Solubility & Stability Screen 2™

HAMPTON RESEARCH
Solutions for Crystal Growth

User Guide **HR2-413 (pg 1)**

Application

Solubility & Stability Screen 2 is designed to assist in the identification of optimal buffer, pH and ionic strength formulations that promote protein solubility and stability.

Features

- Compatible with ThermoFluor (Differential Scanning Fluorimetry, DSF, Protein Thermal Shift) and Dynamic Light Scattering assays
- Formulation of 12 unique buffers, pH 4.5 - 9.5, versus 8 levels of ionic strength
- Evaluate varying ionic strength in the presence and absence of buffer
- Evaluate varying buffer type and pH in different levels of ionic strength
- 96 sterile filtered reagents
- Concentrated reagent formulation

Methodologies

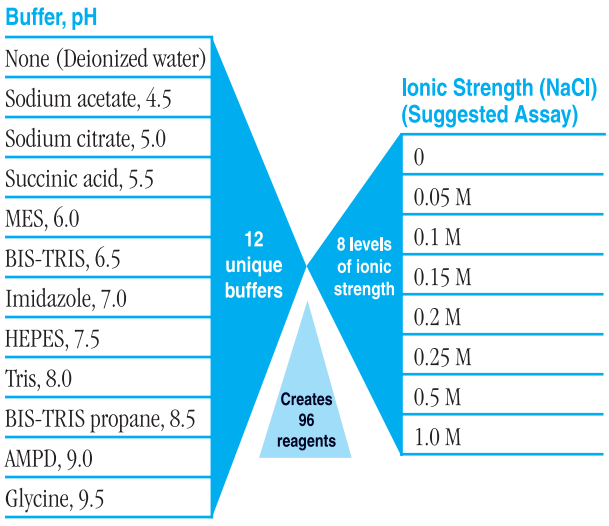
- Solubility assays using Solubility & Stability Screen 2 with Dynamic Light Scattering
- Stability assays using Solubility & Stability Screen 2 with Thermofluor

Discussion

Protein solubility and stability are universally required in a wide range of applications, including general biochemical studies, the preparation of proteins in diagnostics and 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, ionic strength and protein specific additives. pH, buffer type and ionic strength are dominant protein solubility and stability variables that can be evaluated and optimized using the Solubility & Stability Screen 2.

Table 1. Solubility & Stability Screen 2 Variables



It is widely accepted that protein solubility and stability can be maximized by optimizing buffer, pH, ionic strength as well as identifying protein specific additives that may promote protein stability.[5,15] The buffer, pH and ionic strength variables in Table 1 are sampled by Solubility & Stability Screen 2. Each of these variables has been reported as important in improving sample stability and solubility.[2-11, 20-22]

- **Assaying for Optimal Buffer Formulation**

No clear correlation between intrinsic properties of proteins and solubility and stability exist, so systematic screening can help to identify optimal sample buffer conditions. Protein solubility and stability screening, performed using Dynamic Light Scattering and ThermoFluor assays together with the Solubility and Stability Screens and Slice pH can be an extremely data rich, informative, efficient, and cost-effective method for the identification of sample buffer conditions that maximally stabilize a protein for protein purification, formulation, crystallization, and functional characterization.

Dynamic Light Scattering (DLS) is an established technique for determining the size, monodispersity and polydispersity of proteins in solution. DLS with a plate reader allows a multitude of buffers, pH, ionic strength, protein specific additives and temperature to be screened. Combining the Solubility & Stability Screens with DLS, particle size and size distribution, unfolding of proteins, crystallizability, thermal stability, aggregation and solubility behavior can be evaluated in a high throughput format.

The ThermoFluor assay is an established technique for assessing protein thermostability in solution. ThermoFluor allows systematic assessment of many reagents simultaneously, uses only a small amount of protein, and is accessible to anyone with a real-time PCR instrument.

Once an optimal buffer, pH and ionic strength is identified, the sample can be exchanged into the new optimal buffer. Using this optimized sample, further ThermoFluor assays with the Solubility & Stability Screen™, Silver Bullets™, Silver Bullets Bio™ and Additive Screen™ can be performed to identify protein specific additives that further promote solubility and stability of the protein.

