

# XtalTool Soaking Kit

## For *in situ* Ligand Soaking

Cat. No.	Amount
X-XT-102	1 Kit

### Kit Contents

- 1 Needle Holder Pen<sup>[1]</sup>
- 1 Soaking Stand<sup>[2]</sup>
- 4 Test XtalTool Sample Holders<sup>[3]</sup>
- 2 Sets of Paper Wicks
- 20 Cannulas
- 24 Long Extruded Pipette Tips

### Description

The XtalTool Soaking Kit contains all necessary tools for efficient crystal soaking and manipulation of crystals. Please read the entire manual carefully and practice using the supplied test sample holders by performing several dummy-handling tests before starting to manipulate real protein crystals grown on an XtalTool or XtalTool HT.

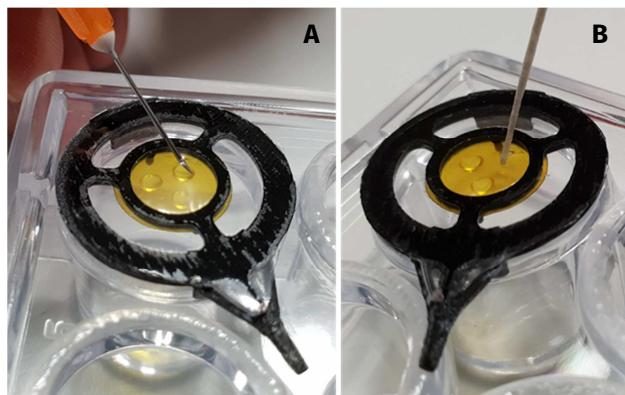
### Ligand Soaking Protocol

After hanging-drop crystallization on an XtalTool or XtalTool HT sample holder, crystal soaking can be performed without direct contact.

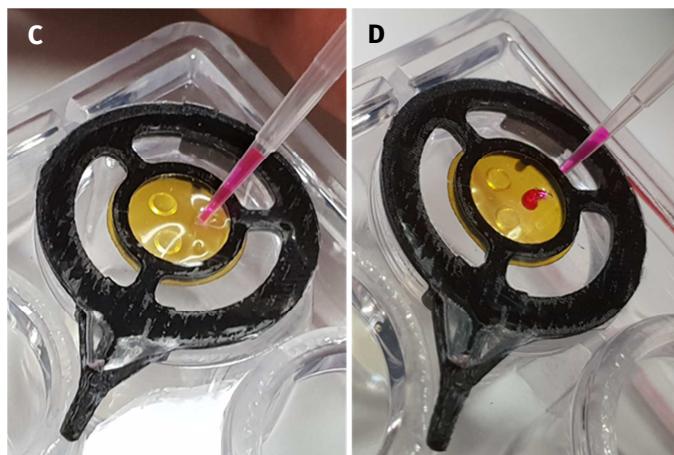
**Note:** **Do not touch** the yellow foil with unprotected fingers to avoid contamination. The usage of protected forceps is recommended.

1. Carefully poke a hole into the upper transparent COC foil at an appropriate position using a cannula connected to the provided needle holder pen (please refer to the chapter “Usage of the Needle Holder Pen”). The position of the puncture should be right next to the desired drop (Figure A).

2. Insert a fine paper wick into the poked hole and carefully push it down until it gently touches the perforated yellow polyimide foil. Keep the paper in contact with the foil to absorb the liquid (Figure B). The required time highly depends on the viscosity of the mother liquor composition (please refer to the chapter “Estimate Soaking Times”). Once done, gently retract the paper wick.



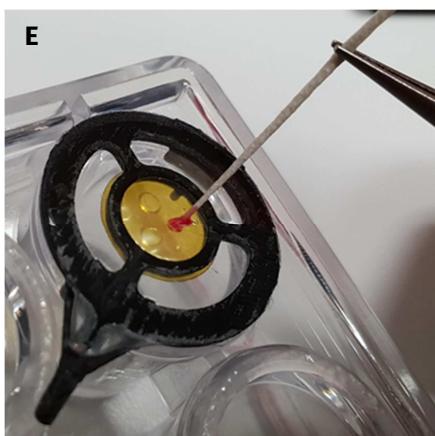
3. Now gently insert a long extruded pipette tip and apply a small volume of a ligand mother liquor solution adjacent to the crystal (Figure C). Efficient soaking is achieved by placing the drop as accurately as possible. Do not puncture the yellow foil. Gently retract the tip (Figure D).



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4. The applied solution will diffuse through the micro pores of the polyimide foil to the crystal growing side. Incubate the plate for the desired soaking time. Remove excess soaking solution with a new fine paper wick (Figure E). You may soak with different compounds subsequently.
5. Transfer a dedicated crystal to the mother liquor. Verify its optimal position within the humid air stream and incubate.
6. For subsequent soaking event the crystal may be transferred into a neighboring drop.



### High-Concentration Ligand-Soaking Protocol

*Note: This procedure requires the availability of a preparative humidity controlled environment. Developed for and limited to extreme high-concentration ligand-soaks, this method requires some experience.*

1. Take the soaking stand and place it on the thin pegs in an ideal position towards the humidity stream.
2. Pipet a small volume (max. 2 µl) of highly concentrated ligand soaking solution in one of the cavities.
3. Gently flip the stand on the thick pegs and adjust the position within the controlled environment.
4. Place up to 1 µl of mother liquor on the opposite site of the cavity just prepared.

