Oct 23, 2024 Version 1

West Nile Virus NS2B-NS3 protease fusion protein expression and purification: large scale 1 L cultures V.1

Forked from Parallel rapid expression and purification of proteins for crystallography (PREPX): large scale 1 L cultures

DOI
dx.doi.org/10.17504/protocols.io.261gerppjl47/v1

Michael Fairhead[1]

[1]university of oxford

ASAP Discovery

Michael Fairhead
university of oxford

DOI: https://dx.doi.org/10.17504/protocols.io.261gerppjl47/v1

External link: https://orcid.org/0000-0001-5361-3933

Protocol Citation: Michael Fairhead 2024. West Nile Virus NS2B-NS3 protease fusion protein expression and purification: large scale 1 L cultures. **protocols.io https://dx.doi.org/10.17504/protocols.io.261gerppjl47/v1**

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

Created: October 23, 2024

Last Modified: October 23, 2024

Protocol Integer ID: 110606

Keywords: parallel protein purification, Recombinant protein, Escherichia coli, purification of west nile virus ns2b, mg of west nile virus ns2b, ns3 protease fusion protein per litre, west nile virus ns2b, ns3 protease fusion protein, ns3 protease fusion protein expression, ns3 protease fusion, fusion protein, cell lysis the fusion protein, tev protease tag cleavage, purification, protein

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 method describes a protocol for the 1 L scale expression and purification of West Nile Virus NS2B-NS3 protease fusion protein in *E. coli* using autoinduction growth media. After enzyme detergent based cell lysis the fusion protein is purified using his tag chromatography, TEV protease tag cleavage, a reverse chromatography step and finally gel filtration. Final yield is 47 mg of West Nile Virus NS2B-NS3 protease fusion protein per litre of culture grown

Attachments

DOCX nyikb5id7.docx 24KB

PDF PAGE22-01614 (1).pdf 2.4MB

Guidelines

Method overview

Expression and purification of West Nile Virus NS2B-NS3 protease fusion protein in *E. coli* using autoinduction growth media followed by purification using his tag chromatography, TEV protease tag cleavage, revIMAC and gel filtration

IMAC = Immobilized Metal Affinity Chromatography commonly used for histidine tagged protein purification

Materials

West Nile Virus NS2B-NS3 fusion protein inactive construct, active site catalytic histidine to alanine mutation (***A***)

- Vector: pNIC (Kanamycin resistance)
- Cell line: E. coli strain BL21(DE3)-RR
- Tags and additions: N-terminal 6His Twin Strep and TEV site

Tagged protein prior to TEV cleavage

- MHHHHHHSSGASWSHPQFEKGGGSGGGSGGSAWSHPQFEKGSGVDLGTENLYFQSMSTDMWIERTADISWESDAEI TGSSERVDVRLDDDGNFQLMNDPGAPWKGGGGSGGGGGVLWDTPSPKEYKKGDTTTGVYRIMTRGLLGSYQAGAGV MVEGVFHTLW***A***TTKGAALMSGEGRLDPYWGSVKEDRLCYGGPWKLQHKWNGQDEVQMIVVEPGKNVKNVQTKPGV FKTPEGEIGAVTLDFPTGTSGSPIVDKNGDVIGLYGNGVIMPNGSYISAIVQGERMDEPIPAGFEPEMLRKK
- MW = 32029.59 Da
- Ext Coeff = 66920 M^{-1} cm^{-1}
- pI = 5.36

Untagged protein after TEV cleavage

- SMSTDMWIERTADISWESDAEITGSSERVDVRLDDDGNFQLMNDPGAPWKGGGGSGGGGGVLWDTPSPKEYKKGDTT TGVYRIMTRGLLGSYQAGAGVMVEGVFHTLW***A***TTKGAALMSGEGRLDPYWGSVKEDRLCYGGPWKLQHKWNGQDE VQMIVVEPGKNVKNVQTKPGVFKTPEGEIGAVTLDFPTGTSGSPIVDKNGDVIGLYGNGVIMPNGSYISAIVQGERMDEPI PAGFEPEMLRKK
- MW = 26335.58 Da
- Ext Coeff = 54430 M^{-1} cm^{-1}
- pI = 4.83

