

Applications

Crystallization screen for biological macromolecules, where Polyethylene glycol 4,000 is the primary reagent, and multiple buffer agents the secondary, sampling a neutral to alkaline pH, or, sampling a broad, acidic to alkaline range of pH when used together with PEG/pH.

Features

- Samples pH 6.4 - 9.6 in 0.2 M buffer
- Samples pH 3.5 - 9.6 in 0.2 M buffer when used with PEG/pH
- Polyethylene glycol 4,000 - primary reagent
- 10 unique buffers, 0.2 M - secondary reagent
- 20 unique buffers, 0.2 M - secondary reagent when used with PEG/pH
- Vapor diffusion, microbatch, free interface diffusion
- Useful for screening samples where a neutral to alkaline pH range is desired

Refer to the enclosed PEG/pH 2 Reagent Formulation for more information.

General Description

pH is an effective solubility, stability and crystallization variable because most proteins demonstrate pH dependent solubility minima and will solubilize, precipitate, or crystallize at particular pH values or in the presence of specific buffers. The solubility minima may correspond with the isoelectric point (pI) of the protein, but this is not always the case. The solubility minima and maxima is often complex and may depend on other chemical and physical variables in the crystallization experiment.

Using PEG/pH 2 one isolates pH, buffer type and relative supersaturation from other chemical and physical variables to screen the effect that pH and buffer type have on the solubility, stability, homogeneity, monodispersity and crystallization of the sample. Varying the pH can alter the protonation state and charge of amino acid residues in the protein, generating different species of the protein for solubility and crystallization screening. The change in pH can have a dramatic effect on inter and intramolecular contacts in the protein and can manipulate how the protein interacts with itself, the surrounding solvent and chemicals in the drop. By screening buffer type and pH in a low ionic strength environment of Polyethylene glycol, PEG/pH 2 simultaneously delivers as a solubility and a crystallization screen for proteins.

Designed as a 96 reagent screen, but offered as two sets of 48 reagents, PEG/pH and PEG/pH 2 samples Polyethylene glycol 4,000 versus 20 different buffers, at 0.2 M, while sampling pH 3.5 - 9.6. PEG/pH 2 is supplied in a x 10 ml tube format. PEG/pH 2 is compatible with vapor diffusion, free interface diffusion, and microbatch crystallization methods. For research use only.

Sample Preparation

The protein sample should be homogenous, as pure as is practically possible (>95%), and free of amorphous material. Remove amorphous material by centrifugation or microfiltration prior to use. The recommended sample concen-

tration is 5 to 25 mg/ml in dilute (25 mM or less) buffer. For initial screens, the sample should be free of unnecessary additives in order to observe the effect of the PEG/pH 2 reagents. However, agents that promote and preserve sample solubility, stability, and homogeneity can and should be included in the sample buffer. For additional sample preparation recommendations see Hampton Research Crystal Growth 101 - Preliminary Sample Preparation.

Manual Method - Sitting Drop Vapor Diffusion

1. Using four 24 well sitting drop crystallization plates, pipet the recommended volume (typically 500 to 1,000 microliters) of crystallization reagent from the tubes into the crystallization plates. PEG/pH 2 reagents 1 through 24 into the first plate and reagents 25 through 48 into the second plate. Use clean pipette tips, a new tip for each of the 48 reagents.

2. Using a clean pipette tip, pipet the desired volume of crystallization reagent (typically 0.5 to 2 microliters) from the crystallization plate reservoir to the sitting drop well.

3. Using a clean pipette tip, pipet the same volume (typically 0.5 to 2 microliters) of sample to the reagent drop in the sitting drop well. Work carefully but quickly to minimize evaporation from the drop and reservoir.

