INSTRUCTION SHEET

WIZARD: Cryo Series

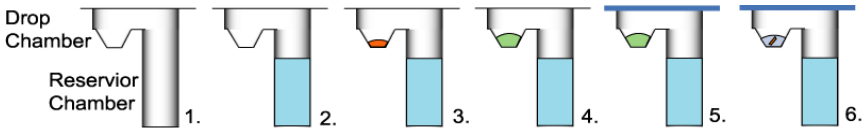
Wizard Cryo 1 Tubes	EB-C1-T
Wizard Cryo 2 Tubes	EB-C2-T
Wizard Cryo 1 & 2 Tubes	EB-C12-T
Wizard Cryo 1 & 2 Block	EB-C12-B

Product Description:
The Wizard: Cryo™ line of random sparse matrix screens is designed for scientists who want to avoid the additional step of optimizing a cryoprotectant condition. Every Wizard: Cryo formulation flash-freezes to a clear, amorphous glass in liquid nitrogen or in a cryo-stream at 100K. Crystals can be frozen directly from their growth drops, avoiding the additional step of pre-equilibration with an artificial cryo-condition that can damage the crystal. Eleven different cryocrystallants and sparing use of glycerol ensures a broad sampling of possible cryo conditions.

Instructions for use:
This screen is intended primarily for use in vapor diffusion crystallizations of biological macromolecules (1,2,3). Before setting up crystallization trials, the biological macromolecule sample should be as highly purified as possible, appearing >97% pure by silver-stained SDS-PAGE. The sample should be in as minimal a buffer as possible to maintain the biological activity of the bimolecule, and at a biomolecule concentration of 5-15 mg/mL.

There are several methods used for protein crystal growth trials. A few of the most popular are outlined here:

Sitting drop crystallization:

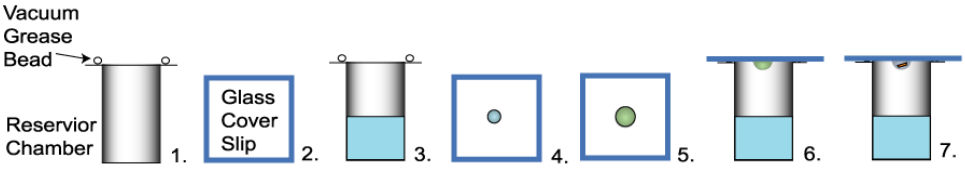


1. Start with a new crystallization plate
2. Add a large volume of Crystallization Solution to the Reservoir Chamber
3. Add a small volume of Protein Solution to the Drop Chamber
4. Transfer a small volume of Reservoir Solution to the Protein Solution in the Drop Chamber
5. Seal the crystallization plate with crystal clear tape
6. Monitor the Drop Chamber by microscopy to identify crystallization results

Notes:

- Sitting drop crystallization plates are available from Rigaku Reagents
    1) Compact, Jr. Plate EB-XJR
    2) CompactClover Plate EB-XPT
    3) CombiClover, Jr Plate EB-CJR
    4) CombiClover Plate EB-CBT
- Typical reservoir solution volumes are 0.1 to 1.0 mL
- Typical drop-chamber solution volumes are 0.5 to 10.0 µL

Hanging drop crystallization:



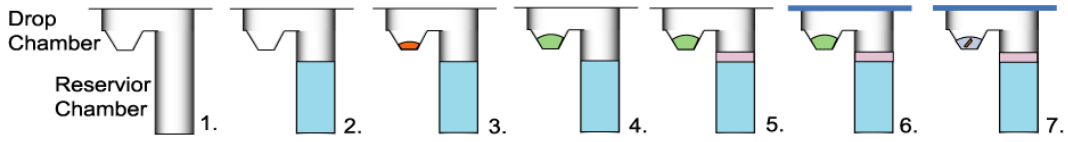
1. Start with a new pre-greased hanging drop crystallization plate
2. Start with siliconized clean Glass Cover Slips for hanging drops
3. Add a large volume Crystallization Solution to the Reservoir Chamber
4. Transfer a small volume of Reservoir Solution to the the Glass Cover Slip
5. Add a small volume of Protein Solution to the Reservior Solution on the Glass Cover Slip
6. Place the Glass Cover Slip onto the Reservoir Chamber with the Crystallization Drop facing the reservoir solution, and press to form a seal with the grease bead
7. Monitor the Crystallization Drop by microscopy to identify crystallization results

Notes:

- Typical reservoir solution volumes are 0.1 to 1.0 mL, depending on plate size
- Typical cover-slip solution volumes are 0.5 to 40.0 µL

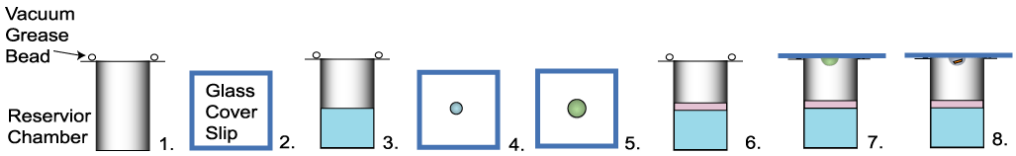
Crystallization using oils

Sitting Drop with Oils:



1. Start with a new crystallization plate
2. Add a large volume of Crystallization Solution to the Reservoir Chamber
3. Add a small volume of Protein Solution to the Drop Chamber
4. Transfer a small volume of Reservoir Solution to the Protein Solution in the Drop Chamber
5. Add a vapor permeable Oil to the Reservoir Chamber
6. Seal the crystallization plate with crystal clear tape
7. Monitor the Drop Chamber by microscopy to identify crystallization results

Hanging Drop with Oils:



1. Start with a new pre-greased hanging drop crystallization plate
2. Start with siliconized clean Glass Cover Slips for hanging drops
3. Add a large volume Crystallization Solution to the Reservoir Chamber
4. Transfer a small volume of Reservoir Solution to the the Glass Cover Slip
5. Add a small volume of Protein Solution to the Reservior Solution on the Glass Cover Slip
6. Add a vapor permeable Oil to the Reservoir Chamber
7. Place the Glass Cover Slip onto the Reservoir Chamber with the Crystallization Drop facing the reservoir solution, and press to form a seal with the grease bead
8. Monitor the Crystallization Drop by microscopy to identify crystallization results

Notes:

- Placing a layer of oil between the crystallization drop and the reservoir helps to control the rate of equilibration *(4).*
- Oils can also be used to seal microbatch plates in the absence of a larger reservoir of crystallizations solutions *(5).*

If the supply of sample permits, it is recommended that crystallizations be set up in duplicate, with one set-up placed at room temperature, and the other one at 4ºC. Regardless of the crystallization method used, the crystallization trials should be stored in a place free of vibrations or mechanical shock, which could result in premature precipitation.

Interpretation of results and optimization

Record observations of crystallization trials every one or two days. The crystallization trials should be viewed under a stereo microscope at 10-100x magnification. Record observations for every condition, even if the crystallization drop remains clear. If less than 10% of the conditions in the crystallization screen do not show heavy precipitate after one day, consider increasing the biomolecule concentration of the sample. If more than 50% of the conditions in the crystallization screen show heavy precipitate after one day, consider reducing the biomolecule concentration.

Crystals suitable for X-ray data collection are generally 0.1 mm or greater in their smallest dimension, and have clean, sharp edges. Typically, less than 5% of the conditions in a crystallization screen will give results worth pursuing. Viewing the crystallization trials between crossed polarizers often aids in distinguishing microcrystals from amorphous precipitate. Except for the rather unusual occurrence of a cubic space group, X-ray diffraction quality biological macromolecule crystals are birefringent (have more than one refractive index), and turn polarized light. When rotated between crossed polarizers, the intensity and/or color of light transmitted through birefringent crystals will change, with a periodicity of 90º. Amorphous precipitates will not transmit and turn polarized light.

It is rare for an initial crystallization screen to yield crystals which are of sufficient size and quality for immediate X-ray diffraction data collection. If small crystals, or crystals which do not grow robustly in all three dimensions (needles and plates), are obtained in an initial screen, the crystallization may be optimized by adjusting the various parameters of the crystallization. Among these parameters are: concentrations of salts and precipitants, pH, biomolecule concentration, use of additives, and temperature. Small crystals can be grown larger by seeding techniques *(6,7)*. Using larger volume crystallization drops may also increase crystal size *(8)*.

Ideally, crystals should be "fresh" for data collection, i.e. harvested straight from the crystallization drop and mounted for data collection without intervening storage. This may not be possible for various reasons, and a suitable mother liquor will be required to stabilize the crystals. For vapor diffusion crystallizations, as a first approximation for mother liquor, assume that the mole quantities of the components in the crystallization drop have not changed during the crystallization, but the volume of the drop has decreased to the volume of the crystallization solution initially used to set up the drop. Some optimization may be required to find the best mother liquor. Cross-linking reagents such as glutaraldehyde may also be used to stabilize biomolecule crystals. If data is to be collected at cryo-temperature *(9)*, a suitable cryo-solvent must be determined, which may also require optimization of the mother liquor.

We wish you great success in your crystallizations. Please contact us if you have any questions or comments.

References

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(4) Chayen, N. E. (1997) *J. Appl. Cryst.* **30**, 198-202.
(5) Chayen, N. E. *et al.* (1990) *J. Appl. Cryst.* **23**, 297-302.
(6) Thaller, C. *et al.* (1985) *Methods in Enzymol.* **114**, 132-135.
(7) Stura, E. A. & Wilson, I. A. (1990) *METHODS: A Companion to Methods in Enzymol.* **1**, 38-49.
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(9) Rodgers, D. W. (1994) *Structure*, **2**, 1135-1140.