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.

Solubility & Stability Screen

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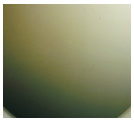
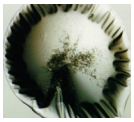
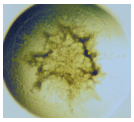
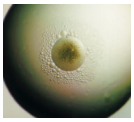
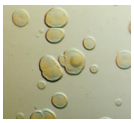
Figure 1
Typical observations in a crystallization experiment

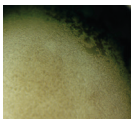

Clear Drop


Skin / Precipitate

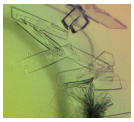

Precipitate


Precipitate / Phase

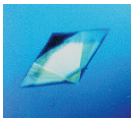

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

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]

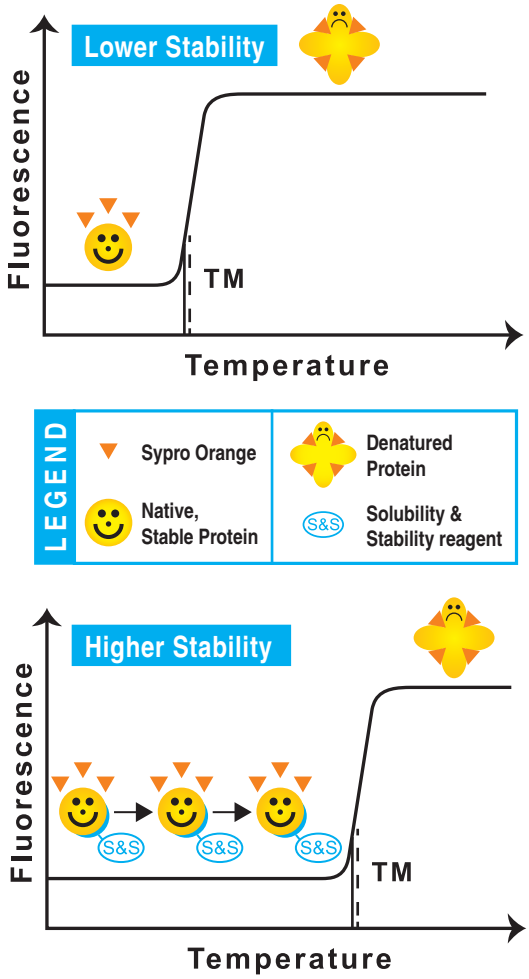


Figure 2. Thermofluor Principle.
The protein in solution is heated in the presence of Solubility & Stability reagent and Sypro® Orange. The fluorescence of the hydrophobic dye Sypro Orange increases significantly when the dye binds to the internal hydrophobic protein patches that become exposed upon 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

HR2-096 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 4:00 p.m. USA Pacific Standard Time.

SYPRO® is a registered trademark of Molecular Probes, Inc.
ThermoFluor® is a registered trademark of Johnson & Johnson.