4. Seal the crystallization plate using an optically clear sealing film or tape.

Examine the Drop

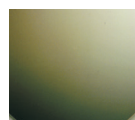
Carefully examine the drops under a stereo microscope (10 to 100x magnification) after setting the screen. Record all observations and be particularly careful to scan the focal plane for small crystals. Observe the drops once each day for the first week, then once a week thereafter for up to 60 days, or until the drop dries out. Records should indicate whether the drop is clear, contains precipitate, and/or crystals. It is helpful to describe the drop contents using descriptive terms. Adding magnitude is also helpful. Example: 4+ yellow/brown fine precipitate, 3+ needle shaped crystals in 1+ white precipitate. One may also employ a numerical scoring scheme (Clear = 0, Crystal = 1. Precipitate = 2). Figure 1 shows typical examples of what one might observe in a crystallization experiment.

Interpreting the Screen Results

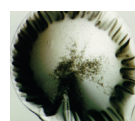
Clear drops indicate that either the relative supersaturation of the sample and reagent is too low or the drop has not yet completed equilibration. If the drop remains clear after 3 to 4 weeks consider repeating the screen condition and doubling the sample concentration. If more than 35 of the 48 drops are clear, then consider doubling the sample concentration and repeating the entire screen.

Drops containing precipitate indicate either the relative supersaturation of the sample and reagent is too high, the sample has denatured, or the sample is heterogeneous. To reduce the relative supersaturation, dilute the sample twofold with sample buffer and repeat the screen condition. If more than 35 of the 48 drops contain precipitate and no crystals are present, then consider dilut-

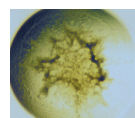
Figure 1
Typical observations in a crystallization experiment



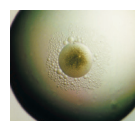
Clear Drop



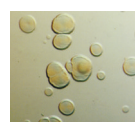
Skin/
Precipitate



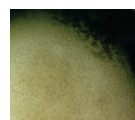
Precipitate



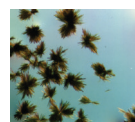
Precipitate/
Phase



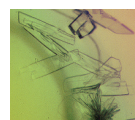
Quasi
Crystals



Microcrystals



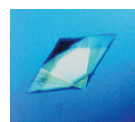
Needle
Cluster



Plates



Rod Cluster



Single
Crystal

ing the sample concentration in half by adding an equal volume of sample buffer to the sample and repeating the entire screen. If sample denaturation is suspect, take measures to stabilize the sample (add reducing agent, ligands, additives, salt, or other stabilizing agents). If the sample is impure, aggregated, or heterogeneous take measures to pursue homogeneity. It is possible to obtain crystals from precipitate so do not discard nor ignore a drop containing precipitate. If possible, examine drops containing precipitate under polarizing or UV optics to differentiate precipitate from microcrystals.

If the drop contains a macromolecular crystal the relative supersaturation of the sample and reagent is appropriate for crystal nucleation and growth. The next step is to optimize the preliminary conditions by varying salt and/or PEG concentration, screen pH, vary temperature between 4 and 30°C, screen additives, and evaluate other crystallization variables including sample construct, purity, stability, and homogeneity in order to achieve the desired crystal size and quality.

When sample quantity permits, set the screen in duplicate (4°C and 25°C) or triplicate (10°C and 20°C and 30°C) to evaluate the effect of temperature on crystallization. Compare the observations between the different temperatures to determine the effect of temperature on sample solubility. Different results in the same drops at different temperatures indicate that sample solubility is temperature dependent and that one should include temperature as a variable in subsequent screens and optimization experiments.

When sample quantity permits, set the screen using multiple drops and drop ratios, such as 1:2, 1:1, and 2:1. See Hampton Research Crystal Growth 101: Drop Ratio for details.

Formulation

Crystallization reagents are formulated using the highest purity chemicals, ultrapure water (Formulated in Type 1+ ultrapure water: 18.2 megaohm-cm resistivity at 25°C, < 5 ppb Total Organic Carbon, bacteria free (<1 Bacteria (CFU/ml)), pyrogen free (<0.03 Endotoxin (EU/ml)), RNase-free (< 0.01 ng/mL) and DNase-free (< 4 pg/μL) and are sterile filtered using 0.22 micron filters into sterile containers (no preservatives added). Store at -20°C. Best if used within 12 months of receipt.

