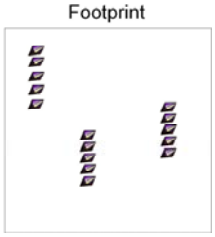
moleculardimensions.com



The Stura Footprint Screen™ + MacroSol™ HT-96 MD1-43

The popular Stura Footprint Screen and MacroSol screen now in HT 96-well format. The footprint screens are based on the concept of screening the protein precipitant solubility curve.

The screen is presented in a deep-well block of 1 x 96ml conditions at acidic, neutral and basic pH's for polyethylene glycols and salts.

Features of Footprint screening

- Screens the protein precipitant solubility curve rather than a crystallization trial.
- Test the relative protein solubility with precipitants that have been used successfully in the crystallization of many proteins.
- Based on reagents of proven success in crystallizing glycoproteins and macromolecular complexes.

Introduction

The Stura Footprint Screen and MacroSol are simple screening methods to analyse the crystallization potential of proteins and their complexes[1]. This method is described as the "First Footprint" as it defines the individual pattern of solubility for a given protein with a range of precipitants and enables the researcher to characterize the types of oils or precipitates obtained. The test is fast and easy to perform as a vapour diffusion experiment. It is highly accurate and reproducible; provided the normal precautions are taken to control the ambient temperature of the experiment. The First Footprint screen (screen #1) is limited to three pH values (5.5, 7.0, and 8.50) and two precipitant classes (PEGs and salts) at four concentrations. The PEG Footprint screen (screen #2) to compliment the original Footprint Screen has subsequently been described[2]. This Footprint screen covers six pH values (4.5 – 8.2) with six PEG's at four concentrations.

MacroSol is a footprint-type screen based on reagents of proven success in crystallizing glycoproteins and macromolecular complexes. This footprint-type screen is based on the concept of screening the protein precipitant solubility curve. When crystallizing complexes, each of the macromolecules that compose the complex will have a different solubility. It is therefore important to find out where each of the macromolecules precipitates as well as determining the precipitation points for the complex. Screening under multiple conditions is important in these cases, in particular when the exact stoichiometry for the complex is unknown or the complex has been mixed from the individual solutions rather than purified as a complex. Depending on how much excess of one particular macromolecule is present in the solution there will be some precipitation under the condition for that uncomplexed macromolecule. Hence, screening under multiple conditions is needed for complexes, but also for glycoproteins and proteins obtained from limited proteolysis where heterogeneity in the protein will be reflected in solubility differences.

The Footprint Principle

The footprint principle can be applied to any of the commercial screens by making different dilutions of the pre-mixed solutions, thus extending their use and increasing the amount of information than can be derived for each precipitant/buffer mixture without having to mix new solutions. By diluting solutions instead of mixing new ones from scratch it is possible to achieve greater precision. This is the concept of working solutions[2].

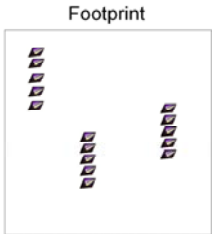
Once initial crystals or crystalline aggregates are obtained from the first screening, streak seeding is recommended to determine the ranges of conditions under which crystal growth can proceed.

MacroSol is an example of how one can extend commercial screens and thus make better use of them. It has been taken from the presentation by Dr. E. Stura at the "EMBO Workshop on the crystallization of Macromolecular Complexes" Grenoble, 8-13 April 2001.

Formulation Notes

Stura Footprint Screen and MacroSol reagents are formulated using ultrapure water (>18.0 MΩ) and are sterile-filtered using 0.22 µm filters. No preservatives are added.

Buffer stock solutions and organic acids are titrated to the specified pH. Final pH may vary from that specified on the datasheet. Molecular Dimensions will



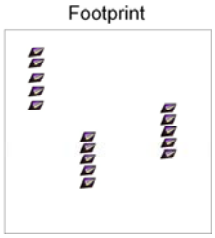
be happy to discuss the precise formulation of individual reagents.
Individual reagents and stock solutions for optimisation are available from Molecular Dimensions.

Enquiries regarding Stura Footprint & MacroSol HT-96 formulation, interpretation of results or optimization strategies are welcome. Please e-mail, fax or phone your query to Molecular Dimensions.

