

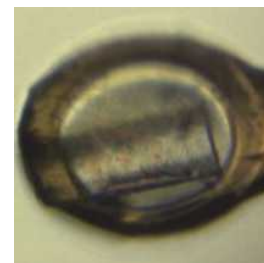
Apr 30, 2025

Version 2

🌐 Crystallization of Enterovirus A71 3C protease in C222₁ apo structure (PDB 8CNY) V.2

DOI

<https://dx.doi.org/10.17504/protocols.io.yxmvm3e89l3p/v2>



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

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Protocol status: In development

We are still developing and optimizing this protocol

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Last Modified: April 30, 2025

Protocol Integer ID: 162060

Keywords: crystallisation, Enterovirus A71, 3C protease, XChem, ASAP, AViDD, CMD, Diamond Light Source, i04-1, crystallization of enterovirus a71 3c protease, enterovirus a71 3c protease crystal, enterovirus a71 3c protease, 3c protease crystal, enterovirus a71, crystallization protocol, crystallization, crystal structure, protein production, rcsb pdb with code 8cny, protease, rcsb pdb, pdb, protein

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Disclaimer

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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**. In this version we added: protein production, solvent tolerance test protocols and the Addgene ID of the construct used.

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

Protocol materials

 Enterovirus A71 3C protease **addgene Catalog #204816**



Protein expression and purification

- 1 The below protocol was used for protein expression and purification of the plasmid:

 Enterovirus A71 3C protease **addgene Catalog #204816**

Protocol

NAME

Enterovirus A71 3C protease small scale expression and purification protocol

CREATED BY

korvus.wang

Preview

Equipment needed

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

Crystallization experiment

1d

- 3 **Protein and buffer requirements:**

 43.2 μ L  1 millimolar (mM)  Sample

 2.88 mL Crystallization screen

- 4 **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.

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

Dispense  150 μL  1 millimolar (mM)  Sample to each lens using the SPT mosquito.

Dispense  150 μL Crystallisation screen to each lens using the SPT mosquito.

Drop ratio: 1:1

Final drop volume: 300 nL

- 6 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.

- 7 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.

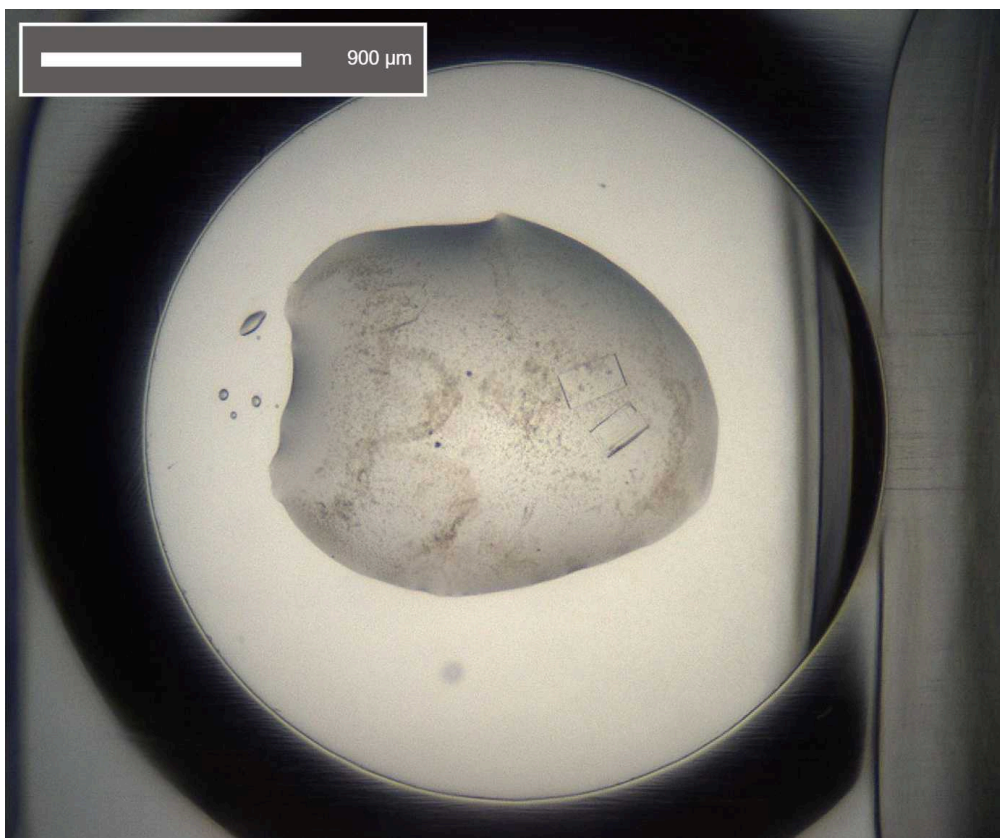
Morphology: typically thin rectangles with pointed ends.

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.

Soaking tolerance

2h 30m



8 Final condition identified: 20% DMSO Duration ⌚ 02:30:00

2h 30m

Protocol



NAME

XChem solvent test

CREATED BY

Peter Marples

Preview

Protocol references

N/A