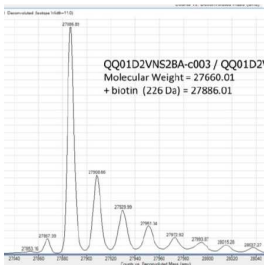
Apr 15, 2025

# Dengue Virus Serotype 2 NS2B-NS3 protease fusion protein Avi-tagged (Biotinylated) protein expression and purification: large scale 1 L cultures




Forked from a private protocol

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

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**Keywords:** parallel protein purification, Recombinant protein, Escherichia coli, ZIKV, Zika, NS2B-NS3 protease, purification of dengue virus serotype, ns3 protease fusion protein avi, dengue virus serotype, ns3 protease fusion protein, recombinant protein, purification, protein expression, protein, biotinylation tag, avitag, virus

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## Disclaimer

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 expression and purification of Dengue Virus Serotype 2 NS2B-NS3 protease fusion protein with a AviTag (biotinylation tag) for biophysical assay at a 1 L culture scale. Recombinant proteins are expressed in *Escherichia coli* using the autoinduction method and then purified in using a IMAC, desalt, tag cleavage, reverse IMAC and gel filtration work flow.

## Attachments

PDF

PAGE24-00124 - AVIDD...

935KB

# Guidelines

### Method overview

Standard workflow is expression via autoinduction followed by purification using IMAC/PD-10/revIMAC and serial gel filtration

## Materials

Dengue Virus Serotype 2 NS2B-NS3 protease fusion protein
Vector: pNIC (Kanamycin resitance)
Cell line: E. coli strain BL21(DE3)-RR + pCDF-BirA plasmid GenBank: JF914075.1 (Streptomycin resistance)
Tags and additions: N-terminal His6 Twin Strep TEV Avitag

Construct protein sequence before tag cleavage
MHHHHHHSSGASWSHPQFEKGGGSGGGSGGSAWSHPQFEKGSGVDLGTENLYFQSGLNDIFEAQKIEWHEMADLELERA
ADVKWEDQAEISGSSPILSITISEDGSMSIKNEEEEQTLGGGGSGGGGAGVLWDVPSPPPMGKAELEDGAYRIKQKGILGYSQ
IGAGVYKEGTFHTMWHVTRGAVLMHKGKRIEPSWADVKKDLISYGGGWKLEGEWKEGEEVQVLALEPGKNPRAVQTKPGL
FKTNAGTIGAVSLDFSPGTSGSPIIDKKGKVVGLYGNGVVTRSGAYVSAIAQTEKSIEDNPEIEDDIFRK

- MW = 27.116 kDa
- Extinction co-efficient = 43430 $M^{-1}$ $cm^{-1}$
- pI = 5.67

Construct protein sequence after tag cleavage
SGLNDIFEAQKIEWHEMADLELERAADVKWEDQAEISGSSPILSITISEDGSMSIKNEEEEQTLGGGGSGGGGAGVLWDVPSP
PPMGKAELEDGAYRIKQKGILGYSQIGAGVYKEGTFHTMWHVTRGAVLMHKGKRIEPSWADVKKDLISYGGGWKLEGEWK
EGEEVQVLALEPGKNPRAVQTKPGLFKTNAGTIGAVSLDFSPGTSGSPIIDKKGKVVGLYGNGVVTRSGAYVSAIAQTEKSIED
NPEIEDDIFRK

- MW = 27.116 kDa, +Biotin MW = 27.886 kDa
- Extinction co-efficient = 47440 $M^{-1}$ $cm^{-1}$
- pI = 4.92