Contact and product details can be found at moleculardimensions.com

References

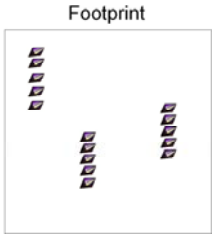
1. Stura E.A., Nemerow G.R., Wilson I.A (1992) Strategies in the crystallization of glycoproteiens and protein complexes. Journal of Crystal Growth 122:273-285
2. Stura E.A. (1999) Strategy 3: Reverse Screening. In "Crystallization of Proteins: Techniques, Strategies and Tips. A laboratory manual" (Bergfors T. ed.) International University Line pp113-124.
3. Stura E.A, Satterthwait, A.C, Calvo, J.C, Kaslow, D.C, Wilson, I.A. (1994) Reverse Screening. Acta Cryst. D50 : 448-455.
4. Stura E.A, at the "EMBO Workshop on the crystallization of Macromolecular Complexes" Grenoble 8-13 April 2001



Stura Footprint Screen Rows A – D

MD1-43

HT#	Salt / Buffer	pH	Precipitant
A1	0.2 M imidazole malate	5.5	15% v/v PEG 600
A2	0.2 M imidazole malate	5.5	24 %v/v PEG 600
A3	0.2 M imidazole malate	5.5	33 % v/v PEG 600
A4	0.2 M imidazole malate	5.5	42 % v/v PEG 600
A5	0.2 M imidazole malate	7.0	10 % w/v PEG 4000
A6	0.2 M imidazole malate	7.0	15 % w/v PEG 4000
A7	0.2 M imidazole malate	7.0	20 % w/v PEG 4000
A8	0.2 M imidazole malate	7.0	25 % PEG w/v 4000
A9	0.2 M imidazole malate	8.5	7.5 % w/v PEG 10,000
A10	0.2 M imidazole malate	8.5	12.5 % w/v PEG 10,000
A11	0.2 M imidazole malate	8.5	17.5 % w/v PEG 10,000
A12	0.2 M imidazole malate	8.5	22.5 % w/v PEG 10,000
B1	0.15 M sodium citrate	5.5	0.75 M ammonium sulfate
B2	0.15 M sodium citrate	5.5	1.0 M ammonium sulfate
B3	0.15 M sodium citrate	5.5	1.5 M ammonium sulfate
B4	0.15 M sodium citrate	5.5	2.0 M ammonium sulfate
B5	0.8 M NaH_2PO_4/K_2HPO_4	7.0	-
B6	1.32 M NaH_2PO_4/K_2HPO_4	7.0	-
B7	1.6 M NaH_2PO_4/K_2HPO_4	7.0	-
B8	2.0 M NaH_2PO_4/K_2HPO_4	7.0	-
B9	0.01 M sodium borate	8.5	0.75 M sodium citrate
B10	0.01 M sodium borate	8.5	1.0 M sodium citrate
B11	0.01 M sodium borate	8.5	1.2 M sodium citrate
B12	0.01 M sodium borate	8.5	1.5 M sodium citrate
C1	0.1 M Na HEPES	8.2	30 % v/v PEG 550 MME
C2	0.1 M Na HEPES	8.2	40 % v/v PEG 550 MME
C3	0.1 M Na HEPES	8.2	50 % v/v PEG 550 MME
C4	0.1 M Na HEPES	8.2	60 % v/v PEG 550 MME
C5	0.1 M Na HEPES	7.5	18 % v/v PEG 600
C6	0.1 M Na HEPES	7.5	27 % v/v PEG 600
C7	0.1 M Na HEPES	7.5	36 % v/v PEG 600
C8	0.1 M Na HEPES	7.5	45 % v/v PEG 600
C9	0.1 M sodium cacodylate	6.5	18 % w/v PEG 2000 MME
C10	0.1 M sodium cacodylate	6.5	27 % w/v PEG 2000 MME
C11	0.1 M sodium cacodylate	6.5	36 % w/v PEG 2000 MME
C12	0.1 M sodium cacodylate	6.5	45 % w/v PEG 2000 MME
D1	0.2 M imidazole malate	6.0	8 % w/v PEG 4000
D2	0.2 M imidazole malate	6.0	15 % w/v PEG 4000
D3	0.2 M imidazole malate	6.0	20 % w/v PEG 4000
D4	0.2 M imidazole malate	6.0	30 % w/v PEG 4000
D5	0.1 M sodium acetate	5.5	12% PEG w/v 5000 MME
D6	0.1 M sodium acetate	5.5	18% PEG w/v 5000 MME
D7	0.1 M sodium acetate	5.5	24% PEG w/v 5000 MME
D8	0.1 M sodium acetate	5.5	36% PEG w/v 5000 MME
D9	0.1 M ammonium acetate	4.5	9 % w/v PEG 10,000
D10	0.1 M ammonium acetate	4.5	15 % w/v PEG 10,000
D11	0.1 M ammonium acetate	4.5	22.5 % w/v PEG 10,000
D12	0.1 M ammonium acetate	4.5	27 % w/v PEG 10,000