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Well #	Chemical	[Assay]	[Formulation]	M_r	Type
1. (A1)	Water (Type 1+ ultrapure grade water)	100% v/v	100% v/v	18.02	Water Control
2. (A2)	Trichloroacetic acid	15% v/v	75% v/v	163.39	Precipitate Control
3. (A3)	L-Arginine	25-125 mM	250 mM	174.20	Amino Acid / Derivative
4. (A4)	L-Arginine, L-Glutamic acid	25-125 mM	250 mM / 250 mM	174.20 / 147.13	Amino Acid / Derivative
5. (A5)	Glycine	50-250 mM	500 mM	75.07	Amino Acid / Derivative
6. (A6)	L-Proline	50-250 mM	500 mM	115.13	Amino Acid / Derivative
7. (A7)	L-Histidine	12-60 mM	120 mM	155.16	Amino Acid / Derivative
8. (A8)	β-Alanine	50-250 mM	500 mM	89.09	Amino Acid / Derivative
9. (A9)	L-Serine	50-250 mM	500 mM	105.09	Amino Acid / Derivative
10. (A10)	L-Arginine ethyl ester dihydrochloride	50-250 mM	500 mM	275.18	Amino Acid / Derivative
11. (A11)	L-Argininamide dihydrochloride	50-250 mM	500 mM	246.14	Amino Acid / Derivative
12. (A12)	6-Aminohexanoic acid	50-250 mM	500 mM	131.18	Amino Acid / Derivative
13. (B1)	Gly-gly	50-250 mM	500 mM	132.12	Peptide
14. (B2)	Gly-gly-gly	20-100 mM	200 mM	189.17	Peptide
15. (B3)	Tryptone	0.5-2.5% w/v	5% w/v	N/A	Peptide
16. (B4)	Betaine monohydrate	250-1,250 mM	2,500 mM	135.16	Osmolyte
17. (B5)	D-(+)-Trehalose dihydrate	75-375 mM	750 mM	378.33	Osmolyte
18. (B6)	Xylitol	200-1,000 mM	2,000 mM	152.15	Osmolyte
19. (B7)	D-Sorbitol	200-1,000 mM	2,000 mM	182.17	Osmolyte
20. (B8)	Sucrose	200-1,000 mM	2,000 mM	342.30	Osmolyte
21. (B9)	Hydroxyectoine	50-250 mM	500 mM	158.16	Osmolyte
22. (B10)	Trimethylamine N-oxide dihydrate	250-1,250 mM	2,500 mM	111.14	Osmolyte
23. (B11)	Methyl α-D-glucopyranoside	200-1,000 mM	2,000 mM	194.18	Osmolyte
24. (B12)	Triethylene glycol	1-5% v/v	10% v/v	150.19	Osmolyte
25. (C1)	Spermine tetrahydrochloride	50-250 mM	500 mM	348.19	Polyamine
26. (C2)	Spermidine	50-250 mM	500 mM	145.25	Polyamine
27. (C3)	5-Aminovaleric acid	50-250 mM	500 mM	117.15	Linker
28. (C4)	Glutaric acid	50-250 mM	500 mM	132.12	Linker
29. (C5)	Adipic acid	8-40 mM	80 mM	146.14	Linker
30. (C6)	Ethylenediamine dihydrochloride	50-250 mM	500 mM	133.02	Linker
31. (C7)	Guanidine hydrochloride	50-250 mM	500 mM	95.53	Chaotrope
32. (C8)	Urea	50-250 mM	500 mM	60.06	Chaotrope
33. (C9)	N-Methylurea	50-250 mM	500 mM	74.08	Chaotrope
34. (C10)	N-Ethylurea	20-100 mM	200 mM	88.11	Chaotrope
35. (C11)	N-Methylformamide	3-15% w/v	30% w/v	59.07	Chaotrope
36. (C12)	Hypotaurine	0.3-1.5% w/v	3% w/v	109.15	Chaotrope
37. (D1)	TCEP hydrochloride	15-75 mM	150 mM	286.65	Reducing Agent
38. (D2)	GSH (L-Glutathione reduced), GSSG (L-Glutathione oxidized)	2-10 mM	20 mM / 20 mM	307.33 / 612.64	Reducing Agent
39. (D3)	Benzamidine hydrochloride	0.5-2.5% w/v	5% w/v	156.62	Inhibitor
40. (D4)	Ethylenediaminetetraacetic acid disodium salt dihydrate	5-25 mM	50 mM	372.24	Chelator
41. (D5)	Magnesium chloride hexahydrate, Calcium chloride dihydrate	10-50 mM	100 mM / 100 mM	203.30 / 147.01	Metal
42. (D6)	Cadmium chloride hydrate, Cobalt(II) chloride hexahydrate	10-50 mM	100 mM / 100 mM	183.32 / 237.93	Metal
43. (D7)	Non Detergent Sulfobetaine 195 (NDSB-195)	100-500 mM	1,000 mM	195.30	Non Detergent
44. (D8)	Non Detergent Sulfobetaine 201 (NDSB-201)	100-500 mM	1,000 mM	201.26	Non Detergent
45. (D9)	Non Detergent Sulfobetaine 211 (NDSB-211)	100-500 mM	1,000 mM	211.30	Non Detergent
46. (D10)	Non Detergent Sulfobetaine 221 (NDSB-221)	100-500 mM	1,000 mM	221.34	Non Detergent
47. (D11)	Non Detergent Sulfobetaine 256 (NDSB-256)	80-400 mM	800 mM	257.37	Non Detergent
48. (D12)	Taurine	50-250 mM	500 mM	125.15	Organic Acid

