

Clear Strategy™ Screen II HT-96 MD1-32

A complimentary screen¹ 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.

MD1-32 is presented as 96 x 1 mL conditions in a deep-well block.

Features of Clear Strategy II HT-96:

- Allows user defined pH.
- Uncoupling of pH from screen.
- Aids rational design of subsequent trials
- Maintains 'folding homogeneity' of protein.
- Provides cryoprotection of crystals.
- Provides potential anomalous scattering centres.
- Interchangeable components.

Introduction

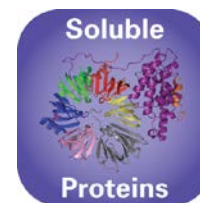
Clear Strategy Screens are designed to offer a more individual and alternative approach to crystallization problems. Their 'inherently simple design and their flexible nature' provide a logical platform for further modification and optimization of crystallization experiments.

Clear Strategy Screen II (CSS-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 user's favourite conditions. In our case it combines PEG 4000 together with calcium acetate.

¹ Developed by Dr. A M Brzozowski and J. Walton from the Structural Biology Laboratory at The University of York.



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+} , Cu^{2+} , 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 available screens, thereby halving the number of initial trials required.

Formulation Notes:

CSS-II reagents are formulated using ultrapure water ($>18.0 \text{ M}\Omega$) and are sterile-filtered using $0.22 \mu\text{m}$ filters. No preservatives are added.

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 optimization are available from Molecular Dimensions.

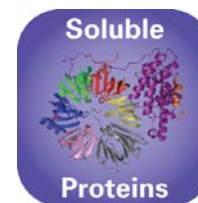
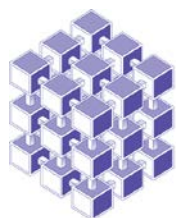
Enquiries regarding CSS-II formulation, interpretation of results or optimization strategies are welcome. Please e-mail, fax or phone your query to Molecular Dimensions.

Contact and product details can be found at www.moleculardimensions.com

Manufacturer's safety data sheets are available to download from our website.

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. (2000), *Acta Cryst.* **D56**, 232 – 237

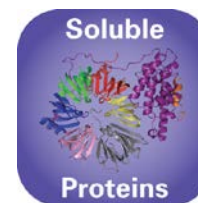
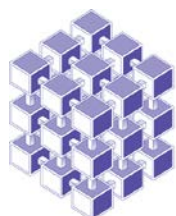


