

Application

Slice pH is a 96 buffer reagent kit designed to be 1) a rapid and convenient assay to evaluate the solubility, homogeneity and monodispersity of a protein over a range of buffer types and pH values and 2) as an independent crystallization screen.

Discussion

Slice pH is a solubility screen, a stability screen, and a crystallization screen. 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.¹⁻⁵ The solubility minima may correspond with the isoelectric point (pI) of the protein, but this is not always the case.⁶ The solubility minima and maxima is often complex and may depend on other chemical and physical variables in the crystallization experiment. Using Slice pH one isolates pH, buffer type and relative supersaturation from other chemical and physical variables and 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 an environment of increasing relative supersaturation, Slice pH simultaneously delivers as a solubility and a crystallization screen for proteins. After the screen, and once the appropriate sample buffer and pH are identified, the sample can be exchanged into the identified buffer and pH for optimal solubility and stability. From this point, the sample in the optimized buffer reagent can be used for crystallization trials or other assays.^{1-5,7,8} Further, crystallization screening and optimization experiments may be more appropriately focused on the optimal pH range and buffer type.⁴⁻⁵

Features

Slice pH is a set of 96 pH titrated high purity buffer reagents. The Slice pH buffer reagents are supplied as 500 µl of 1.0 M solutions spanning the pH range 3.5 to 9.6 in 0.1 pH increments utilizing 20 unique buffers. Each buffer reagent is titrated using hydrochloric acid or sodium hydroxide in high purity water (NCCLS/ASTM Type 1+) at 25°C and sterile filtered (0.22 micron).

Slice pH Buffers

- | | | |
|-------------------------------------|--------------------------------|----------------------|
| • Citric acid | • Sodium cacodylate trihydrate | • HEPES sodium |
| • Sodium citrate tribasic dihydrate | • MES monohydrate | • HEPES |
| • Sodium acetate trihydrate | • BIS-TRIS | • TRIS hydrochloride |
| • DL-Malic acid | • ADA | • Tris |
| • Succinic acid | • Imidazole | • Tricine |
| | • BIS-TRIS propane | • BICINE |
| | • MOPS | • Glycine |
| | | • AMPD |

Instructions -

Vapor Diffusion Assay for Solubility, Stability & Crystallization

A wide variety of methodologies can be administered using the Slice pH reagents to assess the influence of pH and buffer type on sample solubility, stability and crystallization. One of the simpler methodologies is to set a vapor diffusion crystallization experiment with a drop composed of Slice pH reagent and sample in vapor equilibration with a dehydrant such as 3.0 M Sodium chloride. In such an experiment the relative super saturation of the sample and reagent increase as the drop equilibrates with the reservoir. As the concentration of the sample increases in the presence of increasing ionic strength the solubility and stability of the sample is challenged. Clear drops and drops containing crystals may be interpreted as good sample solubility and stability. Precipitated drops can be interpreted as poor sample solubility and stability.

The following describes a vapor diffusion experiment using a 400 nanoliter drop and a 50 microliter reservoir in a 96 well crystallization plate:

1. Allow the Slice pH reagents to equilibrate to the desired experimental temperature. Carefully remove the heat seal film. One piece of AlumaSeal II™ Sealing Film is supplied with the kit to seal the Slice pH plate. Use a new AlumaSeal II film each time to seal the Slice pH plate.
2. Pipette 50 microliters of dehydrant (3.0 M Sodium chloride) into the reservoir.
3. Pipette 200 nanoliters of sample into the drop area.
4. Pipette 200 nanoliters of Slice pH reagent into the sample drop.
5. Repeat for the remaining 95 reagents.
6. Seal the experiment

Examine the Drop

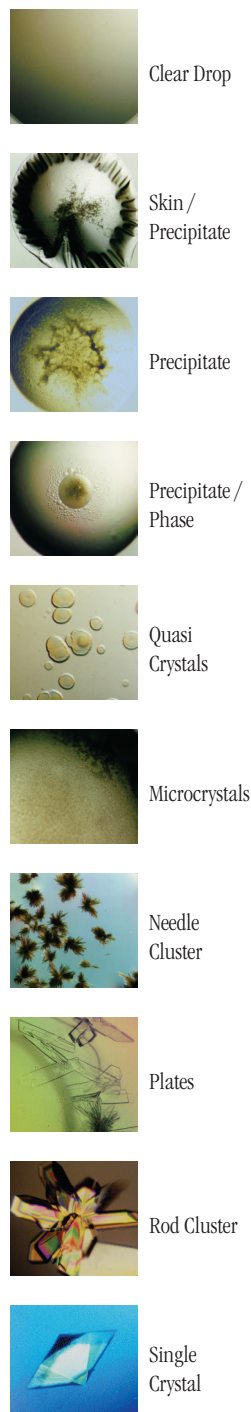
Carefully examine the drops under a stereo microscope (10 to 100x magnification) immediately after setting up the screen and again at 24 hours. Record all observations, scanning the focal plane for results. Solubility and stability results are typically available after 24 hours when using 3.0 M Sodium chloride as the dehydrant with the vapor diffusion assay and a total initial drop size of 1 microliter or less. Larger drop volumes, lower reservoir volumes, different plate configurations or lower dehydrant concentrations may alter equilibration kinetics and require up to a week for equilibration. In such instances, observe the drops once each day for the first week, then once a week there after. Records should indicate whether the drop is clear, contains precipitate, and or crystals. Figure 1 (on page 2) illustrates typical examples of what one might observe when using Slice pH as a crystallization experiment.

