Oct 29, 2024 Version 1

Zika NS2B-NS3 protease co-expression construct small scale expression and purification protocol V.1

Forked from a private protocol

DOI
dx.doi.org/10.17504/protocols.io.3byl49mrrgo5/v1

Korvus Wang[1], Michael Fairhead[1], Eleanor Williams[1]

[1]Centre for Medicines Discovery, University of Oxford

ASAP Discovery

Korvus Wang
CMD

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.3byl49mrrgo5/v1

Protocol Citation: Korvus Wang, Michael Fairhead, Eleanor Williams 2024. Zika NS2B-NS3 protease co-expression construct small scale expression and purification protocol. **protocols.io https://dx.doi.org/10.17504/protocols.io.3byl49mrrgo5/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: October 19, 2024

Last Modified: October 29, 2024

Protocol Integer ID: 110341

Keywords: expression, purification, ASAP, CMD, AViDD, NS3 protease, Zika Virus, Zika Virus NS3 protease, ZVNS2B-NS3 protease, purification of zika ns2b, ns3 protease coexpression construct, zika ns2b, ns3 protease, purification protocol this protocol, purification protocol, purification, protease, ns2b

Funders Acknowledgements:
National Institutes of Health/National Institute Of Allergy and Infectious Diseases (NIH/NIAID)
Grant ID: U19AI171432

Disclaimer

Research was supported in part by NIAID of the U.S National Institutes of Health under award number U19AI171399. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Abstract

This protocol details the co-expression and purification of Zika NS2B-NS3 protease coexpression construct bearing a N-terminal His-GST tag at small scale (<6L).

Attachments

PDF

PAGE24-00691 - XX01Z...

350KB

Guidelines

- **Construct / plasmid resource-name:** ZVNS2B-NS3 protease co-expression construct bearing a N-terminal His-GST tag.

Materials

Plasmid details:

- Vector: pNIC
- Cell line: E. coli Rosetta strain BL21(DE3)-RR
- Tags and additions: N-terminal His- tag
- Construct protein sequence: `
  MHHHHHHSSMSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAII
  RYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPD
  FMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSGGGSENLYFQSM
  GKSVDMYIERAGDITWEKDAEVTGNSPRLDVALDESGDFSLVEE

Expression
TB media, 1mM IPTG

Purification
Chicken hen egg white lysozyme
Benzonase
Imidazole
Ni Sepharose 6 FF resin
Gravity flow column, 2.5cm diameter
Centrifugal concentrators, 10kDa MWCO

On an FPLC system:
Cytiva HiLoad 16/600 Superdex 75 pg
5mL sample loop

SDS-PAGE sample buffer, gel, and gel tank

Lysis buffer:

	A	B
	Hepes (pH 7.5)	50 mM
	NaCl	500 mM
	Glycerol	5%
	TCEP	1 mM
	Lysozyme	0.5 mg/mL
	Benzonase	0.05 mg/mL

Prepare 100L per 1L E.coli expression

Base buffer:

	A	B
	Hepes (pH 7.5)	50 mM
	NaCl	50 mM
	Glycerol	5%
	TCEP	1 mM

Prepare 2L per 6L E.coli expression. Used to prepare the following buffers

Binding buffer: base buffer
Wash buffer 1: base buffer + 1:2000 benzonase + 2mM MgCl2
Wash buffer 2: base buffer + 30mM imidazole
Elution buffer: base buffer, add 500mM imidazole
rIMAC wash buffer: base buffer + 50mM imidazole
Gel filtration buffer: base buffer + 20mM imidazole

SDS-PAGE gel: NuPage 4-12%, Bis-Tris protein gel, 27 well.
Run in MES buffer, 200V 35mins.

Troubleshooting

Abbreviations

1 CV - column volume, total volume of resin in a column
IMAC - immobilised metal affinity chromatography
FT - flow through
ZVNS2B3 - Zika NS2B-NS3 protease

Plasmid Transformation

1d

2 ZVNS2B3 N-terminal His-GST tagged co-expression construct was inoculated from its BL21(DE3)-RR glycerol stock.

Note

The ZVNS2B-NS3 co-expression construct encodes the NS2B and NS3 protease with a N-terminal His-GST tag fusion on a kanamycin resistant plasmid backbone with a T7 promoter.

