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# Clear Strategy Screen™ I HT-96 MD1-31

The first of two high-throughput screens*, that allows easy interpretation of results and optimization of experiments.

The Clear Strategy I 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 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.2

## 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 2K and 4K MW may be cryoprotected using the same PEGs at their concentrations (app. 30%-35% w/v). Potential cryoprotection of the crystals grown with PEG 8K and 20K has been facilitated by the introduction of additional PEGs of smaller molecular weights. Both PEG 1K and 550 MW are good cryoprotectants at higher concentrations.

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


**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.

CSS-I is a simple, flexible and efficient screen that can be used in sitting drop, hanging drop and sandwich experiments.

## 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 formulation, interpretation of results or optimization strategies are welcome. Please e-mail, fax or phone your query to Molecular Dimensions:

enquiries@moleculardimensions.com

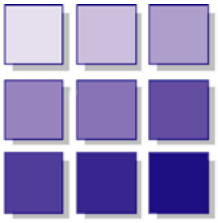
Contact and product details can be found at:

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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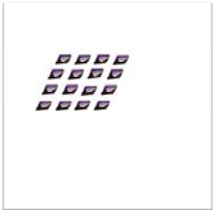

# Crystal Strategy Screen I MD1-31 Rows A – D

| HT-96 Well | Salt | Buffer | Precipitant |
|---|---|---|---|
| A1 | 0.3 M sodium acetate | 0.1 M sodium acetate pH 5.5 | 25% w/v PEG 2K MME |
| A2 | 0.2 M lithium sulfate | 0.1 M sodium acetate pH 5.5 | 25% w/v PEG 2K MME |
| A3 | 0.2 M magnesium chloride | 0.1 M sodium acetate pH 5.5 | 25% w/v PEG 2K MME |
| A4 | 0.2 M potassium bromide | 0.1 M sodium acetate pH 5.5 | 25% w/v PEG 2K MME |
| A5 | 0.2 M potassium thiocyanate | 0.1 M sodium acetate pH 5.5 | 25% w/v PEG 2K MME |
| A6 | 0.8 M sodium formate | 0.1 M sodium acetate pH 5.5 | 25% w/v PEG 2K MME |
| A7 | 0.3 M sodium acetate | 0.1 M sodium acetate pH 5.5 | 15% w/v PEG 4K |
| A8 | 0.2 M lithium sulfate | 0.1 M sodium acetate pH 5.5 | 15% w/v PEG 4K |
| A9 | 0.2 M magnesium chloride | 0.1 M sodium acetate pH 5.5 | 15% w/v PEG 4K |
| A10 | 0.2 M potassium bromide | 0.1 M sodium acetate pH 5.5 | 15% w/v PEG 4K |
| A11 | 0.2 M potassium thiocyanate | 0.1 M sodium acetate pH 5.5 | 15% w/v PEG 4K |
| A12 | 0.8 M sodium formate | 0.1 M sodium acetate pH 5.5 | 15% w/v PEG 4K |
| B1 | 0.3 M sodium acetate | 0.1 M sodium acetate pH 5.5 | 10% w/v PEG 8K + 10% w/v PEG 1K |
| B2 | 0.2 M lithium sulfate | 0.1 M sodium acetate pH 5.5 | 10% w/v PEG 8K + 10% w/v PEG 1K |
| B3 | 0.2 M magnesium chloride | 0.1 M sodium acetate pH 5.5 | 10% w/v PEG 8K + 10% w/v PEG 1K |
| B4 | 0.2 M potassium bromide | 0.1 M sodium acetate pH 5.5 | 10% w/v PEG 8K + 10% w/v PEG 1K |
| B5 | 0.2 M potassium thiocyanate | 0.1 M sodium acetate pH 5.5 | 10% w/v PEG 8K + 10% w/v PEG 1K |