100 mL Thomson SINGLE StEP **Generon** **Catalog #**9452092-100

Ni Sepharose 6 Fast Flow **Cytiva** **Catalog #**17531801

Super Broth **Formedium** **Catalog #**SPB0102

AIM – Terrific Broth Base including Trace elements **Formedium** **Catalog #**AIMTB0210

Ultra Yield 2.5L Flask, Sterile **Generon** **Catalog #**931136-B

AirOtop Enhanced Flask seals **Generon** **Catalog #**899425

SnakeSkin™ Dialysis Tubing, 10K MWCO, 35 mm **Thermo Fisher** **Catalog #**88245

Materials (1 L cultures) for Expression:

- Plates with LB-agar+antibiotics
- 1 L of autoclaved autoinduction TB + 20 g/L glycerol + antibiotics
- 1 mL of 10 % Antifoam 204 (Sigma) in ethanol
- 2.5 L Ultra Yield flasks (fitted with loose foil cover**)

Materials (1 L cultures) for Purification:

- **1L of Base Buffer**

	A	B
	HEPES	10 mM
	Glycerol	5%
	NaCl	500 mM
	TCEP, pH 7.5	0.5 mM

- 100 mL of [M] 3 Molarity (M) imidazole pH 7.5.
- 100 mL of 10 % Triton X-100 in water.
- 100 mg /mL Lysozyme solution (100 x).
- 1 mg /mL homemade benzonase (1000x). Maybe substituted for 10 mg/mL of commercial DNase I

Troubleshooting

Safety warnings

Triton x-100 is currently restricted for use in the EU and cannot be used without an exemption certificate REACH Annex XIV (Jan 2021). It can be readily subsituted with IGEPAL CA-630 (which is likely to be subject to the same restrictions in the near future). Alternatives that also maybe used are Tergitol 15-S-9 or Tween-20 or octyl glucoside.

Expression

12h 20m

1 Transform *BL21 (DE3)* with the appropriate plasmid and spread onto LB-agar plate + 50 µg/mL kanamycin and incubate Overnight 37 °C *. 4h

2 Use several colonies to inoculate 10 mL of superbroth + 1 % glucose + 50 µg/mL kanamycin in a 50 mL tube and 250 rpm, 37°C, 16:00:00 16h

3 Use 10 mL to inoculate 1 L AIM-TB + 50 µg/mL kanamycin + 0.01% Antifoam 204 in a 2.5 L Ultra Yield baffled flask (Thomson) fitted with an AirOtop enhanced flask seal (Thomson)

4 Grow 250 rpm, 37°C, 04:00:00 shaking. 4h

5 Grow 250 rpm, 18°C, 24:00:00 shaking. 1d

6 Harvest at 4000 x g, 4°C, 00:20:00 . 20m

7 Scrape out pellet and place in plastic polygrip bag and place in -80 °C freezer. Final wet cell weight is 45 g/L of culture

Cell lysis

3h 30m

8 Place polygrip bag on flat surface and smash cell pellet into small pieces and pour into 500 mL beaker.

9 Add 4 mL Base Buffer/g cell pellet ([M] 10 millimolar (mM) HEPES, [M] 500 millimolar (mM) NaCl, 5 % Glycerol, [M] 0.5 millimolar (mM) TCEP, pH 7.5)

+ 0.5 mg/mL Lysozyme, 1 µg/ml Benzonase or 10 µg/ml DNase I, 1 % Triton X-100***, [M] 30 millimolar (mM) imidazole.

Note

***Triton x-100 is currently restricted for use in the EU and cannot be used without an exemption certificate REACH Annex XIV (Jan 2021). It can be readily subsituted with IGEPAL CA-630 (which is likely to be subject to the same restrictions in the near future). Alternatives that also maybe used are Tergitol 15-S-9 or Tween-20 or octyl glucoside.

10 Use stripette to dissolve pellet and put up to 45 mL in a 50 mL tube (4 tubes in total).

11 Leave 00:30:00 Room temperature . 30m

12 Place in -80 °C freezer overnight.

13 Thaw in Room temperature water bath 01:00:00 and mix. 1h

14 Centrifuge 4000 x g, 4°C, 01:00:00 . 1h

Purification 3h 30m

15 Apply supernatant to 5 mL Ni Sepharose 6 Fast Flow (Cytiva) in a plastic 100 mL SINGLE StEP column (Thomson) pre-equilibrated in 50 mL Base Buffer + [M] 30 millimolar (mM) Imidazole.