MacroSol Rows E – H

MD1-43

HT#	Reagent 1	Reagent 2	Buffer	pH
E1	10% v/v MPD	0.02 M calcium chloride	0.1 M sodium acetate	4.5
E2	20% v/v MPD	0.02 M calcium chloride	0.1 M sodium acetate	4.5
E3	30% v/v MPD	-	0.1 M sodium acetate	4.5
E4	30% v/v MPD	0.02 M calcium chloride	0.1 M sodium acetate	4.5
E5	1.0 M sodium acetate	0.1 M sodium dihydrogen phosphate	0.1 M sodium cacodylate	6.5
E6	1.4 M sodium acetate	0.1 M sodium dihydrogen phosphate	0.1 M sodium cacodylate	6.5
E7	1.7 M sodium acetate	0.1 M sodium dihydrogen phosphate	0.1 M sodium cacodylate	6.5
E8	1.7 M sodium acetate	-	0.1 M sodium cacodylate	6.5
E9	8% w/v PEG 3350	0.2 M ammonium acetate	0.1 M sodium acetate	4.5
E10	15% w/v PEG 3350	0.2 M ammonium acetate	0.1 M sodium acetate	4.5
E11	20% w/v PEG 3350	0.2 M ammonium acetate	0.1 M sodium acetate	4.5
E12	30% w/v PEG 3350	0.2 M ammonium acetate	0.1 M sodium acetate	4.5
F1	0.75 M ammonium dihydrogen phosphate	-	0.1 M sodium citrate	5.5
F2	1.0 M ammonium dihydrogen phosphate	-	0.1 M sodium citrate	5.5
F3	1.5 M ammonium dihydrogen phosphate	-	0.1 M sodium citrate	5.5
F4	2.0 M ammonium dihydrogen phosphate	-	0.1 M sodium citrate	5.5
F5	10% v/v 2-propanol	0.2 M magnesium chloride	0.1 M Na HEPES	7.5
F6	15% v/v 2-propanol	0.2 M magnesium chloride	0.1 M Na HEPES	7.5
F7	20% v/v 2-propanol	0.2 M magnesium chloride	0.1 M Na HEPES	7.5
F8	30% v/v 2-propanol	0.2 M magnesium chloride	0.1 M Na HEPES	7.5
F9	16% w/v PEG 3350	0.2 M lithium sulfate	0.1 M Tris	8.5
F10	25% w/v PEG 3350	0.2 M lithium sulfate	0.1 M Tris	8.5
F11	30% w/v PEG 3350	0.2 M lithium sulfate	0.1 M Tris	8.5
F12	25% w/v PEG 3350	0.5 M lithium sulfate	0.1 M Tris	8.5
G1	2% w/v PEG 8000	1.0 M lithium sulfate	-	
G2	2% w/v PEG 8000	1.0 M lithium sulfate	0.1 M imidazole malate	5.5
G3	2% w/v PEG 8000	1.0 M lithium sulfate	0.1 M imidazole malate	6.5
G4	2% w/v PEG 8000	1.0 M lithium sulfate	0.1 M imidazole malate	7.5
G5	12% w/v PEG 8000	0.2 M ammonium sulfate	-	-
G6	18% w/v PEG 8000	0.2 M ammonium sulfate	-	-
G7	24% w/v PEG 8000	0.2 M ammonium sulfate	-	-
G8	30% w/v PEG 8000	0.2 M ammonium sulfate	-	-
G9	10% w/v PEG 4000	0.5 M ammonium sulfate	-	-
G10	20% w/v PEG 4000	0.3 M ammonium sulfate	-	-
G11	30% w/v PEG 4000	0.2 M ammonium sulfate	-	-
G12	36% w/v PEG 4000	0.2 M ammonium sulfate	-	-
H1	1.0 M ammonium sulfate	2% v/v PEG 400	0.1 M Na HEPES	7.5
H2	1.5 M ammonium sulfate	2% v/v PEG 400	0.1 M Na HEPES	7.5
H3	2.0 M ammonium sulfate	2% v/v PEG 400	0.1 M Na HEPES	7.5
H4	2.0 M ammonium sulfate	5% v/v PEG 400	0.1 M Na HEPES	7.5
H5	12% w/v PEG 4000	5% v/v 2-propanol	0.1 M sodium citrate	5.5
H6	16% w/v PEG 4000	10% v/v 2-propanol	0.1 M sodium citrate	5.5
H7	20% w/v PEG 4000	15% v/v 2-propanol	0.1 M sodium citrate	5.5
H8	20% w/v PEG 4000	20% v/v 2-propanol	0.1 M sodium citrate	5.5
H9	9% w/v PEG 8000	0.005 M zinc acetate	0.1 M sodium cacodylate	6.5
H10	12% w/v PEG 8000	0.005 M zinc acetate	0.1 M sodium cacodylate	6.5
H11	18% w/v PEG 8000	0.005 M zinc acetate	0.1 M sodium cacodylate	6.5
H12	24% w/v PEG 8000	0.005 M zinc acetate	0.1 M sodium cacodylate	6.5

Abbreviations: Na HEPES; 2-(4-(2-Hydroxyethyl)-1-piperazinyl)ethanesulfonic Acid Sodium Salt, **PEG**; Polyethylene glycol, **Tris**; 2-Amino-2-(hydroxymethyl) propane-1,3-diol. Note: The pH of each final reagent is checked and adjusted back to the stated pH of the buffer (±0.2 pH units) as appropriate.

Manufacturer's datasheets are available on request