Crystal Screen HT™ is a high throughput reagent kit designed to provide a rapid screening method for the crystallization of biological macromolecules. The kit is straightforward, effective, and practical for the determination of preliminary crystallization conditions. The kit is also effective in determining the solubility of a macromolecule in a wide range of reagents and pH.

Crystal Screen HT is supplied in a sterile, polypropylene DeepWell block, each reservoir containing 1 ml of sterile filtered reagent. The block is heat sealed using a special polypropylene backed film.

Crystal Screen and Crystal Screen 2 offer a sparse matrix of trial crystallization reagent conditions based upon the original Jancarik and Kim screen (3). The primary screen variables are salt, pH, and precipitant (salts, polymers, volatile organics, and non-volatile organics).

Sample Preparation
The macromolecular sample should be homogenous, as pure as is practically possible (>95%) and free of amorphous and particulate material. Remove amorphous material by centrifugation or microfiltration prior to use (1, 2, 4).

The recommended sample concentration is 5 to 25 mg/ml in sterile filtered, deionized water or dilute (25 mM or less) buffer. For initial screens, the sample should be free of unnecessary additives in order to observe the effect of the Crystal Screen and Crystal Screen 2 variables. However, agents that promote and preserve sample stability and homogeneity can and should be included in the sample. For additional sample preparation recommendation see Crystal Growth 101 - Preliminary Sample Preparation bulletin from Hampton Research.

Preparing the DeepWell Block for Use
It is recommended the DeepWell block be centrifuged before removing the sealing film. Centrifugation at 500 rpm for five minutes will remove stray reagent from the sealing film. Removing the reagent from the film prevents stray reagent droplets from falling into neighboring wells during film removal. After centrifugation the film can be removed by grasping a corner of the film and gently peeling the film from the plate. Alternatively, the film can be left intact and the pierced for reagent access.

Performing The Screen

Manual Method - Sitting Drop Vapor Diffusion

1. Using a 96 well sitting drop vapor diffusion plate, pipet the recommended volume (typically 100 microliters) of crystallization reagent from the DeepWell block into the reservoirs of the crystallization plate. The DeepWell block is compatible with 8 and 12 channel pipets as well as many automated liquid handling systems. Use clean pipet tips for each reagent set transfer and change pipet tips when changing reagents. For an 8 channel pipet, transfer reagents A1-A8 to reservoirs A1-A8 of the crystallization plate. Repeat this procedure for reagent columns B through L. Change pipet tips when moving between reagent columns. For a 12 channel pipet, transfer reagents A1-L1 to reservoirs A1-L1 of the crystallization plate. Repeat this procedure for reagent rows 1 through 8. See figure 1. Time and pipet tips can be conserved by batch pipetting multiple plates with the same (row or column) of reagent before changing reagent and pipet tips.

2. Using clean pipet tips, pipet 0.05 to 2 microliters of crystallization reagent from the crystallization plate reservoir to the sitting drop well. Some 96 well crystallization plates allow this procedure to be performed using a multichannel pipet where other plates require the use of a single channel pipet. Change the pipet tip between reagents. See figure 2.

3. Using a clean pipet tip, pipet 0.05 to 2 microliters of sample to the reagent drop in the sitting drop well. One may choose to simply dispense the sample with no mixing or dispense with mixing by gently aspirating and dispensing the sample several times, keeping the tip in the drop during mixing to avoid foaming. Work carefully but quickly to minimize evaporation from the crystallization plate. See figure 2.

4. Seal the crystallization plate as per the manufacturer's recommendation. Most 96 well crystallization plates are sealed using a clear sealing tape or film. View and score the experiment as desired. See Hampton Research technical bulletin Crystal Growth 101 - Viewing Crystallization Experiments for additional information on viewing drops.

