



Jun 05, 2025

Version 2

West Nile Virus NS2B-NS3 inactive fusion protease expression and purification: large scale 1 L cultures V.2



Version 1 is forked from [Parallel rapid expression and purification of proteins for crystallography \(PREPX\): large scale 1 L cultures](#)



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Protocol status: Working

We use this protocol and it's working

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Abstract

This method describes a protocol for the 1 L scale expression and purification of West Nile Virus NS2B-NS3 inactive fusion protease 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. In this version, we have added the Addgene id of the plasmid used in the protein expression and purification.



Attachments



[nyikb5id7.docx](#)

24KB



[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

- MHHHHHHSSGASWSHPQFEKGGGSGGGGSAWSHPQFEKGSVDLG TENLYFQSMSTDMWIERTADISWESDAEITGSSERVDVRLDDDGNFQLMNDPGAPWKGGGGSGGGGGVLWDTPSPKEYKKGDTT TGVYRIMTRGLLGSYQAGAGVMVEGVFHTLW**A**TTKGAALMSGEGRLDPYWGSVKEDRLCYGGPWKLQHKWNGQDEVQMIVVEPGKNVKNVQTKPGVFKTPEGEIGAVTLDFPTGTSGSPIVDKNGDVIGLYGNGVIMPNGSYISAIVQGERMDEPIPAGFEPEMLRKK
- MW = 32029.59 Da
- Ext Coeff = 66920 M⁻¹ cm⁻¹
- pI = 5.36

Untagged protein after TEV cleavage

- SMSTDMWIERTADISWESDAEITGSSERVDVRLDDDGNFQLMNDPGAPWKGGGGSGGGGGVLWDTPSPKEYKKGDTT TGVYRIMTRGLLGSYQAGAGVMVEGVFHTLW**A**TTKGAALMSGEGRLDPYWGSVKEDRLCYGGPWKLQHKWNGQDEVQMIVVEPGKNVKNVQTKPGVFKTPEGEIGAVTLDFPTGTSGSPIVDKNGDVIGLYGNGVIMPNGSYISAIVQGERMDEPIPAGFEPEMLRKK
- 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 1M 3 Mass Percent 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

Protocol materials

 WNV NS2B-NS3 protease (catalytically inactive); West Nile NS2B-NS3 fusion **addgene Catalog #204795**

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 substituted 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 *.

WNV NS2B-NS3 protease (catalytically inactive); West Nile NS2B-NS3 fusion **addgene Catalog #204795**
- 2 Use several colonies to inoculate 10 mL of superbrotth + 1 % glucose + 50 µg/mL kanamycin in a 50 mL tube and 250 rpm, 37°C, 16:00:00
- 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.
- 5 Grow 250 rpm, 18°C, 24:00:00 shaking.
- 6 Harvest at 4000 x g, 4°C, 00:20:00 .
- 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 (10 millimolar (mM) HEPES, 500 millimolar (mM) NaCl, 5 % Glycerol, 0.5 millimolar (mM) TCEP, 7.5)



+ 0.5 μ L Lysozyme, 1 μ L Benzonase or 10 μ L DNase I, 1 % Triton X-100***, 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 substituted 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 + 30 millimolar (mM) Imidazole.

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

Purification

3h 30m

- 17 Elute protein with  10 mL of Base Buffer +  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 +  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

