Molecular Dimensions

Soluble Proteins

Clear Strategy™ Screen I MD1-14

A 6 × 4 matrix screen* that offers a more rational, logical and flexible approach to crystallization experiments.

The kit contains 24 stock solutions (10ml) and five pH'd 1M buffers allowing full control of the pH of the screen solutions and facilitating cryoprotection and potential incorporation of anomalous scatters

MD1-14 is presented as a 24 x 10† mL stock conditions + 2 x 10 mL of 5 different pH'd buffers.

Features of Clear Strategy I:

- 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.

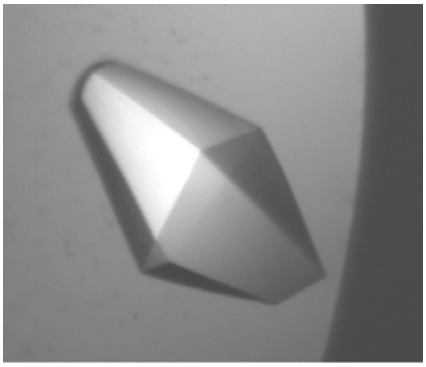
Introduction

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 I (CSS-I) was designed with the following principles in mind:

1. Enzyme proteins as a target.
2. Full control of screen solution pH.
3. Cryoprotection of crystals.
4. Rational planning of further experiments.
5. Provision of potential anomalous scattering centres.

One of the main principles behind the formulation of the CSS-I screen was to increase the rate of successful crystallization of enzymatic proteins. It yielded crystals for several nuclear receptor complexes1, proteins involved in the process of bacterial sporulation, fragments of fibrinogen and growth factors. Crystals of a given protein were often obtained simultaneously in several different conditions. Recently, the ability to control pH was used successfully in the optimization of the crystallization of the 70S ribosome complexed with mRNA and tRNA.



Crystal of the AAA domain of an ATP dependent protease, FtsH, grown using CSS1. Kryzywda *et al* (2002), Acta Cryst. **D58**, 1066

* Developed by Dr. A M Brzozowski and J. Walton from the Structural Biology Laboratory at The University of York and all kits produced are under an exclusive licence from The University of York, UK.

† Our tubes are overfilled to 11 mL.

pH control

One of the most important parameters in the crystallization process is pH. The formulation of both Clear Strategy Screens at 90% of their final volumes leaves the choice of the pH of the screen to the user. Typically the pH of 0.9ml of the screen solution can be adjusted by the addition of 0.1ml of 1M stock buffer.

The starting pH depends upon prior knowledge of each protein's properties, such as purification characteristics, isoelectric point, solubility/stability, pH-aggregation dependence estimated by dynamic light scattering (DLS) and previous crystallization experience with related proteins.

If the optimum pH is unclear, cacodylate buffer at pH 6.5 can be used as a first choice. This covers a broad plateau of pKa values of individual amino acids and provides additional protection against potential specific protein aggregation caused by free –SH groups.

Clear Strategy Screen I shows that the rational use of pH can accelerate successful crystallogenesis through the minimum number of trials.

Cryoprotection

The CSS-I simple but efficient 6 × 4 matrix was designed with some built in provision for the straightforward cryoprotection of any resultant crystals. Crystals obtained with PEGs of 2000 and 4000 MW may be cryoprotected using the same PEGs at their concentrations (app. 30%-35% w/v). Potential cryoprotection of the crystals grown with PEG 8000 and 20,000 has been facilitated by the introduction of additional PEGs of smaller molecular weights. Both PEG 1000 and 550 MW are good cryoprotectants at higher concentrations.

Rational design of further experiments

One of the main aims of the **Clear Strategy I** formulation is that the underlying principles should be very transparent to the user. A simple matrix of different PEGs Vs different salts combined with simultaneous control of pH enables both easy interpretation of results and planning of the next experiments. A new set of conditions can easily be achieved by an increase in the salt or PEG concentration, a shift towards one of the two mixed PEGs or even a change of the pH.

Anomalous scattering centres

The coupling of new crystallization screens with modern methods to solve the crystallographic phase problem is of special importance for high throughput crystallography. One of the easiest ways to implement this[3] is by soaking protein crystals in cryoprotectants containing Br^- or I^-.

To increase the chance of the application of this important approach, one set of **CSS-1** conditions includes potassium bromide. Several well diffracting crystals have been obtained from these conditions and we are currently evaluating whether initial phase estimates can be obtained through location of anomalous scatter sites.

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:

CSSI reagents are formulated using ultrapure water (>18.0 MΩ) 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 CSSI 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

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Clear Strategy Screen I Conditions MD1-14

1 0.3 M Na acetate 25% PEG 2000 MME	2 0.2 M Li_2SO_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_2SO_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_2SO_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_2SO_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; **Li_2SO_4**, lithium sulfate**;** **$MgCl_2$**, 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 Clear Strategy™ Screen stock reagents visit our Optimization page on our website.

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Soluble Proteins

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 × 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 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+}, $Cu2^{+}$, 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.

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

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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 MΩ) 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.

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References

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Clear Strategy Screen II Conditions MD1-15

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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.