Solubility & Stability Screen

HAMPTON RESEARCH
Solutions for Crystal Growth

Application

Solubility & Stability Screen is designed to assist in the identification of solution conditions which promote protein solubility and stability, and minimize protein precipitation. Solubility & Stability Screen is a solubility screen, a stability screen, and may also be used as an additive screen in the presence of a crystallization reagent.

Discussion

Protein solubility and stability are universally required in a wide range of applications, including general biochemical studies, the preparation of proteins in pharmaceuticals, structural biology and crystallization.[1] The preparation of a concentrated, soluble and stable protein sample can often be a difficult task as proteins often aggregate, precipitate or denature.

Protein solubility and stability is affected by many different chemical factors including pH, buffer type and chemical additives. pH and buffer type are dominant protein solubility and stability variables and can be evaluated and optimized using the Hampton Research Slice pH kit (HR2-070). Slice pH evaluates protein solubility, stability and crystallization versus 20 different buffers over the pH range 3.5-9.6. Chemical additives influencing protein solubility and stability can be evaluated using the Solubility & Stability Screen.

It is widely accepted that protein solubility and stability can be increased by the use of chemical additives.[5,15] The classes of reagents in Table 1 are sampled by Solubility & Stability Screen and each of these classes has been reported as important in improving sample solubility and stability.[2-11]

Table 1. Solubility & Stability Screen reagents

- Amino acid / Derivative
- Chaotrope
- Chelator
- Cyclodextrin
- Inhibitor
- Ionic liquid
- Linker
- Metal
- Non detergent sulfobetaine
- Organic acid
- Osmolyte
- Peptide
- Polyamine
- Polymer
- Polyol
- Polyol and Salt
- Salt

The Hampton Research Solubility and Stability screen can evaluate protein solubility, stability and crystallization in the presence 94 different chemical additives sampling 17 different classes of reagents plus two controls.

Features

The Solubility & Stability Screen is a set of 94 high purity reagents formulated in high purity water (NCCLS/ASTM Type 1+) at 25°C and are 0.22 micron sterile filtered. The 94 Solubility & Stability reagents are formulated at 2 to 10 times their recommended working concentration. The remaining two reagents are water and a negative (TCA) control. A water control demonstrates the effect of diluting sample as well as sample buffer concentration. TCA, the negative control, demonstrates total sample precipitation, loss of sample solubility and loss of sample stability. The effects of the Solubility & Stability reagents can be compared with this negative control to assist in the identification of reagents promoting sample solubility and stability. 500 microliters of each reagent is supplied in a sterile 96 well polypropylene deep well block. The Solubility & Stability Screen reagents are compatible with the sitting or hanging drop vapor diffusion, microbatch, free interface diffusion, sandwich drop vapor diffusion, and dialysis crystallization methods utilizing water soluble reagents.

Using Solubility & Stability Screen as an Additive Screen

The Solubility and Stability Screen may be utilized as an additive screen in conjunction with a crystallization reagent. The Screen can be used as part of an optimization strategy with a crystallization reagent that is producing crystals that require further optimization. Additionally, the Solubility & Stability Screen can be used with crystallization reagents that produce promising or interesting precipitates or phase separation. In each instance, the Solubility & Stability Screen is assayed to see if one or more of the reagents will manipulate sample-sample and/or sample-solvent interactions and alter or enhance sample solubility towards improving or promoting crystallization of the sample.

The following describes the use of the Solubility & Stability Screen using the Sitting Drop Vapor Diffusion method and a 50 microliter reservoir volume. Other crystallization methods may also be utilized as well as different reservoir, drop volumes and drop ratios.

Reservoir

1. Pipet 5 microliters of the Solubility & Stability Screen into the reservoir.
2. Pipet and mix 45 microliters of crystallization reagent into the reservoir.