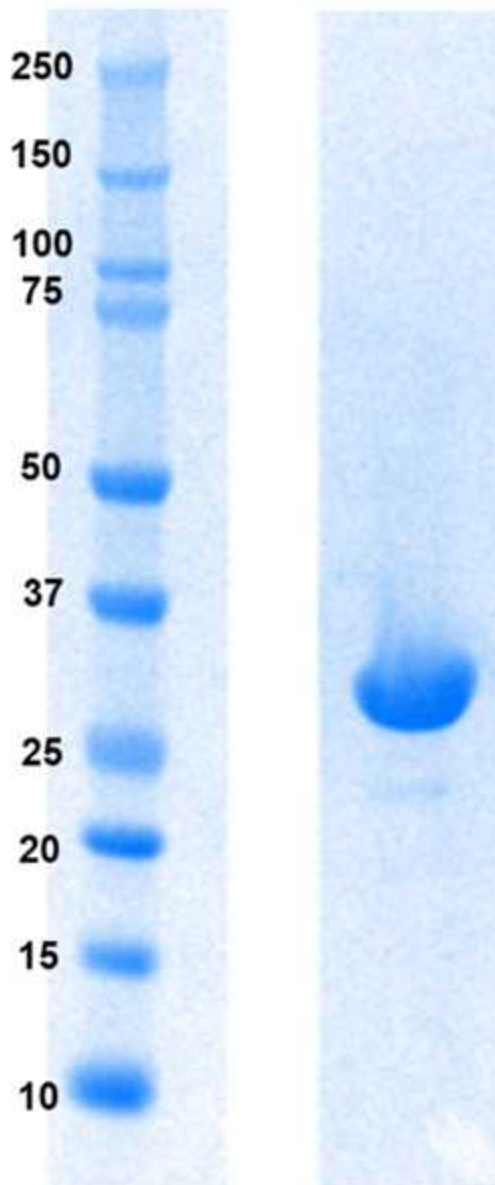


- 22.1 Wash the column with 15 mL of Base Buffer + [M] 30 millimolar (mM) Imidazole and combine with the collected flow through.

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)

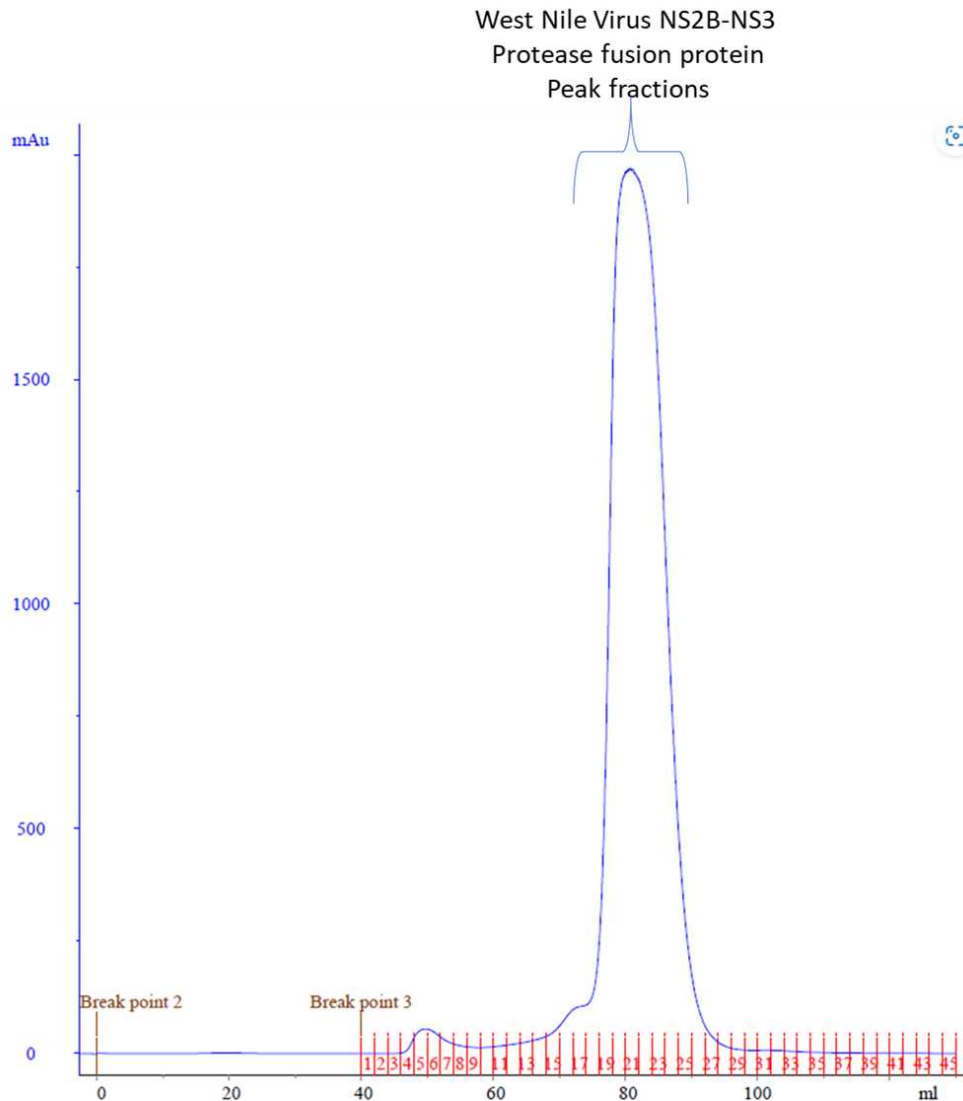
Purification

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.