Interpreting Slice pH Results

When using Slice pH as a crystallization screen one interprets the drops the same as for any other crystallization screen. A simple reagent composed of only buffer and a specific pH can produce crystals of biological macromolecules. When crystals are obtained and optimization is necessary one can

Figure 1

Typical observations in a crystallization experiment



evaluate all crystallization variables including sample concentration, drop size and ratio, additives, temperature, seeding, etc.

When using Slice pH as a solubility screen with the vapor diffusion method to evaluate the effect of buffer type and pH one can use the presence of clear drops or precipitate to identify buffers and pH levels that promote solubility. Clear drops may indicate good solubility and drops with precipitate or phase separation may indicate poor solubility. If multiple buffers or multiple pH ranges show solubility, use challenges and/or diagnostic methods in Table 1 and Table 2 to identify an optimal buffer and pH.

Note: This methodology can be utilized with precipitated or aggregated protein in an effort to identify pH and buffer type that may solubilize the sample. Mix the homogenized precipitated protein sample as described in the vapor diffusion assay. Immediately after set up and after 24 hours view drops. Clear drops are an indication of improved solubility. Drop contents may be assayed diagnostically for monodispersity and aggregation (Table 2).

Using Slice pH Solubility Results in Crystallization Screening

Slice pH can identify buffers and pH levels that promote sample solubility, homogeneity, monodispersity and even result in crystallization. Optimization of the sample buffer solution can promote better results in crystallization screens.⁹ Slice pH results may identify a single buffer and pH range that promotes solubility. With such results one may wish to buffer exchange the sample into the identified buffer at the middle of the soluble pH range and either 1) perform a screen in a general broad pH crystallization screen such as Index or 2) screen a diverse precipitant screen at a focused pH or pH range consistent with the Slice pH results.

Some proteins may show solubility in more than one buffer and pH range. In such instances one may use challenges or diagnostic procedures in Table 1 and Table 2 to better pinpoint the optimal buffer and pH. Otherwise the sample could be split and exchanged into multiple buffers at the pH in the middle of each solubility range and a general broad pH crystallization screen such as Index performed.

Using Slice pH with Other Solubility and Stability Challenges

As an alternative to the vapor diffusion method, a variety of challenges to sample solubility and stability can be utilized with Slice pH, including but not limited to those in Table 1. Each of these challenges can be assayed by a variety of diagnostic methods shown in Table 2.

Table 1. Solubility and Stability Challenges

| | |
|------------------------|--|
| Elevated Temperature | Incubate 24 hours at 37° Celsius |
| Temperature Cycling | Freeze & thaw or warm & cool multiple times |
| Chemical Compatibility | Add chemical challenges such as salts, polymers, volatile organics, or metals. |

Table 2. Diagnostic Assays

| Methodology: | Data: |
|---|--|
| Size Exclusion Chromatography | Sample homogeneity and aggregation |
| Dynamic Light Scattering | Sample homogeneity, polydispersity and aggregation |
| ThermoFluor® | Sample stability |
| Native Gel | Sample homogeneity and aggregation |
| Western, Dot Blot/ELISA | Immunological binding quantity |
| Enzyme Assay | Functional activity |
| Total Protein Assay (UV, Bradford, BCA) | Quantification of soluble protein |
| Fluorescence | Protein function and quantity |
| Filter Plate | Separate soluble from insoluble protein |

Storage

Best if used within 6 months of receipt. Store between -20-4°C. Allow reagents to equilibrate to the room temperature before use.

For research use only.

References

1. Preparation and analysis of protein crystals. Alexander McPherson. 1982 John Wiley and Sons, Inc.
2. Increasing the size of microcrystals by fine sampling of pH limits. Alexander McPherson. J. Appl. Cryst. (1995). 28, 362-365.

3. Protein crystallization techniques, strategies and tips. Edited by Terese Bergfors. 1999. International University Line.
4. Crystallization of Biological Macromolecules. Alexander McPherson. 1991. Cold Spring Harbor Laboratory Press.
5. Current approaches to macromolecular crystallization (review). Alexander McPherson. Eur. J. Biochem. 189, 1-23 (1990).
6. Protein Isoelectric Point as a Predictor for Increased Crystallization screening efficiency. Katherine A. Kantardjieff and Bernhard Rupp. Bioinformatics (2004) 20.
7. 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.
8. Two approaches to the rapid screening of crystallization conditions. Alexander McPherson. Journal of Crystal Growth 122 (1992) 161-167.
9. Optimization of buffer solutions for protein crystallization. R. A. Gosavi, T. C. Mueser and C. A. Schall. Acta Cryst. (2008). D64, 506-514.
10. Buffer Solutions The Basics. R.J. Beynon and J.S. Easterby. 1996. IRL Press.

Related Products

- HR2-134** Index HT™, 1 ml Deep Well Block Format
- HR2-636** 3.0 M Sodium chloride, 200 ml
- HR2-638** 2.0 M Sodium chloride, 200 ml
- HR8-069** AlumaSeal II™ Sealing Film, 100 pack

Technical Support

Inquiries regarding Slice pH reagent formulation, interpretation of screen results, optimization strategies and general inquiries regarding crystallization are welcome. Please e-mail, fax, or telephone your request to Hampton Research. Fax and e-mail Technical Support are available 24 hours a day. Telephone technical support is available 8:00 a.m. to 5:00 p.m. USA Pacific Standard Time.

