Izit Crystal Dye

HAMPTON RESEARCH
Solutions for Crystal Growth

Application
Differentiate protein crystals from salt crystals.

Features
- 0.22 micron sterile filtered solution
- Small molecule dye penetrates solvent channels of macromolecular crystals, coloring the crystals blue.
- Salt crystals do not absorb the dye and remain colorless

Description
Izit is a small molecule dye designed to differentiate biological macromolecule crystals from small molecule and inorganic crystals.[1-2] Biological macromolecule crystals contain large solvent channels allowing Izit to penetrate and color the crystal blue. Small molecule and inorganic crystals do not possess large solvent channels and cannot absorb Izit; hence they will not take up the blue color.

Application
- To an existing drop with crystal(s) to differentiate salt from protein:

Place a small amount of Izit into the drop containing the crystal(s). Example; pipette 1 µl of Izit into a 10 microliter drop or 0.5 µl into a 4 microliter drop. Izit does not need to be added to the reservoir.

For smaller drops (<4 µl), use a Hampton Research Mounted CryoLoop™ to pick up a small amount of Izit within the loop and touch the CryoLoop into the drop to dispense the Izit. This can be repeated to achieve the desired shade of blue color in the drop.

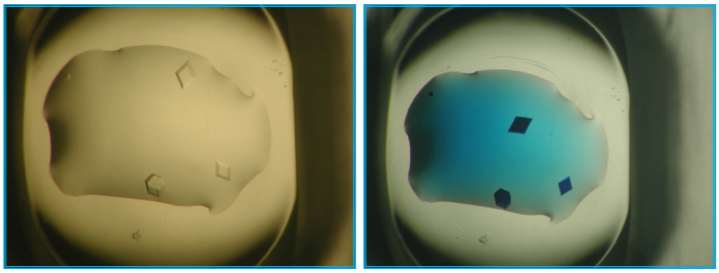
Note: If too much Izit is added to the drop, diluting the drop too much, the crystal(s) may dissolve due to the decreased relative supersaturation (lower sample and reagent concentration). One may attempt to grow again the crystal(s) by simply sealing the experiment and allowing the drop to re-equilibrate with the reservoir.

- As a screening diagnostic:

Izit can be mixed with the crystallization reagent in the reservoir and added to the drop at the time of initial screen set up, before the appearance of a crystal. Try 1 part Izit: 9 parts reagent (10 µl Izit plus 90 µl reagent)

Interpreting Results
Existing protein, peptide, and nucleic acid crystals in the drop will absorb Izit within 1 to 24 hours, taking on a blue color. The blue color will typically intensify within the crystal over time, becoming a darker blue than the remaining solvent in the drop. Precipitate and inorganic crystals will not absorb the Izit and will not become blue. If the blue background color of the mother liquor is too dark, simply dilute Izit 1:10 with deionized water (10 µl Izit plus 90 µl deionized water) or 1:100 (1 µl Izit plus 99 µl deionized water) and repeat the procedure with a different drop containing crystals.

Figure 1
Protein crystals before Izit (left) and after Izit (right)



Thin, needle shaped blue crystals which appear after the addition of Izit may be crystals of the dye in Izit. Crystallization reagents with a high relative supersaturation can promote crystals of the dye. Such false positives can be differentiated from positives by performing a control experiment using Izit plus sample buffer (or water) and the crystallization reagent in a drop versus the crystallization reagent in the reservoir. The appearance of crystals in the control experiment without sample present may be interpreted as dye crystals.

In very few instances Izit will not stain macromolecular crystals.[3]

In very few instances Izit has induced the crystallization of a macromolecule.[4]

Formulation

Name	Methylene blue solution
Synonyms	3,7-bis(Dimethylamino)phenazathionium chloride, Basic Blue 9, Tetramethylthionine chloride
M_r	319.85
CAS Number	[61-73-4]
Linear Formula	$C_{16}H_{18}ClN_3S$

Store at room temperature.

For research use only. For *in vitro* use only.

References
1. Crystalline catalase. Sumner and Dounce Science 1937 85 (2206), p. 366.

2. Preliminary characterization of EcoRI-DNA co-crystals: incomplete factorial design of oligonucleotide sequences. P. A. Wilkosz, K. Chandrasekhar and J. M. Rosenberg. Acta Cryst. (1995). D51, 938-945.

3. Crystallization and preliminary X-ray analysis of Alicyclobacillus acidocaldarius endoglucanase CelA. K. Eckert, H. A. Ernst, E. Schneider, S. Larsen and Lo Leggio. Acta Cryst. (2003). D59, 139-141.

4. Searching for silver bullets: An alternative strategy for crystallizing macromolecules. Alexander McPherson and Bob Cudney. Journal of Structural Biology 156 (2006) 387-406.

Technical Support

Inquiries regarding Izit and general inquiries regarding crystallization are welcome. Please email, 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.

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