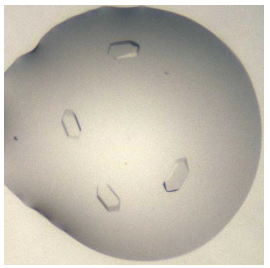
Oct 07, 2024 Version 2

READDI protocol: Crystallisation of CHIKV nsP3 macrodomain V.2



Version 1 is forked from READDI protocol: Crystallisation of CHIKV nsP3 macrodomain

DOI
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ASAP Discovery

Peter Marples
Diamond Light Source

OPEN ACCESS

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External link: https://readdi-ac.org/

Protocol Citation: Jasmin Aschenbrenner, Peter Marples, michael fairhead, Andre Schutzer de Godoy, Daren Fearon 2024. READDI protocol: Crystallisation of CHIKV nsP3 macrodomain. **protocols.io https://dx.doi.org/10.17504/protocols.io.j8nlk8qzdl5r/v2**Version created by **Jasmin Aschenbrenner**

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 crystalllisation 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.

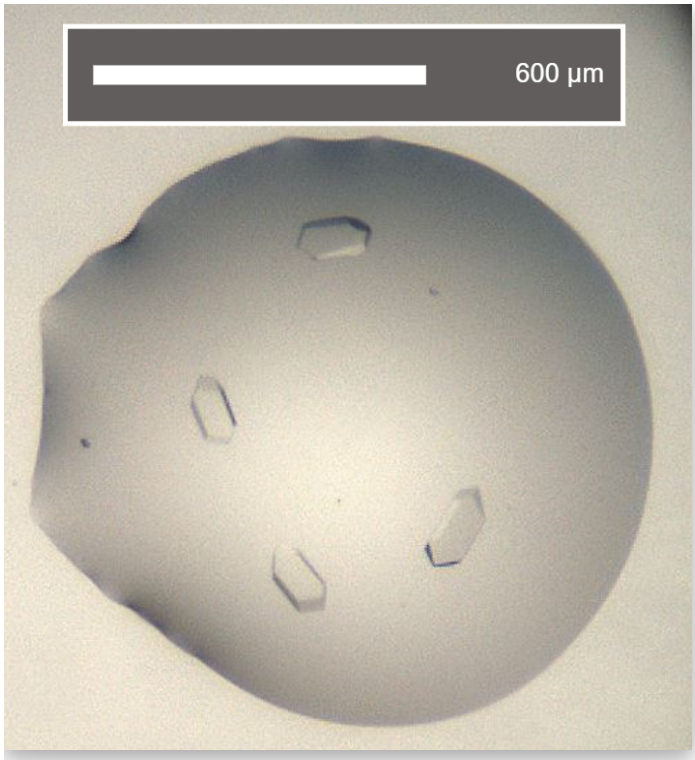
7

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 Å
Space group: $P3_1$
Unit cell: 87 Å, 87 Å, 85 Å
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