

Crystal Former™

Cat. No.	Amount
CF-10	10 pcs.
CF-XL-10	10 pcs.
CF-HT	10 pcs.

Overview

The Crystal Former consists of 16 reaction channels, each providing access to two inlets for loading protein and precipitant solutions. The channels are covered by a thin removable film. The crystallization channel volume is 150 nl; however, it is recommended that users load at least 2-3times the crystallization channel volume to each inlet.

The Crystal Former is set up in the following way:

1. Appropriate volumes of protein solution should be loaded into all the protein inlets. The crystallization channels will fill once the protein solution is loaded.
2. Each precipitant inlet is filled such that the precipitant drop contacts the protein solution in the crystallization channel.
3. The protein and precipitant inlets are sealed and the device is incubated and monitored for the formation of crystals.

The mixing of precipitant and protein solutions occurs primarily by diffusive equilibration based on the concentration difference established at the crystallization channel that faces the precipitant inlet.

The crystallization channel is 10 mm long, allowing for a substantial exploration of protein phase space due to the long equilibration times. For a typical salt, precipitant equilibration will occur over a period of about 1 week, whereas larger polymers will require a longer equilibration. However, note that equilibration is not required for crystal growth, and crystals can form from half a day to three weeks.

As crystals are formed in the Crystal Former they can be obtained from the chip and used for seeding experiments or X-ray diffraction studies.

Instructions for Crystal Maker Operation

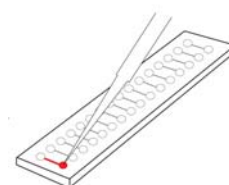
1. Unpacking and labeling the Crystal Former

- Carefully remove the Crystal Former from its sealed aluminum wrapper and place it on a flat surface.
- Label the Crystal Former using a standard lab marker or by affixing a printed label or barcode on the Crystal Former Holder.

Please note: Do not put labels on the film covering the channels, since this film will be removed when channel access is required. Labels should also not be affixed close to inlets, as this may prevent proper sealing prior to incubation.

2. Loading protein solution

- To load the protein solution, first place the Crystal Former on a flat surface with the inlets facing up.
- Aspirate the desired protein volume into the pipette tip. Typically, a protein volume between 0.3-0.5 µl is loaded to each inlet.
- Gently lower the pipette tip into the first inlet. The tip should not touch the bottom of the inlet, and just needs to be lowered far enough for the liquid to enter the inlet when released. If pipetting by hand, one may hold the pipette at a 45-degree angle with respect to the surface of the table.
- Once the pipette tip is lowered sufficiently, the protein can be released into the inlet. Soon after loading, the channels will fill. At this time, the next inlet can be loaded.



Alternatively, the protein inlets can be loaded simultaneously using an eight-channel pipette, or using an automated liquid handler.

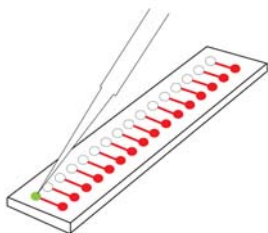
Crystal Former™

3. Loading precipitant solutions

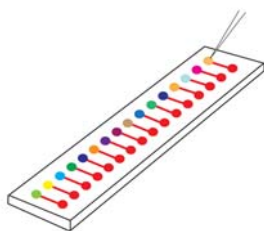
Precipitant solutions are loaded into the inlets opposite of the protein inlets to enable diffusive mixing in the microfluidic channel. The precipitant loading procedure is as follows:

- Aspirate the desired volume of precipitant solution into the pipette tip. Typically, volumes of 0.3-1 μ l are loaded into each inlet. By varying the ratio of protein volume to precipitant volume, different equilibration kinetics will be achieved. If users want to reproduce a particular result, it is critical to reload the same volumes.

- Gently lower the pipette tip into the first inlet. The tip does not need to touch the bottom of the inlet; however it should be close enough to the bottom so that when the drop is released, it contacts the protein solution at the outlet of the microfluidic channel. If pipetting by hand, one may hold the pipette at a 45-degree angle with respect to the surface of the table.



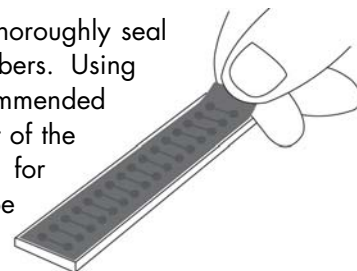
- Once the pipette tip is lowered sufficiently, the contents can be released. A fluidic contact should form between the precipitant solution in the inlet and the protein solution in the channel, enabling the diffusive mixing of the two solutions. At this point, the next inlet can be loaded.



Alternatively, the precipitant inlets can be loaded simultaneously using an eight-channel pipette, or using an automated liquid handler.

4. Sealing the chambers

Use the supplied tape to thoroughly seal inlets and reaction chambers. Using the supplied tape is recommended for keeping the center part of the Crystal Former clear for imaging. The sealing tape is a contact adhesive. Only slight pressure is needed to seal the inlets. Do not press hard when sealing.

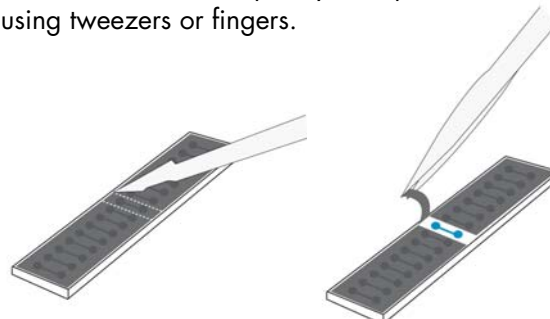


5. Crystal Former storage and incubation

Once the Crystal Former is fully loaded and sealed, it can be stored resting on a flat surface. The humidity of the area in which the Crystal Former is stored will have moderate effects on equilibration over the long term. If the Crystal Former is to be stored for longer than one week, it is recommended that users keep it in a humidified chamber.

6. Harvesting Crystals

- Before harvesting, prepare a cryo-solvent. Turn over the Crystal Former and use a scalpel to cut the peelable film surrounding the reaction chamber(s) where access is desired.
- Once the film is completely cut, peel the film off using tweezers or fingers.



- Once film is removed, a cryo-solvent should be applied to the open reaction chamber to prevent dehydration while manipulating crystals inside chamber.