Drop

3. Pipet 200 nanoliters of sample into the sample well.
4. Pipet 200 nanoliters of the crystallization reagent/Solubility & Stability Screen mixture from the reservoir into the sample drop.
5. Repeat for the remaining reagents.
6. Seal the plate.

Examine the Drop

Carefully examine the drops under a stereo microscope (10 to 100x magnification) immediately after setting up 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 there after. 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, 2+ small bipyramid crystals, clear drop, 3+ needle shaped crystals in 1+ white precipitate. One may also employ a standard numerical scoring scheme (Clear = 0, Precipitate = 1, Crystal = 10, etc). Figure 1 shows typical examples of what one might observe in a crystallization experiment.

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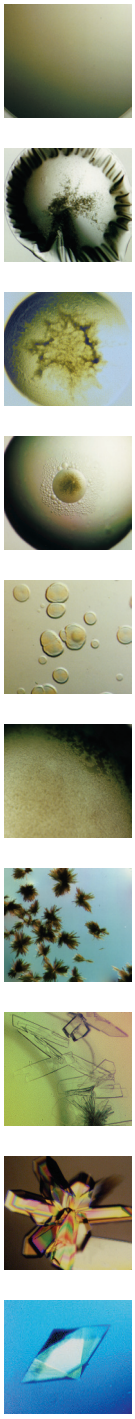
Figure 1
Typical observations in a crystallization experiment



Using Solubility & Stability Screen with Thermofluor® Assay

Thermofluor provides a fluorescence readout measurement of thermally-induced protein melting.[16] The thermofluor assay can be used towards the optimization of solution conditions (pH, ionic strength, solubility & stability reagents, and additives) for protein stabilization, protein preparation and biochemistry, and optimization of crystallization conditions (Figure 2).[12-14, 16-19]

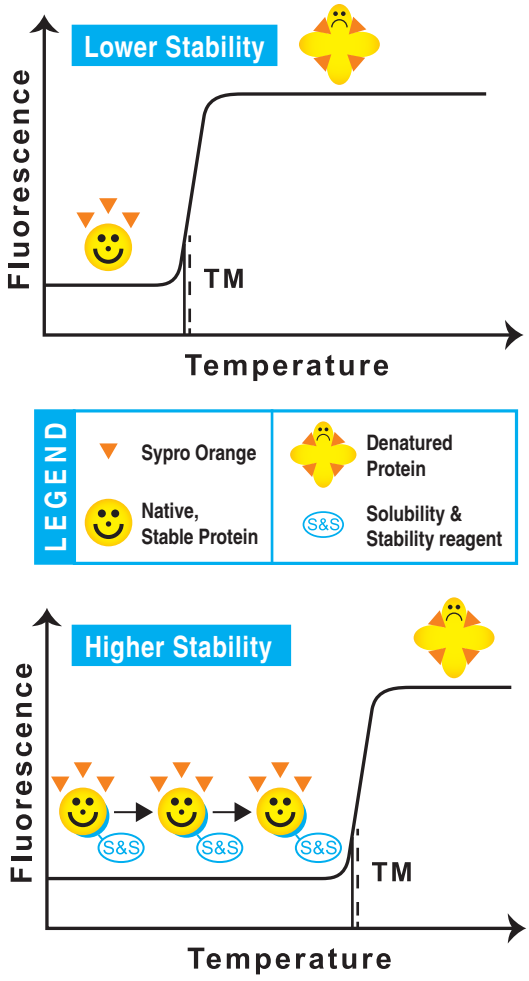


Figure 2. Thermofluor Principle.
The protein in solution is heated in the presence of Solubility & Stability reagent and Sypro® Orange. The fluorescence of the hydrophobic dye Sypro Orange increases significantly when the dye binds to the internal hydrophobic protein patches that become exposed upon protein denaturation. Protein stability, or melting temperature (Tm) can be measured by analyzing the temperature dependence of protein denaturation and subsequent increase in fluorescence intensity. Addition of ligands such as Solubility & Stability Screen reagents that preferentially bind to and stabilize the native protein can by identified by the measured increased Tm.

