

The advanced Cryo Screen**Introduction**

The employment of cryo-techniques in macromolecular crystallography has increased enormously over the last decade and is nowadays routinely used to preserve crystals for X-ray data collection [1].

Cryocooling is not only used to carefully preserve and store crystals for later analysis but also to reduce radiation damage, caused by intense X-ray sources, since the diffusion of active radicals is decelerated. Therefore, cryocooling prolongs crystal lifetime and facilitates straightforward data collection [2]. Cryogenic temperatures also reduce thermal motions of the molecules and allow data collection from very thin or small crystals.

However, the use of cryoprotectants is crucial to prevent crystals from cracking and to protect them from the damaging effects of ice formation during the cryocooling process. The right cryoprotectant will guarantee that the protein and a thin layer of surrounding mother liquor will form an amorphous glass without the formation of water ice. Thus, X-ray data, free of "ice rings", can be collected.

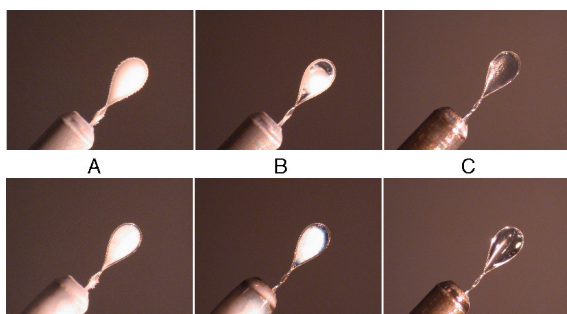


Figure 1: Various results of a cryoprotectant test. Images were taken at cryogenic temperatures. (A) Opaque loop indicates severe ice-formation; (B) translucent loop with a transparent perimeter is still indicative of partial ice formation; (C) transparent loop suggests suitable cryoprotection.

Finding the optimal cryoprotectant solution is often a tedious process of trial and error (Fig.1). It can be accomplished by different approaches:

- ▶ addition of cryoprotectants directly to the drop containing a pre-grown crystal (for more information see the manual of **JBScreen Cryo Pro** [Cat.-No. CC-102])
- ▶ transfer of the crystal from the mother liquor into a cryoprotectant solution
- ▶ screening for crystals using conditions which already contain a sufficient concentration of a cryoprotectant solution

Description

Just like the other **JBScreen** crystal screens, **JBScreen Cryo** is a crystallization screen for proteins, peptides, nucleic acids and macromolecular complexes. It contains preformulated reagents suitable for screening cryo and crystallization conditions using just a single screen.

Based on an extensive data base search [3], the most successful crystallization conditions employing sufficiently large concentrations of cryoprotectants and well suited buffers were chosen for the **JBScreen Cryo** kits.

The 4 screens, offering 96 different crystallization conditions, contain a variety of different cryoprotectants, such as glycerol, ethyleneglycol and PEG. Crystals grown using **JBScreen Cryo** can be directly flash-cooled in liquid nitrogen. The concentration range of the cryoprotectants is sensibly chosen so that the liquid around a crystal will freeze as an amorphous glass avoiding crystal damage and ice formation when placed in the cryostream at 100K.

Usage

Using **JBScreen Cryo**, crystals can be grown under conditions requiring no additional cryoprotectant. **JBScreen Cryo** has been designed for 24-well plates using the hanging or sitting drop vapour diffusion method. If crystals have been obtained they can be directly taken from the drop and flash-cooled in the cryostream. The same applies to the **JBScreen Cryo HTS**, which meets the needs of high-throughput users, with all 96 conditions supplied in a deep-well block.

If crystals have already been obtained using our **JBScreen** crystal screens, simply transfer them into a similar composed solution of **JBScreen Cryo** and allow them to soak for a few seconds before flash-cooling them.

References

- [1] Garman (1999) Cool data: quantity AND quality. *Acta Cryst. D* **55**:1641.
- [2] Garman and Schneider (1997) Macro-molecular cryocrystallography. *J. Appl. Cryst.* **30**:211.
- [3] http://idb.exst.jaxa.jp/db_data/protein/search-e.php