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Version 2

READDI protocol: Crystallisation of CHIKV nsP3 macrodomain V.2

Forked from a private protocol

DOI

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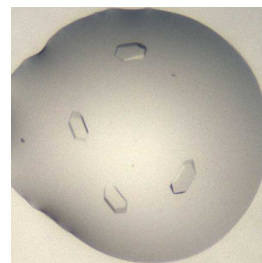
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ASAP Discovery



Peter Marples

Diamond Light Source



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READDI protocol: Crystallisation of CHIKV nsP3 macrodomain. **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.j8nlk8qzdl5r/v2> Version created by **Jasmin Aschenbrenner**

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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 crystallization protocol and buffer conditions used to obtain reproducible Chikungunya Virus NS3 macrodomain crystals suitable for **XChem** fragment screening.

Guidelines

N/A



Materials

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

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

Molecular Dimensions 'The BCS Screen Single Reagent' 2-44:

0.1 M Tris (pH 7.8), 0.1 M Potassium thiocyanate, 0.1 M Sodium bromide, 25 % v/v PEG Smear Broad, Catalog # MDSR-104-2-44

Purified CHIKV Mac protein (11 mg/mL) in 25 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 5 % Glycerol.

Safety warnings

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

Ethics statement

N/A

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

Crystallisation experiment

1d

- 2 **Prepare seed stock:**

17m 40s

Protocol



NAME

Diamond XChem Seeding Protocol

CREATED BY

Peter Marples

Preview

1: 100 dilution  Sample seeds

- 3 **Protein and buffer requirements:**



21.6 µL



[M] 11 mg/mL



Sample



🧪 2.88 mL Crystallisation screen

🧪 10.08 µL 🧴 Sample Seeds, dilution 1:100

4 Crystallisation screen composition:

[M] 0.1 Molarity (M) Tris-NaOH 📏 pH 7.8

[M] 0.1 Molarity (M) Potassium thiocyanate

[M] 0.1 Molarity (M) Sodium bromide

25 % v/v PEG Smear Broad

Stock solutions used:

[M] 1 Molarity (M) Tris adjusted to 📏 pH 7.8 with NaOH

[M] 1 Molarity (M) Potassium thiocyanate

[M] 1 Molarity (M) Sodium bromide

50% v/v PEG Smear Broad

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.

5 Dispense 🧪 30 µL Crystallisation screen into SwissCI 3 lens plate reservoir wells using a 100 µl multi-channel pipette.

10m

Dispense 🧪 75 nL [M] 11 mg/mL 🧴 Sample to each lens using the SPT mosquito.

Dispense 🧪 40 nL Crystallisation screen to each lens using the SPT mosquito.

Dispense 🧪 35 nL CHIKV Mac Seeds to each lens using the SPT mosquito.

Drop ratio: 15:8:7 ratio (75 nl 🧴 Sample : 40 nl Crystallisation solution: 35 nl Seeds)

Final drop volume: 150 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

Expected result

The crystals reach their maximum size after 24-48 h.

Crystals typically form as single crystals at the bottom of the drop or on the drop-air interface.

Morphology: typically thin rectangles with pointed ends.

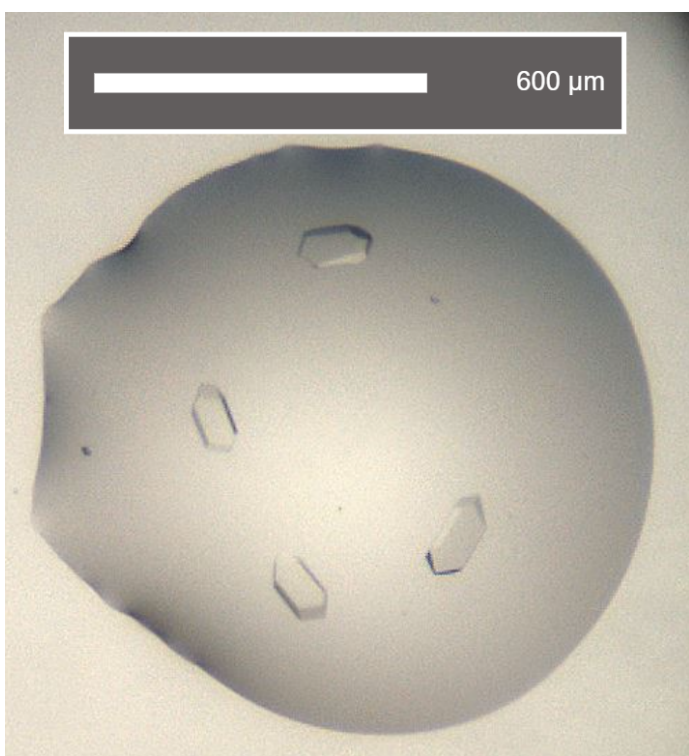
Size: ~100 μm in length and ~50 μm in width, depth of the crystals is ~10 μm

Appearance: glass shard.

Average resolution: 1.5 $\text{\AA}$

Space group: $P3_1$

Unit cell: 87 $\text{\AA}$, 87 $\text{\AA}$, 85 $\text{\AA}$
90.00°, 90.00°, 120.00°



An example of a drop containing CHIKV macrodomain 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): 1.64
Beam Size (μm): 60 X 50
Number of images: 3600
Oscillation: 0.10°
Exposure (s): 0.0020
Transmission (%): 100
Flux (ph/s): 3.80e+12

Protocol references

Identifying novel chemical matter against the Chikungunya virus nsP3 macrodomain through crystallographic fragment screening, <https://doi.org/10.1101/2024.08.23.609196>