

Apr 26, 2024

Crystallization of SARS-CoV-2 nsp15 NendoU

DOI

<https://dx.doi.org/10.17504/protocols.io.36wgqn4yogk5/v1>

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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>

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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, nsp15 endoribonuclease enzyme, crystallization of sar, throughput crystallographic fragment screening at xchem, crystallographic fragment screening, acute respiratory syndrome coronavirus, severe acute respiratory syndrome coronavirus, new antiviral drug, coronavirus disease, causative agent of coronavirus disease, antiviral drug, throughput crystallographic, crystallization, sar, crystal

Funders Acknowledgements:

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

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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

SwissCI 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
50% w/v PEG 6000, 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 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:**

Protocol



NAME

Diamond XChem Seeding Protocol

CREATED BY

Peter Marples

Preview

No dilution Sample seeds

- 3 **Protein and buffer requirements:**



86.4 μ L

[M] 3.4 mg/mL



Sample



🧪 2.88 mL Crystallization screen

🧪 2.88 µL 🧪 Sample seeds, no dilution

4 Crystallisation screen composition:

[M] 0.1 Molarity (M) sodium citrate pH 5

14% w/v PEG 6000

Stock solutions used:

[M] 1 Molarity (M) sodium citrate adjusted to pH 5 with HCl

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.

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

Dispense 🧪 300 µL [M] 3.4 mg/mL 🧪 Sample to each lens using the SPT mosquito.

Dispense 🧪 290 µL Crystallisation screen to each lens using the SPT mosquito.

Dispense 🧪 10 µL 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 🌡️ 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 ~96 h.



Expected result

The crystals reach their maximum size after 96 h.

Crystals typically form as single hexagonal crystals.

Morphology: hexagonal.

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

Appearance: glass shard.

Average resolution: 2.2 $\text{\AA}$

Space group: $P6_3$

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: 0.9127 $\text{\AA}$
Resolution ($\text{\AA}$): 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