Jun 17, 2025 Version 3

SARS-CoV-2 nsp3 macrodomain His-tagged expression and purification protocol for assay V.3

Version 1 is forked from SARS-CoV-2 nsp3 macrodomain His-SUMO tagged expression and purification protocol for crystallization (c004)

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

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Abstract

This protocol details the expression and purification of SARS-CoV-2 nsp3 macrodomain assay construct bearing a N-terminal His tag at small scale (<6L). In this new version, we added the addgene ID of the plasmid used.

Attachments

PAGE22-01678 - SARS-...
264KB

Guidelines

- **Construct / plasmid resource-name:** SARS-CoV-2 nsp3 macrodomain assayconstruct bearing a N-terminal His tag.

Materials

Plasmid details:

- Vector: pDEST?
- Cell line: E. coli Rosetta strain BL21(DE3)-RR

Tags and additions: N-terminal His tag.

- Construct protein sequence:
  MSYYHHHHHHLESTSLYKKAGFLEVLFQGPEVNSFSGYLKLTDNVYIKNADIVEEAKKVKPTVVVNAANVYLKHGGGVA
  GALNKATNNAMQVESDDYIATNGPLKVGGSCVLSGHNLAKHCLHVVGPNVNKGEDIQLLKSAYENFNQHEVLLAPLLS
  AGIFGADPIHSLRVCVDTVRTNVYLAVFDKNLYDKLVSSFLEMKSEK

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, 30kDa 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	0.5 mM
	Lysozyme	0.5 mg/mL
	Benzonase	0.05 mg/mL

Prepare 100L per 1L E.coli expression

Base buffer:

	A	B
	Hepes (pH 7.4)	50 mM
	NaCl	500 mM
	Glycerol	5%
	TCEP	0.5 mM

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

Binding buffer: base buffer
Wash buffer: base buffer + 20 mM imidazole
Elution buffer: base buffer + 500 mM imidazole + 50 mM L-Arginine + 50 mM L-Glutamine
Gel filtration buffer: base buffer

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

Protocol materials

Sars Cov 2 Mac domain **addgene Catalog #**204787

Troubleshooting

Version change log

1 22/10/2024
Fixed mistake with mention of protease tag cleavage

17/06/2025
We added:
Addgene id in step 3
ASAP Discovery Consortium as one of the author's affiliation

Abbreviations

2 CV - column volume, total volume of resin in a column
IMAC - immobilised metal affinity chromatography
FT - flow through
CVNSP3mac1 - SARS-CoV-2 nsp3 macrodomain

Plasmid Transformation

1d

3 CVNSP3mac1 N-terminal His-tagged assay construct was inoculated from its BL21(DE3)-RR glycerol stock. Sars Cov 2 Mac domain **addgene Catalog #**204787

Note

This CVNSP3mac1 construct encodes the SARS-CoV-2 nsp3 macrodomain with a N-terminal His-tag fusion, and truncation of the first methionine residue, in pDEST vector.

Protein expression

2d 10h

4 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 carbenicillin. Grow the starter culture at 37 °C Overnight with 200 rpm shaking.

4h

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

6h

Note

For this protocol 6L of pellet was grown for purification.

6 When the OD_{600} approximately 4.0, lower the temperature and shaker speed to 180 rpm, 16°C . Add IPTG to final concentration of [M] 1 millimolar (mM) . Incubate overnight. 1d

7 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

Protein Purifcation 2d

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

8.1 1h

Note

See Materials tab for buffer compositions.

Note

His-CVNSP3mac1 properties

MW = 22.378 kDa
E (assume all Cys reduced)= 14900 mM-1cm-1
PI = 7.00

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.

8.2 Lyse by sonication 00:00:02 On 00:00:04 Off for a total 'on' time of 00:15:00 at 35% amplitude to fully rupture the cells. Ensure pellet is 0 °C during sonication to prevent overheating. 15m 6s

8.3 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

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

9.1 Dispense 2 mL Nickle affinity resin Ni Sepharose 6 FF - Cytiva into a gravity flow column. Equilibrate resin by first rinsing with ~ 10 µL distilled water, then ~ 10 µL binding buffer to remove the storage solution. 10m

9.2 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

9.3 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.