Reagents formulated in Type 1+ ultrapure grade water, no pH adjustment.

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Well #	Chemical	[Assay]	[Formulation]	M_r	Type
49. (E1)	Acetamide	50-250 mM	500 mM	59.07	Organic Acid
50. (E2)	Oxalic acid dihydrate	50-250 mM	500 mM	126.07	Organic Acid
51. (E3)	Sodium malonate pH 7.0	50-250 mM	500 mM	104.06	Organic Acid
52. (E4)	Succinic acid pH 7.0	50-250 mM	500 mM	118.09	Organic Acid
53. (E5)	Tacsimate pH 7.0	0.5-2.5% v/v	5% v/v	N/A	Organic Acid
54. (E6)	Tetraethylammonium bromide	2.5-12.5% w/v	25% w/v	210.16	Ionic Liquid
55. (E7)	Cholin acetate	2.5-12.5% w/v	25% w/v	163.22	Ionic Liquid
56. (E8)	1-Ethyl-3-methylimidazolium acetate	2.5-12.5% w/v	25% w/v	170.21	Ionic Liquid
57. (E9)	1-Butyl-3-methylimidazolium chloride	2.5-12.5% w/v	25% w/v	174.67	Ionic Liquid
58. (E10)	Ethylammonium nitrate	2.5-12.5% w/v	25% w/v	108.11	Ionic Liquid
59. (E11)	Ammonium sulfate	50-250 mM	500 mM	132.14	Salt
60. (E12)	Ammonium chloride	50-250 mM	500 mM	53.49	Salt
61. (F1)	Magnesium sulfate hydrate	50-250 mM	500 mM	120.37	Salt
62. (F2)	Potassium thiocyanate	50-250 mM	500 mM	97.18	Salt
63. (F3)	Gadolinium(III) chloride hexahydrate	25-125 mM	250 mM	371.70	Salt
64. (F4)	Cesium chloride	25-125 mM	250 mM	168.36	Salt
65. (F5)	4-Aminobutyric acid (GABA)	25-125 mM	250 mM	103.12	Salt
66. (F6)	Lithium nitrate	50-250 mM	500 mM	68.95	Salt
67. (F7)	DL-Malic acid pH 7.0	50-250 mM	500 mM	134.09	Salt
68. (F8)	Lithium citrate tribasic tetrahydrate	50-250 mM	500 mM	281.99	Salt
69. (F9)	Ammonium acetate	25-125 mM	250 mM	77.08	Salt
70. (F10)	Sodium benzenesulfonate	25-125 mM	250 mM	180.16	Salt
71. (F11)	Sodium p-toluenesulfonate	25-125 mM	250 mM	194.18	Salt
72. (F12)	Sodium chloride	100-500 mM	1,000 mM	58.44	Salt