### Preparation and Training

#### Estimate Soaking Times

An important parameter in a soaking experiment is timing. Depending on the viscosity of the solutions used, diffusion times can vary. To investigate and optimize the soaking times before applying the solution on the crystals, the kit contains a soaking stand (patent-pending). With 16 cavities, the stand can be used to test and determine soaking times. The properties of the yellow foil are identical to the one used on XtalTool or XtalTool HT. It allows the determination of the time necessary either to remove mother liquor or to apply soaking solutions.

*Note: Do not touch the yellow foil with unprotected fingers to avoid contamination. The soaking stand should only be touched on the black frame.*

1. Take the soaking stand from its box and place it on a clean surface. The stand features a three-peg stand on either side. To test the diffusion time, place the soaking stand first on the thin pegs. Pipette your liquid drop into one of the cavities.



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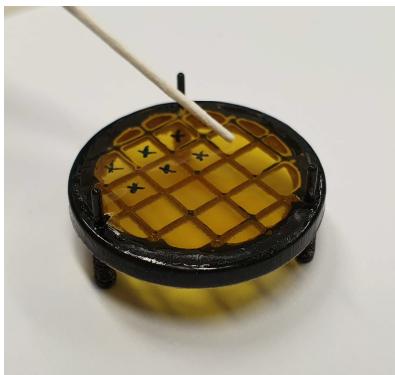
## For *in situ* Ligand Soaking

- Carefully rotate the stand and place it on the thick pegs. Place a paper wick of the size, you intend to use for the XtalTool, gently on the yellow foil and measure the time until all (or most of) the liquid is transferred into the paper wick. Do not pierce the foil from either side.

*Note: The size of the paper wicks will determine the time in most cases. However, the larger it is, the larger the puncture of the COC foil of the sample holder must be. Hence, it may be of advantage to try different paper wick sizes.*



- Mark the used cavities with a regular permanent marker. The usage of the same cavity is not recommended because the components of the mother liquor solutions will dry and clog the pores of the polyimide membrane. This may lead to incorrect conclusions when reusing the same cavity. The design is such that 16 individual solutions can be tested.



### Usage of the Needle Holder Pen

**Note:** **Do not touch the cannula** with unprotected fingers to avoid contamination. The needles are very sharp and all protection regulations regarding the usage of cannulas apply.

- Take the needle holder pen from the box. Its locking system at the front accepts the supplied cannulas as well as others. The mechanism is a screw mechanism similar to the Luer-Lock system. The four red rings on the backside of the pen allow secure handling.



- Take a cannula from its sterile wrap. Gently screw the exposed bottom of the cannula into the tip until you feel resistance to ensure ideal fitting.



- Remove the protection cap of the cannula just before usage. Do not recap needles for safety reasons. The needle holder pen is designed in such a way that the cannula can be removed by using regular needle waist bins. Insert the needle to the drop-off hole and gently twist the pen. The needle will fall off.

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### XtalTool Handling

The kit contains four XtalTool parts, which allow several dummy handling tests in order to be prepared and trained for all the handling steps manipulating real crystals grown on XtalTool or XtalTool HT.

**Note:** **Do not touch** the yellow foil with unprotected fingers to avoid contamination. The provided XtalTool sample holders are not recommended to be used for crystallization trials.

1. Take the XtalTool from its box and place it on a clean surface. Only touch the black parts to avoid contamination.
2. Place the XtalTool with the yellow foil facing up and set up a few dummy drops, e.g. your mother liquor. Flip over the sample holder and place it on a (non-greased) 24-well cavity and observe the drop behavior using a microscope.
3. Prepare the identical solution containing a non-toxic dye, for example a pH indicator.
4. Carefully poke a hole into the upper transparent COC foil at the appropriate position using a cannula connected to the provided needle holder pen (Figure A, please refer to the chapter "Usage of the Needle Holder Pen").

**Note:** **Do not puncture the yellow foil.**

5. Now gently insert a long extruded pipette tip and apply a desired volume (1-2 µl) in the gap between the yellow and the transparent foil. (Figure C) Efficient soaking is achieved by placing the drop as accurately as possible. Observing the dye's diffusion into the "crystal drop" can give an impression of times needed in a soaking experiment<sup>[4]</sup>.
6. Try to remove mother liquor from the same or a different drop using the provided paper wicks.

For efficient removal of mother liquor gently touch and stay in contact with the perforated yellow foil (Figure B,E). The required time to absorb the liquid highly depends on the viscosity of the mother liquor and its composition.

7. Soaking times may greatly vary and highly depend on the degree of association between crystal ligand and protein molecule as demonstrated recently<sup>[4]</sup>.

### Related Products

- XtalTool (Cat. No. X-XT-101)
- XtalTool HT (Cat. No. X-XT-103 and X-XT-104)
- XtalTool Bases (Cat. No. X-XT-105)

### Selected References

- [1] Needle holder: Declaration for utility and design, DE 40 2018 100 962.8, Halter für Kanülen, Christian G. Feiler, Dirk Wallacher, Manfred S. Weiss, registration date: 20.09.2018.
- [2] Soaking Stand: Patent pending, DE 10 2018 123 090.7, Teststand für Kristallbenetzung, Christian G. Feiler, Dirk Wallacher, Manfred S. Weiss, filing date: 20.09.2018, WO application under preparation.
- [3] XtalTool: Patent pending, DE 10 2017 129 761.8, Probenhalter, Christian G. Feiler, Dirk Wallacher, Manfred S. Weiss, filing date: 13.12.2017. An international patent application via the PCT route, using the priority of DE 10 2017 129 761.8 has been filed, PCT/D2018/101007.
- [4] McPherson (2019) Penetration of dyes into protein crystals. *Acta Cryst. F.* **75**:132.