9.4 Wash the column with 10 µL of base buffer, followed by 10 µL of wash buffer twice. Allow wash buffer to pass through completely between washes. 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. 30m

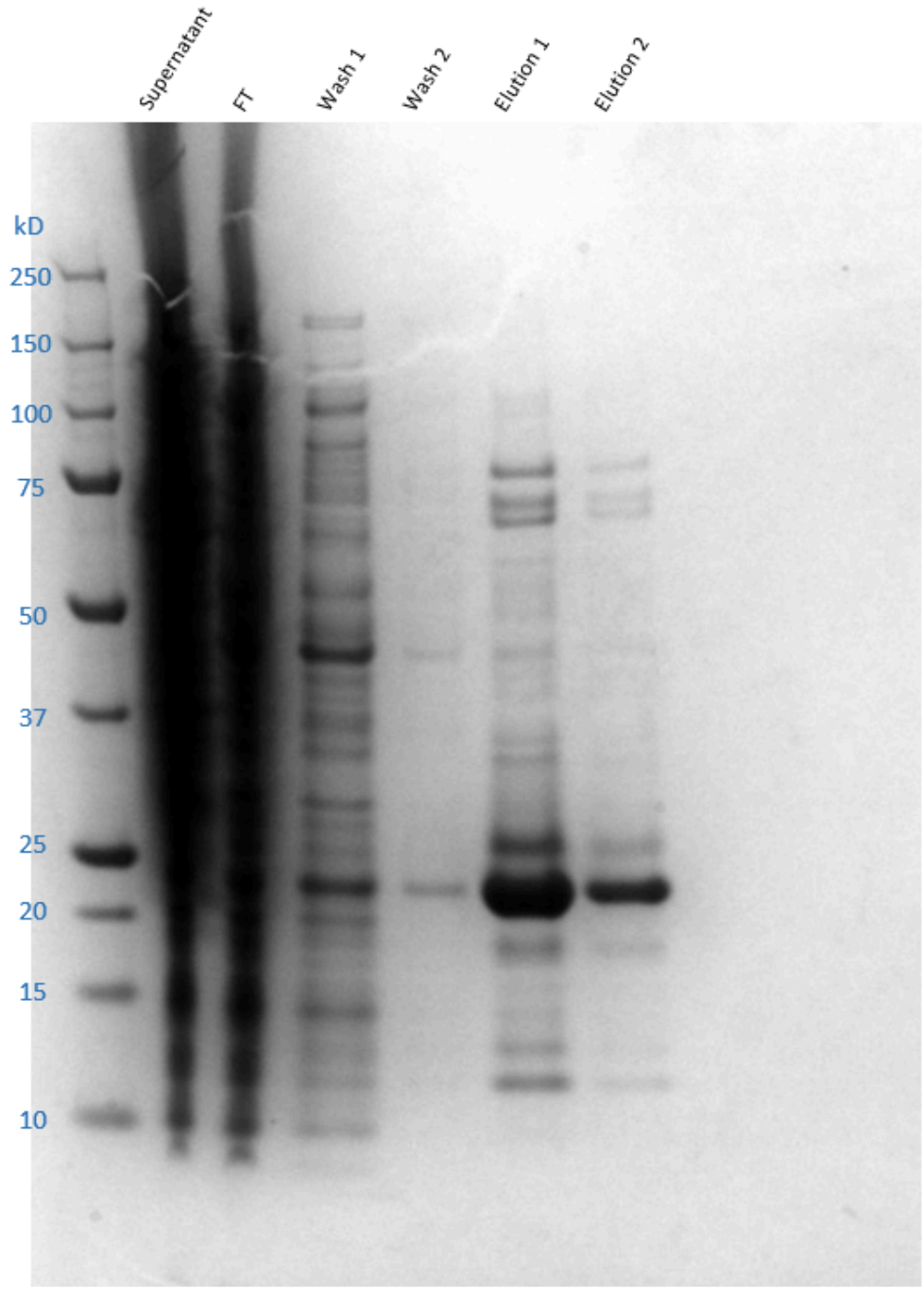
9.5 Elute the protein with 15 mL of elution buffer. 20m

9.6 Repeat step 9.5 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. For example, from 6L TB expression, 2mL Ni Sepharose FF 6 resin, and 15mL elution:

Elution 1: 0.768 mg/mL
Elution 2:0.271 mg/mL

10 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



SDS-PAGE analysis of IMAC fractions. The prominent band in both IMAC elutions corresponds to the correct size of the cleaved target protein (22.378 kDa)

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.