73. (G1)	Potassium chloride	140-700 mM	1,400 mM	74.55	Salt
74. (G2)	Sodium phosphate monobasic monohydrate, Potassium phosphate dibasic	70-350 mM / 130-650 mM	700 mM / 1,300 mM	137.99 / 174.18	Salt
75. (G3)	Sodium sulfate decahydrate	100-500 mM	1,000 mM	322.20	Salt
76. (G4)	Lithium chloride	140-700 mM	1,400 mM	42.39	Salt
77. (G5)	Sodium bromide	100-500 mM	1,000 mM	102.89	Salt
78. (G6)	Glycerol, Lithium chloride	4-20% v/v / 40-200 mM	40% v/v / 400 mM	92.09 / 42.39	Polyol and Salt
79. (G7)	Glycerol	5-25% v/v	50% v/v	92.09	Polyol
80. (G8)	Ethylene glycol	1-5% v/v	10% v/v	62.07	Polyol
81. (G9)	Polyethylene glycol 200	1-5% v/v	10% v/v	~ 200	Polyol
82. (G10)	Polyethylene glycol monomethyl ether 550	0.5-2.5% v/v	5% v/v	~ 550	Polyol
83. (G11)	Polyethylene glycol monomethyl ether 750	0.5-2.5% w/v	5% w/v	~ 750	Polyol
84. (G12)	Formamide	5-25% v/v	50% v/v	45.04	Polyol
85. (H1)	Polyethylene glycol 400	1-5% v/v	10% v/v	380 - 420	Polyol
86. (H2)	Polyethylene glycol 600	1-5% w/v	10% w/v	570 - 630	Polyol
87. (H3)	1,2-Propanediol	1-5% w/v	10% w/v	76.09	Polyol
88. (H4)	Polyethylene glycol monomethyl ether 1,900	0.3-1.5% w/v	3% w/v	~ 1,900	Polymer
89. (H5)	Polyethylene glycol 3,350	0.3-1.5% w/v	3% w/v	3,300 - 3,400	Polymer
90. (H6)	Polyethylene glycol 8,000	0.3-1.5% w/v	3% w/v	7,000 - 9,000	Polymer
91. (H7)	Polyvinylpyrrolidone K 15	0.2-1% w/v	2% w/v	~ 10,000	Polymer
92. (H8)	Polyethylene glycol 20,000	0.2-1% w/v	2% w/v	~ 20,000	Cyclodextrin
93. (H9)	(2-Hydroxypropyl)-β-cyclodextrin	1-5 mM	10 mM	1,396	Cyclodextrin
94. (H10)	α-Cyclodextrin	8-40 mM	80 mM	972.86	Cyclodextrin
95. (H11)	β-Cyclodextrin	1-5 mM	10 mM	1,134.98	Cyclodextrin
96. (H12)	Methyl-β-cyclodextrin	5-25 mM	50 mM	1,320	Cyclodextrin