5. Seal the remaining reagent in the DeepWell block using either tape/film or a mat.

Manual Method - MicroBatch 96 well format

1. Using a 96 well clear polystyrene microplate (U-bottom recommended for best drop centering, flat-bottom recommended for best optics) pipet approximately 150 microliters of microbatch compatible oil into each of the 96 reservoirs. This can be accomplished using an 8 or 12 channel pipet and pipetting the oil from a reagent basin. See figure 3.

2. Seal the crystallization plate as per the manufacturer's recommendation. Most 96 well crystallization plates are sealed using a clear sealing tape or film. View and score the experiment as desired. See Hampton Research technical bulletin Crystal Growth 101 - Viewing Crystallization Experiments for additional information on viewing drops.

3. Seal the remaining reagent in the DeepWell block using either tape/film or a mat.
2. Once the plate is oiled, use an 8 or 12 channel pipet to aspirate reagent from the DeepWell block and dispense the reagent under the oil in the MicroBatch plate. Change tips when changing reagent to prevent cross reagent contamination. To save time and pipet tips, set multiple plates at one time. See figure 4.

3. Using a single channel pipet, aspirate the sample and dispense the sample under oil in the MicroBatch plate. It is not necessary to dispense the sample drop into the reagent drop or mix the drops. See figure 5.

4. After all reagent and sample drops have been dispensed to the MicroBatch plate, place the loose fitting clear cover on the MicroBatch plate and centrifuge the plate for 10 minutes at 500 rpm. Centrifugation will cause the drops to coalesce into a single drop. Note if the drops appear flat or is fragmented into multiple drops, the centrifugation speed is too high and the centrifugation time too long - adjust to obtain a spherical single drop in the center of the well.

5. Store the plates with the loose fitting clear polystyrene cover and observe for crystals. See Hampton Research technical bulletin Crystal Growth 101 - Viewing Crystallization Experiments for additional information on viewing drops.

Crystal Screen and Crystal Screen 2 DeepWell Block and Automated Liquid Handling Systems

The polypropylene DeepWell block is designed to be compatible with the SBS standard 96 microwell format and is therefore compatible with numerous automated liquid handling systems that accept 8x12 96 well assay blocks. Follow the manufacturer’s recommendation for handling deep well microplates.

Examine The Drop

Carefully examine the drops under a stereo microscope (10 to 100x magnification) immediately after setting up the screen. Record all observations and be particularly careful to scan the focal plane for small crystals. Observe the drops once each day for the first week, then once a week thereafter. Records should indicate whether the drop is clear, contains precipitate, or contains crystals. It is helpful to describe the drop contents using descriptive terms. Adding magnitude is also helpful. Example: 4+ yellow/brown fine precipitate, 2+ small bipyramid crystals, clear drop, 3+ needle shaped crystals in 1+ white precipitate. One may also employ a standard numerical scoring scheme (Clear = 0, Precipitate = 1, Crystal = 10, etc). Figure 6 (on page 3) shows typical examples of what one might observe in a crystallization experiment.

Interpreting Crystal Screen HT

Clear drops indicate that either the relative supersaturation of the sample and reagent is too low or the drop has not yet completed equilibration. If the drop remains clear after 3 to 4 weeks consider repeating the screen condition and doubling the sample concentration. If more than 70 of the 96 screen drops are clear, consider doubling the sample concentration and repeating the entire screen.

Drops containing precipitate indicate either the relative supersaturation of the sample and reagent is too high, the sample has denatured, or the sample is heterogeneous. To reduce the relative supersaturation, dilute the sample twofold and repeat the screen condition. If more than 70 of the 96 screen drops contain precipitate and no crystals are present, consider diluting the sample concentration in half and repeating the entire screen. If sample denaturation is suspect, take measures to stabilize the sample (add reducing agent, ligands, glycerol, salt, or other stabilizing agents). If the sample is impure, aggregated, or heterogeneous take measures to pursue homogeneity. It is possible to obtain crystals from precipitate so do not discard nor ignore a drop containing precipitate. If possible, examine drops containing precipitate under polarizing optics to differentiate precipitate from microcrystalline material.

