

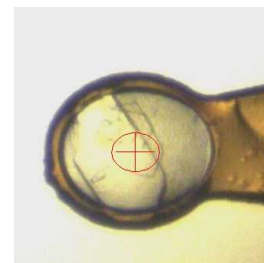
Dec 19, 2023

Version 1

ASAP protocol: Crystallization of Enterovirus D68 3C protease (PDB code 8CNX) V.1

DOI

<https://dx.doi.org/10.17504/protocols.io.n92ldm6jnl5b/v1>



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ASAP Discovery



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External link: <https://asapdiscovery.org/outputs/target-enabling-packages/#ASAP-EVD68-3CLPRO>

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Protocol status: Working

We use this protocol and it's working

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Abstract

The crystallization protocol and buffer conditions used to obtain Enterovirus D68 3C protease crystals suitable for **XChem** fragment screening. The crystal structure was deposited to RCSB PDB with code **8CNX**.

Materials

SwissCI 3 lens crystallization plates <https://swissci.com/product/3-lens-crystallisation-plate/>

1 M Tris-HCl pH 8.14

1 M Ammonium acetate

50% w/v PEG 3350



Equipment needed

- 1 **Formulatrix Rock Imager** (or incubator of choice)
SPT mosquito
P100 multi-channel pipette
SwissCI 3 lens plate

Crystallization experiment

1d

- 2 **Protein and buffer requirements:**

 32 μ L  1 millimolar (mM)  Sample

 2.88 mL Crystallization screen

 16 μ L  Sample seeds, dilution 1:100 000

- 3 **Crystallisation screen composition:**

0.1 M Tris-HCl (pH 8.14)

0.2 M Ammonium acetate

25% w/v PEG 3350

Stock solutions used:

1 M Tris-HCl adjusted to pH 8.14 with HCl

1 M Ammonium acetate

50% w/v PEG 3350

Note

The crystallisation screen can be stored in a duran bottle or aliquoted into 96 deep well block for easy dispensing into SwissCI 3 lens plates.

For long term storage keep the Crystallisation screen in the fridge at 4°C.

- 4 **Prepare seed stock:**

1: 100 000 dilution  Sample seeds

- 5 Dispense  30 μ L Crystallisation screen into SwissCI 3 lens plate reservoir wells using a 100 μ l multi-channel pipette.



Dispense  100 nL  1 millimolar (mM)  Sample to each lens using the SPT mosquito.

Dispense  200 nL Crystallisation screen to each lens using the SPT mosquito.

Dispense  50 nL Seeds to each lens using the SPT mosquito.

Drop ratio: 1:2:05 ratio (100 nL  Sample : 200 nL reservoir solution: 50 nL seeds)

Final drop volume: 350 nL

6 Incubate at  20 °C for  24:00:00 h in Formulatrix Rock Imager.

1d

Imaging Schedule: The first images are taken after 12 h and the imaging schedule follows a Fibonacci sequence of days for further collections.

7 Crystal form after ~12 h.

Expected result

The crystals reach their maximum size after 24 h and most of the precipitant has gone.

Crystals typically form either as single crystals or in small clusters containing 4-6 crystals in a flower-like arrangement. Upon mounting a gentle nudge dissociates the cluster.

Morphology: typically thin rectangles with pointed ends.

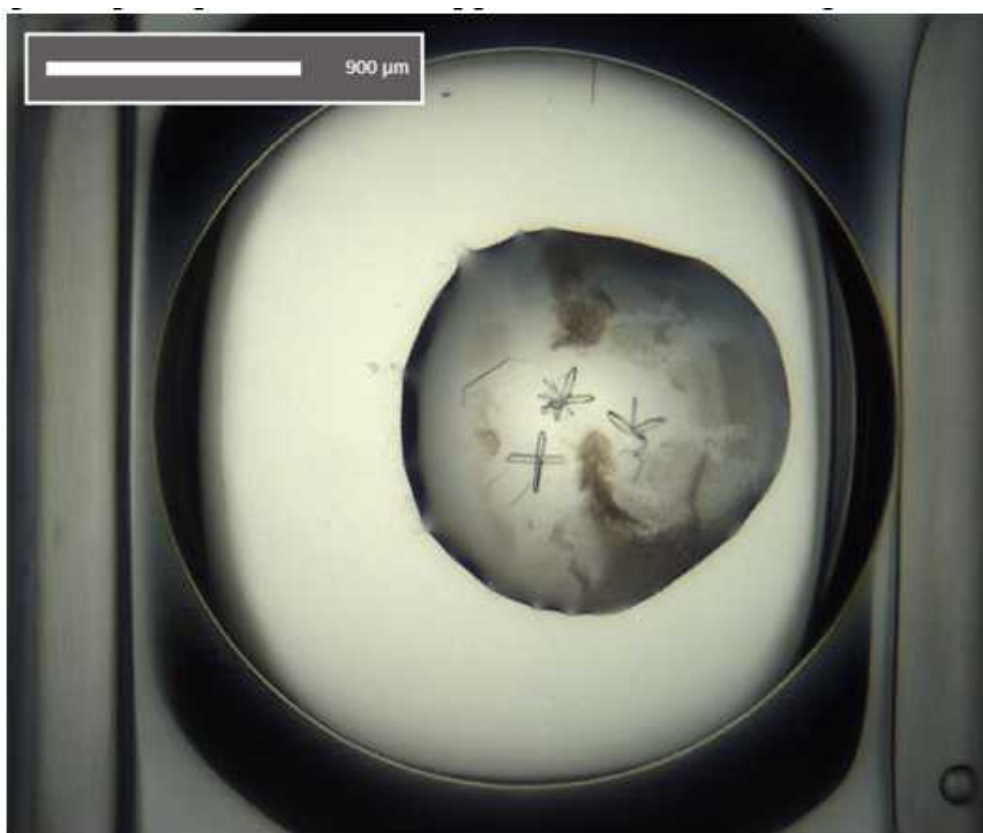
Size: ~100 μm in length and ~50 μm in width, depth of the crystals is ~10 μm

Appearance: glass shard.

Average resolution: 1.5 $\text{\AA}$

Space group: $P2_12_12_1$

Unit cell: 42.82 $\text{\AA}$, 62.53 $\text{\AA}$, 147.36 $\text{\AA}$
90.00°, 90.00°, 90.00°



An example of a drop containing EV D68 3C protease crystals.

Protocol references

N/A