Protein expression

2d 10h

3 Scrape off some of the glycerol stock with a sterile loop and use this to inoculate a 50 mL falcon tube containing 10 mL of LB supplemented with [M] 50 Mass Percent kanamycin. Grow the starter culture at 37 °C Overnight with 200 rpm shaking.

4h

4 Use 10 mL starter culture to inoculate every 1 L TB media (see Materials) supplemented with [M] 50 Mass Percent kanamycin in a baffled flask. 200 rpm, 37°C

6h

Note

For this protocol 2L of pellet was grown for purification

5 When the OD_{600} approximately 1.8, add 1mM IPTG. Lower the temperature and shaker speed to 180 rpm, 18°C. Incubate overnight.

1d

6 Harvest the cell by centrifugation at 4000 x g, 4°C, 00:30:00 . Discard supernatant and store pellet by freezing at -80 °C . 30m

Note

For reference: total pellet weight from 2L TB media was 33g

Protein Purifcation 2d

7 **Lyse cell pellet** 2h 30m

7.1 1h

Note

See Materials tab for buffer compositions.

Note

ZV NS2B-NS3 His-GST fusion protein properties

Before tag cleavage:
MW = 32812.5 Da
E (assume all Cys reduced)= 51340 mM-1cm-1
PI = 5.47

After tag cleavage:
NS2B
MW = 5063.51 Da
E(assume all Cys reduced) = 6990
PI = 3.98

NS3
MW = 18095.66 kDa
E(assume all Cys reduced) = 30940
PI = 6.73

These values are determined by Expasy ProtParam

Thaw and resuspend the pellet in ~7mL of lysis buffer per g of pellet. Stir gently with magnetic stir bar at Room temperature for 00:30:00 to allow lysozyme and bezonase to start breaking down cell components.

7.2 Lyse by sonication 00:00:04 On 00:00:12 Off for a total 'on' time of 00:07:00 at 50% amplitude to fully rupture the cells. Ensure pellet is 0 °C during sonication to prevent overheating. 30m

7.3 Centrifuge the lysed cells for 38000 x g, 4°C, 01:00:00 to remove insoluble cell debris, and collect supernatant in a bottle 4 °C 1h

8 **Perform IMAC to extract target protein from the lysed cell mixture**

8.1 Dispense 3 mL Nickle affinity resin Ni Sepharose 6 FF - Cytiva into a gravity flow column. Equilibrate resin by first rinsing with ~ 10 CV distilled water, then ~ 10 CV binding buffer to remove the storage solution. 10m

8.2 Resuspend the equilibrated resin with some binding buffer and add to the supernatant bottle. Incubate the resin with the supernatant for 00:30:00 while rotating or otherwise mixing gently at 4 °C 30m

8.3 Load the resin/supernatant mix back onto the gravity flow column, retaining the FT separately for SDS-PAGE analysis. 30m

Note

For SDS-PAGE samples, mix 15uL sample with 5uL 4x sample buffer, supplemented with 10mM DTT.

8.4 Wash the column with 10 CV of base buffer, followed by 10 CV of wash buffer 1. Incubate for 10 minutes. This is to digest any DNA that might have bound to the resin. 30m

8.5 Allow wash buffer 1 to drain. Wash resin with 10 CV of wash buffer 2. This is to remove non-specific, weak binding of contaminant proteins from the resin for a cleaner elution.

Collect washes separately for SDS-PAGE analysis.

8.6 Elute the protein with 2.5 CV of elution buffer. 20m

8.7 Repeat step 8.6 one more time, collecting a total of 2 separate elution fractions. This is to ensure maximum retrieval of protein from the resin. 20m

Measure the total protein concentration of the elutions by Nanodrop. Although still a mixture, A280 value can give an estimate of the protein content, which will determine how much protease need to be added to remove the affinity tag.