If the drop contains a macromolecular crystal the relative supersaturation of the sample and reagent is appropriate for crystal nucleation and growth. The next step is to optimize the preliminary conditions (pH, salt type, salt concentration, precipitant type, precipitant concentration, sample concentration, temperature, additives, and other crystallization variables) which produced the crystal in order to improve crystal size and quality.

Compare the observations between the 4°C and room temperature incubation to determine the effect of temperature on sample solubility. Different results in the same drops at different temperatures indicate that sample solubility is temperature dependent and that one should include temperature as a variable in subsequent screens and optimization experiments.

Retain and observe plates until the drops are dried out. Crystal growth can occur within 15 minutes or one year.

Crystal Screen HT Formulation

Crystallization reagents are formulated using the highest purity chemicals, ultrapure water (18.2 Megohm-cm, 5 ppb TOC) and are sterile filtered using 0.22 micron filters into sterile DeepWell blocks (no preservatives added).

Crystallization reagents are readily reproduced using Hampton Research Optimize™ and StockOptions™ stock solutions of salts, polymers and buffers. Optimize and StockOptions stock reagents make reproducing crystallization screen reagents accurate, precise, fast, convenient and easy. Dilutions can be performed directly into the crystallization plate using Optimize and StockOptions stock reagents.

Crystallization reagents containing buffers are formulated by creating a 1.0 M stock buffer, titrated to the desired pH using hydrochloric acid or sodium hydroxide. The buffer is then diluted with the other reagent components and water. No further pH adjustment is required.
Crystallization reagents are stable at room temperature and are best used before the 'Best If Used By'. To enhance reagent stability it is strongly recommended that crystallization reagents be stored at 4°C or -20°C. Avoid ultraviolet light to preserve reagent stability.

If the sample contains phosphate, borate, or carbonate buffers it is possible to obtain inorganic crystals (false positives) when using crystallization reagents containing divalent cations such as magnesium, calcium, or zinc. To avoid false positives use phosphate, borate, or carbonate buffers at concentrations of 10 mM or less or exchange the phosphate, borate, or carbonate buffer with a more soluble buffer that does not complex with divalent cations.

References and Readings

Technical Support
Inquiries regarding Crystal Screen HT reagent formulation, interpretation of screen results, optimization strategies and general inquiries regarding crystallization are welcome. Please e-mail, fax, or telephone your request to Hampton Research. Fax and e-mail Technical Support are available 24 hours a day. Telephone technical support is available 8:00 a.m. to 5:00 p.m. USA Pacific Standard Time.
<table>
<thead>
<tr>
<th>Tube Number</th>
<th>Salt</th>
<th>Tube Number</th>
<th>Buffer</th>
<th>Tube Number</th>
<th>Precipitant</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1.</td>
<td>0.02 M Calcium Chloride dihydrate</td>
<td>A1.</td>
<td>0.1 M Sodium Acetate trihydrate pH 4.6</td>
<td>A1.</td>
<td>30% w/v 2-Methyl-2,4-pentanediol</td>
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<tr>
<td>A2.</td>
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<td>A3.</td>
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<tr>
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<td>0.1 M Tris Hydrochloride pH 8.5</td>
<td>A4.</td>
<td>2.0 M Ammonium Sulfate</td>
</tr>
<tr>
<td>A5.</td>
<td>0.2 M tri-Sodium Citrate dihydrate</td>
<td>A5.</td>
<td>0.1 M HEPES - Na pH 7.5</td>
<td>A5.</td>
<td>30% w/v 2-Methyl-2,4-pentanediol</td>
</tr>
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<td>0.2 M Magnesium Chloride hexahydrate</td>
<td>A6.</td>
<td>0.1 M Tris Hydrochloride pH 8.5</td>
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<tr>
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<td>0.1 M Sodium Cacodylate pH 6.5</td>
<td>A7.</td>
<td>1.4 M Sodium Acetate trihydrate</td>
</tr>
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</tr>
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<td>0.1 M tri-Sodium Citrate dihydrate pH 5.6</td>
<td>A9.</td>
<td>30% w/v Polyethylene Glycol 4000</td>
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<td>30% w/v Polyethylene Glycol 4000</td>
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<td>A11.</td>
<td>0.1 M mono-Ammonium dihydrogen Phosphate</td>
</tr>
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<td>0.1 M HEPES - Na pH 7.5</td>
<td>A12.</td>
<td>30% w/v iso-Propanol</td>
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<td>0.1 M Tris Hydrochloride pH 8.5</td>
<td>B1.</td>
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</tr>
<tr>
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<td>0.1 M Tris Hydrochloride pH 8.5</td>
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<td>30% w/v 2-Methyl-2,4-pentanediol</td>
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<tr>
<td>C3.</td>
<td>0.2 M tri-Sodium Citrate dihydrate</td>
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<td>0.1 M HEPES - Na pH 7.5</td>
<td>C3.</td>
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</tr>
<tr>
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</tr>
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<td>2.0 M Sodium Formate</td>
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<td>D9.</td>
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<td>0.1 M Sodium Cacodylate pH 6.5</td>
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<td>2.0 M Ammonium Sulfate</td>
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<td>0.1 M Tris Hydrochloride pH 8.5</td>
<td>D12.</td>
<td>2.0 M mono-Ammonium dihydrogen Phosphate</td>
</tr>
</tbody>
</table>