| B6 | 0.8 M sodium formate | 0.1 M sodium acetate pH 5.5 | 10% w/v PEG 8K + 10% w/v PEG 1K |
| B7 | 0.3 M sodium acetate | 0.1 M sodium acetate pH 5.5 | 8% w/v PEG 20K + 8% v/v PEG 550 MME |
| B8 | 0.2 M lithium sulfate | 0.1 M sodium acetate pH 5.5 | 8% w/v PEG 20K + 8% v/v PEG 550 MME |
| B9 | 0.2 M magnesium chloride | 0.1 M sodium acetate pH 5.5 | 8% w/v PEG 20K + 8% v/v PEG 550 MME |
| B10 | 0.2 M potassium bromide | 0.1 M sodium acetate pH 5.5 | 8% w/v PEG 20K + 8% v/v PEG 550 MME |
| B11 | 0.2 M potassium thiocyanate | 0.1 M sodium acetate pH 5.5 | 8% w/v PEG 20K + 8% v/v PEG 550 MME |
| B12 | 0.8 M sodium formate | 0.1 M sodium acetate pH 5.5 | 8% w/v PEG 20K + 8% v/v PEG 550 MME |
| C1 | 0.3 M sodium acetate | 0.1 M sodium cacodylate pH 6.5 | 25% w/v PEG 2K MME |
| C2 | 0.2 M lithium sulfate | 0.1 M sodium cacodylate pH 6.5 | 25% w/v PEG 2K MME |
| C3 | 0.2 M magnesium chloride | 0.1 M sodium cacodylate pH 6.5 | 25% w/v PEG 2K MME |
| C4 | 0.2 M potassium bromide | 0.1 M sodium cacodylate pH 6.5 | 25% w/v PEG 2K MME |
| C5 | 0.2 M potassium thiocyanate | 0.1 M sodium cacodylate pH 6.5 | 25% w/v PEG 2K MME |
| C6 | 0.8 M sodium formate | 0.1 M sodium cacodylate pH 6.5 | 25% w/v PEG 2K MME |
| C7 | 0.3 M sodium acetate | 0.1 M sodium cacodylate pH 6.5 | 15% w/v PEG 4K |
| C8 | 0.2 M lithium sulfate | 0.1 M sodium cacodylate pH 6.5 | 15% w/v PEG 4K |
| C9 | 0.2 M magnesium chloride | 0.1 M sodium cacodylate pH 6.5 | 15% w/v PEG 4K |
| C10 | 0.2 M potassium bromide | 0.1 M sodium cacodylate pH 6.5 | 15% w/v PEG 4K |
| C11 | 0.2 M potassium thiocyanate | 0.1 M sodium cacodylate pH 6.5 | 15% w/v PEG 4K |
| C12 | 0.8 M sodium formate | 0.1 M sodium cacodylate pH 6.5 | 15% w/v PEG 4K |
| D1 | 0.3 M sodium acetate | 0.1 M sodium cacodylate pH 6.5 | 10% w/v PEG 8K + 10% w/v PEG 1K |
| D2 | 0.2 M lithium sulfate | 0.1 M sodium cacodylate pH 6.5 | 10% w/v PEG 8K + 10% w/v PEG 1K |
| D3 | 0.2 M magnesium chloride | 0.1 M sodium cacodylate pH 6.5 | 10% w/v PEG 8K + 10% w/v PEG 1K |
| D4 | 0.2 M potassium bromide | 0.1 M sodium cacodylate pH 6.5 | 10% w/v PEG 8K + 10% w/v PEG 1K |
| D5 | 0.2 M potassium thiocyanate | 0.1 M sodium cacodylate pH 6.5 | 10% w/v PEG 8K + 10% w/v PEG 1K |
| D6 | 0.8 M sodium formate | 0.1 M sodium cacodylate pH 6.5 | 10% w/v PEG 8K + 10% w/v PEG 1K |
| D7 | 0.3 M sodium acetate | 0.1 M sodium cacodylate pH 6.5 | 8% w/v PEG 20K + 8% v/v PEG 550 MME |
| D8 | 0.2 M lithium sulfate | 0.1 M sodium cacodylate pH 6.5 | 8% w/v PEG 20K + 8% v/v PEG 550 MME |
| D9 | 0.2 M magnesium chloride | 0.1 M sodium cacodylate pH 6.5 | 8% w/v PEG 20K + 8% v/v PEG 550 MME |
| D10 | 0.2 M potassium bromide | 0.1 M sodium cacodylate pH 6.5 | 8% w/v PEG 20K + 8% v/v PEG 550 MME |
| D11 | 0.2 M potassium thiocyanate | 0.1 M sodium cacodylate pH 6.5 | 8% w/v PEG 20K + 8% v/v PEG 550 MME |
| D12 | 0.8 M sodium formate | 0.1 M sodium cacodylate pH 6.5 | 8% w/v PEG 20K + 8% v/v PEG 550 MME |