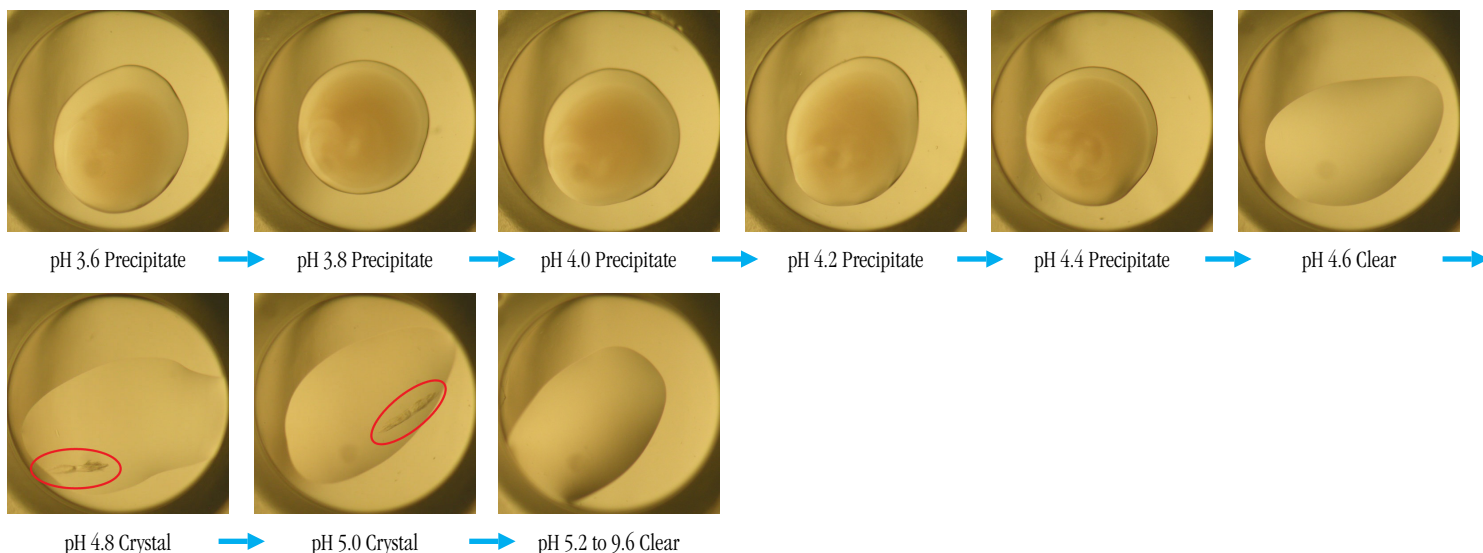
ThermoFluor® is a registered trademark of Johnson & Johnson.

pH Case Study: Papain

- Papain, 20 mg/ml in deionized water. Drop: 0.5 µl Papain + 0.5 µl Slice pH / Reservoir: 50 µl 3.0 M Sodium chloride
- Sitting Drop Vapor Diffusion MRC 2 Well Crystallization Plate (Swissci) HR3-083
- pH 3.5 to 4.4 Precipitate; pH 4.6 Clear, pH 4.8 and 5.0 Crystal; pH 5.2 to 9.6 Clear

Recommendation:

Crystallization screen should focus on pH 4.5-9.6, specifically pH 4.8-5.0 and the sample buffer should be Sodium acetate trihydrate pH 4.9. **Previously reported in the literature:** Crystallization at pH is 5 to 6, all using a volatile organic solvent. (M. Charles, S. Veesler and F. Bonneté (2006) MPCD: a new interactive on-line crystallization data bank for screening strategies, Acta Cryst. D, 62, 1311-1318).



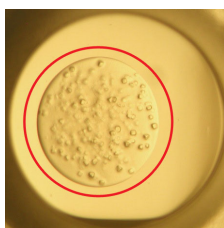
pH Case Study: Lipase B

- Lipase B, 20 mg/ml in deionized water. Drop: 0.5 µl Lipase B + 0.5 µl Slice pH / Reservoir: 50 µl 3.0 M Sodium chloride
- Sitting Drop Vapor Diffusion MRC 2 Well Crystallization Plate (Swissci) HR3-083
- Results:
 - Three different results using three different buffers at the same pH.
pH 4.5 DL-Malic acid Crystals; pH 4.5 Sodium acetate Clear; pH 4.5 Sodium potassium phosphate Precipitate
 - Glycine pH 8.6 Clear; Glycine pH 8.8-9.5 Precipitate/Phase Separation; pH 9.6 Crystals/Precipitate

Recommendation:

Crystallization screen should focus on DL-Malic acid as a buffer / precipitant near pH 4.5 as well as Glycine as a buffer/precipitant between pH 8.6-9.6.

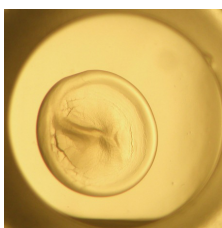
Previously reported in the literature: Lipase is most stable between pH 5 to 7 with an isoelectric point of 6.0. Typical pH range for crystallization is 4 to 6. (The sequence, crystal structure determination and refinement of two crystal forms of lipase B from *Candida antarctica*. Uppenberg, J., Hansen, M.T., Patkar, S., Jones, T.A. Structure v2 pp. 293-308, 1994; Crystallization and preliminary X-ray studies of lipase B from *Candida antarctica*. Uppenberg J., Patkar S.; Bergfors T., Jones, T. A.. Journal of Molecular Biology, 1994, vol. 235, no2, pp. 790-792).