*Buffer pH is that of a 1.0 M stock prior to dilution with other reagent components. pH with HCl or NaOH.*

Crystal Screen (DeepWell Block) contains forty-eight unique reagents beginning at position A1. To determine the formulation of each reagent, simply read across the page.
<table>
<thead>
<tr>
<th>Tube Number</th>
<th>Salt</th>
<th>Buffer 1</th>
<th>Precipitant</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>2.0 M Sodium chloride</td>
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<td>E1. 10% w/v Polyethylene Glycol</td>
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<tr>
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<td></td>
<td></td>
<td>0.01 M Magnesium Chloride hexahydrate</td>
</tr>
<tr>
<td>E3</td>
<td>None</td>
<td>E3. None</td>
<td>E3. 25% v/v Ethylene Glycol</td>
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<td>E4</td>
<td>None</td>
<td>E4. None</td>
<td>E4. 35% v/v Dioxane</td>
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<tr>
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<td>E5. 5% v/v iso-Propanol</td>
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<td>E6. None</td>
<td>E6. 1.0 M Imidazole pH 7.0</td>
</tr>
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<td>E7</td>
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<td>E7. 10% w/v Polyethylene Glycol 1000</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>10% w/v Polyethylene Glycol 8000</td>
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<td>E8</td>
<td>1.5 M Sodium Chloride</td>
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<td>E8. 10% w/v Ethanol</td>
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<td>None</td>
<td>E9. None</td>
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<td>F1. 30% v/v Polyethylene Glycol Monomethyl Ether 2000</td>
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<td>F2. None</td>
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<td>F4</td>
<td>0.5 M Sodium Chloride</td>
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<td>G8. 1.6 M Magnesium Sulfate heptahydrate</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>G10</td>
<td>0.05 M Cadmium Sulfate hydrate</td>
<td>G10. None</td>
<td></td>
</tr>
<tr>
<td>G11</td>
<td>None</td>
<td>G11. None</td>
<td></td>
</tr>
<tr>
<td>G12</td>
<td>None</td>
<td>G12. None</td>
<td></td>
</tr>
<tr>
<td>H2</td>
<td>None</td>
<td>H2. None</td>
<td></td>
</tr>
<tr>
<td>H3</td>
<td>0.2 M Magnesium Chloride hexahydrate</td>
<td>H3. None</td>
<td></td>
</tr>
<tr>
<td>H4</td>
<td>None</td>
<td>H4. None</td>
<td></td>
</tr>
<tr>
<td>H5</td>
<td>0.01 M Nickel(II) Chloride hexahydrate</td>
<td>H5. None</td>
<td></td>
</tr>
<tr>
<td>H6</td>
<td>1.5 M Ammonium Sulfate</td>
<td>H6. None</td>
<td></td>
</tr>
<tr>
<td>H7</td>
<td>0.2 M mono Ammonium dihydrogen Phosphate</td>
<td>H7. None</td>
<td></td>
</tr>
<tr>
<td>H8</td>
<td>None</td>
<td>H8. None</td>
<td></td>
</tr>
<tr>
<td>H9</td>
<td>0.01 M Nickel(II) Chloride hexahydrate</td>
<td>H9. None</td>
<td></td>
</tr>
<tr>
<td>H10</td>
<td>0.1 M Sodium Chloride</td>
<td>H10. None</td>
<td></td>
</tr>
<tr>
<td>H11</td>
<td>None</td>
<td>H11. None</td>
<td></td>
</tr>
<tr>
<td>H12</td>
<td>2% v/v Dioxane</td>
<td>H12. None</td>
<td></td>
</tr>
</tbody>
</table>

