

Detergent Screen 2™

User Guide

HR2-411

Detergent Screen 2™ Kit is designed to allow the rapid and convenient evaluation of 24 unique detergents and their ability to influence the crystallization of the sample. The screen is designed to be compatible with most popular crystallization reagents including all reagents utilized in Crystal Screen™ and Crystal Screen 2™.

Formulation

Each of the detergents is preformulated in deionized, sterile water. The supplied concentration of each detergent is listed on the opposite side of this document under the column “[Actual]”.

Storage

Recommended long term storage for the detergent screening kit is -20° to -70°C . The kit is stable at 4°C for up to 30 days and for up to 72 hours at room temperature. For enhanced stability it is recommended the tubes be flooded with an inert gas such as argon prior to storage.

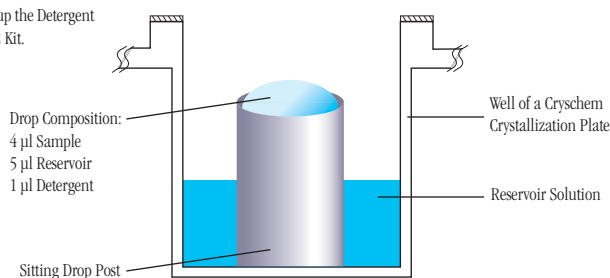
Instructions

The instructions described below demonstrate the use of Detergent Screen 2 using the Sitting Drop methodology. Other methods such as Hanging Drop crystallization may also be utilized. Recommended final detergent concentration after immediate mixing (prior to equilibration) is 1 to 3x the CMC.

1. Pipet 4 microliters of sample onto the sitting drop post of a Cryschem plate.
2. Pipet 1 microliter of detergent 1 into the sample drop.
3. Pipet 5 microliters of the selected precipitant into the sample/detergent drop. When mixing use caution to avoid bubbles and to minimize spreading of the drop. Some drop spreading will occur due to the nature of the detergent.
4. Seal the reservoir with clear sealing tape. Repeat for the remaining detergents. It is recommended a control experiment without detergent (use water) be set up for comparison.
5. Observe drops immediately and at the following intervals: 24 hours, each day for 7 days, and every week thereafter.

Figure 1

Setting up the Detergent Screen 2 Kit.



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. Re-

ports should indicate whether the drop is clear, contains precipitate, and/or 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).

Interpreting the Results

The detergents in the Detergent Screen 2 are capable of manipulating hydrophobic sample-sample interactions which can lead to non-specific aggregation and prevent or interfere with sample crystallization. The detergents may also perturb water structure which might play a role in sample crystallization. Results with crystals or improved crystals should be pursued with further optimization of the primary crystallization variables as well as consideration of detergent type, concentration, and the potential evaluation of amphipathic additives. Note: differences in solubility between the control sample and the detergents experiments. Pay particular attention to results changing from precipitate to clear or precipitate to crystalline.

References

1. Calbiochem-Novabiochem Corporation's technical booklet: "A Guide to the Properties and Uses of Detergents in Biology and Biochemistry", 1987.
2. Crystallization of membrane proteins. Edited by Hartmut Michel, CRC Press, 1991.
3. Crystallization of nucleic acids and proteins, Edited by A. Ducruix and R. Giegé, The Practical Approach Series, Oxford Univ. Press, 1992 175-191.
4. The effects of neutral detergents on the crystallization of soluble proteins. A. McPherson et al. J. Crystal Growth, 76, 1986, 547-553.
5. Kuhlbrand, W., Quarterly Rev. Biophys., 21, 429, 1988.
6. Garavito, R.M., & Picot, D., Methods, A Companion to Methods in Enzymology, 1, 57, 1990.
7. Garavito, R.M. et al, J. Crystal Growth 76, 1986, 701-709.
8. Cudney, B. et al, ActaCryst., D50, 1994, 414-423.

Technical Support

Inquiries regarding Detergent Screen 2 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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