a) pH 4.5 DL-Malic acid / Crystals



pH 4.5 Sodium acetate / Clear



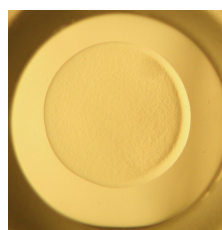
pH 4.5 Na potassium phosphate / Precipitate



b) pH 8.6 Glycine / Clear



pH 8.8 Glycine Precipitate / Phase Separation



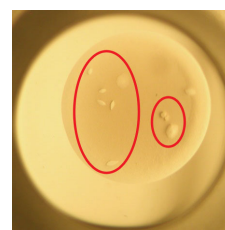
pH 9.0 Glycine Precipitate / Phase Separation



pH 9.3 Glycine Precipitate / Phase Separation



pH 9.5 Glycine Precipitate / Phase Separation



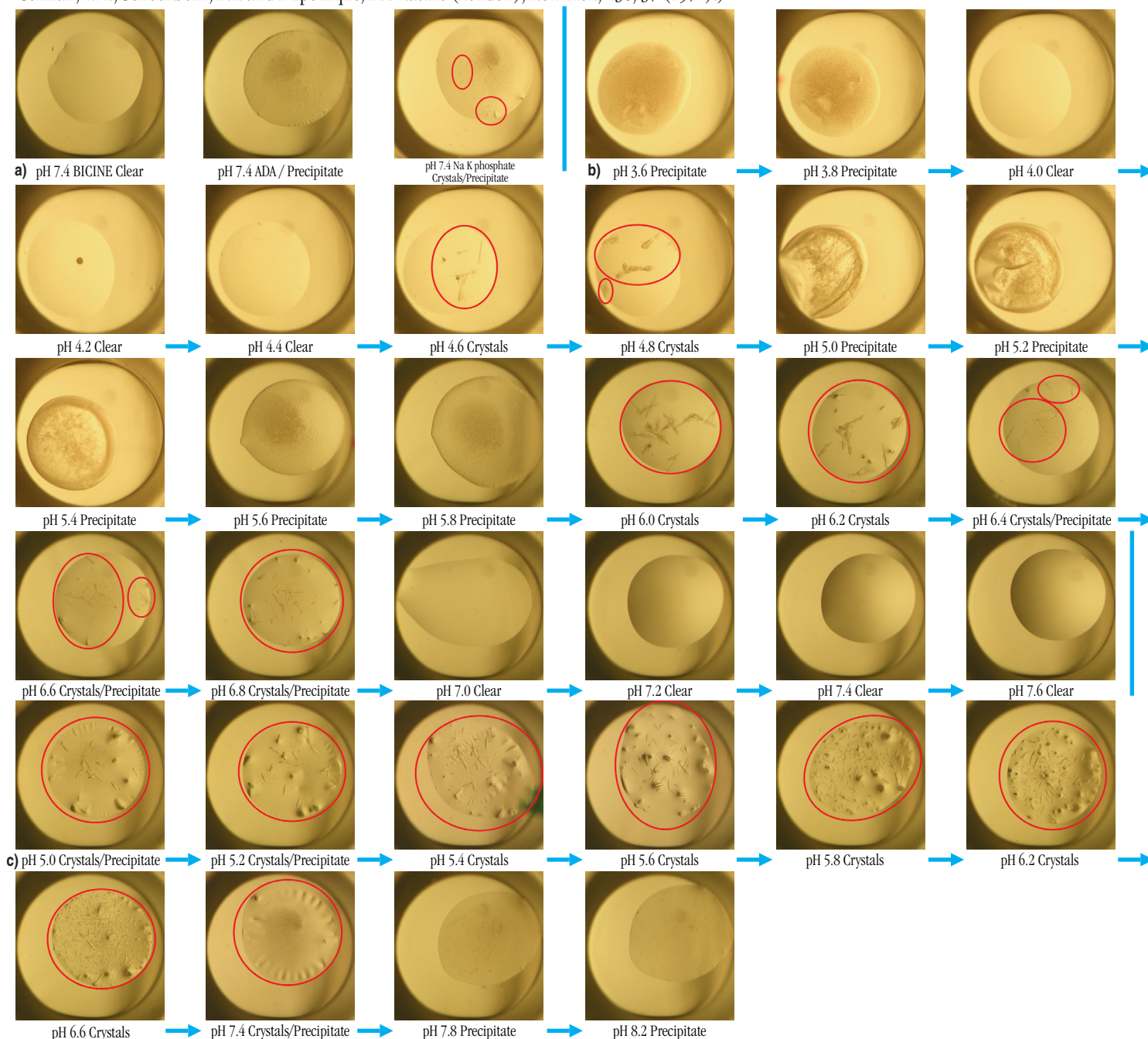
pH 9.6 Glycine Crystals / Precipitate

pH Case Study: Thermolysin

- Thermolysin, 20 mg/ml in deionized water/NaOH. Drop: 0.5 μ l Thermolysin + 0.5 μ l Slice pH / Reservoir: 50 μ l 3.0 M Sodium chloride
- Sitting Drop Vapor Diffusion MRC 2 Well Crystallization Plate (Swissci) HR3-083
- Results: a) Three different results at pH 7.4 using three different buffers. Bicine Clear; ADA Precipitate; Na/K Phosphate Crystals/Precipitate.
b) pH 3.6 to 3.8 Precipitate (Citric acid); pH 4.0 to 4.4 Clear (Sodium acetate); pH 4.6 to 4.8 Crystals (Sodium citrate); pH 5.4 to 5.8 Precipitate (Sodium citrate); pH 6.0 to 6.8 Crystals (Sodium cacodylate); pH 7.0 to 7.6 Clear (HEPES sodium).
c) pH 5.0 to 7.4 Crystals; pH 7.8 to 8.2 Precipitate using the Sodium potassium phosphate buffer system

Recommendation:

Crystallization screen should focus between pH 4.6 to 7.4 with special attention buffer; preference for Sodium citrate, Sodium potassium phosphate and Sodium cacodylate. **Previously reported in the literature:** Thermolysin crystallizes and is most stable between 4.6 to 8.5. (Matthews, B.W., Jansonius, J.N., Colman, P.M., Schoenborn, B.P. and Dupourque, D. : Nature (London), New Biol., 238, 37 (1972).)

