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Grid

Clear Strategy Screen™ II HT-96 MD1-32

A complimentary high-throughput screen* to CSS-I, with flexible scaffolding of parameters, easily modified to the specific needs of a project. Easily interpret results and optimize your experiments with this versatile screening kit.

The Clear Strategy II kit reagents are premixed at 4 pH values (pH's 5.5, 6.5, 7.5 and 8.5) in 96 × 1ml deep well blocks.

Features of Clear Strategy Screens (CSS):

- Limit number of trials.
- Aid rational design of subsequent trials.
- User defined pH.
- Use protein information.
- Maintain 'folding homogeneity' of protein.
- Cryoprotection of crystals.
- Provision of potential anomalous scattering centres.
- Interchangeable components.

Introduction

Clear Strategy Screens are designed to offer a more individual and alternative approach to crystallization problems. Their 'inherently simple design and their flexible nature' provide a logical platform for further modification and optimization of crystallization experiments.

Clear Strategy Screen II

Although CSS-I has wide applications, there is a need for a complementary 6 × 4 set of conditions that fills gaps in the network of crystallization parameters. The basic principles of CSS-I were also applied to CSS-II. Its two-dimensional layout is very simple and can be divided into several integral areas (A-E).

- **Area A: Conditions (1 - 4) and (7 - 10)** represent single salt screening to provide additional information about protein solubility. Each salt is represented by two conditions, thus giving clearer insight into protein/salt solubility dependence. At the same time, the risk of overlooking a positive condition in cases where only one (e.g. heavily precipitating) salt concentration is applied is minimised.

- **Area B: Conditions (13 - 14) and (19 - 20)** function as an 'organic' solution screen.

- **Area C: Conditions 15 and 21** evaluate the influence of heavier cations on protein crystallization properties.

- **Section D: Conditions (16 -18) and (22 - 24)** supplements the CSS-I with other PEGS mixed with KSCN.

- **Area E: Conditions (5 – 6) and (11 – 12)** or 'creativity corner'. Symbolises part of the screening matrix that can be biased towards the users favourite conditions. In our case it combines PEG 4000 together with calcium acetate.

CSS-II Flexibility

Contrary to the more rigid and precisely defined CSS-I, CSS-II gives each individual investigator a wide range of tools and parameters, to reflect both personal experience and the specificity of the project.

For example, conditions 1 - 2,4, 7 - 8, and 10 should not be used if calcium is required for the protein activity or integrity; instead they may be replaced by using other salts that do not crystallize as easily in the presence of Ca^{2+}, Cd^{2+},or Ni^{2+}, and may be replaced by cations more specific for a particular protein (e.g. Zn^{2+}, $Cu2^{+}$, Mn^{2+} , *etc.*) or even heavy metals such as Hg or U albeit at much lower (1 - 2mM) concentrations; calcium acetate (conditions 5 - 6, 11 - 12) may also be replaced by another salt if necessary.

CSS-II should therefore be considered as a flexible scaffolding of parameters that can be easily modified by the individual user, but preferably, in the context of conditions available in CSS-I.

Although driven by slightly different principles, CSS-I and CSS-II should be seen as self-complementary screens that may be used instead of other commercially

* Developed by Dr. A M Brzozowski and J. Walton from the Structural Biology Laboratory at The University of York.

available screens, thereby halving the number of initial trials required.

Formulation Notes

To set up a screen:
Clear Strategy Screens reagents are formulated using ultrapure water (>18.0 MΩ) and are sterile-filtered using 0.22 µm filters. No preservatives are added.

Molecular Dimensions will be happy to discuss the precise formulation of individual reagents.

Individual reagents and stock solutions for optimization are available from Molecular Dimensions.

Enquiries regarding Clear Strategy Screen II HT-96 formulation, interpretation of results or optimization strategies are welcome. Please e-mail, fax or phone your query to Molecular Dimensions:

enquiries@moleculardimensions.com

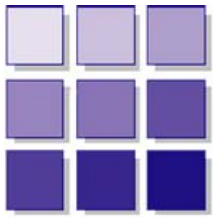
Contact and product details can be found at:

www.moleculardimensions.com

These kits are produced by Molecular Dimensions Ltd under an exclusive license from University of York.

References

1. Brzozowski and Walton (2001) J. Appl. Cryst. **34**, 97 – 101.
2. Selmer *et al* (2006), Science **313**, 1935 – 1942.
3. Dauter, Z, Dauter, M & Rajashankar, K. R. (2K), *Acta Cryst*. **D56**, 232 – 237.