† Buffer pH is that of a 1.0 M stock (0.5 M for MES) prior to solution with other reagent components, pH with HCl or NaOH.

Crystal Screen 2 (DeepWell Block) contains forty-eight unique reagents beginning at position E1.

To determine the formulation of each reagent, simply read across the page.
## Crystal Screen HT - Scoring Sheet

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample Buffer</th>
<th>Reservoir Volume</th>
<th>Drop Volume</th>
<th>Sample Concentration</th>
<th>Date:</th>
<th>Date:</th>
<th>Date:</th>
<th>Date:</th>
<th>Date:</th>
<th>Date:</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1.</td>
<td>30% MPD, 0.1 M Na Acetate pH 4.6, 0.02 M Calcium Chloride</td>
<td>A2.</td>
<td>0.4 M K, Na Tartrate</td>
<td>A3.</td>
<td>0.4 M Ammonium Phosphate</td>
<td>A4.</td>
<td>2.0 M Ammonium Sulfate, 0.1 M Tris HCl pH 8.5</td>
<td>A5.</td>
<td>30% MPD, 0.1 M Na Hapes pH 7.5, 0.2 M Sodium Citrate</td>
<td>A6.</td>
</tr>
</tbody>
</table>
Crystal Screen 2 HT - Scoring Sheet

Date: Date: Date: Date: Date:

1. Clear Drop
2. Phase Separation
3. Regular Granular Precipitate
4. Birefringent Precipitate or Microcrystals
5. Rosettes or Sphenoliths
6. Needles (1D Growth)
7. Plates (2D Growth)
8. Single Crystals (3D Growth < 0.2mm)
9. Single Crystals (3D Growth > 0.2mm)

Sample: Sample Buffer: Reservoir Volume: Sample Concentration: Date:

Clear Drop: Phase Separation: Regular Granular Precipitate: Birefringent Precipitate or Microcrystals: Rosettes or Sphenoliths: Needles (1D Growth): Plates (2D Growth): Single Crystals (3D Growth < 0.2mm): Single Crystals (3D Growth > 0.2mm):

<table>
<thead>
<tr>
<th>Drop Volume:</th>
<th>Sample Volume:</th>
<th>Reservoir Volume:</th>
<th>Additive Volume:</th>
</tr>
</thead>
</table>

Additive:

Sample:

Sample Buffer:

Reservoir Volume:

Date:

Temperature:

Sample:

Sample Buffer:

Reservoir Volume:

Sample:

Sample Buffer:

Reservoir Volume:

Sample:

Sample Buffer:

Reservoir Volume:

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