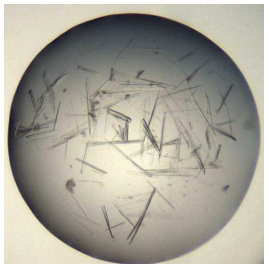
Oct 02, 2024

Crystallisation of MERS-CoV Mpro



Forked from Crystallization of MERS-CoV Mpro

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

ASAP Discovery

Peter Marples
Diamond Light Source

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.8epv5rn84g1b/v1

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 ([M] 17 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 crystalllisation 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 µL [M] 17 mg/mL Sample

3.36 mL Crystallisation screen

14.4 µL Sample seeds, dilution 1:1000

5 **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.

6 Dispense 35 µL Crystallisation screen into SwissCI 3 lens plate reservoir wells using a 100 µl multi-channel pipette. 10m

Dispense 150 nL [M] 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: ~100 µm in length and ~2 µm in width, depth of the crystals is ~2 µm
Appearance: glass shard.
Average resolution: 2.2 Å
Space group: $C222_1$
Unit cell: 87 Å, 94 Å, 155 Å
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