Clear Strategy II HT-96

Conditions A1 – D12

MD1-32

Tube #	Well #	Conc.	Salt	Conc.	Buffer	pH	Conc.	Precipitant1	Conc.	Precipitant2
1	A1	1.5 M	Ammonium sulfate	0.1 M	Sodium acetate	5.5				
2	A2	0.8 M	Lithium sulfate	0.1 M	Sodium acetate	5.5				
3	A3	2.0 M	Sodium formate	0.1 M	Sodium acetate	5.5				
4	A4	0.5 M	Potassium phosphate monobasic	0.1 M	Sodium acetate	5.5				
5	A5	0.2 M	Calcium acetate hydrate	0.1 M	Sodium acetate	5.5	25 % w/v	PEG 2000 MME		
6	A6	0.2 M	Calcium acetate hydrate	0.1 M	Sodium acetate	5.5	15 % w/v	PEG 4000		
7	A7	2.7 M	Ammonium sulfate	0.1 M	Sodium acetate	5.5				
8	A8	1.8 M	Lithium sulfate	0.1 M	Sodium acetate	5.5				
9	A9	4.0 M	Sodium formate	0.1 M	Sodium acetate	5.5				
10	A10	1.0 M	Potassium phosphate monobasic	0.1 M	Sodium acetate	5.5				
11	A11	0.2 M	Calcium acetate hydrate	0.1 M	Sodium acetate	5.5	10 % w/v	PEG 8000		10 % w/v PEG 1000
12	A12	0.2 M	Calcium acetate hydrate	0.1 M	Sodium acetate	5.5	8 % w/v	PEG 20,000		8 % v/v PEG 500 MME
13	B1			0.1 M	Sodium acetate	5.5	40 % v/v	MPD		
14	B2			0.1 M	Sodium acetate	5.5	40 % v/v	1,4-Butanediol		
15	B3	0.005 M	Cadmium chloride hemi(pentahydrate)	0.1 M	Sodium acetate	5.5	20 % w/v	PEG 4000		
16	B4	0.15 M	Potassium thiocyanate	0.1 M	Sodium acetate	5.5	20 % v/v	PEG 500 MME		
17	B5	0.15 M	Potassium thiocyanate	0.1 M	Sodium acetate	5.5	20 % v/v	PEG 600		
18	B6	0.15 M	Potassium thiocyanate	0.1 M	Sodium acetate	5.5	20 % w/v	PEG 1500		
19	B7			0.1 M	Sodium acetate	5.5	35 % v/v	2-Propanol		
20	B8			0.1 M	Sodium acetate	5.5	30 % v/v	Jeffamine® M-600 pH 7.0		
21	B9	0.005 M	Nickel(II) chloride hexahydrate	0.1 M	Sodium acetate	5.5	20 % w/v	PEG 4000		
22	B10	0.15 M	Potassium thiocyanate	0.1 M	Sodium acetate	5.5	18 % w/v	PEG 3350		
23	B11	0.15 M	Potassium thiocyanate	0.1 M	Sodium acetate	5.5	18 % w/v	PEG 5000 MME		
24	B12	0.15 M	Potassium thiocyanate	0.1 M	Sodium acetate	5.5	15 % w/v	PEG 6000		
25	C1	1.5 M	Ammonium sulfate	0.1 M	Sodium cacodylate	6.5				
26	C2	0.8 M	Lithium sulfate	0.1 M	Sodium cacodylate	6.5				
27	C3	2.0 M	Sodium formate	0.1 M	Sodium cacodylate	6.5				
28	C4	0.5 M	Potassium phosphate monobasic	0.1 M	Sodium cacodylate	6.5				
29	C5	0.2 M	Calcium acetate hydrate	0.1 M	Sodium cacodylate	6.5	25 % w/v	PEG 2000 MME		
30	C6	0.2 M	Calcium acetate hydrate	0.1 M	Sodium cacodylate	6.5	15 % w/v	PEG 4000		
31	C7	2.7 M	Ammonium sulfate	0.1 M	Sodium cacodylate	6.5				
32	C8	1.8 M	Lithium sulfate	0.1 M	Sodium cacodylate	6.5				
33	C9	4.0 M	Sodium formate	0.1 M	Sodium cacodylate	6.5				
34	C10	1.0 M	Potassium phosphate monobasic	0.1 M	Sodium cacodylate	6.5				
35	C11	0.2 M	Calcium acetate hydrate	0.1 M	Sodium cacodylate	6.5	10 % w/v	PEG 8000		10 % w/v PEG 1000
36	C12	0.2 M	Calcium acetate hydrate	0.1 M	Sodium cacodylate	6.5	8 % w/v	PEG 20,000		8 % v/v PEG 500 MME
37	D1			0.1 M	Sodium cacodylate	6.5	40 % v/v	MPD		
38	D2			0.1 M	Sodium cacodylate	6.5	40 % v/v	1,4-Butanediol		
39	D3	0.005 M	Cadmium chloride hemi(pentahydrate)	0.1 M	Sodium cacodylate	6.5	20 % w/v	PEG 4000		
40	D4	0.15 M	Potassium thiocyanate	0.1 M	Sodium cacodylate	6.5	20 % v/v	PEG 500 MME		
41	D5	0.15 M	Potassium thiocyanate	0.1 M	Sodium cacodylate	6.5	20 % v/v	PEG 600		
42	D6	0.15 M	Potassium thiocyanate	0.1 M	Sodium cacodylate	6.5	20 % w/v	PEG 1500		
43	D7			0.1 M	Sodium cacodylate	6.5	35 % v/v	2-Propanol		
44	D8			0.1 M	Sodium cacodylate	6.5	30 % v/v	Jeffamine® M-600 pH 7.0		
45	D9	0.005 M	Nickel(II) chloride hexahydrate	0.1 M	Sodium cacodylate	6.5	20 % w/v	PEG 4000		
46	D10	0.15 M	Potassium thiocyanate	0.1 M	Sodium cacodylate	6.5	18 % w/v	PEG 3350		
47	D11	0.15 M	Potassium thiocyanate	0.1 M	Sodium cacodylate	6.5	18 % w/v	PEG 5000 MME		
48	D12	0.15 M	Potassium thiocyanate	0.1 M	Sodium cacodylate	6.5	15 % w/v	PEG 6000		

