

XtalTool HT

Sample Holder for Crystal Growth, *in situ* Ligand Soaking and *in situ* Data Collection with a Robot Assisted Sample Mounting

Cat. No.	Amount
X-XT-103	20 XtalTool HT Sample Holder (22 mm)
X-XT-104	20 XtalTool HT Sample Holder (18 mm)

Application

XtalTool HT is a sample holder for crystal growth, soaking and X-ray data collection at ambient and cryogenic temperature without the need of direct crystal manipulation or mounting^[1]. It is compatible with robot assisted sample mounting systems following SPINE standard and therefore suitable for high-throughput (HT) applications.

Kit Contents

20 XtalTool HT Sample Holder
1 XtalTool HT Dummy Sample Holder to test individual robot compatibility

Description

The XtalTool HT is a patent-pending^[2-4] sample holder consisting of a plastic support with an outer transparent COC film and an inner X-ray transparent yellow polyimide film with 5 µm pores. The design allows the setup of two crystallization drops on one XtalTool HT. Crystal growth can be monitored either by a transmission microscope or an automated inspection system. All intended manipulative steps on the crystal are carried out *in-situ*. Before data collection the outer ring can easily be broken off at the designated respective break points. Mounted on an XtalTool Base (Cat. No. X-XT-105)^[5], the XtalTool HT then fits into a SPINE standard magnetic CryoVial and thereby facilitates robot assisted sample mounting. Its geometry meets the 18 mm SPINE standard and it's compatible with most synchrotron and in-house beamlines dedicated to macromolecular crystallography. XtalTool HT is available in two sizes

(22 and 18 mm diameter) compatible with the two standard 24 well crystallization plate formats. Despite the different outer diameter, the geometry of the inner sample holder is identical.

Usage

Note 1: Do not touch the yellow foil with unprotected fingers to avoid contamination. The usage of protected forceps is recommended.

Note 2: It is highly recommended to ensure full robot/beamline compatibility using the provided XtalTool HT Dummy Sample Holder. If in doubt, please contact the local beamline scientist.

1. Take one XtalTool HT from its box and place it with the outer transparent film on a smooth surface to avoid damage and unwanted puncture of the film. Setup your crystallization drops on the yellow polyimide film, as you would do on a regular cover slide. The optimal number of two drops is recommended as depicted. Do not pierce the film.



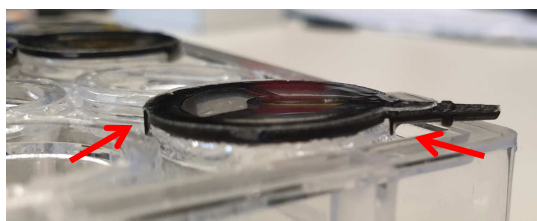
2. Flip over the sample holder and place it onto a greased cavity of a 24 well plate. The design is such that 24 sample holders will fit onto the plate.

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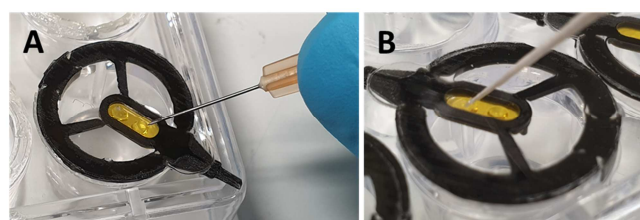
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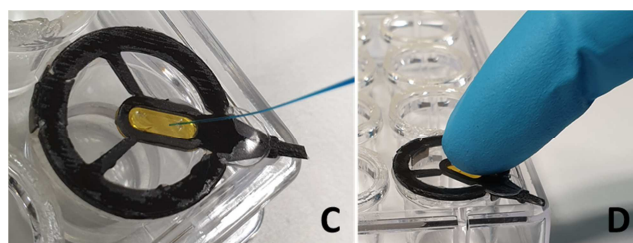
- Seal the cavity with the sample holder. Ensure that XtalTool HT is placed correctly indicated by the two outer positioning guides on either side.