- **Buffer Optimization for Crystallization**

Solubility & Stability Screen 2 can be used to optimize the protein's sample buffer prior to crystallization screening. Finding buffer formulations that optimally solubilize and stabilize proteins in solution can aid the formation of crystals for X-ray structure determination, since crystallization involves the assembly of a three dimensional lattice from structurally identical objects. Proteins are intrinsically dynamic structures that can undergo conformational flexibility as a result of thermal fluctuations. Finding buffer formulations that maximize protein solubility and thermal stability has the effect of reducing the conformational heterogeneity of the protein, leading to improved crystal formation.[17,18,25]

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Formulation

Solubility & Stability Screen 2 is a set of 96 high purity, sterile filtered (0.22 micron) reagents formulated in high purity water (NCCLS/ASTM Type 1+) at 25°C. The concentrated reagent formulation provides the means for evaluating a wide range of buffer concentration and ionic strength when diluting the reagents between 1:1 and 1:10 in the assay. The formulation pH is the final, actual measured pH at 25°C. Buffered reagents are titrated with NaOH or HCl. The water control and reagents without buffer are not titrated. The measured pH range of the eight unbuffered reagents A1-H1 is 5.6 - 6.2. 500 microliters of each reagent is supplied in a sterile 96 well polypropylene deep well block. The reagent block is sealed with a peelable aluminum polypropylene composite thermal film. Equilibrate the reagents and block to room temperature before peeling back the film for complete seal removal. After removing the thermal film, the block can be sealed with AlumaSeal II film. Two AlumaSeal II films are supplied with Solubility & Stability Screen 2. Additional AlumaSeal II films (HR8-069) are available from Hampton Research.

Using Solubility & Stability Screen 2 with the ThermoFluor Assay

The ThermoFluor assay provides a fluorescence readout measurement of thermally-induced protein melting.[16,24] A ThermoFluor assay with the protein in the presence of varying buffer, pH and ionic strength can be used to help in the identification of sample buffer formulations that increase Tm and relative stability of the protein. (Figure 2).[12-14, 16-19]

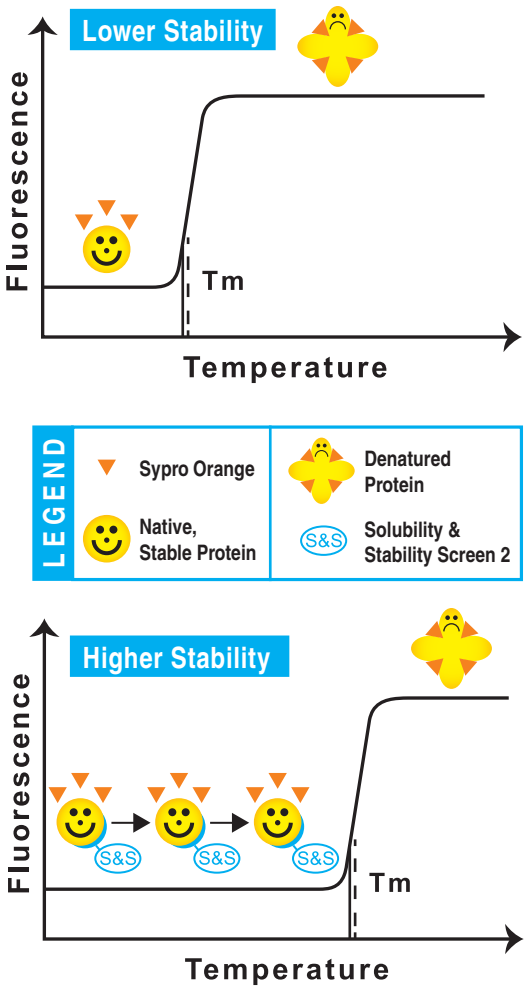
Sample Preparation for ThermoFluor

Sample purity should be 75% or greater for reliable ThermoFluor experiments. It is desirable to remove affinity tags from the protein sample before ThermoFluor. Tags can have high dynamic structure and destabilize the protein during ThermoFluor or have their own contribution to the melting curve. A tag can decrease protein thermal stability as well as complicate ThermoFluor data and interpretation of the results

The combined salt and buffer concentration in the sample prior to adding water, Solubility & Stability Screen 2 reagent and Sypro Orange should be no more than 0.2 M and of neutral pH. The sample is diluted by a factor of 10 in the ThermoFluor assay described in this user guide and ideally the concentration of chemicals in the sample should be lower than that employed in the Solubility & Stability Screen 2 assay. Higher concentrations of sample buffer or elution agents (Imidazole) can alter and interfere with the pH and ionic strength of the experiment. If possible, avoid glycerol, reducing agents, or detergents since these chemicals can influence protein stability and at high concentrations interfere with fluorescence measurements. If the use of glycerol, reducing agents or detergents is necessary, use the lowest possible concentration and run additional ThermoFluor assays, varying the concentration of these additives to study their influence on the assay.