Crystallization reagents can be reproduced using Hampton Research Optimize™ polyethylene glycols and buffers.

Recommended Reading

1. Introduction to protein crystallization. Alexander McPherson and Jose A. Gavira. Acta Crystallographica Section F Volume 70, Issue 1, pages 2–20, January 2014.
2. Optimization of crystallization conditions for biological macromolecules. Alexander McPherson and Bob Cudney. Acta Crystallographica Section F Volume 70, Issue 11, pages 1445–1467, November 2014.
3. Protein Isoelectric Point as a Predictor for Increased Crystallization screening efficiency. Katherine A. Kantardjieff and Bernhard Rupp. Bioinformatics (2004) 20.
4. A protein crystallization strategy using automated grid searches on successively finer grid screens. Patricia C. Weber. Methods: A Companion to Methods in Enzymology. Vol. 1, No. 1, August, pp. 31-37, 1990.
5. Two approaches to the rapid screening of crystallization conditions. Alexander McPherson. Journal of Crystal Growth 122 (1992) 161-167.
6. Optimization of buffer solutions for protein crystallization. R. A. Gosavi, T. C. Mueser and C. A. Schall. Acta Cryst. (2008). D64, 506-514.
7. Buffer Solutions The Basics. R.J. Beynon and J.S. Easterby. 1996. IRL Press.

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34 Journey

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Website: www.hamptonresearch.com

Tube #	Buffer	pH ◇	Titrant	Precipitant
1.	0.2 M BIS-TRIS propane	6.4	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
2.	0.2 M BIS-TRIS propane	6.7	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
3.	0.2 M BIS-TRIS propane	7.0	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
4.	0.2 M BIS-TRIS propane	7.3	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
5.	0.2 M MOPS	6.5	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
6.	0.2 M MOPS	6.8	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
7.	0.2 M MOPS	7.1	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
8.	0.2 M MOPS	7.4	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
9.	0.2 M MOPS	7.7	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
10.	0.2 M HEPES sodium	6.6	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
11.	0.2 M HEPES sodium	6.9	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
12.	0.2 M HEPES sodium	7.2	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
13.	0.2 M HEPES sodium	7.5	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
14.	0.2 M HEPES	6.8	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
15.	0.2 M HEPES	7.1	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
16.	0.2 M HEPES	7.4	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
17.	0.2 M HEPES	7.7	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
18.	0.2 M TRIS hydrochloride	7.2	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
19.	0.2 M TRIS hydrochloride	7.5	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
20.	0.2 M TRIS hydrochloride	7.8	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
21.	0.2 M TRIS hydrochloride	8.1	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
22.	0.2 M Tris	7.3	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
23.	0.2 M Tris	7.6	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
24.	0.2 M Tris	7.9	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
25.	0.2 M Tris	8.2	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
26.	0.2 M Tris	8.5	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
27.	0.2 M Tricine	7.4	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
28.	0.2 M Tricine	7.7	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
29.	0.2 M Tricine	8.0	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
30.	0.2 M Tricine	8.3	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
31.	0.2 M Tricine	8.6	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
32.	0.2 M BICINE	7.5	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
33.	0.2 M BICINE	7.8	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
34.	0.2 M BICINE	8.1	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
35.	0.2 M BICINE	8.4	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
36.	0.2 M BICINE	8.7	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
37.	0.2 M BIS-TRIS propane	8.5	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
38.	0.2 M BIS-TRIS propane	8.8	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
39.	0.2 M BIS-TRIS propane	9.1	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
40.	0.2 M BIS-TRIS propane	9.4	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
41.	0.2 M Glycine	8.6	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
42.	0.2 M Glycine	8.9	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
43.	0.2 M Glycine	9.2	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
44.	0.2 M Glycine	9.5	Sodium hydroxide	20% w/v Polyethylene glycol 4,000
45.	0.2 M AMPD	8.7	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
46.	0.2 M AMPD	9.0	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
47.	0.2 M AMPD	9.3	Hydrochloric acid	20% w/v Polyethylene glycol 4,000
48.	0.2 M AMPD	9.6	Hydrochloric acid	20% w/v Polyethylene glycol 4,000

