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Crystallization of MERS-CoV Mpro

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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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We use this protocol and it's working

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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 () in HEPES, ,
 NaCl, 5% glycerol, 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:**

17m 40s

Protocol



NAME

Diamond XChem Seeding Protocol

CREATED BY

Peter Marples

Preview

1: 1000 dilution  Sample seeds

- 3 **Protein and buffer requirements:**



32 µL

[M] 17 mg/mL



Sample



🧪 3.36 mL Crystallization screen

🧪 14.4 µL 🧪 Sample seeds, dilution 1:1000

4 Crystallisation screen composition:

[M] 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.

5 Dispense 🧪 35 µL Crystallisation screen into SwissCI 3 lens plate reservoir wells using a 100 µl multi-channel pipette.

10m

Dispense 🧪 150 µL [M] 17 mg/mL 🧪 Sample to each lens using the SPT mosquito.

Dispense 🧪 20 µL Seeds to each lens using the SPT mosquito.

Dispense 🧪 130 µL 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

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 Crystal form after ~24 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

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): 1.78
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