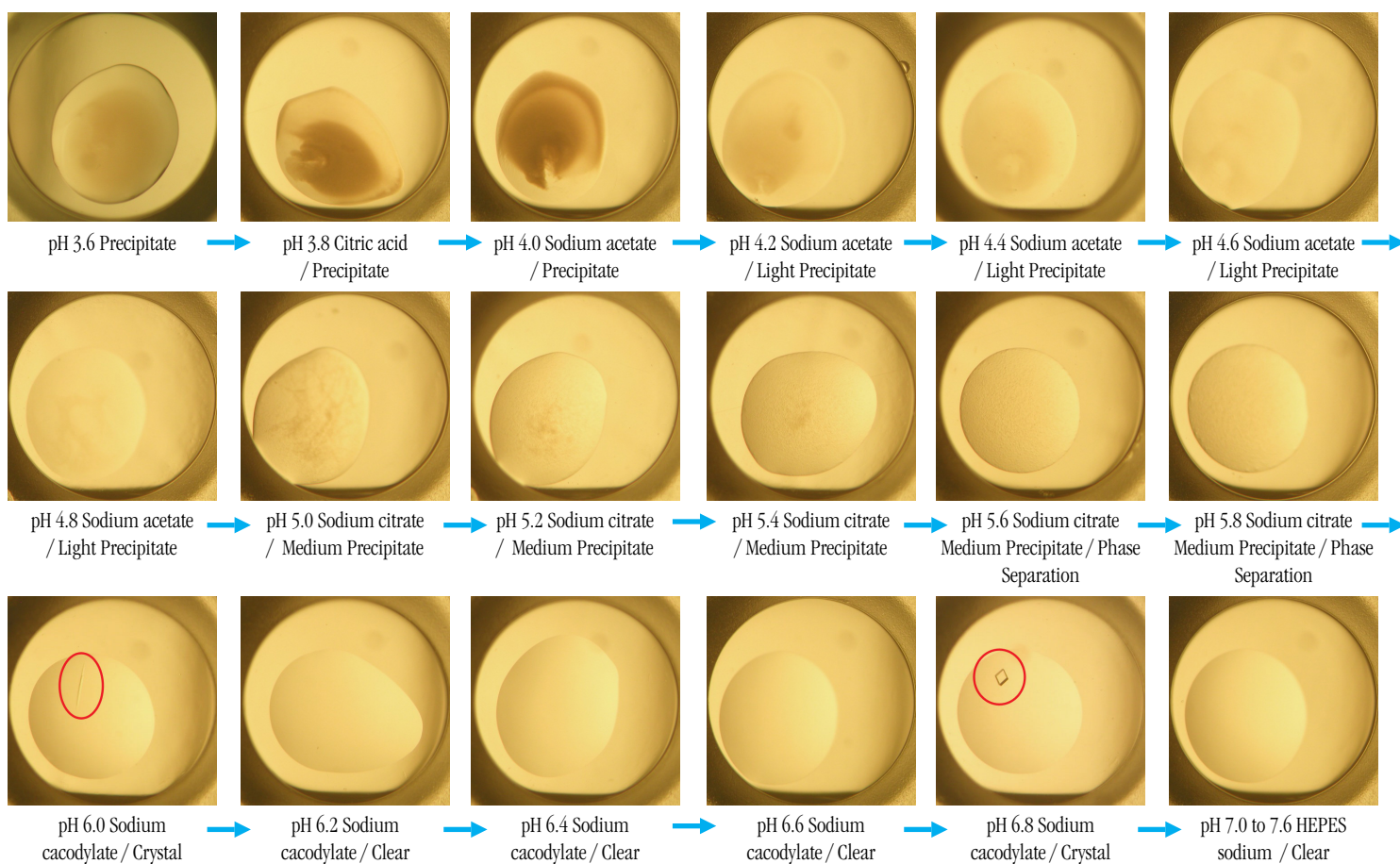


pH Case Study: Glucose Isomerase

- Glucose isomerase, 20 mg/ml in deionized water. Drop: 0.5 µl Glucose isomerase + 0.5 µl Slice / pH Reservoir: 50 µl 3.0 M Sodium chloride
- Sitting Drop Vapor Diffusion MRC 2 Well Crystallization Plate (Swissci) HR3-083
- Results:
pH 3.5 to 4.0 Heavy Precipitate; pH 4.2 to 4.8 Light Precipitate with Sodium acetate pH pH 5.0 and 5.8 Medium Precipitate/Phase Separation with Sodium citrate; pH 6.0 and 6.8 with Sodium cacodylate Crystal, pH 6.2-6.6 Clear with Sodium cacodylate. pH 7.0 to 7.6 Clear HEPES sodium.

Recommendation:

Crystallization screen should focus between pH 6 to 9.6 with special attention to pH 6 to 7. **Previously reported in the literature:** Glucose isomerase rapidly denatures below pH 5 and is most stable between pH 6 and 8. (Carrell, H. L. et al., Proc. Natl. Acad. Sci. USA, Vol 86, pp 4440-4444, June 1989.)



