Ionic Liquid Screen

User Guide

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Application

Ionic Liquid Screen is a kit designed to allow the rapid and convenient evaluation of 24 unique ionic liquids and their ability to influence the crystallization of the sample. The screen is designed to be compatible with most crystallization reagents including all reagents utilized in all of the Hampton Research screens. Each of the additives are preformulated in deionized water and sterile filtered using a 0.2 micron filter. Recommended storage for the Ionic Liquid Screen is room temperature.

Discussion

Ionic liquids have been found effective as additives in protein crystallization, with different ionic liquids used to increase crystallization rates and crystal size. The inclusion of ionic liquids in crystallization experiments has been reported to lead to less crystal polymorphism as well as less precipitation at higher precipitant concentrations. Ionic liquids have been used as additives to produce crystals in reagents that had previously not resulted in crystallization and results suggest ionic liquids may be applicable for the solubilization and crystallization of membrane proteins.

Ionic liquids are organic salts with melting points below 100°Celsius. They are thermally stable, nonflammable and demonstrate very low vapor pressure. Ionic liquids are soluble in a variety of organic and inorganic reagents and can be highly water soluble. Ionic liquids can demonstrate a degree of localized structuring about each ion compared to materials composed of disassociated ions, setting them apart from salt solutions. Ionic liquids can participate in ionic, hydrophobic and hydrogen bond interactions. Ionic liquids are often chaotropic, composed of low symmetry ions with charge delocalization and weak intermolecular interactions. These organic salts generally consist of combinations of organic cations and either an organic or inorganic anion. Ionic liquids have been demonstrated to suppress protein aggregation and significantly increase protein folding yields. Ionic liquids have been reported to stabilize protein activity and structure.

The inclusion of the ionic liquid 1-n-Butyl-3-methylimidazolium tetrafluoroborate (included in this kit) improved the thermal stability and solubility of integral membrane proteins for membrane proteomics study.

Some ionic liquids, such as ethylammonium nitrate have water-like characteristics, including the capacity for hydrogen bonding and the promotion of micelle formation by some surfactants. Many ionic liquids are also organic acids and have ionic character in addition to the hydrophobic behavior, which makes them unique and useful solvents in protein chemistry.

Variation of the anion and the cation as well as the utilization of both soft (formate and acetate) and hard anions (nitrate) in the Ionic Liquid Screen reagents provides an additional dimension for evaluating the effects on ionic liquids on the solubility and crystallization of proteins. The Ionic Liquid Screen contains a set of 24 water soluble ionic liquids that comprise different cation (imidazolium, phosphonium, ammonium) and anion (borate, halides, sulfates, acetates, sulfonates, nitrates) structures for a diverse ionic liquid additive screen for use in improving the crystallization behavior and X-ray diffraction resolution of proteins.

Features

The Ionic Liquid Screen kit is designed to provide a rapid screening method for the manipulation of sample-sample and sample solvent interactions to enhance or alter sample solubility.

The Ionic Liquid Screen evaluates the manipulation factors of imidazolium tetrafluoroborate, imidazolium chloride, imidazolium based triflates, phosphonium, ammonium, pyridinium, imidazolium based sulfate, imidazolium based trifluoracetate, imidazolium based dicyanamide, imidazolium based acetate, imidazolium phosphate, cholin, and imidazolium based thiocyanate ionic liquids.

The Ionic Liquid Screen kit is to be used before and during the optimization of preliminary crystallization conditions.

Each Ionic Liquid Screen kit contains 0.5 milliter of 24 unique ionic liquids formulated to allow one to rapidly screen with a minimum amount of sample.

Instructions for Ionic Liquid Screen

This guide will describe the use of the Ionic Liquid Screen kit using the Sitting Drop Vapor Diffusion method with a 2 microliter drop volume and a 1 milliliter reservoir volume. Other methods such as Hanging Drop Vapor Diffusion, MicroBatch, Free Interface Diffusion, Dialysis and Sandwich Drop may also be utilized as well as smaller reservoir and drop volumes. A complete description of the Hanging, Sitting, Sandwich Drop, Dialysis and other crystallization methods are available from the Hampton Research Crystal Growth 101 Library.

Reservoir Setup

- Use 9 parts crystallization reagent plus 1 part liquid.
- Pipet 900 microliters of crystallization reagent into the reservoir.
- Pipet and mix 100 microliters of the ionic liquid into the reservoir.

Drop Setup

- Use 1 part sample plus 1 part crystallization reagent/ionic liquid mixture from reservoir. Other drop ratios such as 1 part sample plus 2 parts reagent or 2 parts sample plus 1 part reagent should be evaluated during optimization.
- Pipet 1 microliter of sample onto a sitting drop post.
- Pipet 1 microliter of the crystallization reagent/ionic liquid mixture from the reservoir into the sample drop.
- Seal the reservoir with tape or grease and slides.
- Repeat for the remaining ionic liquids.
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 crystals. It is helpful to describe the drop contents using descriptive terms. Adding magnitude is also helpful. Example: ++ yellow/brown fine precipitate, + small bi-pyramid crystals, clear drop, + needle shaped crystals in 1+ white precipitate. One may also employ a standard numerical scoring scheme (Clear = 0, Precipitate = 1, Crystal = 10, etc.)

Figure 1 (left side of page 2) shows typical examples of what one might observe in a crystallization experiment.

References and Readings

Technical Support
Inquiries regarding Ionic Liquid Screen 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 4:30 p.m. USA Pacific Standard Time.