

## Description

Detergent Screen HT is a convenient, ready to use formulation of mild detergent reagents appropriate for use in protein solubility and crystallization studies.

Detergent Screen HT is a kit composed of 96 unique biological detergents, solubilizing reagents, and includes the following classes of reagents:

- Ionic detergents
  - Non-ionic detergents
  - Zwitterionic detergents
  - Non-detergent Sulfobetaines
  - Synthetic lipids
- **Ionic detergents** contain a head group with a net charge and are either anionic (negative charge) or cationic (positive charge). Ionic detergents are useful for dissociating sample-sample interactions. The CMC of ionic detergents is reduced by increasing the ionic strength of the reagent and is unaffected by temperature changes.
- **Non-ionic detergents** contain an uncharged hydrophilic head group. Non-ionic detergents manipulate sample-sample, sample-reagent, and sample-solvent interactions and are widely used in protein crystallization. Non-ionic detergents are non-denaturing and allow the solubilized protein to retain native structure, enzymatic activity and function. The CMC of non-ionic detergents is not significantly altered by changes in ionic strength although the CMC increases with increasing temperature.
- **Zwitterionic detergents** contain combined properties of ionic and non-ionic detergents and are useful for disrupting and manipulating sample-sample interactions.
- **Non-detergent Sulfobetaines (NDSB)** are non-denaturing protein solubilizing reagents used successfully for both the solubilization and crystallization of proteins<sup>1</sup>. NDSB reagents are zwitterionic, possess good solubility in water, do not alter significantly the pH or viscosity of reagent, and can easily be removed by dialysis since they do not form micelles. NDSB reagents are efficient in preventing non-specific interactions between proteins. They will not however, disrupt strongly aggregated proteins.
- **Synthetic lipids** are water soluble lipids that have detergent properties. These compounds are unique in that they contain a nonpolar, uncharged spacer between the hydrophilic head group and the hydrophobic tail. Synthetic lipids have been used in a number of different membrane protein applications including protein purification and structure determination.

## Formulation

The Detergent Screen HT reagent formulations are designed to be compatible with most crystallization reagents and yet allow one to screen the detergent at an appropriate concentration for solubility and crystallization studies.

Each solution is formulated in sterile filtered, deionized Type 1 reagent grade water and filled (0.250 milliliter per reagent) into sterile, thermal sealed polypropylene blocks.

The kit should be stored at minus 20°C. The block and reagents must be allowed to equilibrate to room temperature and mixed prior to removal of the sealing film and use of the reagents.

Individual Detergent Screen HT reagents are available through the Hampton Research Custom Shop in 0.5 ml volumes at the same concentration supplied in the kit. Visit [www.hamptonresearch.com](http://www.hamptonresearch.com) and Search for “detergent” and follow the link to Detergent Screen HT Custom Shop. For larger volumes or to obtain solid starting material e-mail your inquiry to [tech@hrmail.com](mailto:tech@hrmail.com) with the reagent number and lot number.

## Application

For crystallization to take place, a protein must be soluble in aqueous solution. Ideally the sample should be homogenous, monodisperse, and in a state of aggregation conducive to interactions which will promote the nucleation and subsequent growth of a crystal. Unfavorable aggregation can compete with, obstruct, and prevent the normal ordering of the sample into a crystal. Many proteins, typically membrane proteins, membrane associated proteins, and soluble proteins contain hydrophobic residues on the surface which can lead to non-specific aggregation, a deterrent to solubility and crystallization. Mild, biological detergents can perturb and manipulate hydrophobic sample-sample and sample-solvent interactions. Incorporating detergents into the solubilization or crystallization reagent during screening and optimization is a popular and effective strategy for identifying conditions which promote and enhance solubility and crystallization<sup>3</sup>. Besides improving the crystallization properties for some proteins and nucleic acids, detergents have also been shown to alleviate problems of twinning and secondary nucleation and can also produce different crystal forms<sup>4,5</sup>.

Since it can be difficult to impossible a priori to predict and select which detergent or solubilizing agent will minimize the general nonspecific interactions yet at the same time sustain the specific interactions necessary for crystallization, the screening of a portfolio of detergent and solubilizing reagents is an efficient and effective strategy to identify the appropriate and best detergent reagent. The Detergent Screen HT is a set of solubilization and crystallization specific, mild, biological detergent reagents in a ready to use format at concentrations appropriate for solubility and crystallization screens. The Detergent Screen HT format allows one to identify a detergent effect as well as help select the best detergent reagent.