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| Well # | Buffer ◇ | pH | Titrant |
|-----------|---|-----|-------------------|
| 1. (A1) | 1.0 M Citric acid | 3.5 | Sodium hydroxide |
| 2. (A2) | 1.0 M Citric acid | 3.8 | Sodium hydroxide |
| 3. (A3) | 1.0 M Citric acid | 4.1 | Sodium hydroxide |
| 4. (A4) | 1.0 M Citric acid | 4.4 | Sodium hydroxide |
| 5. (A5) | 1.0 M Sodium citrate tribasic dihydrate | 3.6 | Hydrochloric acid |
| 6. (A6) | 1.0 M Sodium citrate tribasic dihydrate | 3.9 | Hydrochloric acid |
| 7. (A7) | 1.0 M Sodium citrate tribasic dihydrate | 4.2 | Hydrochloric acid |
| 8. (A8) | 1.0 M Sodium citrate tribasic dihydrate | 4.5 | Hydrochloric acid |
| 9. (A9) | 1.0 M Sodium acetate trihydrate | 3.7 | Hydrochloric acid |
| 10. (A10) | 1.0 M Sodium acetate trihydrate | 4.0 | Hydrochloric acid |
| 11. (A11) | 1.0 M Sodium acetate trihydrate | 4.3 | Hydrochloric acid |
| 12. (A12) | 1.0 M Sodium acetate trihydrate | 4.6 | Hydrochloric acid |
| 13. (B1) | 1.0 M Sodium acetate trihydrate | 4.9 | Hydrochloric acid |
| 14. (B2) | 1.0 M DL-Malic acid | 4.7 | Sodium hydroxide |
| 15. (B3) | 1.0 M DL-Malic acid | 5.0 | Sodium hydroxide |
| 16. (B4) | 1.0 M DL-Malic acid | 5.3 | Sodium hydroxide |
| 17. (B5) | 1.0 M DL-Malic acid | 5.6 | Sodium hydroxide |
| 18. (B6) | 1.0 M DL-Malic acid | 5.9 | Sodium hydroxide |
| 19. (B7) | 1.0 M Succinic acid | 4.8 | Sodium hydroxide |
| 20. (B8) | 1.0 M Succinic acid | 5.1 | Sodium hydroxide |
| 21. (B9) | 1.0 M Succinic acid | 5.4 | Sodium hydroxide |
| 22. (B10) | 1.0 M Succinic acid | 5.7 | Sodium hydroxide |
| 23. (B11) | 1.0 M Succinic acid | 6.0 | Sodium hydroxide |
| 24. (B12) | 1.0 M Sodium cacodylate trihydrate | 5.2 | Hydrochloric acid |
| 25. (C1) | 1.0 M Sodium cacodylate trihydrate | 5.5 | Hydrochloric acid |
| 26. (C2) | 1.0 M Sodium cacodylate trihydrate | 5.8 | Hydrochloric acid |
| 27. (C3) | 1.0 M Sodium cacodylate trihydrate | 6.1 | Hydrochloric acid |
| 28. (C4) | 1.0 M Sodium cacodylate trihydrate | 6.4 | Hydrochloric acid |
| 29. (C5) | 1.0 MES monohydrate | 5.3 | Sodium hydroxide |
| 30. (C6) | 1.0 MES monohydrate | 5.6 | Sodium hydroxide |
| 31. (C7) | 1.0 MES monohydrate | 5.9 | Sodium hydroxide |
| 32. (C8) | 1.0 MES monohydrate | 6.2 | Sodium hydroxide |
| 33. (C9) | 1.0 MES monohydrate | 6.5 | Sodium hydroxide |
| 34. (C10) | 1.0 M BIS-TRIS | 5.7 | Hydrochloric acid |
| 35. (C11) | 1.0 M BIS-TRIS | 6.0 | Hydrochloric acid |
| 36. (C12) | 1.0 M BIS-TRIS | 6.3 | Hydrochloric acid |
| 37. (D1) | 1.0 M BIS-TRIS | 6.6 | Hydrochloric acid |
| 38. (D2) | 1.0 M BIS-TRIS | 6.9 | Hydrochloric acid |
| 39. (D3) | 1.0 M ADA | 5.8 | Sodium hydroxide |
| 40. (D4) | 1.0 M ADA | 6.1 | Sodium hydroxide |
| 41. (D5) | 1.0 M ADA | 6.4 | Sodium hydroxide |
| 42. (D6) | 1.0 M ADA | 6.7 | Sodium hydroxide |
| 43. (D7) | 1.0 M ADA | 7.0 | Sodium hydroxide |
| 44. (D8) | 1.0 M Imidazole | 6.2 | Hydrochloric acid |
| 45. (D9) | 1.0 M Imidazole | 6.5 | Hydrochloric acid |
| 46. (D10) | 1.0 M Imidazole | 6.8 | Hydrochloric acid |
| 47. (D11) | 1.0 M Imidazole | 7.1 | Hydrochloric acid |
| 48. (D12) | 1.0 M Imidazole | 7.4 | Hydrochloric acid |