34 Journey
Aliso Viejo, CA 92656-3317 U.S.A.
Tel: (949) 425-1321 • Fax: (949) 425-1611
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Website: hamptonresearch.com

Reagents formulated in Type 1+ ultrapure grade water, no pH adjustment.

HAMPTON RESEARCH
Solutions for Crystal Growth

Sample: _____ Sample Concentration: _____

Sample Buffer: _____ Date: _____

Reservoir Volume: _____ Temperature: _____

Drop Volume: Total _____ µl Sample _____ µl Reservoir _____ µl Additive _____ µl

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)

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Solubility and Stability Screen - HR2-072 Scoring Sheet		Type:	Date:	Date:	Date:
1. (A1)	100% v/v Water (Type 1+ ultrapure grade water)	Water Control			
2. (A2)	75% v/v Trichloroacetic acid	Precipitate Control			
3. (A3)	250 mM L-Arginine	Amino Acid / Derivative			
4. (A4)	250 mM L-Arginine, 250 mM L-Glutamic acid	Amino Acid / Derivative			
5. (A5)	500 mM Glycine	Amino Acid / Derivative			
6. (A6)	500 mM L-Proline	Amino Acid / Derivative			
7. (A7)	120 mM L-Histidine	Amino Acid / Derivative			
8. (A8)	500 mM β-Alanine	Amino Acid / Derivative			
9. (A9)	500 mM L-Serine	Amino Acid / Derivative			
10. (A10)	500 mM L-Arginine ethyl ester dihydrochloride	Amino Acid / Derivative			
11. (A11)	500 mM L-Argininamide dihydrochloride	Amino Acid / Derivative			
12. (A12)	500 mM 6-Aminohexanoic acid	Amino Acid / Derivative			
13. (B1)	500 mM Gly-gly	Peptide			
14. (B2)	200 mM Gly-gly-gly	Peptide			
15. (B3)	5% w/v Tryptone	Peptide			
16. (B4)	2,500 mM Betaine monohydrate	Osmolyte			
17. (B5)	750 mM D-(+)-Trehalose dihydrate	Osmolyte			
18. (B6)	2,000 mM Xylitol	Osmolyte			
19. (B7)	2,000 mM D-Sorbitol	Osmolyte			
20. (B8)	2,000 mM Sucrose	Osmolyte			
21. (B9)	500 mM Hydroxyectoine	Osmolyte			
22. (B10)	2,500 mM Trimethylamine N-oxide dihydrate	Osmolyte			
23. (B11)	2,000 mM Methyl α-D-glucopyranoside	Osmolyte			
24. (B12)	10% v/v Triethylene glycol	Osmolyte			
25. (C1)	500 mM Spermine tetrahydrochloride	Polyamine			
26. (C2)	500 mM Spermidine	Polyamine			
27. (C3)	500 mM 5-Aminovaleric acid	Linker			
28. (C4)	500 mM Glutaric acid	Linker			
29. (C5)	80 mM Adipic acid	Linker			
30. (C6)	500 mM Ethylenediamine dihydrochloride	Linker			
31. (C7)	500 mM Guanidine hydrochloride	Chaotrope			
32. (C8)	500 mM Urea	Chaotrope			
33. (C9)	500 mM N-Methylurea	Chaotrope			
34. (C10)	200 mM N-Ethylurea	Chaotrope			
35. (C11)	30% w/v N-Methylformamide	Chaotrope			
36. (C12)	3% w/v Hypotaurine	Chaotrope			
37. (D1)	150 mM TCEP hydrochloride	Reducing Agent			
38. (D2)	20 mM GSH (L-Glutathione reduced), 20 mM GSSG (L-Glutathione oxidized)	Reducing Agent			
39. (D3)	5% w/v Benzamidine hydrochloride	Inhibitor			
40. (D4)	50 mM Ethylenediaminetetraacetic acid disodium salt dihydrate	Chelator			
41. (D5)	100 mM Magnesium chloride hexahydrate, 100 mM Calcium chloride dihydrate	Metal			
42. (D6)	100 mM Cadmium chloride hydrate, 100 mM Cobalt(II) chloride hexahydrate	Metal			
43. (D7)	1,000 mM Non Detergent Sulfobetaine 195 (NDSB-195)	Non Detergent			
44. (D8)	1,000 mM Non Detergent Sulfobetaine 201 (NDSB-201)	Non Detergent			
45. (D9)	1,000 mM Non Detergent Sulfobetaine 211 (NDSB-211)	Non Detergent			
46. (D10)	1,000 mM Non Detergent Sulfobetaine 221 (NDSB-221)	Non Detergent			
47. (D11)	800 mM Non Detergent Sulfobetaine 256 (NDSB-256)	Non Detergent			
48. (D12)	500 mM Taurine	Organic Acid			

Sample: _____ Sample Concentration: _____

Sample Buffer: _____ Date: _____

Reservoir Volume: _____ Temperature: _____

Drop Volume: Total _____ µl Sample _____ µl Reservoir _____ µl Additive _____ µl

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)