8.8 Wash used IMAC resin with 10CV of base buffer, and leave in the column submerged in a small amount of base buffer such that the resin is kept moist.
This washed IMAC resin will later be reused for reverse IMAC (rIMAC)

9 Run SDS-PAGE of all samples from total lysis supernatant to final elution. Stain gel with protein staining solution Coomasssie Blue and determine which fractions contain the target protein by finding the band corresponding to the target molecular weight. 40m

Note

The target protein is expected to be present mostly in the elution samples, although small amounts may be found in the FT and washes.
If that is not the case, then further troubleshooting is required.

10 **Elution de-salting, tag cleavage and reverse IMAC** 1d

10.1 Pool and desalt the two elutions using HiPrep 26/10 deasalting columns, run on AKTA pure at the maximum flow rate of 10mL/min. 30m

Note

Sample can be injected into the desalting column using a Cytiva Superloop or sample pump.

Note

This is to reduce imidazole concentration in the sample. High concentration of imidazole will inhibit protease activity during tag cleavage and removal.

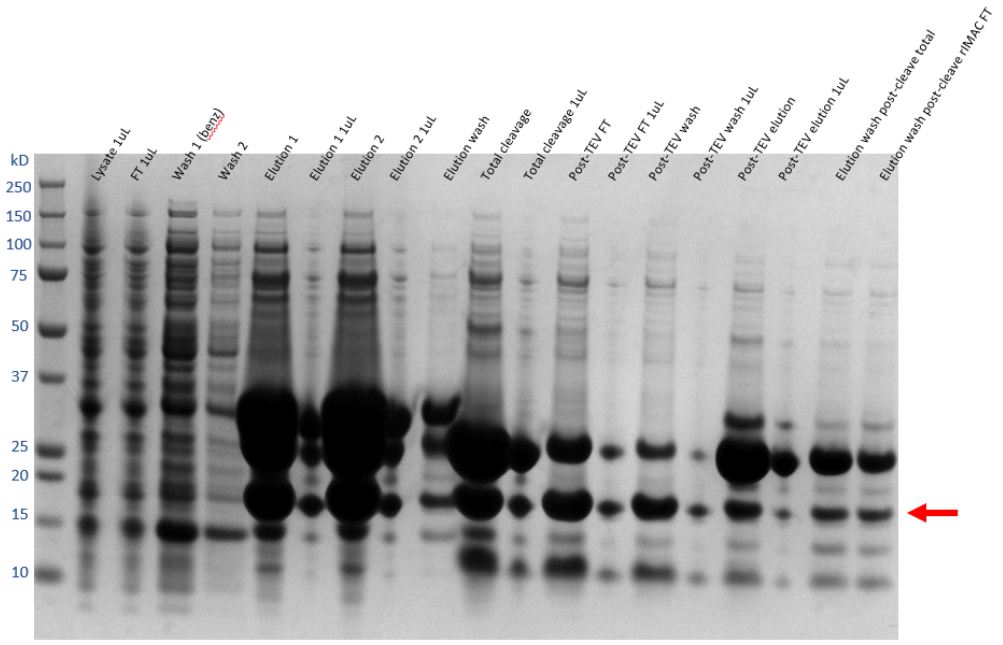
10.2 For tag removal, His-TEV was added in 1:100 ratio to the total protein content of the desalted sample, as determined by nanodrop. The mixture was left in the cold room at 4 °C Overnight 1d

10.3 In morning, pour the cleavage mixture over the washed resin three times and collect final FT. 30m

Note

This step will remove the cleaved tag and any uncleaved target from the sample. If the protease used is His-tagged, then the protease is removed from sample too.

10.4 Wash rIMAC resin with 2 CV rIMAC wash buffer to remove any target protein still bound to the resin. 30m
Take samples of the FT and wash, characterise content by SDS-PAGE



SDS-PAGE analysis of IMAC and cleavage fractions. The band highlighted by red arrow agrees with the size of the cleaved NS3 construct (18095.66 kDa)

10.5 (Optional) elute rIMAC resin with 2 CV elution buffer to confirm if the protein shows non-specific binding to the resin used. 5m

> Note
>
> This will help determine if the protein is "sticky" to the Ni resin matrix material, and help in further troubleshooting if the final yield is lower than expected.