## Use - Definitions

### Sample

The protein, peptide, nucleic acid, or other biological macromolecule target for solubilization or crystallization.

### Detergent Reagent

Any one of the 96 Detergent Screen HT reagents.

### Crystallization Reagent

Any mixture of chemicals used to crystallize the sample.

## Example 1

### • Detergent reagent in drop only

Vapor Diffusion Crystallization: Detergent Reagents need only appear in the crystallization drop.

Detergent Reagents DO NOT need to be dispensed into the reservoir for vapor diffusion. It is recommended the crystallization drop be built by adding Sample first, followed by Detergent Reagent and finally the Crystallization Reagent. This procedure allows the Detergent Reagent to interact with the sample prior to the addition of Crystallization Reagent to the drop.

### • Reservoir

Pipette the appropriate volume of Crystallization Reagent into the plate reservoir.

### • Drop

The recommended drop ratio is:

5 parts Sample : 1 part Detergent Reagent : 4 parts Crystallization Reagent

Example: 500 nl protein + 100 nl Detergent Reagent + 400 nl Crystallization Reagent. Seal the plate.

## Example 2

### • Detergent reagent in drop and reservoir

Vapor Diffusion Crystallization: Detergent Reagents need only appear in the crystallization drop. However, some prefer to mix the drop in two steps and this requires adding the Detergent Reagent to the Crystallization Reagent in the reservoir.

### • Reservoir

It is recommended the initial or final concentration of Detergent Reagent in the drop be 10 to 30% of the concentration supplied in the Detergent Screen HT. Pipette the appropriate volume of crystallization reagent into the plate reservoir, for example 90 ul. Pipette 10 ul of Detergent Reagent to the reservoir.

### • Drop

The recommended drop ratio for initial screens is:

1 part sample: 1 part Detergent Reagent / Crystallization Reagent, although other drop ratios might be worth evaluating during optimization.

Example 500 nl protein + 500 nl Detergent Reagent / Crystallization Reagent. Seal the plate.

## Examine the Drop

After setting the screen, carefully examine the drops under 10 to 100 x magnifications. Examine drops each day for seven days and then for once each week thereafter. Record all observations and be particularly careful to scan the focal plane of the drop for the presence of small crystals.

There are many different ways to score observations, but one should primarily be concerned whether the drop is clear, contains a precipitate or phase separation, or crystals. Adding magnitude to observations can also prove useful, such as 1+ yellow/brown fine precipitate, 2+ phase separation, or 3+ small bipyramid crystals. One may also employ a numerical scoring scheme such as 0 = Clear, 1 = skin/precipitate, 2 = light precipitate, 3 = granular precipitate, 4 = precipitate/phase separation, 5 = phase separation, 6 = quasicrystals, 7 = micro crystals, 8 = needle clusters, 9 = plates, 10 = single crystal(s).

## Interpretation

Detergent Screen HT results with crystals or improved crystals should be pursued for further optimization. In the absence of crystals, or when performing solubility experiments, one should look at differences in solubility between the control drops and those drops with the Detergent Reagent – paying particular attention to results changing from precipitate to clear (increased solubility) and precipitate to crystalline. During optimization one should evaluate, in addition to the primary crystallization variables, the Detergent Reagent class and concentration; the variation of the type, the type and concentration of the primary Crystallization Reagent; the potential evaluation of amphipathic additives (such as 1,2,3-Heptanetriol) as well as salts and polyethylene glycols; pH and buffer type; temperature; and other chemical and physical crystallization variables.

Group the results of the Detergent Screen HT reagents by the appearance of the drop compared to that of the original / control drop. One might group detergents by clear drops, drops with precipitate, drops with phase separation, and drops with crystals. Within each group, review the Detergent Reagent and look for similarities in class, structure, chain length, head group, or CMC. If one or more specific detergents or classes of detergents or a CMC can be identified as producing a desirable change in solubility or crystallization, one should pursue further optimization with this detergent or group of detergents. This may mean varying detergent concentration,

drop ratio, Crystallization Reagent type and concentration, pH, buffer type, temperature, and other crystallization variables to determine the best use of the Detergent Reagent.

## References and Further Reading

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## Technical Support

Inquiries regarding Detergent 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.

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