

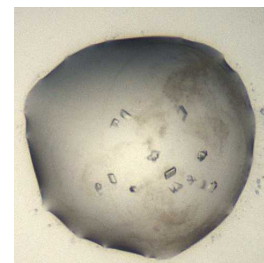
Apr 26, 2024

Version 1

Crystallization of Enterovirus D68 3C protease V.1

DOI

<https://dx.doi.org/10.17504/protocols.io.5qpvoky29l4o/v1>



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ASAP Discovery



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Protocol Citation: Ryan Lithgo, Peter Marples, Lizbé Koekemoer, Daren Fearon 2024. Crystallization of Enterovirus D68 3C protease. protocols.io <https://dx.doi.org/10.17504/protocols.io.5qpvoky29l4o/v1>

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Protocol status: Working

We use this protocol and it's working

Created: April 26, 2024

Last Modified: April 26, 2024

Protocol Integer ID: 98840

Keywords: crystallisation, 3C protease, XChem, ASAP, AViDD, CMD, Diamond Light Source, i04-1, D68 3C protease, crystallization of enterovirus d68 3c protease, enterovirus d68 3c protease, pathogen enterovirus d68, emerging pathogen enterovirus d68, enterovirus d68, d68 3c prob crystal, spectrum antiviral, antiviral drug, 3c protease of ev, essential role in the viral life cycle, 3c protease, viral life cycle, crystallization, pathogen, d68, throughput crystallographic, protease, d68 3c

Funders Acknowledgements:

National Institutes of Health/National Institute Of Allergy and Infectious Diseases (NIH/NIAD)

Grant ID: Grant ID: U19AI171399

Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Acknowledgements:

Diamond Light Source Ltd, Harwell Science and Innovation Campus, Didcot OX11 0QX, UK
Research Complex at Harwell, Harwell Science and Innovation Campus, Didcot OX11 0FA, UK
Oxford Lab Technologies crystal shifter <https://doi.org/10.1107/S2059798320014114>

Abstract

The development of effective broad-spectrum antivirals forms an important part of preparing for future pandemics. A cause for concern is the currently emerging pathogen Enterovirus D68 (EV-D68) which primarily spreads through respiratory routes causing mostly mild to severe respiratory illness but, in severe cases, acute flaccid myelitis. The 3C protease of EV-D68 is a potential target for the development of antiviral drugs due to its essential role in the viral life cycle and high sequence conservation. This protocol was used to grow D68 3C ProB crystals that were applied high-throughput crystallographic follow up compound screening on D68 3C.



Materials

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

Midi: UVXPO-3LENS 3W96T-PS 3W96T-UVP

[M] 1 Molarity (M) Tris adjusted to pH 7.8 with NaOH, Molecular Dimensions, Catalog # MD2-027-PH 7.8

[M] 1 Molarity (M) Ammonium acetate, Molecular Dimensions, Catalog # MD2-002-PH

50% w/v PEG 3350, Molecular Dimensions, Catalog # MD2-250-9

Purified D683C protein ([M] 35 mg/mL) in [M] 10 millimolar (mM) HEPES, pH 7.5 , [M] 0.5 Molarity (M) NaCl,
5% glycerol, [M] 0.5 millimolar (mM) TCEP

Safety warnings

⚠ Follow all handling warning for the chemicals used in the crystallisation screen composition.



Equipment needed

- 1 **Formulatrix Rock Imager** (or incubator of choice)
SPT mosquito

Equipment

Mosquito HV

NAME

High Volume 16-Channel Robotic Liquid Handler

TYPE

SPT LabTech

BRAND

3097-01057

SKU

<https://www.sptlabtech.com/products/liquid-handling/mosquito-hv/>^{LINK}

P100 8 multi-channel pipette

SwissCI 3 lens plate

Crystallization experiment

1d

- 2 **Prepare seed stock:**

Protocol



NAME

Diamond XChem Seeding Protocol

CREATED BY

Peter Marples

Preview

1: 1 000 000 dilution  Sample seeds

- 3 **Protein and buffer requirements:**



14.4 µL



[M] 35 mg/mL



Sample

🧪 2.88 mL Crystallization screen

🧪 7.2 µL seeds, dilution 1:1 000 000

4 Crystallisation screen composition:

[M] 0.1 Molarity (M) Tris

⬆️

 pH 7.8

[M] 0.2 Molarity (M) Ammonium acetate

26% w/v PEG 3350

Stock solutions used:

[M] 1 Molarity (M) Tris adjusted to

⬆️

 pH 7.8 with NaOH

[M] 1 Molarity (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.

