Apr 26, 2024

Crystallization of SARS-CoV-2 nsp15 NendoU

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

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ASAP Discovery

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OPEN ACCESS

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**

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
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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 crystalllisation 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 µL [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 undetermined [M] 3.4 mg/mL Sample to each lens using the SPT mosquito.

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

Dispense 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 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 Å
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 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