

# Crystal Former™

## Frequently Asked Questions

### What is the minimum volume of sample per channel?

The total volume of the channel is 150 nL. While some liquid handling robots are capable of dispensing such small volumes, drop sizes of at least 0.2 µL each for the protein and precipitant are recommended. For total drop volumes of 0.4 µL, the sample consumption for the Crystal Formers is comparable to most automated screening methods.

### What are the recommended sample volumes?

For the 16- and 96-channel Crystal Formers, a protein volume between 0.3-0.5 µL is recommended. The corresponding volume for the crystallization solution typically ranges between 0.3-1 µL. When automation is employed, a volume as low as 0.2 µL for each sample inlet can be utilized. As a rule of thumb, we recommend the use of 0.5 µL for manual pipetting and 0.3 µL for set-up with a liquid handling robot. The microchannels of the Scale-up chip are larger, thus 1.5 µL of protein sample with 0.1-1.5 µL of crystallization solution should be loaded per channel.

### How do the microchannels vary between the Crystal Former formats?

For all chips, the length of the microchannels is 10 mm. Both the 16- and 96-channel original Crystal Formers have channels that are 150 nL in total volume. In contrast, the microchannels of the Scale-up Crystal Former are 1.1 µL in total volume.

### Can I use an alternative sealing method?

Sealing tape is provided in a dual-strip format for sealing only the inlet wells. This design minimizes potential imaging difficulties that arise from air bubbles trapped between the tape and the microchannels when larger tape strips are used. When sealing the Crystal Formers, the user should avoid pressing with excessive force, as this will cause mixing of the reactions and dissipate the gradient. Microlytic's format for sealing tape thus also avoids placing undue pressure over the microchannels. Any other sealing film regularly utilized for sealing crystallization trials is, in principle, compatible with the Crystal Formers, though it is recommended that the substitute tape be trimmed such that it only covers the sample inlets.

### Can the Crystal Formers be used to crystallize my integral membrane protein?

Yes. The materials used to manufacture the Crystal Formers are fully compatible with high concentrations of detergents used in the solubilization of membrane proteins. Keep checking back for the final published reference highlighting the successful crystallization of an integral membrane protein with the Crystal Former.

### Can I use organic solvents in the Crystal Former?

Yes. To date, we have not identified any common organic compounds used in protein crystallization that are incompatible with the Crystal Formers.

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## How should I store the Crystal Formers?

Crystal Formers do not require any special storage conditions. For extended incubation periods, higher humidity storage (>60%) is recommended to minimize evaporation. This can be easily achieved by storing the Crystal Formers in a small container that also includes a moist napkin.

## Can the Crystal Formers be used for in situ testing?

All formats of the Crystal Former are compatible with in situ diffraction testing, providing that a suitable chip mount is available. The 96-channel Crystal Formers are compatible with most experimental set-ups that permit whole plate mounting for in situ screening. The Crystal Formers are also compatible with the PX Scanner from Oxford Diffraction.

## Are the Crystal Formers compatible with my UV imaging system?

Yes. The materials used in Crystal Former manufacturing are fully compatible with UV imaging.

## Are the Crystal Formers automatable?

Both the 16- and 96-channel Crystal Formers are fully compatible with the Mosquito from TTP Labtech (Cambridge, MA). The plate files are available on request. The Crystal Formers are also compatible with a variety of crystal imaging systems, including the Rock Imager from Formulatrix (Waltham, MA). Please contact our technical support line for additional details regarding product integration.

## How long does equilibration of the channel take?

Equilibration of ions in the microchannel takes approximately 1 week. Larger polymers and macromolecules will diffuse more slowly and may thus take up to two weeks for full equilibration. Equilibration is not necessary, however, for crystal growth and many crystals appear rapidly (18-72 hours) within the microchannels.

## How do I harvest crystals from the microchannels?

Crystals are harvested from the back of the Crystal Former. Use a sharp blade to cut the sealing film on either side of the microchannel and peel the film away. To facilitate crystal manipulation, use a loop that is no larger than 100 µm, as this will permit complete submersion of the loop into the channel. The addition of a stabilization solution on exposure of the channel to air is recommended. The stabilization solution can be estimated based on the history of crystal appearance along the channel and the incubation time. As equilibration of the channel typically takes 1 week (for a salt), samples that are at least 1 week old can be stabilized at approximately 50% of the starting crystallization solution.

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## Are there any recommended protocols for crystal cryoprotection?

The harvesting and cryoprotection of a protein crystal grown in any given system frequently requires some degree of carefully consideration and optimization. Once crystals have been harvested from the microchannels of the Crystal Former, they may be manipulated using standard methods. As mentioned above, crystals may be stabilized using a solution that approximates the equilibrated condition. Alternatively, the cryoprotectant or cryoprotectant-mother liquor combination may be applied directly over the channel and the crystals flash frozen immediately on extraction. When the conditions for cryoprotection are unclear, mineral oil or paratone oil may be used to cover the exposed microchannel and the crystals again flash frozen after extraction through the oil layer.

The design of the Crystal Former also presents a unique opportunity for the gentle cryoprotection and/or dehydration of crystals. The cryoprotectant can be applied to one of the sample inlets and allowed to diffuse through the microchannel, thus permitting slow diffusion of the cryoprotectant into the crystal channels prior to harvesting.

## How do I optimize a crystallization hit? Can crystallization conditions be translated to other crystallization systems?

Many of the crystallization hits can be translated into vapor diffusion systems by systematic grid screening using the initial crystallization condition as a starting point. We also offer the Crystal Former XL, in which the larger channels place less restriction on the maximum growth of the crystals. Many users have successfully optimized their crystals for data collection by altering the ratio of protein to crystallization solution in the Crystal Former. The resultant change in the crystallization gradient has direct impact on the nucleation and crystallization rate.

## Why are there only 96 available crystallization conditions? I usually start with many more than that for my initial crystallization trials?

The Crystal Former is designed to enable gently diffusive mixing of protein and precipitant inside microchannels resulting in improved mixing kinetics and significantly higher crystallization hit rates. Liquid-liquid diffusion, harnessed in the Crystal Former, is an orthogonal approach to other methods (e.g. Vapor diffusion, microbatch and dialysis) and samples shift to significantly different regions of the protein phase diagram. When coupled with crystallization conditions that have been optimized for liquid-liquid diffusion approaches, the Crystal Former system provides a robust and efficient method for crystallization screening. The user therefore requires fewer crystallization conditions with significantly increased probability of crystallization success!

## How do the SH-1 and SH-2 Crystal Former holders differ from one another?

The SH-1 holder offers a snap-fit mechanism that holds the individual 16-channel Crystal Formers tightly in place. It is fully compatible with many automated systems. The SH-2 holder is a low profile design that is compatible with the visible light objective of the Rock Imager from Formulatrix and is also compatible with all automated systems previously using the SH-1 holder. The SH-1 holder is NOT compatible with the Rock Imager. The SH-2 holder design also affords 2 major advantages: (1) manual manipulation of the Crystal Formers is facilitated by the drop-in design and (2) the reduction in mechanical stress decreases the likelihood of damage to the Crystal Formers during staging.