- PD-10 desalting columns packed with Sephadex G-25 resin **Cytiva Catalog #**17085101
- PD-10 Spin Adapter **Cytiva Catalog #**28923245
- LabMate PD-10 Buffer Reservoir **Cytiva Catalog #**18321603
- His GraviTrap **Cytiva Catalog #**11003399
- Super Broth **Formedium Catalog #**SPB0102
- AirOtop Enhanced Flask seals **Generon Catalog #**899425
- Nalgene™ Unwire™ Test Tube Racks: Resmer™ Manufacturing Technology, for 30mm tubes, white **Thermo Fisher Catalog #**5970-0030
- AIM – Terrific Broth Base including Trace elements **Formedium Catalog #**AIMTB0210
- Ultra Yield 2.5L Flask, Sterile **Generon Catalog #**931136-B

**Optional but useful**

BENCHMIXER™ XL MULTI-TUBE VORTEXER **Benchmark Scientific Catalog #**BV1010

**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 Mass Percent imidazole pH 7.5.
- 100 mL of 10 % Triton X-100 in water.
- 10 mL Lysozyme solution (100 x).
- 1 mL homemade benzonase (1000x). Maybe substituted for 10 mg/mL of commercial DNase I
- 2 x His GraviTrap column per litre of culture to be purified (Cytiva) fitted with LabMate extender (Cytiva) and PD-10 spin adapter (Cytiva) in 24 place Nalgene rack.
- 2 x PD-10 desalting column per litre of culture to be purified  fitted with LabMate extender and PD-10 spin adapter in 24 place Nalgene rack.
- 2 × 50 mL centrifuge tubes per litre of culture to be purified in a 24 place Nalgene rack.

## Protocol materials

Dengue virus serotype 2 NS2B-NS3 protease fusion with non cleavable linker and non cleavable avi-tag **addgene Catalog #**228649

## 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

1d 19h

1 Transform *BL21 (DE3)* with the appropriate plasmid

Dengue virus serotype 2 NS2B-NS3 protease fusion with non cleavable linker and non cleavable avi-tag **addgene Catalog #**228649

and spread onto LB-agar plate + 50 µg/mL kanamycin + 50 µg/mL streptomycin and incubate Overnight 37 °C *.

2 Prepare a starter culture by using several colonies to inoculate 10 mL of superbroth + 1 % glucose+ 50 µg/mL kanamycin + 50 µg/mL streptomycin in a 50 mL tube and incubate at 250 rpm, 37°C, 16:00:00 16h

3 Use 10 mL started culture to inoculate 1 L AIM-TB + 50 µg/mL kanamycin + 50 µg/mL streptomycin + 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 Add 1 mL [M] 500 millimolar (mM) D-Biotin dissolved in DMSO

6 Gow 250 rpm, 18°C, 01:00:00 shaking. 1h

7 Add 1 mL [M] 500 millimolar (mM) D-Biotin dissolved in DMSO

Add 1 mL [M] 100 millimolar (mM) IPTG

8 Grow 250 rpm, 18°C, 22:00:00 shaking. 22h

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

10 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

11 Place the polygrip bag on a flat surface and smash cell pellet into small pieces and transfer the cell pellet into 500 mL beaker.

12 Add 3 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 [M] 0.5 millimolar (mM) D-Biotin

> 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 could be used are Tergitol 15-S-9 or Tween-20 or octyl glucoside.

13 Use stripette to dissolve pellet and transfer 45 mL (maximum) to a 50 mL tube (4 tubes in total).

14 Leave 00:30:00 Room temperature .

30m

15 Place tubes in -80 °C freezer for 2h or overnight if preferred.

16 Thaw in Room temperature water bath for 01:00:00 and mix.

1h

17 Centrifuge at 4000 x g, 4°C, 01:00:00 .

1h

## Purification

3h 30m

18 Apply supernatant from 2 × 50 mL tubes to 1 mL His GraviTrap column (Cytiva) fitted with a LabMate PD-10 Buffer Reservoir (Cytiva). His GraviTrap columns are pre-equilibrated in Base Buffer + [M] 30 millimolar (mM) Imidazole.

19 Wash with 20 mL Base Buffer + [M] 30 millimolar (mM) Imidazole
Repeat wash a total of 3 times

20 Slot His GraviTrap column into PD-10 desalting column (Cytiva) fitted with a LabMate PD-10 Buffer Reservoir. PD-10 desalting columns are pre-equilibrated in Base Buffer w/o Imidazole.