Reagents formulated in Type 1+ ultrapure grade water

◇ Measured pH at 25°C after titration

Sample: _____ Sample Concentration: _____
 Sample Buffer: _____ Date: _____
 Reservoir Volume: _____ Temperature: _____
 Drop Volume: Total _____ μ l Sample _____ μ l Reservoir _____ μ l Additive _____ μ l

- 1 Clear Drop
- 2 Phase Separation
- 3 Regular Granular Precipitate
- 4 Birefringent Precipitate or Microcrystals
- 5 Posettes or Spherulites
- 6 Needles (1D Growth)
- 7 Plates (2D Growth)
- 8 Single Crystals (3D Growth < 0.2 mm)
- 9 Single Crystals (3D Growth > 0.2 mm)

PEG/pH™ 2 - HR2-081 Scoring Sheet

Date: Date: Date: Date:

1.	0.2 M BIS-TRIS propane pH 6.4, 20% w/v Polyethylene glycol 4,000				
2.	0.2 M BIS-TRIS propane pH 6.7, 20% w/v Polyethylene glycol 4,000				
3.	0.2 M BIS-TRIS propane pH 7.0, 20% w/v Polyethylene glycol 4,000				
4.	0.2 M BIS-TRIS propane pH 7.3, 20% w/v Polyethylene glycol 4,000				
5.	0.2 M MOPS pH 6.5, 20% w/v Polyethylene glycol 4,000				
6.	0.2 M MOPS pH 6.8, 20% w/v Polyethylene glycol 4,000				
7.	0.2 M MOPS pH 7.1, 20% w/v Polyethylene glycol 4,000				
8.	0.2 M MOPS pH 7.4, 20% w/v Polyethylene glycol 4,000				
9.	0.2 M MOPS pH 7.7, 20% w/v Polyethylene glycol 4,000				
10.	0.2 M HEPES sodium pH 6.6, 20% w/v Polyethylene glycol 4,000				
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17.	0.2 M HEPES pH 7.7, 20% w/v Polyethylene glycol 4,000				
18.	0.2 M TRIS hydrochloride pH 7.2, 20% w/v Polyethylene glycol 4,000				
19.	0.2 M TRIS hydrochloride pH 7.5, 20% w/v Polyethylene glycol 4,000				
20.	0.2 M TRIS hydrochloride pH 7.8, 20% w/v Polyethylene glycol 4,000				
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36.	0.2 M BICINE pH 8.7, 20% w/v Polyethylene glycol 4,000				
37.	0.2 M BIS-TRIS propane pH 8.5, 20% w/v Polyethylene glycol 4,000				
38.	0.2 M BIS-TRIS propane pH 8.8, 20% w/v Polyethylene glycol 4,000				
39.	0.2 M BIS-TRIS propane pH 9.1, 20% w/v Polyethylene glycol 4,000				
40.	0.2 M BIS-TRIS propane pH 9.4, 20% w/v Polyethylene glycol 4,000				
41.	0.2 M Glycine pH 8.6, 20% w/v Polyethylene glycol 4,000				
42.	0.2 M Glycine pH 8.9, 20% w/v Polyethylene glycol 4,000				
43.	0.2 M Glycine pH 9.2, 20% w/v Polyethylene glycol 4,000				
44.	0.2 M Glycine pH 9.5, 20% w/v Polyethylene glycol 4,000				
45.	0.2 M AMPD pH 8.7, 20% w/v Polyethylene glycol 4,000				
46.	0.2 M AMPD pH 9.0, 20% w/v Polyethylene glycol 4,000				
47.	0.2 M AMPD pH 9.3, 20% w/v Polyethylene glycol 4,000				
48.	0.2 M AMPD pH 9.6, 20% w/v Polyethylene glycol 4,000				