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Version 1

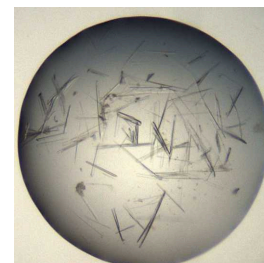
🌐 Crystallisation of MERS-CoV Mpro V.1



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

DOI

<https://dx.doi.org/10.17504/protocols.io.8epv5rn84g1b/v1>



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

ASAP Discovery



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

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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. 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.



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 (ⓘ 17 mg/mL) in ⓘ 10 millimolar (mM) HEPES, ⓘ pH 7.5 ,
 ⓘ 0.5 Molarity (M) NaCl, 5% glycerol, ⓘ 0.5 millimolar (mM) TCEP

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.

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

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: 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 20 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: $\sim 100\ \mu\text{m}$ in length and $\sim 2\ \mu\text{m}$ in width, depth of the crystals is $\sim 2\ \mu\text{m}$

Appearance: glass shard.

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

Space group: $C222_1$

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

Protocol references

N/A