0) 0.05M, PEG 3350 12% | | | | | |
| 3. | 96376 | Na-citrate (pH 3.0) 0.05M, PEG 3350 20% | | | | | |
| 4. | 95918 | Na-citrate (pH 3.0) 0.05M, PEG 3350 28% | | | | | |
| 5. | 94191 | Na-citrate (pH 4.0) 0.05M, PEG 3350 4% | | | | | |
| 6. | 96711 | Na-citrate (pH 4.0) 0.05M, PEG 3350 12% | | | | | |
| 7. | 75402 | Na-citrate (pH 4.0) 0.05M, PEG 3350 20% | | | | | |
| 8. | 78667 | Na-citrate (pH 4.0) 0.05M, PEG 3350 28% | | | | | |
| 9. | 80654 | Na-citrate (pH 4.5) 0.05M, PEG 3350 4% | | | | | |
| 10. | 71586 | Na-citrate (pH 4.5) 0.05M, PEG 3350 12% | | | | | |
| 11. | 80089 | Na-citrate (pH 4.5) 0.05M, PEG 3350 20% | | | | | |
| 12. | 80552 | Na-citrate (pH 4.5) 0.05M, PEG 3350 28% | | | | | |
| 13. | 73291 | Na-citrate (pH 5.0) 0.05M, PEG 3350 4% | | | | | |
| 14. | 78067 | Na-citrate (pH 5.0) 0.05M, PEG 3350 12% | | | | | |
| 15. | 80749 | Na-citrate (pH 5.0) 0.05M, PEG 3350 20% | | | | | |
| 16. | 77657 | Na-citrate (pH 5.0) 0.05M, PEG 3350 28% | | | | | |
| 17. | 77652 | Na-citrate (pH 5.5) 0.05M, PEG 3350 4% | | | | | |
| 18. | 80538 | Na-citrate (pH 5.5) 0.05M, PEG 3350 12% | | | | | |
| 19. | 87007 | Na-citrate (pH 5.5) 0.05M, PEG 3350 20% | | | | | |
| 20. | 91788 | Na-citrate (pH 5.5) 0.05M, PEG 3350 28% | | | | | |
| 21. | 86309 | MES-Na (pH 6.0) 0.05M, PEG 3350 4% | | | | | |
| 22. | 86287 | MES-Na (pH 6.0) 0.05M, PEG 3350 12% | | | | | |
| 23. | 89781 | MES-Na (pH 6.0) 0.05M, PEG 3350 20% | | | | | |
| 24. | 92179 | MES-Na (pH 6.0) 0.05M, PEG 3350 28% | | | | | |
| 25. | 91341 | Bis-TRIS-HCl (pH 6.5) 0.05M, PEG 3350 4% | | | | | |
| 26. | 89146 | Bis-TRIS-HCl (pH 6.5) 0.05M, PEG 3350 12% | | | | | |
| 27. | 89776 | Bis-TRIS-HCl (pH 6.5) 0.05M, PEG 3350 20% | | | | | |
| 28. | 87316 | Bis-TRIS-HCl (pH 6.5) 0.05M, PEG 3350 28% | | | | | |
| 29. | 86313 | Imidazole-HCl (pH 7.0) 0.05M, PEG 3350 4% | | | | | |
| 30. | 95479 | Imidazole-HCl (pH 7.0) 0.05M, PEG 3350 12% | | | | | |
| 31. | 74563 | Imidazole-HCl (pH 7.0) 0.05M, PEG 3350 20% | | | | | |
| 32. | 94774 | Imidazole-HCl (pH 7.0) 0.05M, PEG 3350 28% | | | | | |
| 33. | 92538 | HEPES Na-salt (pH 7.5) 0.05M, PEG 3350 4% | | | | | |
| 34. | 86461 | HEPES Na-salt (pH 7.5) 0.05M, PEG 3350 12% | | | | | |
| 35. | 92021 | HEPES Na-salt (pH 7.5) 0.05M, PEG 3350 20% | | | | | |
| 36. | 92146 | HEPES Na-salt (pH 7.5) 0.05M, PEG 3350 28% | | | | | |
| 37. | 92154 | TRIS-HCl (pH 8.0) 0.05M, PEG 3350 4% | | | | | |
| 38. | 80866 | TRIS-HCl (pH 8.0) 0.05M, PEG 3350 12% | | | | | |
| 39. | 89785 | TRIS-HCl (pH 8.0) 0.05M, PEG 3350 20% | | | | | |
| 40. | 95129 | TRIS-HCl (pH 8.0) 0.05M, PEG 3350 28% | | | | | |
| 41. | 92477 | TRIS-HCl (pH 8.5) 0.05M, PEG 3350 4% | | | | | |
| 42. | 94611 | TRIS-HCl (pH 8.5) 0.05M, PEG 3350 12% | | | | | |
| 43. | 94586 | TRIS-HCl (pH 8.5) 0.05M, PEG 3350 20% | | | | | |
| 44. | 94845 | TRIS-HCl (pH 8.5) 0.05M, PEG 3350 28% | | | | | |
| 45. | 93209 | Glycine Na-salt (pH 9.0) 0.05M, PEG 3350 4% | | | | | |
| 46. | 92636 | Glycine Na-salt (pH 9.0) 0.05M, PEG 3350 12% | | | | | |
| 47. | 96261 | Glycine Na-salt (pH 9.0) 0.05M, PEG 3350 20% | | | | | |
| 48. | 75289 | Glycine Na-salt (pH 9.0) 0.05M, PEG 3350 28% | | | | | |
| 49. | 77584 | Glycine Na-salt (pH 10.0) 0.05M, PEG 3350 4% | | | | | |
| 50. | 88194 | Glycine Na-salt (pH 10.0) 0.05M, PEG 3350 28% | | | | | |

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