Slice pH™

User Guide

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. 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 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. Further, crystallization screening and optimization experiments may be more appropriately focused on the optimal pH range and buffer type.

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 citrate tribasic dihydrate
- Sodium acetate trihydrate
- DL-Malic acid
- Succinic acid
- Sodium cacodylate trihydrate
- MES monohydrate
- BIS-TRIS
- ADA
- Imidazole
- BIS-TRIS propane
- MOPS
- HEPES sodium
- HEPES
- TRIS hydrochloride
- Tris
- Tricine
- BICINE
- 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 made 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 supersaturation 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™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 thereafter. 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
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. 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 and 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>
<thead>
<tr>
<th>Table 1. Solubility and Stability Challenges</th>
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<tr>
<td>Elevated Temperature</td>
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<td>Chemical Compatibility</td>
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<th>Table 2. Diagnostic Assays</th>
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<tr>
<td>Methodology:</td>
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<td>Size Exclusion Chromatography</td>
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<td>Enzyme Assay</td>
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<td>Total Protein Assay (UV, Bradford, BCA)</td>
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<td>Fluorescence</td>
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<td>Filter Plate</td>
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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


**Related Products**

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Description</th>
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<tbody>
<tr>
<td>HR2-134</td>
<td>Index HT™, 1 ml Deep Well Block Format</td>
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<tr>
<td>HR2-636</td>
<td>3.0 M Sodium chloride, 200 ml</td>
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<tr>
<td>HR2-638</td>
<td>2.0 M Sodium chloride, 200 ml</td>
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<tr>
<td>HR8-069</td>
<td>AlumaSeal II™ Sealing Film, 100 pack</td>
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</table>

**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.

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**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:
  a) 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
  b) 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).
**Slice pH™**

**pH Case Study: Thermolysin**

- Thermolysin, 20 mg/ml in deionized water/NaOH. Drop: 0.5 μl Thermolysin + 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: 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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◊ Buffer pH is that of a 1.0 M stock prior to dilution. pH titrated with HCl or NaOH.
### Slice pH™ - HR2-070 Scoring Sheet

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**Slice pH™ - HR2-070 Scoring Sheet**

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