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Crystal Strategy Screen II MD1-32 Rows A – D

HT-96 Well	Salt	Buffer	Precipitant
A1	1.5 M ammonium sulfate	0.1 M sodium acetate pH 5.5	None
A2	0.8 M lithium sulfate	0.1 M sodium acetate pH 5.5	None
A3	2.0 M sodium formate	0.1 M sodium acetate pH 5.5	None
A4	0.5 M potassium dihyd. phosphate	0.1 M sodium acetate pH 5.5	None
A5	0.2 M calcium acetate	0.1 M sodium acetate pH 5.5	25% w/v PEG 2K MME
A6	0.2 M calcium acetate	0.1 M sodium acetate pH 5.5	15% w/v PEG 4K
A7	2.7 M ammonium sulfate	0.1 M sodium acetate pH 5.5	None
A8	1.8 M lithium sulfate	0.1 M sodium acetate pH 5.5	None
A9	4.0 M sodium formate	0.1 M sodium acetate pH 5.5	None
A10	1.0 M potassium dihyd. phosphate	0.1 M sodium acetate pH 5.5	None
A11	0.2 M calcium acetate	0.1 M sodium acetate pH 5.5	10% w/v PEG 8K + 10% w/v PEG 1K
A12	0.2 M calcium acetate	0.1 M sodium acetate pH 5.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
B1	None	0.1 M sodium acetate pH 5.5	40% v/v MPD
B2	None	0.1 M sodium acetate pH 5.5	40% v/v 1,4-Butanediol
B3	0.005 M cadmium chloride	0.1 M sodium acetate pH 5.5	20% w/v PEG 4K
B4	0.15 M potassium thiocyanate	0.1 M sodium acetate pH 5.5	20% v/v PEG 550 MME
B5	0.15 M potassium thiocyanate	0.1 M sodium acetate pH 5.5	20% v/v PEG 600
B6	0.15 M potassium thiocyanate	0.1 M sodium acetate pH 5.5	20% w/v PEG 1.5K
B7	None	0.1 M sodium acetate pH 5.5	35% v/v 2-Propanol
B8	None	0.1 M sodium acetate pH 5.5	30% v/v Jeffamine M-600
B9	0.005 M nickel chloride	0.1 M sodium acetate pH 5.5	20% w/v PEG 4K
B10	0.15 M potassium thiocyanate	0.1 M sodium acetate pH 5.5	18% w/v PEG 3350
B11	0.15 M potassium thiocyanate	0.1 M sodium acetate pH 5.5	18% w/v PEG 5K MME
B12	0.15 M potassium thiocyanate	0.1 M sodium acetate pH 5.5	15% w/v PEG 6K
C1	1.5 M ammonium sulfate	0.1 M sodium cacodylate pH 6.5	None
C2	0.8 M lithium sulfate	0.1 M sodium cacodylate pH 6.5	None
C3	2.0 M sodium formate	0.1 M sodium cacodylate pH 6.5	None
C4	0.5 M potassium dihyd. phosphate	0.1 M sodium cacodylate pH 6.5	None
C5	0.2 M calcium acetate	0.1 M sodium cacodylate pH 6.5	25% w/v PEG 2K MME
C6	0.2 M calcium acetate	0.1 M sodium cacodylate pH 6.5	15% w/v PEG 4K
C7	2.7 M ammonium sulfate	0.1 M sodium cacodylate pH 6.5	None
C8	1.8 M lithium sulfate	0.1 M sodium cacodylate pH 6.5	None
C9	4.0 M sodium formate	0.1 M sodium cacodylate pH 6.5	None
C10	1.0 M potassium dihyd. phosphate	0.1 M sodium cacodylate pH 6.5	None
C11	0.2 M calcium acetate	0.1 M sodium cacodylate pH 6.5	10% w/v PEG 8K + 10% w/v PEG 1K
C12	0.2 M calcium acetate	0.1 M sodium cacodylate pH 6.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
D1	None	0.1 M sodium cacodylate pH 6.5	40% v/v MPD
D2	None	0.1 M sodium cacodylate pH 6.5	40% v/v 1,4-Butanediol
D3	0.005 M cadmium chloride	0.1 M sodium cacodylate pH 6.5	20% w/v PEG 4K
D4	0.15 M potassium thiocyanate	0.1 M sodium cacodylate pH 6.5	20% v/v PEG 550 MME
D5	0.15 M potassium thiocyanate	0.1 M sodium cacodylate pH 6.5	20% v/v PEG 600
D6	0.15 M potassium thiocyanate	0.1 M sodium cacodylate pH 6.5	20% w/v PEG 1.5K
D7	None	0.1 M sodium cacodylate pH 6.5	35% v/v 2-Propanol
D8	None	0.1 M sodium cacodylate pH 6.5	30% v/v Jeffamine M-600
D9	0.005 M nickel chloride	0.1 M sodium cacodylate pH 6.5	20% w/v PEG 4K
D10	0.15 M potassium thiocyanate	0.1 M sodium cacodylate pH 6.5	18% w/v PEG 3350
D11	0.15 M potassium thiocyanate	0.1 M sodium cacodylate pH 6.5	18% w/v PEG 5K MME
D12	0.15 M potassium thiocyanate	0.1 M sodium cacodylate pH 6.5	15% w/v PEG 6K