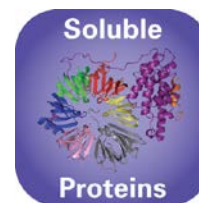
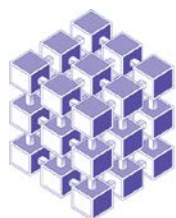


Clear Strategy II HT-96

Conditions E1 – H12

MD1-32

Well #	Conc.	Salt	Conc.	Buffer	pH	Conc.	Precipitant1	Conc.	Precipitant2
E1	1.5 M	Ammonium sulfate	0.1 M	Tris	7.5				
E2	0.8 M	Lithium sulfate	0.1 M	Tris	7.5				
E3	2.0 M	Sodium formate	0.1 M	Tris	7.5				
E4	0.5 M	Potassium phosphate monobasic	0.1 M	Tris	7.5				
E5	0.2 M	Calcium acetate hydrate	0.1 M	Tris	7.5	25 % w/v	PEG 2000 MME		
E6	0.2 M	Calcium acetate hydrate	0.1 M	Tris	7.5	15 % w/v	PEG 4000		
E7	2.7 M	Ammonium sulfate	0.1 M	Tris	7.5				
E8	1.8 M	Lithium sulfate	0.1 M	Tris	7.5				
E9	4.0 M	Sodium formate	0.1 M	Tris	7.5				
E10	1.0 M	Potassium phosphate monobasic	0.1 M	Tris	7.5				
E11	0.2 M	Calcium acetate hydrate	0.1 M	Tris	7.5	10 % w/v	PEG 8000	10 % w/v	PEG 1000
E12	0.2 M	Calcium acetate hydrate	0.1 M	Tris	7.5	8 % w/v	PEG 20,000	8 % v/v	PEG 500 MME
F1			0.1 M	Tris	7.5	40 % v/v	MPD		
F2			0.1 M	Tris	7.5	40 % v/v	1,4-Butanediol		
F3	0.005 M	Cadmium chloride hemi(pentahydrate)	0.1 M	Tris	7.5	20 % w/v	PEG 4000		
F4	0.15 M	Potassium thiocyanate	0.1 M	Tris	7.5	20 % v/v	PEG 500 MME		
F5	0.15 M	Potassium thiocyanate	0.1 M	Tris	7.5	20 % v/v	PEG 600		
F6	0.15 M	Potassium thiocyanate	0.1 M	Tris	7.5	20 % w/v	PEG 1500		
F7			0.1 M	Tris	7.5	35 % v/v	2-Propanol		
F8			0.1 M	Tris	7.5	30 % v/v	Jeffamine® M-600 pH 7.0		
F9	0.005 M	Nickel(II) chloride hexahydrate	0.1 M	Tris	7.5	20 % w/v	PEG 4000		
F10	0.15 M	Potassium thiocyanate	0.1 M	Tris	7.5	18 % w/v	PEG 3350		
F11	0.15 M	Potassium thiocyanate	0.1 M	Tris	7.5	18 % w/v	PEG 5000 MME		
F12	0.15 M	Potassium thiocyanate	0.1 M	Tris	7.5	15 % w/v	PEG 6000		
G1	1.5 M	Ammonium sulfate	0.1 M	Tris	8.5				
G2	0.8 M	Lithium sulfate	0.1 M	Tris	8.5				
G3	2.0 M	Sodium formate	0.1 M	Tris	8.5				
G4	0.5 M	Potassium phosphate monobasic	0.1 M	Tris	8.5				
G5	0.2 M	Calcium acetate hydrate	0.1 M	Tris	8.5	25 % w/v	PEG 2000 MME		
G6	0.2 M	Calcium acetate hydrate	0.1 M	Tris	8.5	15 % w/v	PEG 4000		
G7	2.7 M	Ammonium sulfate	0.1 M	Tris	8.5				
G8	1.8 M	Lithium sulfate	0.1 M	Tris	8.5				
G9	4.0 M	Sodium formate	0.1 M	Tris	8.5				
G10	1.0 M	Potassium phosphate monobasic	0.1 M	Tris	8.5				
G11	0.2 M	Calcium acetate hydrate	0.1 M	Tris	8.5	10 % w/v	PEG 8000	10 % w/v	PEG 1000
G12	0.2 M	Calcium acetate hydrate	0.1 M	Tris	8.5	8 % w/v	PEG 20,000	8 % v/v	PEG 500 MME
H1			0.1 M	Tris	8.5	40 % v/v	MPD		
H2			0.1 M	Tris	8.5	40 % v/v	1,4-Butanediol		
H3	0.005 M	Cadmium chloride hemi(pentahydrate)	0.1 M	Tris	8.5	20 % w/v	PEG 4000		
H4	0.15 M	Potassium thiocyanate	0.1 M	Tris	8.5	20 % v/v	PEG 500 MME		
H5	0.15 M	Potassium thiocyanate	0.1 M	Tris	8.5	20 % v/v	PEG 600		
H6	0.15 M	Potassium thiocyanate	0.1 M	Tris	8.5	20 % w/v	PEG 1500		
H7			0.1 M	Tris	8.5	35 % v/v	2-Propanol		
H8			0.1 M	Tris	8.5	30 % v/v	Jeffamine® M-600 pH 7.0		
H9	0.005 M	Nickel(II) chloride hexahydrate	0.1 M	Tris	8.5	20 % w/v	PEG 4000		
H10	0.15 M	Potassium thiocyanate	0.1 M	Tris	8.5	18 % w/v	PEG 3350		
H11	0.15 M	Potassium thiocyanate	0.1 M	Tris	8.5	18 % w/v	PEG 5000 MME		
H12	0.15 M	Potassium thiocyanate	0.1 M	Tris	8.5	15 % w/v	PEG 6000		