| Well # | Buffer ◇ | pH | Titrant |
|-----------|--------------------------|-----|-------------------|
| 49. (E1) | 1.0 M BIS-TRIS propane | 6.4 | Hydrochloric acid |
| 50. (E2) | 1.0 M BIS-TRIS propane | 6.7 | Hydrochloric acid |
| 51. (E3) | 1.0 M BIS-TRIS propane | 7.0 | Hydrochloric acid |
| 52. (E4) | 1.0 M BIS-TRIS propane | 7.3 | Hydrochloric acid |
| 53. (E5) | 1.0 M MOPS | 6.5 | Sodium hydroxide |
| 54. (E6) | 1.0 M MOPS | 6.8 | Sodium hydroxide |
| 55. (E7) | 1.0 M MOPS | 7.1 | Sodium hydroxide |
| 56. (E8) | 1.0 M MOPS | 7.4 | Sodium hydroxide |
| 57. (E9) | 1.0 M MOPS | 7.7 | Sodium hydroxide |
| 58. (E10) | 1.0 M HEPES sodium | 6.6 | Hydrochloric acid |
| 59. (E11) | 1.0 M HEPES sodium | 6.9 | Hydrochloric acid |
| 60. (E12) | 1.0 M HEPES sodium | 7.2 | Hydrochloric acid |
| 61. (F1) | 1.0 M HEPES sodium | 7.5 | Hydrochloric acid |
| 62. (F2) | 1.0 M HEPES | 6.8 | Sodium hydroxide |
| 63. (F3) | 1.0 M HEPES | 7.1 | Sodium hydroxide |
| 64. (F4) | 1.0 M HEPES | 7.4 | Sodium hydroxide |
| 65. (F5) | 1.0 M HEPES | 7.7 | Sodium hydroxide |
| 66. (F6) | 1.0 M TRIS hydrochloride | 7.2 | Sodium hydroxide |
| 67. (F7) | 1.0 M TRIS hydrochloride | 7.5 | Sodium hydroxide |
| 68. (F8) | 1.0 M TRIS hydrochloride | 7.8 | Sodium hydroxide |
| 69. (F9) | 1.0 M TRIS hydrochloride | 8.1 | Sodium hydroxide |
| 70. (F10) | 1.0 M Tris | 7.3 | Hydrochloric acid |
| 71. (F11) | 1.0 M Tris | 7.6 | Hydrochloric acid |
| 72. (F12) | 1.0 M Tris | 7.9 | Hydrochloric acid |
| 73. (G1) | 1.0 M Tris | 8.2 | Hydrochloric acid |
| 74. (G2) | 1.0 M Tris | 8.5 | Hydrochloric acid |
| 75. (G3) | 1.0 M Tricine | 7.4 | Sodium hydroxide |
| 76. (G4) | 1.0 M Tricine | 7.7 | Sodium hydroxide |
| 77. (G5) | 1.0 M Tricine | 8.0 | Sodium hydroxide |
| 78. (G6) | 1.0 M Tricine | 8.3 | Sodium hydroxide |
| 79. (G7) | 1.0 M Tricine | 8.6 | Sodium hydroxide |
| 80. (G8) | 1.0 M BICINE | 7.5 | Sodium hydroxide |
| 81. (G9) | 1.0 M BICINE | 7.8 | Sodium hydroxide |
| 82. (G10) | 1.0 M BICINE | 8.1 | Sodium hydroxide |
| 83. (G11) | 1.0 M BICINE | 8.4 | Sodium hydroxide |
| 84. (G12) | 1.0 M BICINE | 8.7 | Sodium hydroxide |
| 85. (H1) | 1.0 M BIS-TRIS propane | 8.5 | Hydrochloric acid |
| 86. (H2) | 1.0 M BIS-TRIS propane | 8.8 | Hydrochloric acid |
| 87. (H3) | 1.0 M BIS-TRIS propane | 9.1 | Hydrochloric acid |
| 88. (H4) | 1.0 M BIS-TRIS propane | 9.4 | Hydrochloric acid |
| 89. (H5) | 1.0 M Glycine | 8.6 | Sodium hydroxide |
| 90. (H6) | 1.0 M Glycine | 8.9 | Sodium hydroxide |
| 91. (H7) | 1.0 M Glycine | 9.2 | Sodium hydroxide |
| 92. (H8) | 1.0 M Glycine | 9.5 | Sodium hydroxide |
| 93. (H9) | 1.0 M AMPD | 8.7 | Hydrochloric acid |
| 94. (H10) | 1.0 M AMPD | 9.0 | Hydrochloric acid |
| 95. (H11) | 1.0 M AMPD | 9.3 | Hydrochloric acid |
| 96. (H12) | 1.0 M AMPD | 9.6 | Hydrochloric acid |

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)