Figure 2. ThermoFluor Principle

The protein in solution is heated in the presence of Solubility & Stability Screen 2 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. Solubility & Stability Screen 2 reagents that stabilize or destabilize the protein can be identified by the measured increased or decreased Tm.



ThermoFluor Assay for Buffer Optimization

ThermoFluor Assay for Solubility & Stability Screen 2 with a final well volume of 20 µL. With the PCR plate on ice, add the following to each well.

1. Pipette 2 µL of 1 mg/mL protein per well (2 µg of protein per well).
2. Pipette 11 µL of deionized water per well.
3. Pipette 5 µL of Solubility & Stability Screen 2 per well.*
4. Pipette 2 µL of 50x Sypro Orange per well. ***Add this last*** (Note: To make 200 µl of 50x Sypro Orange add 2µl of 5000x Sypro Orange to 198 µl of deionized water.)

5. Repeat 1-4 for each well of the PCR reaction plate.
6. Seal the PCR reaction plate with transparent sealing film.
7. Centrifuge the PCR reaction plate at 4°C at 2,500 g for 30 seconds to bring all of the solution to the bottom of the well and to rid each well of any bubbles.
8. Place the sealed plate in the RT-PCR instrument equilibrated to 4°C or 25°C, programmed for a 5 minute equilibration in order for the temperature of the plate and reagents to equilibrate with the block and as well as allow the reagents to diffuse and mix.
9. Perform the ThermoFluor assay from either 4 to 95°C or 25 to 95°C with a temperature ramp of 1°C per minute.

* Step 3 is a 1:4 dilution, generating the Suggested Assay Concentration formulation. Other dilutions can and should be assayed to find buffer concentration and ionic strength. Analyze the ThermoFluor data to identify the optimal buffer environment for the sample.[20-22] Exchange the sample into the optimized buffer. Using this optimized sample, perform the ThermoFluor assay with the Solubility & Stability Screen, Silver Bullets, Silver Bullets Bio and Additive Screen to identify protein specific additives that stabilize the protein in the presence of the optimized buffer.[22]

Dynamic Light Scattering Assay for Buffer Optimization

DLS measurements can be performed on any single or multi-angle light scattering instrument. The volume required will depend upon how the instrument is equipped as well as the type and volume of cuvette or plate. The minimum protein concentration required for a good DLS signal will depend upon the molecular weight of the protein. An often mentioned concentration is 1 mg/ml, however a protein of 15 kD might perform well at 3 mg/ml, a 100 kd protein at 0.5 mg/ml. Follow the manufacturer's recommendation for setting up the instrument, temperature options and general experimental protocol. For the DLS assay using the Solubility & Stability Screen 2 reagents, the suggested initial assay concentration is to dilute the reagents 1:4. For example, if your assay volume is 4 µl, add 1 µl of reagent to 3 µl of protein. If the assay volume is 40 µl, add 10 µl of reagent to 30 µl of protein. To evaluate the effect of varying buffer concentration and ionic strength, consider screening multiple dilutions, between 1:1 and 1:10. If possible, perform measurements at temperatures between 4 - 37°C as the dispersity profile can vary with temperature. Evaluate the data to identify reagents that promote a monidisperse DLS profile. In general, good sample monodispersity is a radius <5 nm and polydispersity <25%. Exchange the sample into the optimal buffer. Using this optimized sample, perform the DLS assay with the Solubility & Stability Screen, Silver Bullets, Silver Bullets Bio and Additive Screen to identify protein specific additives that produce a monodisperse DLS profile.[26-28]

Using Solubility & Stability Screen 2 in Other Assays

A variety of challenges to sample stability and solubility can be utilized with Solubility & Stability Screen 2, 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.

Table 2. Solubility and Stability Challenges

Elevated Temperature	Incubate 24 hours at 37° Celsius
Temperature Cycling	Freeze & thaw or warm & cool multiple times

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
HR2-072	Solubility & Stability Screen
HR2-088	Silver Bullets Bio HT
HR2-096	Silver Bullets HT
HR2-138	Additive Screen HT
HR4-525	Sealing Film Applicator, each
HR8-069	AlumaSeal II Sealing Film, 100 pack

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