Crystal Strategy Screen II HT-96 MD1-32 Rows E – H

HT-96 Well	Salt	Buffer	Precipitant
E1	1.5 M ammonium sulfate	0.1M Tris pH 7.5	None
E2	0.8 M lithium sulfate	0.1M Tris pH 7.5	None
E3	2.0 M sodium formate	0.1M Tris pH 7.5	None
E4	0.5 M potassium dihyd. phosphate	0.1M Tris pH 7.5	None
E5	0.2 M calcium acetate	0.1M Tris pH 7.5	25% w/v PEG 2K MME
E6	0.2 M calcium acetate	0.1M Tris pH 7.5	15% w/v PEG 4K
E7	2.7 M ammonium sulfate	0.1M Tris pH 7.5	None
E8	1.8 M lithium sulfate	0.1M Tris pH 7.5	None
E9	4.0 M sodium formate	0.1M Tris pH 7.5	None
E10	1.0 M potassium dihyd. phosphate	0.1M Tris pH 7.5	None
E11	0.2 M calcium acetate	0.1M Tris pH 7.5	10% w/v PEG 8K + 10% w/v PEG 1K
E12	0.2 M calcium acetate	0.1M Tris pH 7.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
F1	None	0.1M Tris pH 7.5	40% v/v MPD
F2	None	0.1M Tris pH 7.5	40% v/v 1,4-Butanediol
F3	0.005 M cadmium chloride	0.1M Tris pH 7.5	20% w/v PEG 4K
F4	0.15 M potassium thiocyanate	0.1M Tris pH 7.5	20% v/v PEG 550 MME
F5	0.15 M potassium thiocyanate	0.1M Tris pH 7.5	20% w/v PEG 600
F6	0.15 M potassium thiocyanate	0.1M Tris pH 7.5	20% w/v PEG 1.5K
F7	None	0.1M Tris pH 7.5	35% v/v 2-Propanol
F8	None	0.1M Tris pH 7.5	30% v/v Jeffamine M-600
F9	0.005 M nickel chloride	0.1M Tris pH 7.5	20% w/v PEG 4K
F10	0.15 M potassium thiocyanate	0.1M Tris pH 7.5	18% w/v PEG 3350
F11	0.15 M potassium thiocyanate	0.1M Tris pH 7.5	18% w/v PEG 5K MME
F12	0.15 M potassium thiocyanate	0.1M Tris pH 7.5	15% w/v PEG 6K
G1	1.5 M ammonium sulfate	0.1M Tris pH 8.5	None
G2	0.8 M lithium sulfate	0.1M Tris pH 8.5	None
G3	2.0 M sodium formate	0.1M Tris pH 8.5	None
G4	0.5 M potassium dihyd. phosphate	0.1M Tris pH 8.5	None
G5	0.2 M calcium acetate	0.1M Tris pH 8.5	25% w/v PEG 2K MME
G6	0.2 M calcium acetate	0.1M Tris pH 8.5	15% w/v PEG 4K
G7	2.7 M ammonium sulfate	0.1M Tris pH 8.5	None
G8	1.8 M lithium sulfate	0.1M Tris pH 8.5	None
G9	4.0 M sodium formate	0.1M Tris pH 8.5	None
G10	1.0 M potassium dihyd. phosphate	0.1M Tris pH 8.5	None
G11	0.2 M calcium acetate	0.1M Tris pH 8.5	10% w/v PEG 8K + 10% w/v PEG 1K
G12	0.2 M calcium acetate	0.1M Tris pH 8.5	8% w/v PEG 20K + 8% v/v PEG 550 MME
H1	None	0.1M Tris pH 8.5	40% v/v MPD
H2	None	0.1M Tris pH 8.5	40% v/v 1,4-Butanediol
H3	0.005 M cadmium chloride	0.1M Tris pH 8.5	20% w/v PEG 4K
H4	0.15 M potassium thiocyanate	0.1M Tris pH 8.5	20% v/v PEG 550 MME
H5	0.15 M potassium thiocyanate	0.1M Tris pH 8.5	20% v/v PEG 600
H6	0.15 M potassium thiocyanate	0.1M Tris pH 8.5	20% w/v PEG 1.5K
H7	None	0.1M Tris pH 8.5	35% v/v 2-Propanol
H8	None	0.1M Tris pH 8.5	30% v/v Jeffamine M-600
H9	0.005 M nickel chloride	0.1M Tris pH 8.5	20% w/v PEG 4K
H10	0.15 M potassium thiocyanate	0.1M Tris pH 8.5	18% w/v PEG 3350
H11	0.15 M potassium thiocyanate	0.1M Tris pH 8.5	18% w/v PEG 5K MME
H12	0.15 M potassium thiocyanate	0.1M Tris pH 8.5	15% w/v PEG 6K

PEG, polyethylene glycol (concentrations quoted as w/v %); MME, monomethyl ether; 1K, 2K, 4K, 8K and 20K correspond to the molecular weight, in thousands of Daltons, of PEG.

Manufacturer's safety data sheets are available upon request.