CrystalChip

Protocol

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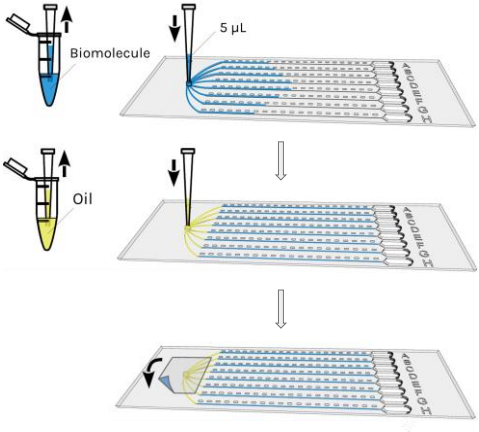
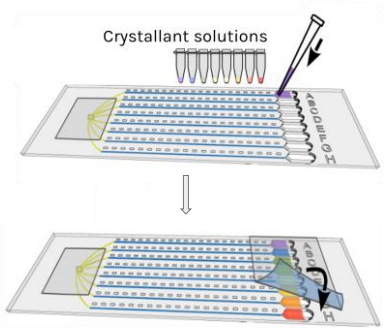
Protocol overview

1 Sample loading

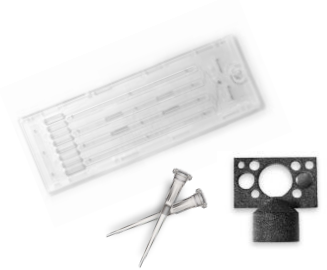


2 Crystallant solution loading



3 Crystal detection & X-ray analysis

1. Kit contents



Provided in the kit:

- Microfluidic crystallization chip(s)
- 1x Chip holder for mounting on the beamline goniometer
- Pipette tips for sample injection (2 per chip)
- Crystallant solutions (screening kits only)*

To be provided by the user:

- Adhesive tape
- Standard 10 µL pipette for injection of crystallization solutions
- (Optional) Paraffin oil

*40 crystallant solutions are provided with screening kits, including 5 different crystallant compositions provided at pHs ranging from 3 to 10.

The composition of each set of solutions can be identified using the 2-letter code printed at the end of the lids (Table 1).

Numbers from 3 to 10 printed along the lids indicate the pH of each solution. For each set, the tube with the lowest pH (pH = 3) is marked with a colored tape.

Mix composition	Code
30% PEG 400	P1
30% PEG 4000	P2
30% PEG 8000	P3
20% PEG 400 + 15% PEG 4000 + 10% PEG 8000	P4
Ammonium Sulfate 3M	AS

Table 1: list of 2-letter codes used to identify each crystallant solution

Up to 8 different crystallization solutions can be loaded in a single chip (one per channel). For an initial screening, all 40 solutions provided can be loaded in 5 chips: each chip will screen one crystallant composition at 8 different pHs and with a continuous gradient of concentrations created by liquid-liquid diffusion.

2. Before you start

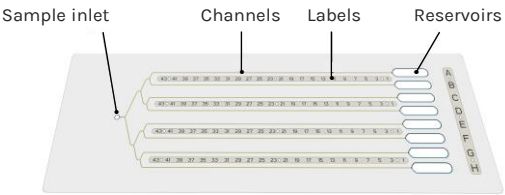


Figure 1: Schematic diagram showing the crystallization chip design

- We strongly recommend using the crystallant solutions provided in the screening kits when using CrystalChip for an initial screening of biomolecules. These solutions, made of the most successful crystallizing agents typically employed in protein crystallization, were specifically optimized for the counter-diffusion setup and demonstrated their efficiency at screening crystallization conditions of various soluble and membrane proteins, as well as an RNA duplex.
- Alternatively, you can load your own crystallization solutions. The counter-diffusion method will work best with crystallants concentrated up to the maximum of their solubility at 4°C, which is not the case with standard commercial kits designed for vapor diffusion or batch methods. If initial crystallization conditions were obtained by vapor diffusion, the crystallant concentration should be increased by a factor of 1.5-2.
- Each channel should be used only once. We do not recommend reusing the channels as this can lead to incomplete liquid diffusion.
- Once formed, crystals can be safely kept in the chip for several months while preserving their intrinsic quality.
- The protein can be labelled with a fluorescent compound before being loaded in the chip to facilitate the detection of crystals in the chip using fluorescence microscopy.
- A detailed video showing the step-by-step procedure for molecule crystallization and analysis in CrystalChip, as well as protocols for protein labeling with fluorescent compounds, are available online. Scan the QR code to check it out:

3. Loading the sample molecule in the chip

- Take the chip out of its storage bag.
- Collect 5 µL of the molecule solution using a standard 10 µL micropipette and the pipette tips provided for sample injection.

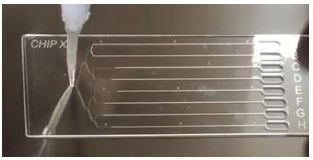


Figure 2: correct positioning of the tip for sample injection through the chip inlet

Make sure you use the provided tips for sample injection as slight changes in tip dimensions may compromise liquid diffusion in the channel. If needed, Labcon™ 1151-965-006-9 and VWR® 732-3630P tip references are also compatible with the sample inlet.

- Place your pipette perpendicular to the chip and insert the tip vertically into the sample inlet located on the left side of the chip (Figure 2).

When properly inserted, you should hear a "click" and the tip shouldn't move. Do not apply too much pressure to avoid damaging the chip.

- Slowly inject the protein solution into the inlet until all eight channels are filled up to their opposite end.