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

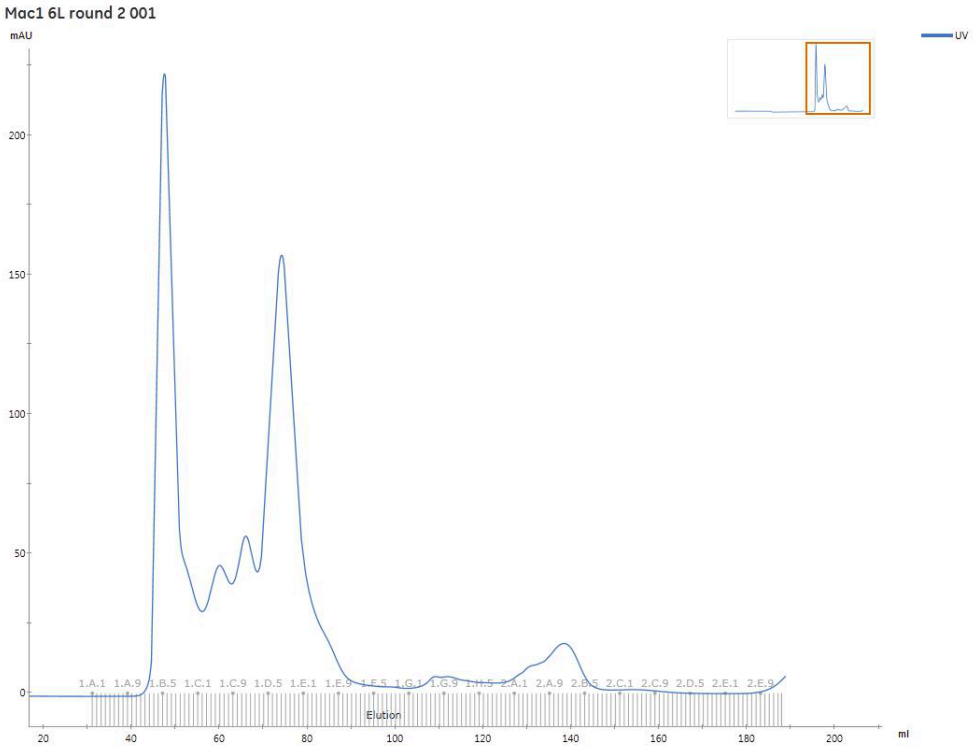
This is to remove as much solid particles from the injection sample as possible, so as to not clog the in-line filter or frit of the column.