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Solubility and Stability Screen - HR2-072 Scoring Sheet	Type:	Date:	Date:	Date:
49. (E1) 500 mM Acetamide	Organic Acid			
50. (E2) 500 mM Oxalic acid dihydrate	Organic Acid			
51. (E3) 500 mM Sodium malonate pH 7.0	Organic Acid			
52. (E4) 500 mM Succinic acid pH 7.0	Organic Acid			
53. (E5) 5% v/v Tacsimate pH 7.0	Organic Acid			
54. (E6) 25% w/v Tetraethylammonium bromide	Ionic Liquid			
55. (E7) 25% w/v Cholin acetate	Ionic Liquid			
56. (E8) 25% w/v 1-Ethyl-3-methylimidazolium acetate	Ionic Liquid			
57. (E9) 25% w/v 1-Butyl-3-methylimidazolium chloride	Ionic Liquid			
58. (E10) 25% w/v Ethylammonium nitrate	Ionic Liquid			
59. (E11) 500 mM Ammonium sulfate	Salt			
60. (E12) 500 mM Ammonium chloride	Salt			
61. (F1) 500 mM Magnesium sulfate hydrate	Salt			
62. (F2) 500 mM Potassium thiocyanate	Salt			
63. (F3) 250 mM Gadolinium(III) chloride hexahydrate	Salt			
64. (F4) 250 mM Cesium chloride	Salt			
65. (F5) 250 mM 4-Aminobutyric acid (GABA)	Salt			
66. (F6) 500 mM Lithium nitrate	Salt			
67. (F7) 500 mM DL-Malic acid pH 7.0	Salt			
68. (F8) 500 mM Lithium citrate tribasic tetrahydrate	Salt			
69. (F9) 250 mM Ammonium acetate	Salt			
70. (F10) 250 mM Sodium benzenesulfonate	Salt			
71. (F11) 250 mM Sodium p-toluenesulfonate	Salt			
72. (F12) 1,000 mM Sodium chloride	Salt			
73. (G1) 1,400 mM Potassium chloride	Salt			
74. (G2) 700 mM Sodium phosphate monobasic monohydrate, 1,300 mM Potassium phosphate dibasic	Salt			
75. (G3) 1,000 mM Sodium sulfate decahydrate	Salt			
76. (G4) 1,400 mM Lithium chloride	Salt			
77. (G5) 1,000 mM Sodium bromide	Salt			
78. (G6) 40% v/v Glycerol, 400 mM Lithium chloride	Polyol and Salt			
79. (G7) 50% v/v Glycerol	Polyol			
80. (G8) 10% v/v Ethylene glycol	Polyol			
81. (G9) 10% v/v Polyethylene glycol 200	Polyol			
82. (G10) 5% v/v Polyethylene glycol monomethyl ether 550	Polyol			
83. (G11) 5% w/v Polyethylene glycol monomethyl ether 750	Polyol			
84. (G12) 50% v/v Formamide	Polyol			
85. (H1) 10% v/v Polyethylene glycol 400	Polyol			
86. (H2) 10% w/v Polyethylene glycol 600	Polyol			
87. (H3) 10% w/v 1,2-Propanediol	Polyol			
88. (H4) 3% w/v Polyethylene glycol monomethyl ether 1,900	Polymer			
89. (H5) 3% w/v Polyethylene glycol 3,350	Polymer			
90. (H6) 3% w/v Polyethylene glycol 8,000	Polymer			
91. (H7) 2% w/v Polyvinylpyrrolidone K 15	Polymer			
92. (H8) 2% w/v Polyethylene glycol 20,000	Cyclodextrin			
93. (H9) 10 mM (2-Hydroxypropyl)-β-cyclodextrin	Cyclodextrin			
94. (H10) 80 mM α-Cyclodextrin	Cyclodextrin			
95. (H11) 10 mM β-Cyclodextrin	Cyclodextrin			
96. (H12) 50 mM Methyl-β-cyclodextrin	Cyclodextrin			