Abbreviations: PEG, polyethylene glycol (concentrations quoted as w/v %); MME, monomethyl ether; MPD, 2-methyl-2,4-pentanediol; butanediol, 1,4-butanediol; Jeffamine M-600 is at pH 7.

Manufacturer's safety data sheets are available from our website or by scanning the QR code here:



Ordering details:

Catalogue Description

Catalogue Code

Clear Strategy Screen I	(24 x 10 mL + 5 x 10 mL buffers)	MD1-14
Clear Strategy Screen II	(24 x 10 mL + 5 x 10 mL buffers)	MD1-15
Clear Strategy Screen I & II (Combination Screen)	(48 x 10 mL kit + 10 x 10 mL buffers)	MD1-16
Clear Strategy I HT-96	(96 x 1 mL)	MD1-31
Clear Strategy II HT-96	(96 x 1 mL)	MD1-32

ECO Screens

Clear Strategy Screen I	(24 x 10 mL + 5 x 10 mL buffers)	MD1-14-ECO
Clear Strategy Screen II	(24 x 10 mL + 5 x 10 mL buffers)	MD1-15-ECO
Clear Strategy Screen I & II (Combination Screen)	(48 x 10 mL kit + 10 x 10 mL buffers)	MD1-16-ECO
Clear Strategy I HT-96	(96 x 1 mL)	MD1-31-ECO
Clear Strategy II HT-96	(96 x 1 mL)	MD1-32-ECO

Single Reagents

Clear Strategy Screen I	(100 mL)	MDSR-14 - tube number
Clear Strategy Screen II	(100 mL)	MDSR-15 - tube number
Clear Strategy I HT-96	(100 mL)	MDSR-31 - well number
Clear Strategy II HT-96	(100 mL)	MDSR-32 - well number

For Clear Strategy™ Screen stock reagents visit our Optimization page on our website.