12 Using the AKTA Pure system: 2h

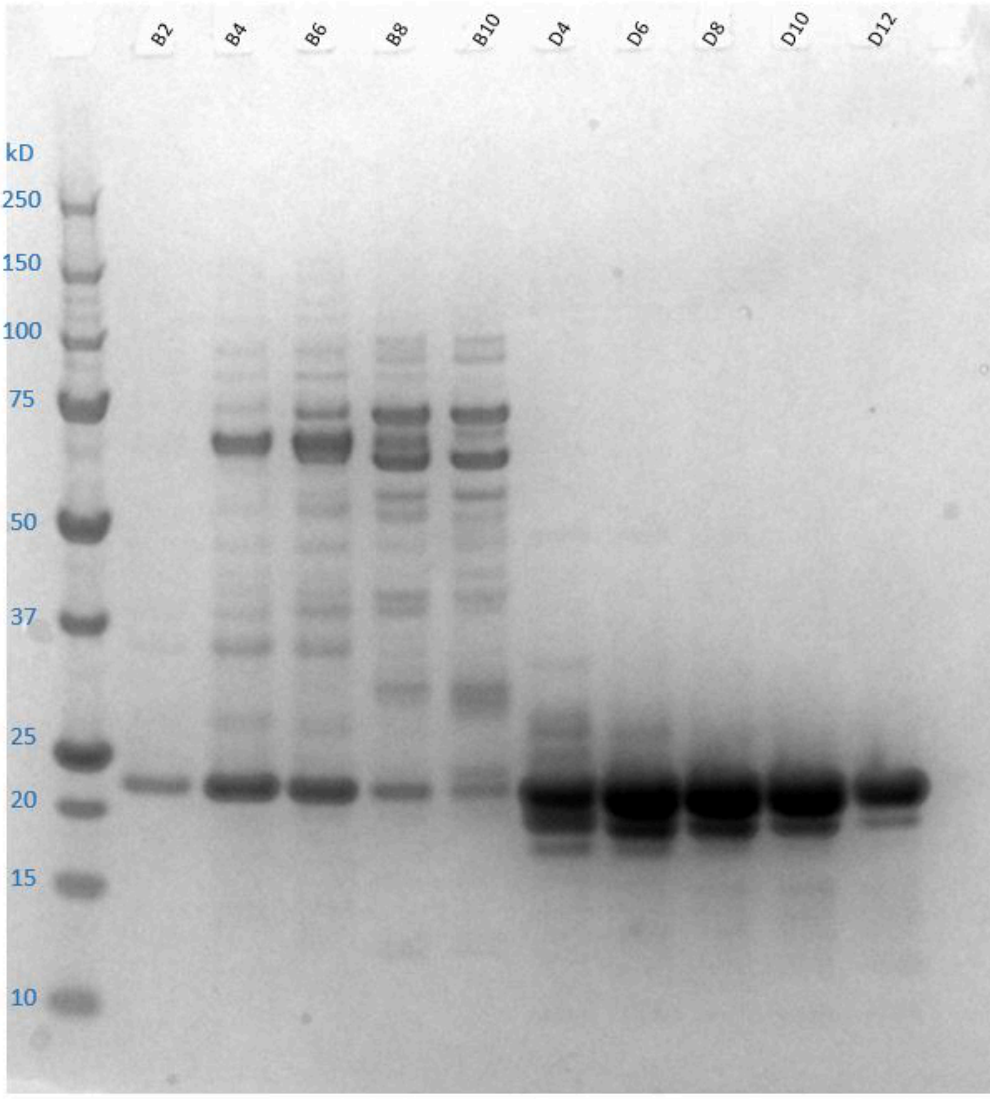
Inject the sample onto a 5mL sample loop.

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



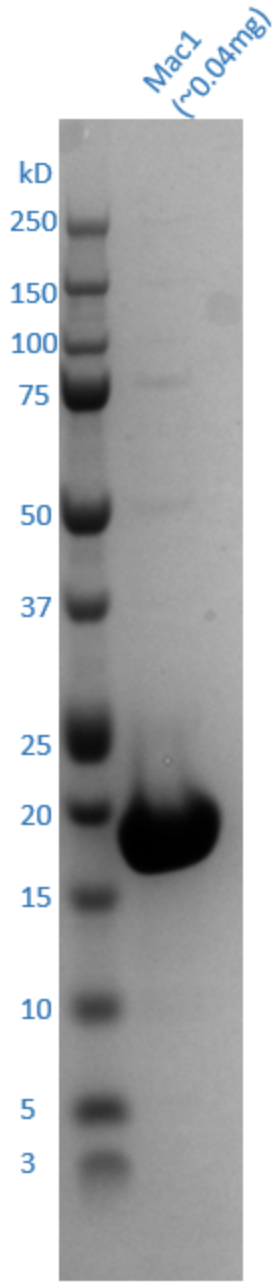
Chromatogram of the cleaved His-CVNSP3mac1 SEC run. Fractions B2-B10 and D4-D12 were analysed by SDS-PAGE to see which contained the target protein



SDS-PAGE analysis of SEC fraction B2-B10 and D4-D12. Fractions D4-D11 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 D4-D11. Concentrate the final sample in Vivaspin 500 10kda MWCO centrifugal concentrator until the concentration reaches > [M] 6 mg/mL . 30m

Take 1 µL of the final sample for SDS-PAGE. Intact MS can also be carried out to confirm sample purity.



SDS-PAGE analysis of final sample

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