16 Wash 50 mL Base Buffer + [M] 30 millimolar (mM) Imidazole.

Purification

3h 30m

16.1 repeat step 16 a total of 3 times, i.e. wash column with a total of 150 mL Base Buffer + [M] 30 millimolar (mM) Imidazole.

Purification

3h 30m

17 Elute protein with 10 mL of Base Buffer + [M] 500 millimolar (mM) Imidazole and collect elution.

Purification

3h 30m

17.1 repeat step 17 and combine the two elution's, giving a final volume of 20 mL.

Purification

3h 30m

18 Measure A280 of elution, should be around 20 mL with an A280 of 16 or approximately a total of 178 mg of West Nile Virus NS2B-NS3 protease fusion protein.

19 Add 1 OD unit of TEV protease for every 10 OD units target, specifically 18 mg of TEV was added to the 178 mg of West Nile Virus NS2B-NS3 protease fusion protein

Purification

3h 30m

20 Dialyse TEV cleavage reaction Overnight 4 °C against 3 L of Base Buffer using a 10,000 MWCO SnakeSkin dialysis membrane (ThermoFisher).

1h

21 Equilibrate 5 mL of Ni Sepharose 6 Fast Flow (Cytiva) in a plastic 100 mL SINGLE StEP column (Thomson) with 50 mL Base Buffer + [M] 30 millimolar (mM) Imidazole.

Purification

3h 30m

22 Tun the TEV cleavage reaction over the 5 mL of Ni Sepharose 6 Fast Flow (Cytiva) and collect the flow through.

Purification

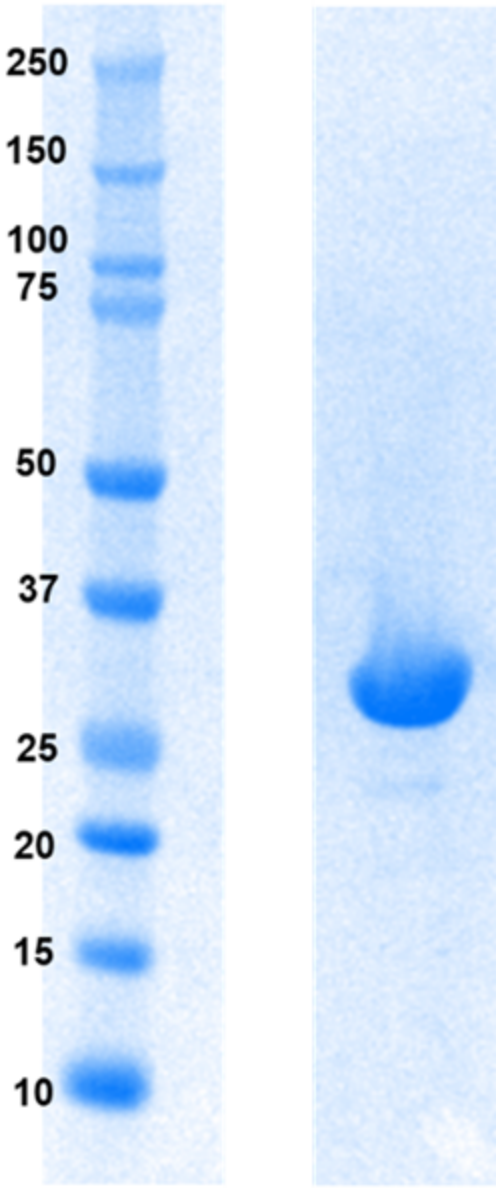
3h 30m

22.1 Wash the column with 15 mL of Base Buffer + [M] 30 millimolar (mM) Imidazole and combine with the collected flow through.

Purification

3h 30m

23 Check purity of cleaved West Nile Virus NS2B-NS3 protease fusion protein on SDS-PAGE, 136 mg of cleaved West Nile Virus NS2B-NS3 protease fusion protein should be obtained



SDS-PAGE West Nile Virus NS2B-NS3 protease fusion protein TEV cleavage and reverse IMAC Samples run on NuPAGE Bis-Tris 4-12% gels (Invitrogen), 200V 40 minutes using MES running buffer (Invitrogen)

