

Clear Strategy™ Screen II MD1-15

A complimentary 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 contains 24 x 10⁺ mL stock solutions and five (2 x 10 mL) pH'd 1M buffers allowing full control of the pH of the screen solutions and facilitating cryoprotection and potential incorporation of anomalous scatters.

Features of Clear Strategy II:

- 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

Although Clear Strategy Screen I (CSSI) has wide applications, there is a need for a complementary 6 x 4 set of conditions that fills gaps in the network of crystallization parameters. The basic principles of CSSI were also applied to Clear Strategy Screen II (CSSII). 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.

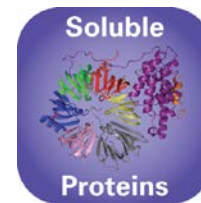
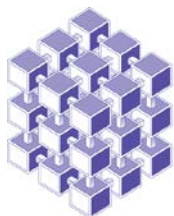
- **Area B: Conditions (13 - 14) and (19 - 20)** function as an 'organic' solution screen.
- **Area C: Conditions 15 and 21** evaluate the influence of heavier cations on protein crystallization properties.
- **Section D: Conditions (16 -18) and (22 - 24)** supplements the CSS-I with other PEGS mixed 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 CSSI, CSSII gives each individual investigator a wide range of tools and parameters, to reflect both personal experience and the specificity of the project.

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

[†] Our tubes are overfilled to 11 mL.



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.

CSSII 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-CF.

Although driven by slightly different principles, CSSI and CSSII 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.

To set up a screen:

Typically the pH of 0.9ml of the screen solution can be adjusted by the addition of 0.1ml of 1M stock buffer. Therefore, 10 × concentrate (1M) buffer should be added to a stock solution in the proportions of 1:9.

e.g. 50 μL buffer to 450 μL stock solution

100 μL buffer to 900 μL stock solution.

Each kit contains 24 stock solutions and the following buffers (1M):

Sodium acetate – pH 4.5

Sodium acetate – pH 5.5

Sodium cacodylate – pH 6.5

Tris – pH 7.5

Tris – pH 8.5

All buffers are titrated to specified pH using glacial acetic acid.

Formulation Notes:

CSS II reagents are formulated using ultrapure water (>18.0 M Ω) and are sterile-filtered using 0.22 μm 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.

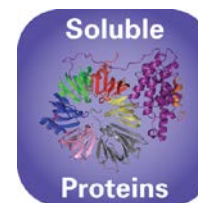
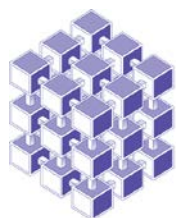
Enquiries regarding CSS II formulation, interpretation of results or optimization strategies are welcome. Please e-mail, fax or phone your query to Molecular Dimensions.

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 Screen II Conditions

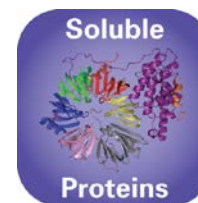
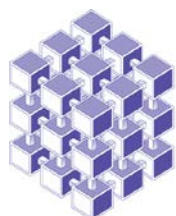
MD1-15

1 0.3 M Na acetate 25% PEG 2000 MME	2 0.2 M Li ₂ SO ₄ 25% PEG 2000 MME	3 0.2 M MgCl ₂ 25% PEG 2000 MME	4 0.2 M KBr 25% PEG 2000 MME	5 0.2 M KSCN 25% PEG 2000 MME	6 0.8 M Na formate 25% PEG 2000 MME
7 0.3 M Na acetate 15% PEG 4000	8 0.2 M Li ₂ SO ₄ 15% PEG 4000	9 0.2 M MgCl ₂ 15% PEG 4000	10 0.2 M KBr 15% PEG 4000	11 0.2 M KSCN 15% PEG 4000	12 0.8 M Na formate 15% PEG 4000
13 0.3 M Na acetate 10% PEG 8000+ 10% PEG 1000	14 0.2 M Li ₂ SO ₄ 10% PEG 8000+ 10% PEG 1000	15 0.2 M MgCl ₂ 10% PEG 8000+ 10% PEG 1000	16 0.2 M KBr 10% PEG 8000+ 10% PEG 1000	17 0.2 M KSCN 10% PEG 8000+ 10% PEG 1000	18 0.8 M Na formate 10% PEG 8000+ 10% PEG 1000
19 0.3 M Na acetate 8% PEG 20,000 + 8% PEG 500 MME	20 0.2 M Li ₂ SO ₄ 8% PEG 20,000 + 8% PEG 500 MME	21 0.2 M MgCl ₂ 8% PEG 20,000 + 8% PEG 500 MME	22 0.2 M KBr 8% PEG 20,000 + 8% PEG 500 MME	23 0.2 M KSCN 8% PEG 20,000 + 8% PEG 500 MME	24 0.8 M Na formate 8% PEG 20,000 + 8% PEG 500 MME

Abbreviations: **Na acetate**, Sodium acetate trihydrate; **Li₂SO₄**, lithium sulfate; **MgCl₂**, Magnesium chloride hexahydrate; **KBr**, Potassium bromide; **KSCN**, Potassium thiocyanate; **Na formate**, sodium formate; **PEG**, polyethylene glycol (concentrations quoted as w/v %); **MME**, monomethyl ether.

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

Cacodylate-free versions

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

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