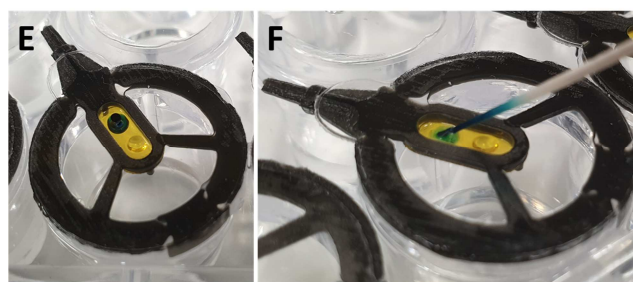
- Ligand soaking: Remove mother liquor in a two-step procedure:
 - First, carefully poke a hole into the upper COC film at the appropriate position using a fine needle. The puncture should be selected such that the hole is slightly next to the desired drop (Figure A).
 - Second, insert a fine paper wick into the poked hole and carefully push it down until it gently touches the polyimide film. Keep the paper in contact with the perforated film to absorb the liquid. The required time highly depends on the viscosity of the mother liquor composition. Once done, gently retract the paper wick (Figure B).



Now gently insert a long extruded pipet tip and apply a small volume (3 μ l maximum) of the ligand containing 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 film. Retract the tip and gently touch the film with protected warm finger for 2 seconds and slide across. (Figure D) The film will reseal itself.



The applied solution will diffuse through the micropores to the crystal growing side. Incubate for the desired soaking time (Figure E). Then create a new entry hole for another paper wick to remove excess soaking solution (Figure F). You may soak with different compounds subsequently.



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Note 1: Suitable material for crystal soaking on an XtalTool HT is provided in the XtalTool Soaking Kit (Cat. No. X-XT-102).

Note 2: The ligand containing solution can also be applied without prior removal of the mother liquor solution. This setup allows for more gentle soaking conditions where the high ligand concentration slowly exchanges. Hence, soaking time needs to be extended and adjusted accordingly.

- Crystal cryo-protection: Apply the cryo solution in the gap between the outer COC and the inner polyimide film. After the desired incubation time, remove the solution with a fine paper wick. The time to attain cryo-protected crystals is highly dependent on the employed components.

Note: The removal of mother liquor and/or cryo solution before data collection is recommended to minimize solvent scattering. If diffraction data are collected at ambient temperature, residual mother liquor solution can also be removed using a paper wick within the humidity on the goniometer.

- X-ray data collection: Gently remove the transparent COC back film in the same manner as a lid of a yoghurt cup. Gently lift the indicated edge and remove it.



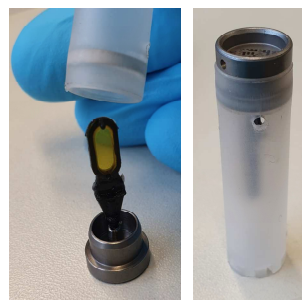
Take the sample holder off the cavity and screw it into an XtalTool Base (Cat. No. X-XT-105) until the indication marker is reached.



Apply gentle pressure on the outer ring to break the inner part free using the pre-defined breakpoints. Discard the outer parts.



For data collection at ambient temperature, mount the sample holder directly onto a goniometer. For data collection at cryogenic temperature, plunge the sample holder into liquid nitrogen and place it into a SPINE standard magnetic CryoVial. The sample holder can now be handled by most sample mounting robots.



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Related Products

- 24 Well SuperClear Plates (Cat. No. CPL-130, CPL-132) for 22 mm XtalTool HT Sample Holder
- Greiner ComboPlates (Cat. No. CPL-119, CPL-131) for 18 mm XtalTool HT Sample Holder
- XtalTool Soaking Kit (Cat. No. X-XT-102)
- XtalTool Bases (Cat. No. X-XT-105)
- XtalTool (Cat. No. X-XT-101)

References

[1] Feiler *et al.* (2019) An All-in-one Sample Holder for Macromolecular X-ray Crystallography with Minimal Background Scattering. *J. Vis. Exp.* **149**:e59722.

[2] XtalTool: Patent DE 10 2017 129 761.8, Christian G. Feiler, Dirk Wallacher, Manfred S. Weiss, filing date: 13.12.2017, An international patent application via the PCT route, WO 2019114879 A1 using the priority of DE 10 2017 129 761.8 has been filed, PCT/DE2018/101007.

[3] XtalToolHT: Patent pending, DE 10 2018 129 125.6, Christian G. Feiler, Dirk Wallacher, Manfred S. Weiss, filing date: 20.11.2018.

[4] XtalToolHT: Patent pending, DE 10 2018 125 129.7, Christian G. Feiler, Dirk Wallacher, Manfred S. Weiss, filing date: 11.10.2018.

[5] XtalToolHT and XtalToolBases: Registration of utility model, DE 20 2018 106 955.1, Christian G. Feiler, Dirk Wallacher, Manfred S. Weiss, filing date: 06.12.2018.