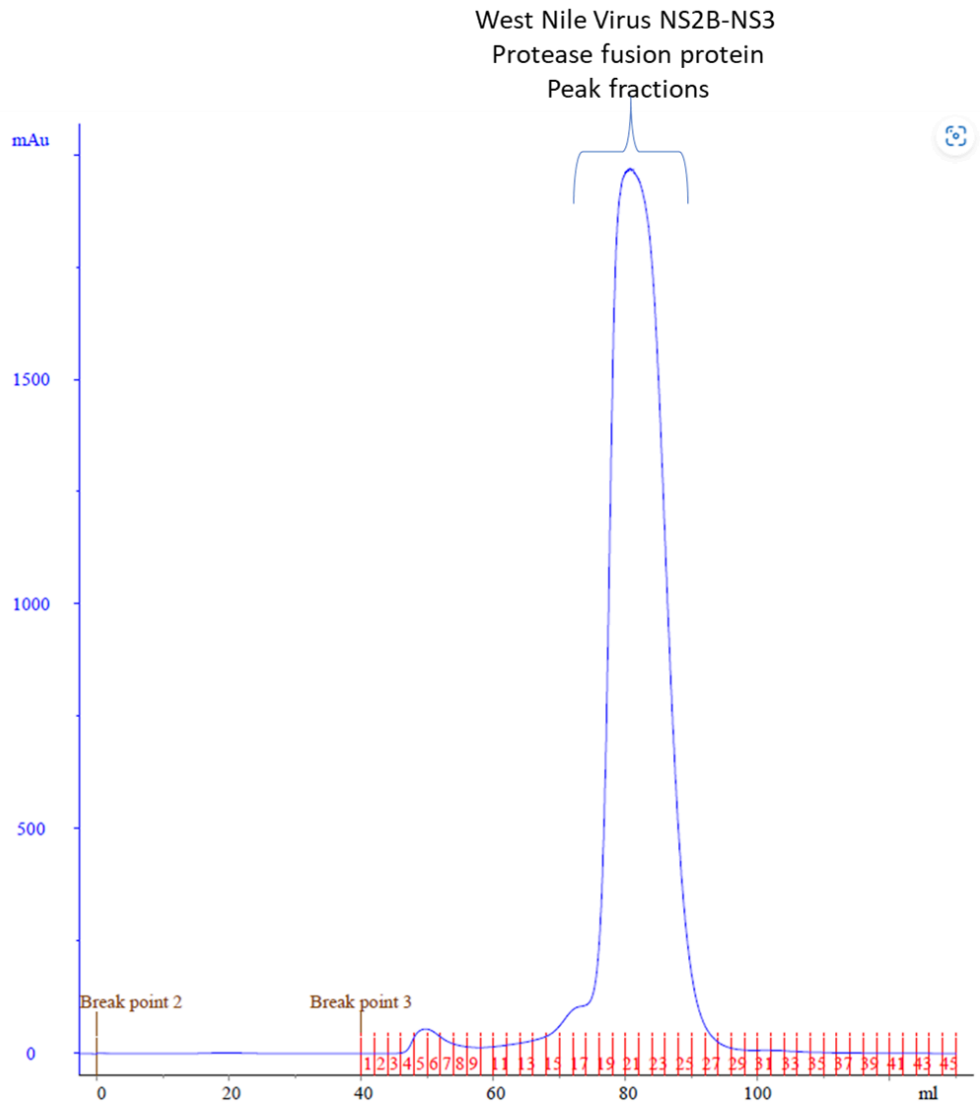
Purification

3h 30m

24 Concentrate to 25 mg/mL using a 10,000 MWCO centrifugal filter (Amicon, Merck-Millipore) and inject the sample (5 mL) onto 125 mL superose 12 pg column pre-equilibrated in Base Buffer. Collect 2 mL fractions using a flow rate at 1 mL/minute.

A superdex 75 pg column or equivalent may also be used.

Chromatogram using Base Buffer as the mobile phase



West Nile Virus NS2B-NS3 protease fusion protein SEC chromatogram profile on Superose 12 pg 125 mL using Base Buffer as the mobile phase

25 Pool the peak fraction(s) (e.g. 19-24 in the chromatogram above) and concentrate to 10 mg/mL using a 10,000 MWCO centrifugal filter (Amicon, Merck-Millipore)
Snap freeze protein using liquid nitrogen as 0.1 mL aliquots and store the sample at -80°C until use
Final yield is 47 mg of West Nile Virus NS2B-NS3 protease fusion protein per litre of culture grown.