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Version 1

Crystallisation of SARS-CoV-2 Mpro V.1

 Forked from [Crystallization of SARS-CoV-2 Mpro](#)

DOI

<https://dx.doi.org/10.17504/protocols.io.261ge5emyg47/v1>



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blake.h.balcomb : The principle crystallographer on the SARS Mpro project.;

ASAP Discovery



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Protocol status: Working

We use this protocol and it's working

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Disclaimer

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

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Abstract

The COVID-19 pandemic has highlighted the need to identify novel therapeutic interventions and strategies for pandemic preparedness against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). This protocol outlines the crystallization protocol and buffer conditions used to obtain reproducible SARS-CoV-2 Mpro crystals suitable for **XChem** fragment screening.



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) MES adjusted to [pH] 6.7 with HCl, Molecular Dimensions, Catalog # MD2-013-PH 6.7
50% w/v PEG 4000, Molecular Dimensions, Catalog # MD2-250-11
99.9% DMSO, Molecular Dimensions, Catalog # MD2-250-159

Purified SARS-CoV-2 Mpro protein ([M] 5 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.



SARS-CoV-2 Mpro expression and purification

- 1 The protein used for crystallisation was expressed and purified using the following protocol.

Protocol

NAME

SARS-CoV-2 Mpro small scale expression and purification protocol

CREATED BY

korvus.wang

Preview

Equipment needed

- 2 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

Crystallisation experiment

1d



3 Prepare seed stock:

17m 40s

Protocol



NAME

Diamond XChem Seeding Protocol

CREATED BY

Peter Marples

[Preview](#)

1: 250 dilution Sample seeds

4 Protein and buffer requirements:

 43.2 μ L 5 mg/mL Sample

1.92 mL Crystallisation screen

 14.4 μ L Sample seeds, dilution 1:250

5 Crystallisation screen composition:

0.1 Molarity (M) MES 6.7

11% w/v PEG 4000

5% DMSO

Stock solutions used:

1 Molarity (M) MES adjusted to 6.7 with HCl

50% w/v PEG 4000

99.9% DMSO

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.

6 Dispense 20 μ L Crystallisation screen into SwissCI 3 lens plate reservoir wells using a 100 μ l multi-channel pipette.

10m



Dispense 150 nL 5 mg/mL Sample to each lens using the SPT mosquito.

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

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

Drop ratio: 3:3:1 ratio (150 nl Sample : 150 nl reservoir solution: 50 nl seeds)

Final drop volume: 350 nl

7 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.

8 Crystal form after ~24 h.

Expected result

The crystals reach their maximum size after 48 h.

Crystals typically form either as stacked plates or in small clusters containing 4-6 crystals.

Morphology: plates.

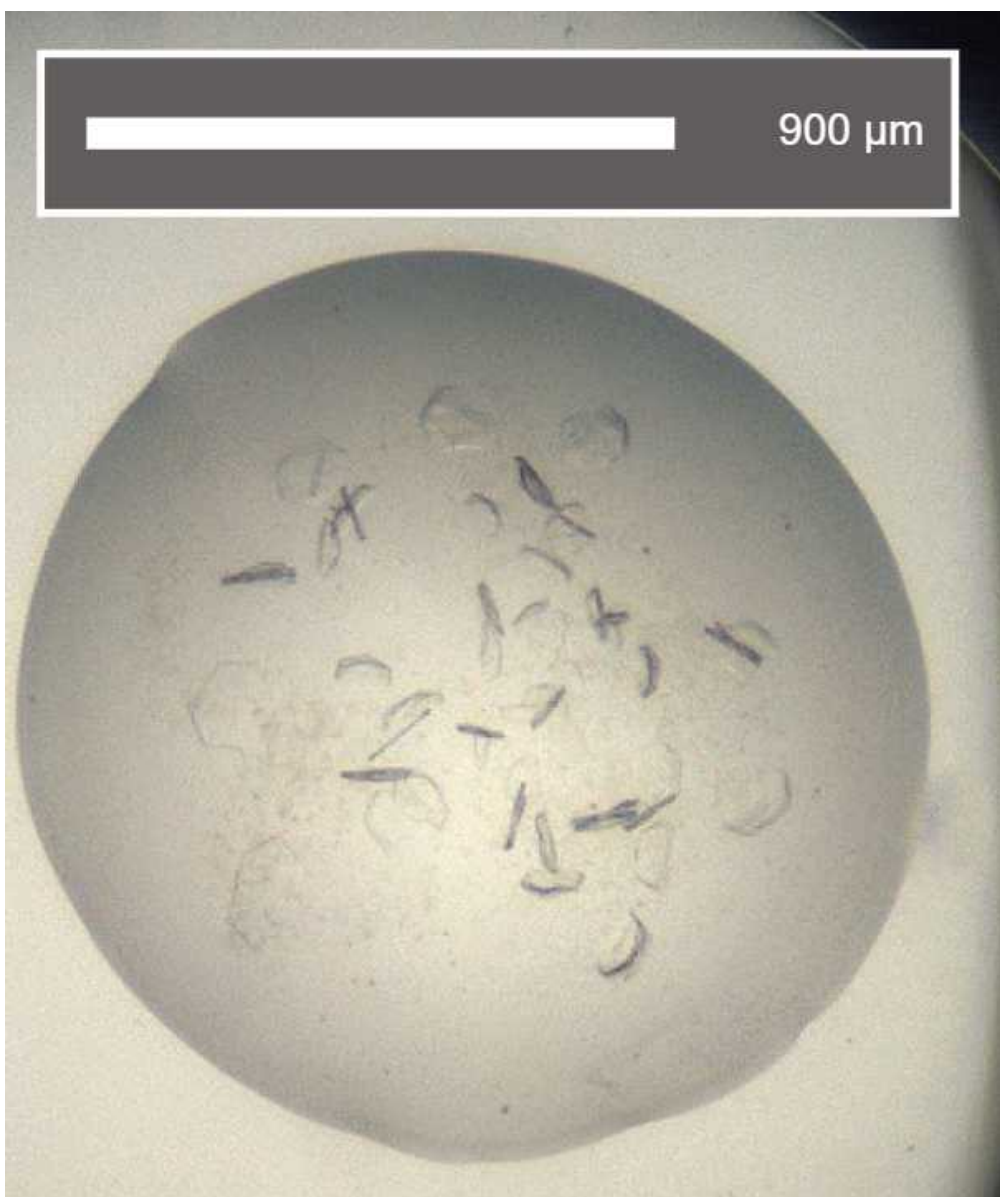
Size: ~100 μm in length and ~20 μm in width, depth of the crystals is ~10 μm

Appearance: glass shard.

Average resolution: 1.8 $\text{\AA}$

Space group: $P2_12_12_1$

Unit cell: 67 $\text{\AA}$, 99 $\text{\AA}$, 102 $\text{\AA}$
90.00°, 90.00°, 90.00°



An example of a drop containing SARS-CoV-2 Mpro protease crystals.



Data collection at Synchrotron

- 9 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): 1.78
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