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

Dispense 🧪 50 µL [M] 35 mg/mL

🧪

 Sample to each lens using the SPT mosquito.

Dispense 🧪 100 µL Crystallisation screen to each lens using the SPT mosquito.

Dispense 🧪 25 µL Seeds to each lens using the SPT mosquito.

Drop ratio: 2:4:1

Final drop volume: 175 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

Expected result

Crystals typically appear after 24 hours and reach their maximum size after ~24 h with some precipitation often remaining.

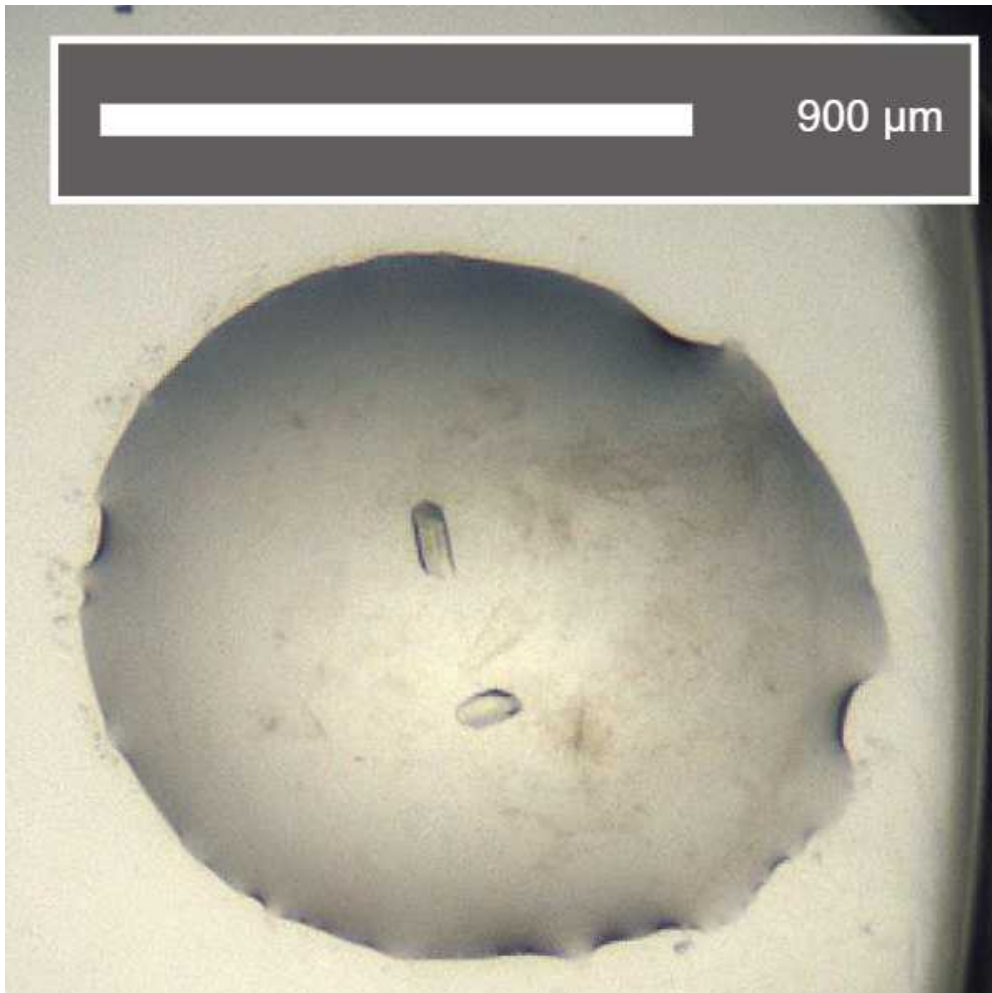
Morphology: small shards.

Size: ~40 μm in length and ~40 μm in width, depth of the crystals is ~20 μm , giving a glass shard appearance

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

Space group: $P2_1$

Unit cell: 39.7 $\text{\AA}$, 105 $\text{\AA}$, 43.5 $\text{\AA}$
90.00°, 110.00°, 90.00°



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



Data collection at Synchrotron

8 Diamond Light Source
Unattended Data Collection (UDC)
Data Collection Temperature: 100K
Detector: DECTRIS EIGER2 X 9M
Beamline: I04-1
Wavelength: 0.9212 Å
Resolution (Å): 1.62
Beam Size (μm): 60 X 50
Number of images: 3600
Oscillation: 0.10°
Exposure (s): 0.0020
Transmission (%): 100
Flux (ph/s): 9.50e+11

Protocol references

N/A