Thermofluor Assay for Solubility & Stability Buffer Optimization

1. Add 20 µl of 10 mg/ml protein to 1.2 ml sample buffer in a microcentrifuge tube. Add 1.2 µl Sypro orange dye. Protein concentration of this solution is ~ 0.15 mg/ml with Sypro orange dye diluted ~ 1:1000.

Note: If you have not yet identified an appropriate sample buffer try 100 mM HEPES, 150 mM NaCl, pH 7.5.

Note: Based on the initial protein concentration, adjust the amount of protein added so that the concentration at the end of Step 1 is approximately 0.15 mg/ml.

2. Pipette 10 µl of the protein-Sypro orange dye solution to each well of a 96 well qPCR microplate.
3. Add 10 µl of each of the 96 Solubility & Stability Screen reagents to the corresponding well of the 96 well qPCR microplate (Clear, non-skirted, low profile, optical reading-compatible).
4. Seal the plate with transparent sealing film.
5. Centrifuge the 96 well microplate for 1 minute at 500xg at 25 degrees Celsius.
6. Perform the Thermofluor assay and analyze the data to identify the reagents that promote sample stability. [13]

Note: In addition to the Solubility & Stability Screen also consider performing the Thermofluor Assay with the Additive Screen HT (HR2-138), Silver Bullets HT (HR2-096), Silver Bullets Bio HT (HR2-088) and Slice pH (HR2-070).

Using the Solubility & Stability Screen as a Solubilization Assay

Precipitated protein can contain reversibly aggregated protein that can be brought back into solution using pH and buffer type (Slice pH HR2-070) as well as reagents found in the Solubility & Stability Screen.

1. Pipette 50 µl of each of the 96 Solubility & Stability Screen reagents into each the respective reservoirs of a 96 well sitting drop crystallization plate.
2. Pipette 200 nl of protein sample to each of the 96 drop wells.
3. Pipette 200 nl of each of the respective 96 Solubility & Screen reagents from the reservoir to the drop.
4. Seal the plate with optically clear tape or film.
5. Observe and score each drop for the presence or absence of precipitate immediately after setting the plate. Observe and score each drop again for the presence or absence of precipitate after 24 hours. Use the Solubility & Stability Screen

reagent formulation to identify reagents that yield clear drops (soluble protein). Transfer sample into a new sample buffer containing the identified Solubility & Stability reagent.

Note: Remember that reagent A1 is a water control and A2 is a total precipitate control (15% v/v TCA)

Note: To identify buffers and pH that promote solubility use the Hampton Research Slice pH Screen (HR2-070).

Using Solubility & Stability Screen with Other Solubility and Stability Challenges

A variety of challenges to sample solubility and stability can be utilized with Solubility & Stability Screen, including but not limited to those in Table 2. Each of these challenges can be assayed by a variety of diagnostic methods shown in Table 3.

When using Solubility & Stability Screen with Dynamic Light Scattering (DLS) as a diagnostic assay, look for ideal sample monodispersity (radius <5 nm and polydispersity <25%). If the Solubility & Stability reagents do not produce ideal sample monodispersity, run the sample with Slice pH (HR2-070) to identify the optimal pH and buffer type for sample monodispersity. Then exchange the sample into the optimal pH and buffer, concentrate the sample to 2 mg/ml or higher and then repeat the DLS with Solubility & Stability Screen to identify the reagent that promotes ideal monodispersity of the sample.

Table 2. 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 3. 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 12 months of receipt. Store between minus 20 and 4°C. Allow reagents to equilibrate to the room temperature before use.

For research use only.

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Related Products

HR2-070 Slice pH

HR2-088 Silver Bullets Bio HT

HR2-096 Silver Bullets HT

HR2-138 Additive Screen HT

Technical Support

Inquiries regarding Solubility & Stability Screen 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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