Slice pH™ - HR2-070 Scoring Sheet

Date: **Date:** **Date:**

1. (A1) 1.0 M Citric acid pH 3.5

2. (A2) 1.0 M Citric acid pH 3.8

3. (A3) 1.0 M Citric acid pH 4.1

4. (A4) 1.0 M Citric acid pH 4.4

5. (A5) 1.0 M Sodium citrate tribasic dihydrate pH 3.6

6. (A6) 1.0 M Sodium citrate tribasic dihydrate pH 3.9

7. (A7) 1.0 M Sodium citrate tribasic dihydrate pH 4.2

8. (A8) 1.0 M Sodium citrate tribasic dihydrate pH 4.5

9. (A9) 1.0 M Sodium acetate trihydrate pH 3.7

10. (A10) 1.0 M Sodium acetate trihydrate pH 4.0

11. (A11) 1.0 M Sodium acetate trihydrate pH 4.3

12. (A12) 1.0 M Sodium acetate trihydrate pH 4.6

13. (B1) 1.0 M Sodium acetate trihydrate pH 4.9

14. (B2) 1.0 M DL-Malic acid pH 4.7

15. (B3) 1.0 M DL-Malic acid pH 5.0

16. (B4) 1.0 M DL-Malic acid pH 5.3

17. (B5) 1.0 M DL-Malic acid pH 5.6

18. (B6) 1.0 M DL-Malic acid pH 5.9

19. (B7) 1.0 M Succinic acid pH 4.8

20. (B8) 1.0 M Succinic acid pH 5.1

21. (B9) 1.0 M Succinic acid pH 5.4

22. (B10) 1.0 M Succinic acid pH 5.7

23. (B11) 1.0 M Succinic acid pH 6.0

24. (B12) 1.0 M Sodium cacodylate trihydrate pH 5.2

25. (C1) 1.0 M Sodium cacodylate trihydrate pH 5.5

26. (C2) 1.0 M Sodium cacodylate trihydrate pH 5.8

27. (C3) 1.0 M Sodium cacodylate trihydrate pH 6.1

28. (C4) 1.0 M Sodium cacodylate trihydrate pH 6.4

29. (C5) 1.0 M MES monohydrate pH 5.3

30. (C6) 1.0 M MES monohydrate pH 5.6

31. (C7) 1.0 M MES monohydrate pH 5.9

32. (C8) 1.0 M MES monohydrate pH 6.2

33. (C9) 1.0 M MES monohydrate pH 6.5

34. (C10) 1.0 M BIS-TRIS pH 5.7

35. (C11) 1.0 M BIS-TRIS pH 6.0

36. (C12) 1.0 M BIS-TRIS pH 6.3

37. (D1) 1.0 M BIS-TRIS pH 6.6

38. (D2) 1.0 M BIS-TRIS pH 6.9

39. (D3) 1.0 M ADA pH 5.8

40. (D4) 1.0 M ADA pH 6.1

41. (D5) 1.0 M ADA pH 6.4

42. (D6) 1.0 M ADA pH 6.7

43. (D7) 1.0 M ADA pH 7.0

44. (D8) 1.0 M Imidazole pH 6.2

45. (D9) 1.0 M Imidazole pH 6.5

46. (D10) 1.0 M Imidazole pH 6.8

47. (D11) 1.0 M Imidazole pH 7.1

48. (D12) 1.0 M Imidazole pH 7.4

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)

Slice pH™ - HR2-070 Scoring Sheet

Date: **Date:** **Date:**

| | | | | |
|-----------|---------------------------------|--|--|--|
| 49. (E1) | 1.0 M BIS-TRIS propane pH 6.4 | | | |
| 50. (E2) | 1.0 M BIS-TRIS propane pH 6.7 | | | |
| 51. (E3) | 1.0 M BIS-TRIS propane pH 7.0 | | | |
| 52. (E4) | 1.0 M BIS-TRIS propane pH 7.3 | | | |
| 53. (E5) | 1.0 M MOPS pH 6.5 | | | |
| 54. (E6) | 1.0 M MOPS pH 6.8 | | | |
| 55. (E7) | 1.0 M MOPS pH 7.1 | | | |
| 56. (E8) | 1.0 M MOPS pH 7.4 | | | |
| 57. (E9) | 1.0 M MOPS pH 7.7 | | | |
| 58. (E10) | 1.0 M HEPES sodium pH 6.6 | | | |
| 59. (E11) | 1.0 M HEPES sodium pH 6.9 | | | |
| 60. (E12) | 1.0 M HEPES sodium pH 7.2 | | | |
| 61. (F1) | 1.0 M HEPES sodium pH 7.5 | | | |
| 62. (F2) | 1.0 M HEPES pH 6.8 | | | |
| 63. (F3) | 1.0 M HEPES pH 7.1 | | | |
| 64. (F4) | 1.0 M HEPES pH 7.4 | | | |
| 65. (F5) | 1.0 M HEPES pH 7.7 | | | |
| 66. (F6) | 1.0 M TRIS hydrochloride pH 7.2 | | | |
| 67. (F7) | 1.0 M TRIS hydrochloride pH 7.5 | | | |
| 68. (F8) | 1.0 M TRIS hydrochloride pH 7.8 | | | |
| 69. (F9) | 1.0 M TRIS hydrochloride pH 8.1 | | | |
| 70. (F10) | 1.0 M Tris pH 7.3 | | | |
| 71. (F11) | 1.0 M Tris pH 7.6 | | | |
| 72. (F12) | 1.0 M Tris pH 7.9 | | | |
| 73. (G1) | 1.0 M Tris pH 8.2 | | | |
| 74. (G2) | 1.0 M Tris pH 8.5 | | | |
| 75. (G3) | 1.0 M Tricine pH 7.4 | | | |
| 76. (G4) | 1.0 M Tricine pH 7.7 | | | |
| 77. (G5) | 1.0 M Tricine pH 8.0 | | | |
| 78. (G6) | 1.0 M Tricine pH 8.3 | | | |
| 79. (G7) | 1.0 M Tricine pH 8.6 | | | |
| 80. (G8) | 1.0 M BICINE pH 7.5 | | | |
| 81. (G9) | 1.0 M BICINE pH 7.8 | | | |
| 82. (G10) | 1.0 M BICINE pH 8.1 | | | |
| 83. (G11) | 1.0 M BICINE pH 8.4 | | | |
| 84. (G12) | 1.0 M BICINE pH 8.7 | | | |
| 85. (H1) | 1.0 M BIS-TRIS propane pH 8.5 | | | |
| 86. (H2) | 1.0 M BIS-TRIS propane pH 8.8 | | | |
| 87. (H3) | 1.0 M BIS-TRIS propane pH 9.1 | | | |
| 88. (H4) | 1.0 M BIS-TRIS propane pH 9.4 | | | |
| 89. (H5) | 1.0 M Glycine pH 8.6 | | | |
| 90. (H6) | 1.0 M Glycine pH 8.9 | | | |
| 91. (H7) | 1.0 M Glycine pH 9.2 | | | |
| 92. (H8) | 1.0 M Glycine pH 9.5 | | | |
| 93. (H9) | 1.0 M AMPD pH 8.7 | | | |
| 94. (H10) | 1.0 M AMPD pH 9.0 | | | |
| 95. (H11) | 1.0 M AMPD pH 9.3 | | | |
| 96. (H12) | 1.0 M AMPD pH 9.6 | | | |