

Apr 26, 2024

_

Crystallization of SARS-CoV-2 nsp15 NendoU

DOI

dx.doi.org/10.17504/protocols.io.36wgqn4yogk5/v1

Peter Marples^{1,2}, Daren Fearon^{1,2}, Lizbé Koekemoer³, Andre Schutzer de Godoy⁴

¹Diamond Light Source; ²Research Complex at Harwell; ³Centre of Medicines Discovery, University of Oxford;

⁴University of Sao Paulo

ASAP Discovery



Lizbé Koekemoer

University of Oxford





DOI: dx.doi.org/10.17504/protocols.io.36wgqn4yogk5/v1

External link: https://asapdiscovery.org/outputs/target-enabling-packages/#ASAP-SARS-COV-2-ENDORIBONUCLEASE

Protocol Citation: Peter Marples, Daren Fearon, Lizbé Koekemoer, Andre Schutzer de Godoy 2024. Crystallization of SARS-CoV-2 nsp15 NendoU. **protocols.io** https://dx.doi.org/10.17504/protocols.io.36wgqn4yogk5/v1

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's

working

Created: April 26, 2024

Last Modified: April 26, 2024

Protocol Integer ID: 98842

Keywords: crystallisation, XChem, ASAP, AViDD, CMD, Diamond Light Source, i04-1, SARS CoV-2 NSP15, NSP15



Funders Acknowledgement:
National Institutes of
Health/National Institute Of
Allergy and Infectious
Diseases (NIH/NIAID)
Grant ID: Grant ID:
U19AI171399

Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Acknowledgements:

Diamond Light Source Ltd, Harwell Science and Innovation Campus, Didcot OX11 0QX, UK Sao Carlos Institute of Physics, University of Sao Paulo, Av. Joao Dagnone, 1100 - Jardim Santa Angelina, Sao Carlos, 13563-120, Brazil

Research Complex at Harwell, Harwell Science and Innovation Campus, Didcot OX11 0FA, UK Oxford Lab Technologies crystal shifter https://doi.org/10.1107/S2059798320014114

Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of coronavirus disease 2019 (COVID-19). The NSP15 endoribonuclease enzyme, known as NendoU, is highly conserved and plays a critical role in the ability of the virus to evade the immune system. NendoU is a promising target for the development of new antiviral drugs. This protocol was used to grow SARS CoV-2 NSP15 crystals that were applied high-throughput crystallographic fragment screening at XChem.

Materials

SwissCl 3 lens crystallization plates https://swissci.com/product/3-lens-crystallisation-plate/ Codes: Midi: UVXPO-3LENS 3W96T-PS 3W96T-UVP

[M] 1 Molarity (M) sodium citrate adjusted to PH 5 with HCl, Molecular Dimensions, Catalog # 133235 with HCl, Molecular Dimensions, Catalog # MD2-250-12

Purified SARS CoV-2 NSP15 protein ([M] 3.4 mg/mL) in [M] 20 millimolar (mM) HEPES PH 7.5 ,

[M] 150 millimolar (mM) NaCl, 5% (v/v) glycerol and [M] 0.5 millimolar (mM) TCEP



Safety warnings

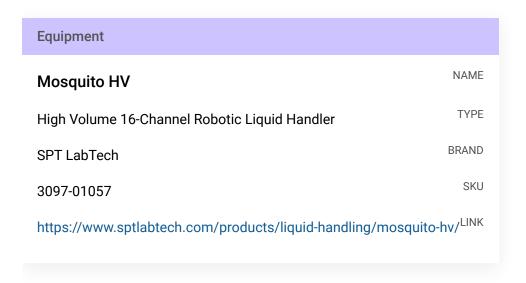


• Follow all handling warning for the chemicals used in the crystalllisation screen composition.



Equipment needed

Formulatrix Rock Imager (or incubator of choice) **SPT mosquito**

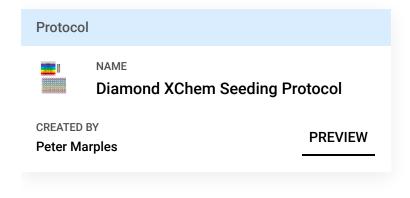


P100 8 multi-channel pipette

SwissCI 3 lens plate

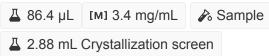
Crystallization experiment

2 Prepare seed stock:



No dilution 🔊 Sample seeds

3 Protein and buffer requirements:







4 Crystallisation screen composition:

[M] 0.1 Molarity (M) sodium citrate 6 5 14% w/v PEG 6000

Stock solutions used:

[M] 1 Molarity (M) sodium citrate adjusted to the with HCI 50% w/v PEG 6000

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.

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

Dispense 300 undetermined M3.4 mg/mL Sample to each lens using the SPT mosquito.

Dispense 🚨 290 undetermined Crystallisation screen to each lens using the SPT mosquito.

Dispense \perp 10 undetermined Seeds to each lens using the SPT mosquito.

Drop ratio: 30:29:1 ratio (300 nl ♦ Sample : 290 nl reservoir solution: 10 nl seeds)

Final drop volume: 600 nl

6 Incubate at \$\colon 20 \colon C for 24:00:00 h in Formulatrix Rock Imager.

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 ~96 h.

1d



Expected result

The crystals reach their maximum size after 96 h.

Crystals typically form as single hexagonal crystals.

Morphology: hexagonal.

Size: \sim 100 μ m in length and \sim 50 μ m in width, depth of the crystals is \sim 10 μ m

Appearance: glass shard. Average resolution: 2.2 Å

Space group: P63

Data collection at Synchrotron

8 Diamond Light Source

> **Unattended Data Collection (UDC) Data Collection Temperature:** 100K **Detector:** DECTRIS EIGER2 X 9M

Beamline: 104-1

Wavelength: 0.9127 Å Resolution (Å): 2.00 **Beam Size (µm):** 60 X 50 Number of images: 1500

Oscillation: 0.12° **Exposure (s):** 0.0400 Transmission (%): 100 Flux (ph/s): 93.50e+11

Protocol references

N/A