

May 13, 2025

Version 2

🌐 Crystallisation of MERS-CoV Mpro in C222₁ used for compound soaking V.2

🔗 Version 1 is forked from [Crystallization of MERS-CoV Mpro](#)

DOI

<https://dx.doi.org/10.17504/protocols.io.8epv5rn84g1b/v2>

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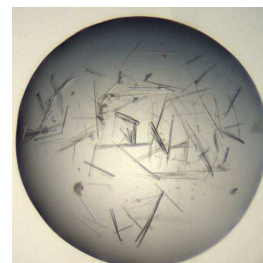
Blake Balcomb: The principle crystallographer on the MERS-CoV Mpro project.;

ASAP Discovery



Peter Marples

Diamond Light Source



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External link: <https://asapdiscovery.org/outputs/target-enabling-packages/#ASAP-COV-MPRO>

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<https://dx.doi.org/10.17504/protocols.io.8epv5rn84g1b/v2> Version created by **Mary-Ann Xavier**

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Protocol status: Working

We use this protocol and it's working

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Protocol Integer ID: 123150

Keywords: crystallisation, XChem, ASAP, AVIDD, CMD, Diamond Light Source, i04-1, Research complex at Harwell, 8R5J, MERS, MERS Mpro, Mpro, severe acute respiratory syndrome coronavirus, several human coronavirus, other than severe acute respiratory syndrome coronavirus, cov mpro in c2221, therapeutic intervention for mer, zoonotic virus, essential for viral replication, cov mpro, crystallisation of mer, cov, protein purification protocol, covid, virus, strategies for pandemic preparedness, viral replication, mer, pandemic concern, pandemic preparedness, main protease, pandemic, middle eastern respiratory syndrome, protein, wise to sar, sar

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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. Other than Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), there are several human coronaviruses that are of pandemic concern, these include SARS-CoV and Middle Eastern Respiratory Syndrome (MERS-CoV). MERS-CoV is a zoonotic virus that was first discovered in 2012. The disease has spread rapidly with large outbreaks as recent as 2015 and 2018. Currently there is no therapeutic intervention for MERS-CoV with 35% of reported cases resulting in human death. Like-wise to SARS-CoV-2, MERS-CoV produces a main protease (Mpro) which is essential for viral replication and therefore an attractive target to inhibit the virus. In this new version we added the protein purification protocol and Addgene id, together with the solvent test and compound soaking conditions.

Materials

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

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

JCSG+ condition 2-30, Molecular Dimensions, Catalog # MDSR-37-250-2-30

Purified MERS-CoV Mpro protein () in HEPES, ,
 NaCl, 5% glycerol, TCEP

Protocol materials

 MERS-CoV Mpro strain HCoV-EMC 3CL protease domain **addgene** Catalog #228646

Safety warnings

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



MERS-CoV Mpro expression and purification

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



MERS-CoV Mpro strain HCoV-EMC 3CL protease domain [addgene Catalog #228646](#)

Protocol

NAME

MERS-CoV Mpro large scale 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

Crystallisation experiment

1d

- 3 **Prepare seed stock:**

17m 40s

Protocol



NAME

Diamond XChem Seeding Protocol

CREATED BY

Peter Marples

Preview



1: 1000 dilution  Sample seeds

4 **Protein and buffer requirements:**

 32 μ L  17 mg/mL  Sample

 3.36 mL Crystallisation screen

 14.4 μ L  Sample seeds, dilution 1:1000

5 **Crystallisation screen composition:**

 0.2 Molarity (M) Sodium malonate dibasic monohydrate

20% w/v PEG 3350

Stock solutions used:

JCSG+ condition 2-30

Note

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

6 Dispense  35 μ L Crystallisation screen into SwissCI 3 lens plate reservoir wells using a 100 μ l multi-channel pipette.

10m

Dispense  150 nL  17 mg/mL  Sample to each lens using the SPT mosquito.

Dispense  25 nL Seeds to each lens using the SPT mosquito.

Dispense  130 nL Crystallisation screen to each lens using the SPT mosquito.

Drop ratio: 15:13:2 ratio (150 nL  Sample : 150 nL reservoir solution: 20 nL seeds)

Final drop volume: 300 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 ~12 h.

Expected result

The crystals reach their maximum size after 24 h.

Morphology: typically thin needles or rectangles with pointed ends.

Size: ~100 μm in length and ~2 μm in width, depth of the crystals is ~2 μm

Appearance: glass shard.

Average resolution: 2.2 $\text{\AA}$

Space group: C222₁

Unit cell: 87 $\text{\AA}$, 94 $\text{\AA}$, 155 $\text{\AA}$
90.00°, 90.00°, 90.00°



An example of a drop containing MERS-CoV Mpro 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

Soaking Tolerance

- 10 Crystals can tolerate up to 20% DMSO, 1-2h

Protocol



NAME

XChem solvent test

CREATED BY

Peter Marples

[Preview](#)

Compound Soaking

- 11 Crystals can tolerate up to 20% DMSO, 1-2h



Protocol



NAME

XChem crystallographic fragment screening

CREATED BY

Peter Marples

Preview

Protocol references

N/A