Make sure your tip is properly inserted and that you inject the solution slowly to avoid forming bubbles. If bubbles have formed, repeat the injection with the same volume and collect the extra solution in the reservoirs.

Successful loading will result in all 8 channels being filled with liquid and solutions coming out in the crystallant reservoirs. Placing the chip over a dark background will help checking for proper channel filling.

- (Optional) Inject 1 µL of paraffin oil in the sample inlet, using a similar technique as described for sample injection, until the oil reaches the horizontal part of the channels.

This step will ensure the crystallization solutions remain disconnected from each other and is recommended to avoid cross-contamination when screening various crystallant solutions. If paraffin oil is not available, any other solution that is immiscible in water or air can be injected instead.

- Dry any remaining oil with a soft towel if needed and seal the sample inlet using a 1 cm x 1 cm piece of adhesive tape.

- Collect the remaining sample solution coming out in the reservoirs at the extremity of each channel using a standard 10 µL pipette.

4. Loading the crystallant solutions

- Collect 5 µL of the crystallant solution to be tested using a standard 10 µL pipette tip and place your tip in one of the reservoirs located on the right side of the chip.

- Orient the tip toward the channel entry in the funnel shaped part of the reservoir and slowly inject the crystallization solution (Figure 3).

! To avoid bubbles and ensure proper crystallant diffusion into the channel, do not place the pipette tip directly against the channel entry (Figure 3).

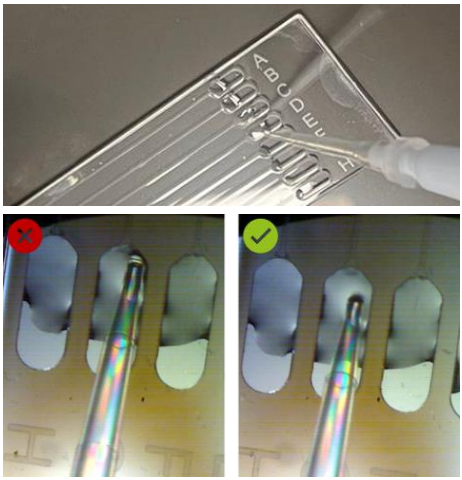


Figure 3: correct positioning of the tip when injecting crystallant solutions through the reservoirs

This step can be performed under a binocular magnifier for an easier visualization.

- After loading all solutions in the chip, seal the reservoirs with a 2.5 cm x 1 cm piece of adhesive tape.
- Incubate the chip at room temperature (18-25°C) until crystals have formed.

Optimal incubation temperature may vary depending on the molecule target to be crystallized, and can be adjusted between 4°C and 37°C.

Total time needed for successful crystal formation can range from a few minutes to several weeks depending on the target molecule and the crystallant solution(s) used. Chips are typically checked over a period of 2 to 4 weeks to track crystal growth.

Once crystals have formed, ligands, enzyme substrates or heavy atoms can be introduced into the crystals by diffusion following the same procedure as when loading the crystallization solutions. Additional compounds should be introduced at least 24-48h before X-ray analysis to allow for proper diffusion along the channels and into the crystals.

5. Observing the crystals formed in the chip

The chip material is transparent to visible light and compatible with the use of any stereomicroscopes, polarizers or UV illumination.

- Place your chip on any microscope stage equipped with a slide holder compatible with standard microscope slides (25 mm x 75 mm).
- Screen each microfluidic channel starting from the crystallization solution reservoirs to the sample inlet (highest to lowest crystallant concentration).
- Keep track of the obtained crystals using the labels present along the channels, or by manually marking crystal locations using a permanent marker.

6. *In situ* crystal X-ray analysis

These indications are based on use at X06D1 beamline (SLS, Villigen, Switzerland) and may be adjusted depending on the beamline setup and the diffraction properties of the crystals obtained.

- Turn off the beamline cryo-jet to carry out analysis at room temperature.
- Insert your chip into the dedicated holder, making sure the channels containing the crystals to be analyzed are positioned at the center of the holder.
- Attach the holder to the goniometer electromagnet.
- Orient the thick top layer of the chip towards the direct beam and the thin lower layer behind the crystal to maximize the diffracted signal.

! **Restrict goniometer movements in the range of -30°C / + 30°C (0° corresponding to the channels being perpendicular to the X-ray beam) to prevent the chip from colliding with the surrounding material.**

- Select a crystal position using the labels present along the channels.
- Center the crystal either by standard low dose grid/raster screening or 1-click procedure.
- Collect diffraction data within the range -30°C / + 30°C.

If performing serial crystallography, relocate the chip and repeat the last 3 steps on another crystal located in the same channel.

- To analyze another channel, realign the chip on the holder so that another channel is placed at the center and proceed to data collection on crystal(s) present in this channel.
- Data can be processed, merged and the structure can be solved and refined using standard crystallographic packages and procedures.

The CrystalChip technology was developed by Claude Sauter (Institut de Biologie Moléculaire et Cellulaire IBMC) – Strasbourg, France.

Publishing a study using CrystalChip? Please cite their original publication:

A simple and versatile microfluidic device for efficient biomacromolecule crystallization and structural analysis by serial crystallography. R. de Wijn, O. Hennig, J. Roche, S. Engilberge, K. Rollet, P. Fernandez-Millan, K. Brillet, H. Betat, M. Mörl, A. Roussel, E. Girard, C. Mueller-Dieckmann, G.C. Fox, V. Oliéric, J.A. Gavira, B. Lorber & C. Sauter. IUCrJ (2019), 6: 454–464.

CRYSTALCHIP ONLINE

Find out detailed technical information, FAQ, result examples and much more at:

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