21 Elute protein, with 2.5 mL of Base Buffer + [M] 500 millimolar (mM) Imidazole, directly onto PD-10 column.

22 Remove His GraviTrap column.

23 Place PD-10 desalting column into 50 mL falcon tube and add 3.5 mL Base Buffer w/o Imidazole and collect the flow through from the column.

24 Run TEV digested sample over His GraviTrap column to remove protease, tag and un-cleaved protein
Wash column with 2.5 mL Base Buffer + [M] 30 millimolar (mM) Imidazole

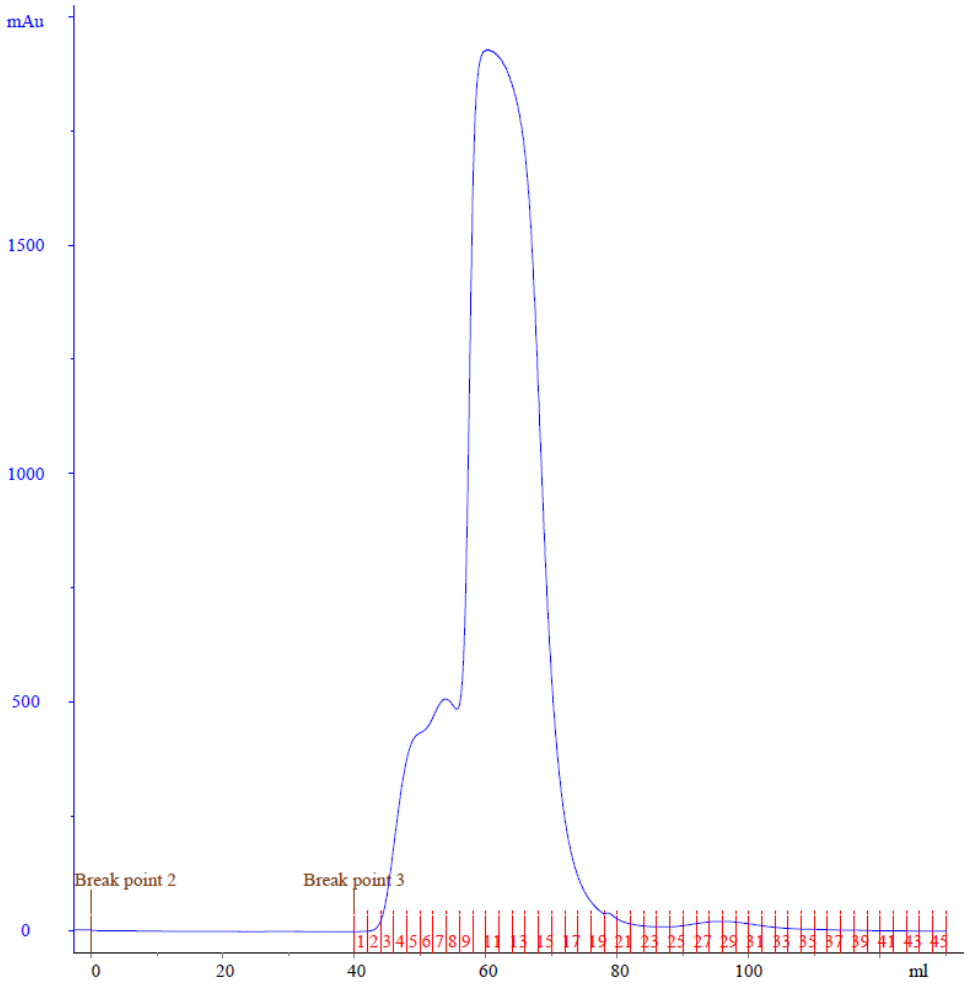
25 Concentrate to 10 mg/mL using a 10,000 MWCO centrifugal filter (Amicon, Merck-Millipore) and inject the sample (5 mL) onto 125 mL supdex 75 pg column pre-equilibrated in Base Buffer. Collect 2 mL fractions using a flow rate at 1 mL/minute.