# Crystal Strategy Screen I HT-96 MD1-31 Rows E – H

| HT-96 Well | Salt | Buffer | Precipitant |
|---|---|---|---|
| E1 | 0.3 M sodium acetate | 0.1 M Tris pH 7.5 | 25% w/v PEG 2K MME |
| E2 | 0.2 M lithium sulfate | 0.1 M Tris pH 7.5 | 25% w/v PEG 2K MME |
| E3 | 0.2 M magnesium chloride | 0.1 M Tris pH 7.5 | 25% w/v PEG 2K MME |
| E4 | 0.2 M potassium bromide | 0.1 M Tris pH 7.5 | 25% w/v PEG 2K MME |
| E5 | 0.2 M potassium thiocyanate | 0.1 M Tris pH 7.5 | 25% w/v PEG 2K MME |
| E6 | 0.8 M sodium formate | 0.1 M Tris pH 7.5 | 25% w/v PEG 2K MME |
| E7 | 0.3 M sodium acetate | 0.1 M Tris pH 7.5 | 15% w/v PEG 4K |
| E8 | 0.2 M lithium sulfate | 0.1 M Tris pH 7.5 | 15% w/v PEG 4K |
| E9 | 0.2 M magnesium chloride | 0.1 M Tris pH 7.5 | 15% w/v PEG 4K |
| E10 | 0.2 M potassium bromide | 0.1 M Tris pH 7.5 | 15% w/v PEG 4K |
| E11 | 0.2 M potassium thiocyanate | 0.1 M Tris pH 7.5 | 15% w/v PEG 4K |
| E12 | 0.8 M sodium formate | 0.1 M Tris pH 7.5 | 15% w/v PEG 4K |
| F1 | 0.3 M sodium acetate | 0.1 M Tris pH 7.5 | 10% w/v PEG 8K + 10% w/v PEG 1K |
| F2 | 0.2 M lithium sulfate | 0.1 M Tris pH 7.5 | 10% w/v PEG 8K + 10% w/v PEG 1K |
| F3 | 0.2 M magnesium chloride | 0.1 M Tris pH 7.5 | 10% w/v PEG 8K + 10% w/v PEG 1K |
| F4 | 0.2 M potassium bromide | 0.1 M Tris pH 7.5 | 10% w/v PEG 8K + 10% w/v PEG 1K |
| F5 | 0.2 M potassium thiocyanate | 0.1 M Tris pH 7.5 | 10% w/v PEG 8K + 10% w/v PEG 1K |
| F6 | 0.8 M sodium formate | 0.1 M Tris pH 7.5 | 10% w/v PEG 8K + 10% w/v PEG 1K |
| F7 | 0.3 M sodium acetate | 0.1 M Tris pH 7.5 | 8% w/v PEG 20K + 8% v/v PEG 550 MME |
| F8 | 0.2 M lithium sulfate | 0.1 M Tris pH 7.5 | 8% w/v PEG 20K + 8% v/v PEG 550 MME |
| F9 | 0.2 M magnesium chloride | 0.1 M Tris pH 7.5 | 8% w/v PEG 20K + 8% v/v PEG 550 MME |
| F10 | 0.2 M potassium bromide | 0.1 M Tris pH 7.5 | 8% w/v PEG 20K + 8% v/v PEG 550 MME |
| F11 | 0.2 M potassium thiocyanate | 0.1 M Tris pH 7.5 | 8% w/v PEG 20K + 8% v/v PEG 550 MME |
| F12 | 0.8 M sodium formate | 0.1 M Tris pH 7.5 | 8% w/v PEG 20K + 8% v/v PEG 550 MME |