11 **Purify sample further by size exclusion chromatography**. 6h

11.1 Using 10,000 MWCO spin concentrators, concentrate the rIMAC step containing fractions of the target protein to a final volume of under 5 mL . 1h

11.2 Remove any solid aggregates from the sample by centrifugation at 17200 x g, 4°C, 00:10:00 , then immediately draw up the supernatant with a 5mL syringe and a blunt-tip fill needle, taking care not to disturb the pellet. 15m

Note

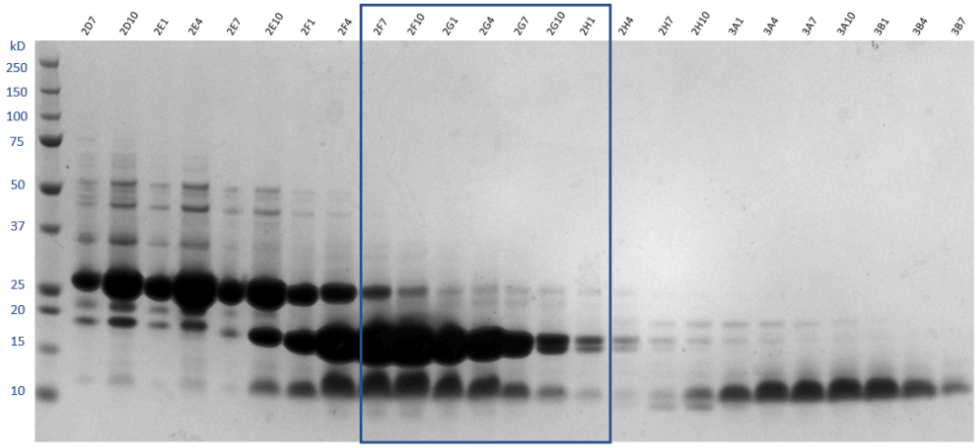
This is to remove as much solid particles from the injection sample as possible, so as to not clog the in-line filter or frit of the column.

12 Using the AKTA Pure system: 2h

Inject the sample onto a 5mL sample loop.

Run the sample down HiLoad 16/60 Superdex 75 pg gel filtration column at 1mL/min in gel filtration buffer, collecting 1mL aliquots.

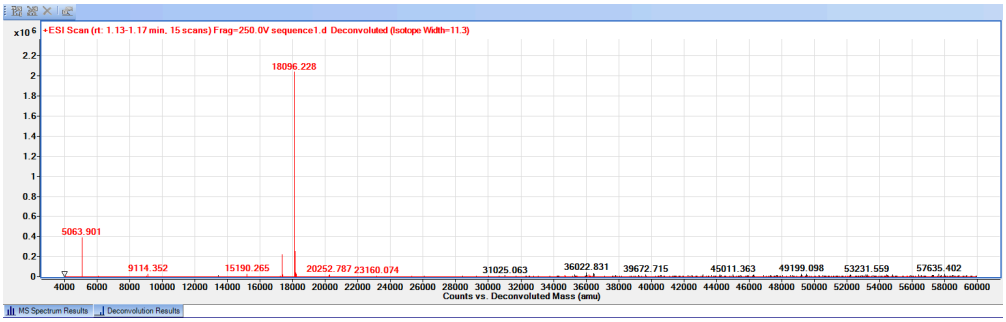
13 From the chromatogram, fraction F9-H8 analyse by SDS-PAGE. 1h



SDS-PAGE analysis of SEC fraction 2D7-3B7. Fractions 2F7-H1 were pooled as they contain majority target protein in comparison to contaminants.

13.1 Take the fractions that contain the target protein, which in this case are fraction E10-F4. Concentrate the final sample in Vivaspin 500 10kda MWCO centrifugal concentrator until the concentration reaches > [M] 23 mg/mL or [M] 1 millimolar (mM) . 30m

Take 1 µL of the final sample for SDS-PAGE(result not shown here), and another for mass spectroscopy (MS).



Intact MS of the final protein sample. The main deconvoluted mass, 5069 Da and 18096 Da, agrees with the size of the cleaved NS2B and NS3 construct.

13.2 Aliquot into appropriate volumes for future usage to minimise freeze/thaw cycles. Flash-freeze in liquid nitrogen, and store at -80 °C until required. 10m