26 Pool the peak fraction(s) (e.g. 10-16 in the chromatogram below) 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
Approximate final yield is 55 mg

## Purification Results

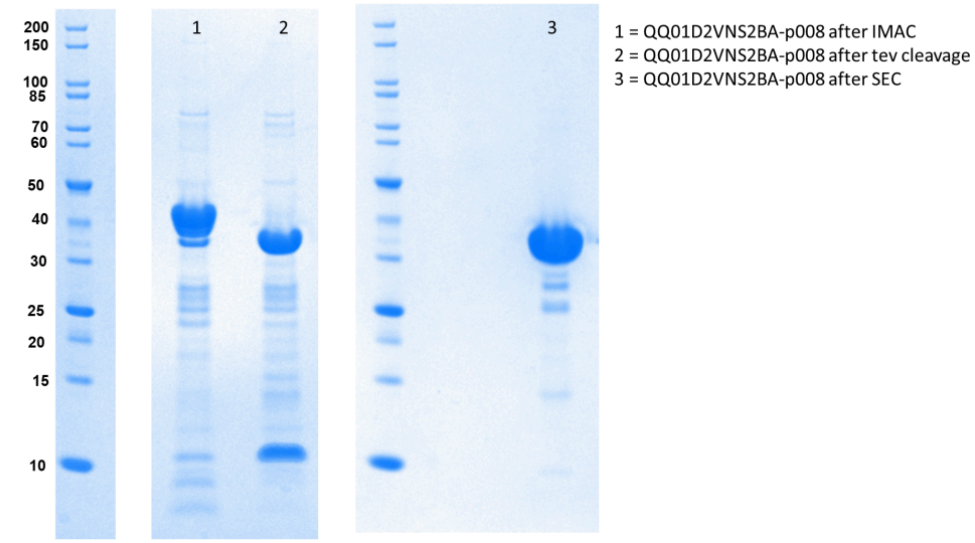
3h 30m

26.1 Chromatogram using Base Buffer as the mobile phase



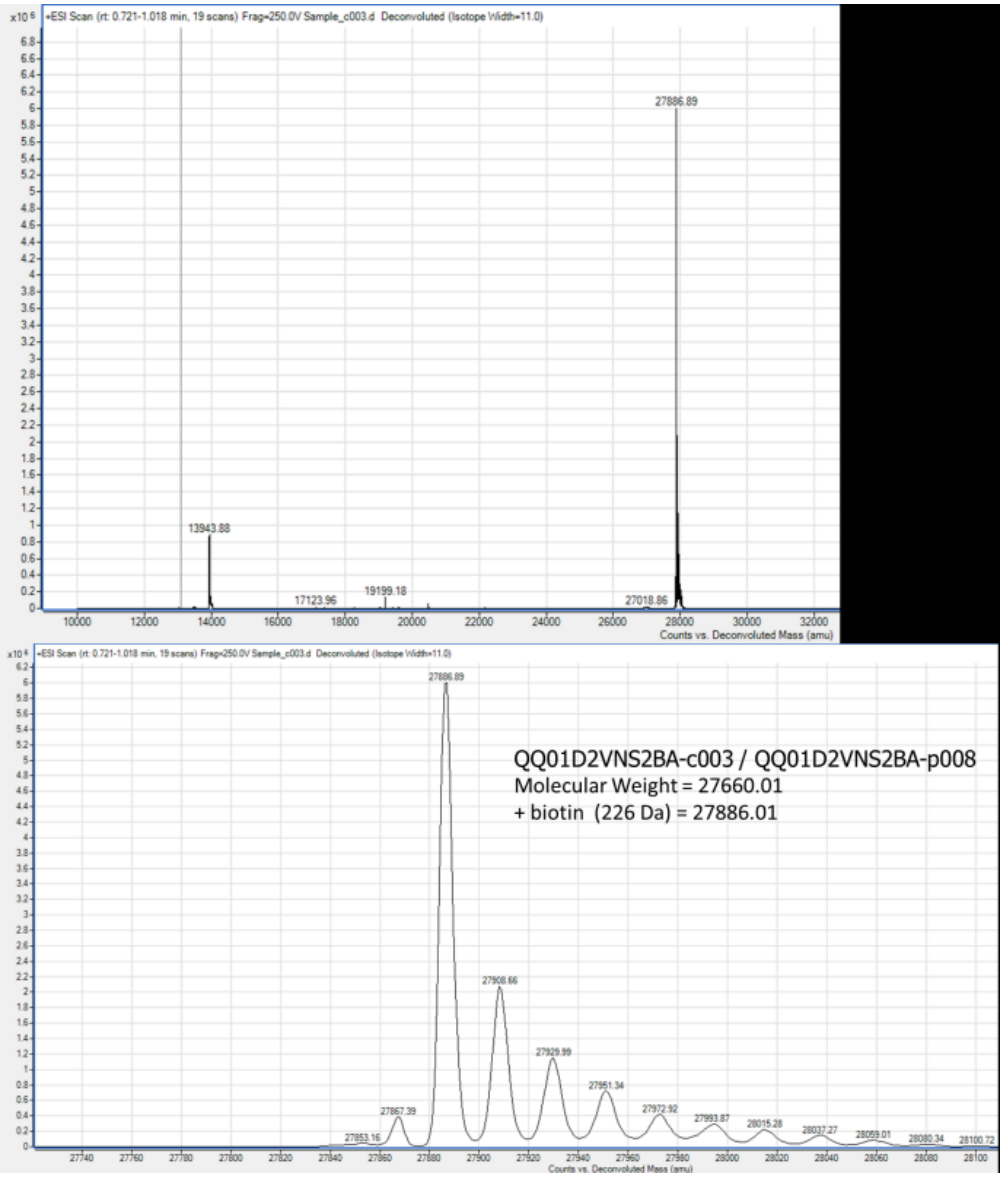

QQ01D2VNS2BA-c003 SEC profile on 125 mL Superdex 75 pg

26.2 SDS PAGE on 4-12% Bis-TRIS gel




QQ01-c003 SEC profile on 125 mL Superdex 75 pg SDS PAGE of samples during purification

27 Mass spectrometry of final sample to confirm biotinylation




QQ01-c003 Mass spectrometry showing the final sample has the expected MW for biotinylated protein

## Column regeneration: PD-10

28 Wash PD-10 columns with 50 mL - 100 mL of Milli-Q water.

29 Store all columns in water at 4 °C. For long term storage, use 20 % Ethanol

## Column regeneration: His GraviTrap

30 Wash the IMAC columns with 40 mL Milli-Q.

31 Wash IMAC columns 10 mL 20 % Ethanol + [M] 0.1 Mass Percent EDTA*.

*PUT NICKEL WASTE IN APPROPRIATE CONTAINER FOR DISPOSAL!

32 Wash IMAC columns 40 mL Milli-Q.

33 Wash IMAC columns 10 mL [M] 1 Mass Percent NaOH.

34 Wash IMAC columns 40 mL Milli-Q.

35 Wash IMAC columns 10 mL [M] 1 Mass Percent Acetic Acid + 1 % Triton X-100.

36 Wash IMAC columns 40 mL Milli-Q.

37 Wash IMAC columns with 0.5 mL [M] 100 millimolar (mM) Nickel Sulfate + [M] 20 millimolar (mM) Tris.HCl pH 8*.

Note

*PUT NICKEL WASTE IN APPROPRIATE CONTAINER FOR DISPOSAL!

38 Wash IMAC colums with 40 mL Milli-Q.

39 Store all columns in water at 4 °C. For long term storage use 20 % Ethanol

## Protocol references

dx.doi.org/10.17504/protocols.io.4r3l22jkxl1y/v1