| G1 | 0.3 M sodium acetate | 0.1 M Tris pH 8.5 | 25% w/v PEG 2K MME |
| G2 | 0.2 M lithium sulfate | 0.1 M Tris pH 8.5 | 25% w/v PEG 2K MME |
| G3 | 0.2 M magnesium chloride | 0.1 M Tris pH 8.5 | 25% w/v PEG 2K MME |
| G4 | 0.2 M potassium bromide | 0.1 M Tris pH 8.5 | 25% w/v PEG 2K MME |
| G5 | 0.2 M potassium thiocyanate | 0.1 M Tris pH 8.5 | 25% w/v PEG 2K MME |
| G6 | 0.8 M sodium formate | 0.1 M Tris pH 8.5 | 25% w/v PEG 2K MME |
| G7 | 0.3 M sodium acetate | 0.1 M Tris pH 8.5 | 15% w/v PEG 4K |
| G8 | 0.2 M lithium sulfate | 0.1 M Tris pH 8.5 | 15% w/v PEG 4K |
| G9 | 0.2 M magnesium chloride | 0.1 M Tris pH 8.5 | 15% w/v PEG 4K |
| G10 | 0.2 M potassium bromide | 0.1 M Tris pH 8.5 | 15% w/v PEG 4K |
| G11 | 0.2 M potassium thiocyanate | 0.1 M Tris pH 8.5 | 15% w/v PEG 4K |
| G12 | 0.8 M sodium formate | 0.1 M Tris pH 8.5 | 15% w/v PEG 4K |
| H1 | 0.3 M sodium acetate | 0.1 M Tris pH 8.5 | 10% w/v PEG 8K + 10% w/v PEG 1K |
| H2 | 0.2 M lithium sulfate | 0.1 M Tris pH 8.5 | 10% w/v PEG 8K + 10% w/v PEG 1K |
| H3 | 0.2 M magnesium chloride | 0.1 M Tris pH 8.5 | 10% w/v PEG 8K + 10% w/v PEG 1K |
| H4 | 0.2 M potassium bromide | 0.1 M Tris pH 8.5 | 10% w/v PEG 8K + 10% w/v PEG 1K |
| H5 | 0.2 M potassium thiocyanate | 0.1 M Tris pH 8.5 | 10% w/v PEG 8K + 10% w/v PEG 1K |
| H6 | 0.8 M sodium formate | 0.1 M Tris pH 8.5 | 10% w/v PEG 8K + 10% w/v PEG 1K |
| H7 | 0.3 M sodium acetate | 0.1 M Tris pH 8.5 | 8% w/v PEG 20K + 8% v/v PEG 550 MME |
| H8 | 0.2 M lithium sulfate | 0.1 M Tris pH 8.5 | 8% w/v PEG 20K + 8% v/v PEG 550 MME |
| H9 | 0.2 M magnesium chloride | 0.1 M Tris pH 8.5 | 8% w/v PEG 20K + 8% v/v PEG 550 MME |
| H10 | 0.2 M potassium bromide | 0.1 M Tris pH 8.5 | 8% w/v PEG 20K + 8% v/v PEG 550 MME |
| H11 | 0.2 M potassium thiocyanate | 0.1 M Tris pH 8.5 | 8% w/v PEG 20K + 8% v/v PEG 550 MME |
| H12 | 0.8 M sodium formate | 0.1 M Tris pH 8.5 | 8% w/v PEG 20K + 8% v/v PEG 550 MME |

**Abbreviations:**

**Tris**; 2-Amino-2-(hydroxymethyl)propane-1,3-diol, **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.