

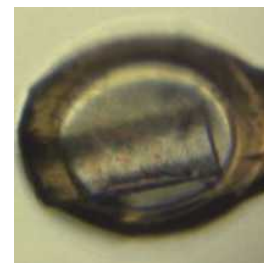
Dec 19, 2023

Version 1

🌐 ASAP protocol: Crystallization of Enterovirus A71 3C protease (PDB code 8CNY) V.1

DOI

<https://dx.doi.org/10.17504/protocols.io.yxmvm3e89l3p/v1>



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ASAP Discovery



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External link: <https://asapdiscovery.org/outputs/target-enabling-packages/#ASAP-EVA71-3CLPRO>

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Protocol status: Working

We use this protocol and it's working

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Protocol Integer ID: 87512

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Abstract

The crystallization protocol and buffer conditions used to obtain Enterovirus A71 3C protease crystals. The crystal structure was deposited to RCSB PDB with code **8CNY**.

Materials

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

1 M Tris-HCl pH 8.5

1 M Magnesium Chloride Hexahydrate supplier

50% PEG 4000



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:**

43.2 µL 1 millimolar (mM) Sample

2.88 mL Crystallization screen

- 3 **Crystallisation screen composition:**

0.1 M Tris-HCl (pH 8.50)

0.2 M Magnesium chloride hexahydrate

30% w/v PEG 4000

Stock solutions used:

1 M Tris-HCl adjusted to pH 8.5 with HCl

1 M $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$

50% w/v PEG 4000

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 Dispense 30 µL Crystallisation screen into SwissCI 3 lens plate reservoir wells using a 100 µl multi-channel pipette.
Dispense 150 nL 1 millimolar (mM) Sample to each lens using the SPT mosquito.
Dispense 150 nL Crystallisation screen to each lens using the SPT mosquito.

Drop ratio: 1:1**Final drop volume:** 300 nl



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

1d

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

- 6 Crystal typically form after ~24hrs

Expected result

Crystals typically reach their maximum size after ~24 h with precipitation often remaining.

They typically form without seeding and are sturdy enough to be manipulated in solution readily.

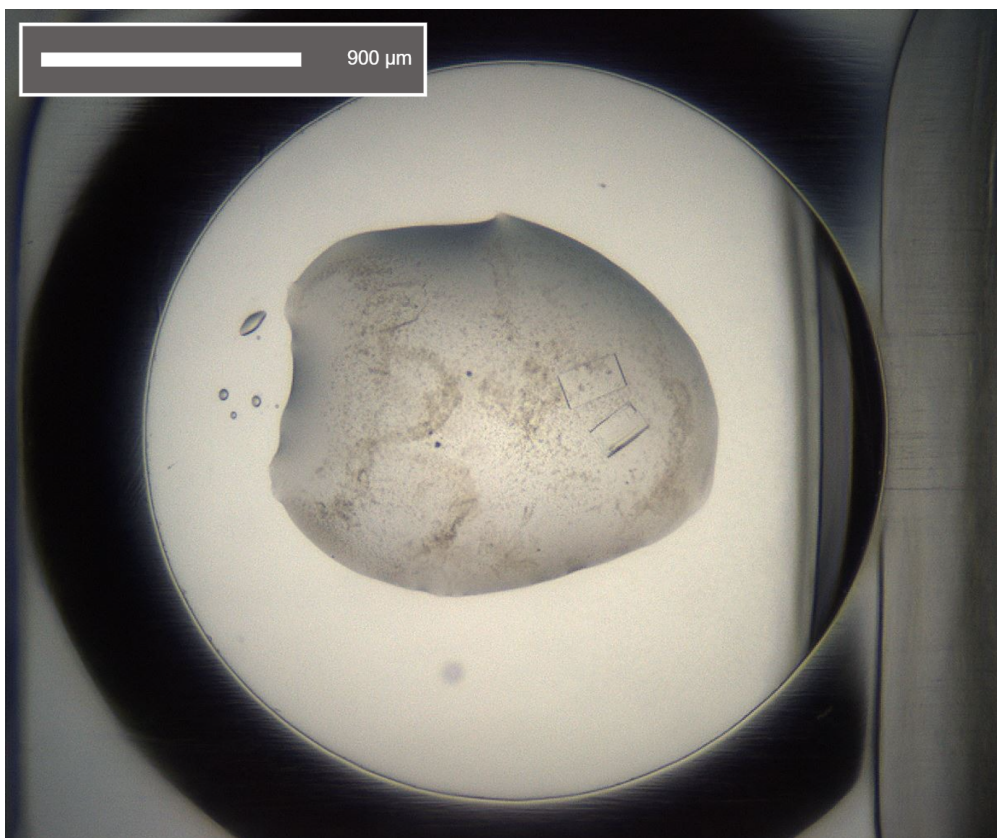
Morphology: typically thin rectangles with pointed ends.

Size: ~50 μm in length and ~25 μm in width, depth of the crystals is ~5 μm , giving a glass shard appearance

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

Space group: C222₁

Unit cell: 64.90 $\text{\AA}$, 65.73 $\text{\AA}$, 76.27 $\text{\AA}$
90.00°, 90.00°, 90.00°



An example of a drop containing EV A71 3C protease crystals.

Protocol references

N/A